Associations of various healthy dietary patterns with biological age acceleration and the mediating role of gut microbiota: results from the China Multi-Ethnic Cohort study

Simplified title

Diet, biological age and gut microbiota

Hongmei Zhang[#1], Haojiang Zuo[#1], Yi Xiang[1], Jiajie Cai[1], Ning Zhang[1], Fen Yang[1], Shourui Huang[1], Yuan Zhang[1], Hongxiang Chen[1], Sicheng Li[2], Tingting Yang[3], Fei Mi[4], Liling Chen[5], Mingming Han[6], Jingzhong Li*[7], Xiong Xiao*[1], Xing Zhao[1]

[1]West China School of Public Health and West China Fourth Hospital, Sichuan University, Chengdu, China

[2]Xiamen Cardiovascular Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, China

[3]School of Public Health, the Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, Guizhou Medical University, Guiyang, China

[4]School of Public Health, Kunming Medical University, Kunming, China

[5]Chongqing Municipal Center for Disease Control and Prevention, Chongqing, China

[6] Chengdu Center for Disease Control and Prevention, Chengdu, China

[7]Tibet Center for Disease Control and Prevention, Tibet, China

[#]Joint first authorship

*Joint corresponding author: Xiong Xiao xiaoxiong.scu@scu.edu.cn, Jingzhong Li 13908996200@139.com

Abbreviations:

aMED: alternative Mediterranean diets

This peer-reviewed article has been accepted for publication but not yet copyedited or typeset, and so may be subject to change during the production process. The article is considered published and may be cited using its DOI
10.1017/S0007114524002733
The British Journal of Nutrition is published by Cambridge University Press on behalf of The Nutrition Society

CMEC: China Multi-Ethnic Cohort study

DASH: Dietary Approaches to Stop Hypertension

FFQ: food frequency questionnaire

HDS: healthy diet score

hPDI: healthful plant-based diet index

KDM-BA: biological age based on Klemera and Doubal's method

KDM-AA: KDM-BA acceleration

LEMRs: less-developed ethnic minority regions

PDI: plant-based diet index

QGC: quantile G-computation

uPDI: unhealthful plant-based diet index

Abstract

To investigate the associations between dietary patterns and biological aging, identify the most recommended dietary pattern for coping with biological aging and explore the potential mediating role of gut microbiota in less-developed ethnic minority regions (LEMRs). This prospective cohort study included 8288 participants aged 30-79 years from the China Multi-Ethnic Cohort study (CMEC). Anthropometric measurements and clinical biomarkers were utilized to construct biological age based on Klemera and Doubal's method (KDM-BA) and KDM-BA acceleration (KDM-AA). Dietary information was obtained through the baseline food frequency questionnaire (FFQ). Six dietary patterns were constructed: plant-based diet index (PDI), healthful plant-based diet index (hPDI), unhealthful plant-based diet index (uPDI), healthy diet score (HDS), Dietary Approaches to Stop Hypertension (DASH), and alternative Mediterranean diets (aMED). Follow-up adjusted for baseline analysis were employed to assess the associations between dietary patterns and KDM-AA. Additionally, quantile G-computation was utilized to evaluate the significant beneficial and harmful food groups. In the subsample of 764 participants with gut microbiota data obtained through 16S rRNA gene sequencing, we used causal mediation model to explore the mediating role of gut microbiota in the associations between dietary patterns and KDM-AA. The results showed that all dietary patterns were associated with KDM-AA. Transitioning from non-compliance to compliance, DASH exhibited the strongest negative association with KDM-AA [β = -0.91, 95%CI (-1.19, -0.63)]. The component analyses revealed that tea and soybean products were the significant beneficial food groups, while salt, preserved vegetables, red and processed meats were identified as the major harmful food groups. In mediation analysis, the decreased abundance of *Synergistetes* phylum and *Pyramidobacter* genus possibly mediated the negative associations between plant-based diets and KDM-AA (5.61%-9.19%). Overall, healthy dietary patterns, especially DASH, are negatively associated with biological aging in LEMRs. The *Synergistetes* and *Pyramidobacter* may mediate the associations between plant-based diets and biological aging. Developing appropriate strategies may promote healthy aging in LEMRs.

Keywords: diet; biological aging; gut microbiota; mediation analysis; follow-up adjusted for baseline analysis

Introduction

Aging has emerged as a significant global challenge, with the population aged 60 and older projected to reach 1.4 billion by 2030 and 2.1 billion by 2050[1]. This demographic shift may be accompanied by a rapid increase in the prevalence of age-related diseases. Biological age (BA) serves as a promising indicator for assessing the biological processes of aging[2]. The complex mechanisms underlying aging are closely linked to the development of age-related diseases[3, 4]. BA can be evaluated through various measures, including epigenetic clocks, telomere length, frailty, and composite biomarkers, each reflecting different aspects of the aging process[5-7]. Among these measures, the composite biomarker BA derived from clinical biochemical markers offers the advantages of affordability and accessibility, making it particularly suitable for routine screening. It provides an effective pathway for the early identification and intervention of age-related diseases.

A healthy dietary pattern plays a crucial role in coping with biological aging [8, 9]. Beyond traditional dietary patterns, such as the Dietary Approaches to Stop Hypertension (DASH)[10, 11] and alternative Mediterranean diets(aMED)[12, 13], emerging dietary indices like the plant-based diet index (PDI) and healthy diet score (HDS) have demonstrated beneficial associations with healthy aging[14-18]. Among various recommended dietary patterns, the optimal dietary pattern for coping with biological aging is currently unclear. There is limited comprehensive research comparing associations between traditional and emerging dietary patterns and composite biomarker BA. Moreover, as the majority of previous comparative studies have been conducted in developed regions, the findings should be generalized with caution[8, 13, 19]. Significant disparities in dietary habits, living environments, and socioeconomic status (SES) exist between developed and less-developed regions, particularly in less-developed ethnic minority regions (LEMRs). Therefore, further research is necessary to explore the association between the advocated traditional and emerging dietary patterns and composite biomarker BA in LEMRs, along with identifying the most recommended dietary pattern to guide the rational utilization of limited food resources for enhanced health benefits.

Previous studies have proposed potential mechanisms linking healthy dietary patterns with biological aging. For example, specific food components (e.g., polyphenols) in healthy dietary patterns may exerting anti-inflammatory effects and modulating gut microbiota composition or activity, thereby reducing the risk of adverse health outcomes[20, 21]. The gut microbiome, closely associated with diet, is recognized as one of the twelve primary mechanisms of aging[5]. However, population-based evidence exploring the mediating role of gut microbiota in the association between dietary patterns and BA remains limited. Similar to dietary pattern, the characteristics of the gut microbiota vary significantly across different geographical regions [22-27], which may lead to distinct mediation effects of the same microbial measurements. In LEMRs, investigating the mediating role of specific gut microbiota measurements in the associations between dietary patterns and composite biomarker BA potentially provides appropriate microbial strategies for addressing aging.

The China Multi-Ethnic Cohort (CMEC) Study is a large-scale epidemiological investigation conducted in Southwest China, a region characterized by significant diversity in SES, ethnicity, habitual diet, and living environments [28]. It provides an ideal opportunity to explore the associations between dietary patterns and composite biomarker BA in LEMRs. Utilizing baseline and follow-up data from the CMEC, this study aims to investigate the associations between various dietary patterns [including DASH, aMED, PDI, healthful plant-based diet index(hPDI), unhealthful plant-based diet index(uPDI), HDS] and composite biomarker BA based on Klemera and Doubal's method (KDM-BA), and to identify the most recommended dietary pattern for coping with biological aging. Additionally, we propose to explore the potential mediating role of the gut microbiome in these associations.

Method

Study population

The CMEC study is an ongoing community-based prospective cohort study covering five provinces in Southwest China, including Sichuan, Yunnan, Guizhou, Tibet, and Chongqing. A multistage, stratified cluster sampling method was employed to select the study population. Detailed information on the recruitment of the population in the CMEC study has been described in our previous study[28, 29]. The selected study population exhibits diverse SES, racial composition, population size, and disease patterns. The baseline survey was conducted from May 2018 to September 2019, involving data collection from 99,556 participants. The first follow-up survey took place from August 2020 to July 2021, including nearly 10% of the baseline participants. The study collected data through face-to-face interviews, medical examinations, laboratory tests, and obtained questionnaire data, physical examination data, biological samples, as well as disease occurrence and diagnosis information[28]. This study adhered to the ethical principles outlined in the Declaration of Helsinki, with all procedures involving human subjects approved by the Ethics Review Committee of Sichuan University (K2016038, K2020022) and local ethics committees at each participating site. Written informed consent was obtained from all subjects.

In the present study, for the longitudinal associations between dietary patterns and KDM-BA, individuals with abnormal total energy intake (n = 227), implausible body mass index (BMI) (n = 52) and missing covariates (n = 475) were excluded. Finally, we included 8,288 study participants with complete diet related data and covariates in baseline survey, and biomarkers for constructing KDM-BA in follow-up survey. For the mediation analysis of gut microbiota, we excluded individuals who had an abnormal total energy intake (n = 44), implausible BMI (n = 3), had used antibiotics within one month prior to baseline (n = 553), self-reported baseline digestive conditions such as ulcers, gastritis, gallstones, and cholecystitis (n = 236), or had missing covariate data (n = 3). Ultimately, 764 study participants were included in the analysis, all of whom had fecal sample data, complete diet-related data, biomarkers for constructing the KDM-BA, and associated covariates. Detailed information on the enrollment process is provided in **Supplementary Figure 1**.

Dietary measurement

We obtained self-reported information on participants' dietary habits in the year before the baseline survey through a semi-quantitative food frequency questionnaire (FFQ). The reproducibility and validity of the FFQ were evaluated by conducting repeated FFQs and 24-hour dietary recalls[30]. The FFQ comprehensively assessed the intake of major food groups [include rice, wheat products, coarse grain, tubers, meat, poultry, fish/sea food, eggs, fresh vegetables, soybean products, preserved vegetables, fresh fruit, dairy products, alcohol, tea, sugar sweetened beverages (SSBs), vegetable oil, animal oil, and salt]. The consumption of each food group was measured using intake quantity (standard portion/grams) and intake frequency (daily, weekly, monthly, yearly). Ultimately, all consumption amounts for food groups were converted into weekly grams.

Dietary pattern measurement

Based on the consumption of food groups, we constructed six dietary patterns, including PDI, hPDI, uPDI, HDS, DASH and aMED. Detailed information on the scoring criteria for each dietary pattern can be found in **Supplementary Methods, Supplementary Tables 1**. In brief, plant-based diet indices were calculated based on 15 food groups [include tubers, fresh vegetables, soybean products, fresh fruits, coarse grain, tea, vegetable oil, preserved vegetables, fine grain (rice and wheat products), red and processed meats, poultry, fish/sea food, eggs, dairy products, animal oil], categorized into healthy plant foods, unhealthy plant foods, and animal foods according to their varying health effects[18, 31, 32]. The scoring criteria for healthy plant foods, unhealthy plant foods, and animal foods differ among the three plant-based diet indices. In general, each food group was assigned a score from 1 to 5. The total scores ranged from 15 to 75 for the three plant-based diet indices, reflecting adherence to a plant-based diet. The HDS[16] was determined by evaluating 5 specific healthy dietary groups (fresh vegetables, soybean products, fresh fruits, fish/seafood, and dairy products), with each food group assigned a score ranging from 1 to 5. The total HDS score ranged from 5 to 25, where higher scores reflect a healthier diet. We calculated the DASH score based on seven food groups (fresh fruit, fresh vegetables, soybean products, dairy products, coarse grain, red and processed meats, and salt) to evaluate adherence to the DASH diet, and the aMED score was calculated based on eight food groups (fresh vegetables, soybean products, fresh fruit, coarse grain, fish/seafood, MUFA: SFA (the ratio of monounsaturated fatty acids to saturated fatty acids), red and processed meats, and alcohol) to assess adherence to the Mediterranean diet among non-Mediterranean populations[30]. Each food group was assigned a value from 1 to 5. The theoretical range for total DASH scores was 7-35, while the theoretical range for total aMED scores was 8-40.

Composite biomarker BA measurement

We utilized clinical biomarkers, anthropometric measurements to construct biological age based on Klemera and Doubal's method (KDM-BA)[33] , which has been well validated for predicting biological aging and age-related health status.[34-36]. The KDM-BA constructed

based on the CMEC population has been described and validated in our previous study[29]. In summary, we selected eligible biomarkers based on the assumptions and selection criteria necessary for constructing KDM-BA. A total of 15 indicators were included: systolic blood pressure (SBP), waist-to-hip ratio (WHR), peak expiratory flow, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), glycated hemoglobin (HBA1C), triglyceride (TG), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), albumin (ALB), alkaline phosphatase (ALP), creatinine, urea, mean corpuscular volume (MCV), and platelet count. Subsequently, we employed linear regression to predict chronological age (CA) using the selected biomarkers. This approach allowed us to obtain the regression coefficients, which were then integrated with the biological age (BA) formula to compute the KDM-BA[33].

Additionally, we calculated the KDM-BA acceleration (KDM-AA) by subtracting CA from KDM-BA. A positive KDM-AA indicates that the individual is physiologically older than expected in the reference population, while a negative KDM-AA suggests that the individual is physiologically younger than expected.

Gut microbiota measurement

We collected fecal samples and stored in -80°C biobank freezer prior to testing. DNA extraction from the samples was performed using the Mag-Bind® Soil DNA Kit (M5635, Omega Bio-tek, Georgia, USA), while concentration and quality measurements were conducted using a fluorescence spectrophotometer (E6090 QuantiFluor, Promega, Wis-consin, USA) and 1% agarose gel electropho-resis. The amplification of the 16S rRNA gene fragment was carried out using universal primers (338F and 806R). Following PCR amplification, gel purification, and quantification, we constructed the required DNA library and sequenced it using the Illumina Novaseq 6000 PE250 sequencing platform. The resulting raw sequencing data were subjected to corresponding analysis to obtain the operational taxonomic unit (OUT) data, which serves as the foundational dataset for constructing gut microbial measurements. Further details regarding fecal sample collection, DNA extraction, and sequencing can be found in our previous study [37].

Covariates measurement

Covariates information was obtained from questionnaires. We constructed directed acyclic graphs (DAGs) based on the protocol of "Evidence Synthesis for Constructing Directed Acyclic Graphs" (ESC-DAGs) (DAGs is presented in **Supplementary Figure 2**). Following causal diagrams and the backdoor criterion, the following covariates were included in the main analysis: age, sex, ethnicity, marital status, education, annual household income, occupation, family history of cardiovascular metabolic diseases, urbanicity, smoking status, total energy intake, physical activity, BMI, insomnia symptom, dietary supplement, depressive symptom, anxiety symptom, beverage consumption. Detailed information can be found in the **Supplementary Methods.** See **Supplementary Table 2** for missing information on covariates.

Statistical analysis

We described the baseline characteristics across various categories of dietary pattern scores in the study populations. Continuous variables were presented as median (25th, 75th percentile), while categorical variables were indicated as numbers (percentages). T-tests and chi-square tests were conducted to assess the differences between the current complete-cases data and the corresponding entire-population with missing covariates.

We employed the follow-up adjusted for baseline analysis by applying multiple linear regression model to assess the association between baseline dietary pattern scores (quintiles) and the follow-up KDM-AA (continuous). We further adjusted for potential confounding factors as identified in the DAG and the baseline KDM-AA to reduce reverse causation and minimize potential residual confounding[38] (**Supplementary Methods**). Additionally, to further elucidate the significant beneficial and detrimental food groups, we employed the quantile G-computation (QGC) method[39] (**Supplementary Methods**) to evaluate the relative contribution of each food group in the association of each dietary pattern with KDM-AA, as well as the relative weights of all food groups associated with KDM-AA. The QGC method evaluates the positive or negative relative contributions of each food group, and has been widely used in epidemiological studies[40, 41].

In the microbiota-related analysis, the original OTU data were rarefied to 38,000 reads[42] (the rarefaction curve is provided in **Supplementary Figure 3**). Shannon, Simpson, Chao1, ACE, and Obs indices were computed using the rarified counts to assess α-diversity of the gut microbiota. We excluded taxa with low relative abundance and retained those with a relative abundance exceeding 10^{-5} in at least 5% of the samples[43]. Ultimately, we incorporated 5 α-diversity indices, 16 phylum-level taxa, and 304 genus-level taxa. Additionally, we conducted the regression-based causal mediation model[44] to explore the mediating role of gut microbiota in the associations between dietary patterns and KDM-AA. It primarily consisted of two steps. First, regressing gut microbiota on dietary patterns. Then, regressing KDM-AA on dietary patterns and gut microbiota. Considering the potential correlations among taxa, we did not consider each test for taxa as independent test. Therefore, for taxa with $|r| > 0.3$ and $P < 0.05$ in Spearman correlation analysis, we did not correct for multiple testing[45, 46](**Supplementary Methods, Supplementary Figure 4**). Furthermore, we utilized Spearman correlation analysis to assess the correlations between dietary patterns and gut microbiota, as well as between KDM-AA and gut microbiota.

To explore potential effect modification on the associations between dietary patterns and KDM-AA, we included dietary indicators as continuous variables and conducted stratified analysis based on sex, age, ethnicity, physical activity, BMI, education level, and smoking status.

To test the robustness of our findings, the following sensitivity analyses were performed in association analysis: 1) conducting the association analysis with entire-population dataset imputed missing covariates rather than the current complete-case data. 2) repeating the

analysis after excluding individuals with KDM-AA values greater than 4 standard deviations. 3) inclusion of dietary indicators as binary, ternary, and quaternary variables in the model. 4) performing a cross-sectional analysis of the association between dietary patterns and KDM-AA based on baseline diet-related data and baseline KDM-AA. 5) we excluded self-reported chronic diseases at baseline (diabetes, hypertension, hyperlipidemia, coronary heart disease and stroke) to reduce reverse causation.

Two-sided *P* value < 0.05 were considered statistically significant. In microbiota-related analysis, we utilized the False Discovery Rate (FDR) method for multiple test correction. All statistical analyses were conducted using R version 4.2.1.

Result

General characteristics

According to the percentile classification of dietary indicators, we described the baseline characteristics of 8,288 participants in the association analysis (see **Table 1**). The median age of the study population was 51 years (44, 59), with the majority being female (61.6%), Han ethnicity (60.6%), and residing in rural areas (64.3%). The PDI, uPDI, and hPDI dietary patterns exhibited similar baseline characteristics. Participants with higher compliance with these dietary patterns tended to prefer living in rural areas, be of non-Han ethnicity, and have relatively lower levels of education. Among these individuals, those with higher compliance with uPDI also showed a tendency to work in primary industry-related occupations and have lower economic status. Similarly, the HDS, DASH, and aMED dietary patterns shared comparable baseline characteristics. Participants with higher compliance to these dietary patterns tended to be Han ethnicity, reside in urban areas, work in tertiary industry-related occupations, and have higher levels of education and economic status. A comparison of the characteristics of the association analysis samples and the corresponding entire-population dataset can be found in **Supplementary Table 3**. The results showed that there were no significant differences in the above two samples.

Associations between dietary patterns with KDM-AA

Table 2 presents the estimated associations between various dietary patterns and KDM-AA after adjusting for potential confounding variables. Overall, all dietary patterns were found to be associated with KDM-AA. The healthy dietary patterns (PDI, hPDI, HDS, DASH, aMED) exhibited negative association with KDM-AA, with DASH demonstrating the strongest negative association. Conversely, uPDI showed positive association with KDM-AA. When comparing the highest and lowest quantiles of the dietary indicator, the strongest negative association was observed between DASH and KDM-AA [β = -0.91 (-1.19, -0.63)], while the weakest was observed for hPDI [β = -0.41 (-0.67, -0.15)]. Additionally, uPDI demonstrated a positive association with KDM-AA [β= 0.68(0.39,0.97)] as participants transitioned from non-compliance to compliance. Consistent results were obtained when dietary indicators were included as continuous variables in the model. A 1-point increase in the DASH score

was associated with a 0.33-year decrease in KDM-AA, whereas a 1-point increase in the hPDI score was associated with a mere 0.15-year decrease in KDM-AA. Conversely, each 1-point increase in the uPDI score was associated with a 0.25-year increase in KDM-AA. Notably, all trend *P* values were less than 0.001.

For the varying associations between different dietary patterns and KDM-AA, component analysis may provide corresponding explanations (**Figure 1**). Overall, the results of component analysis showed that tea and soybean products may be the significant beneficial food groups, while salt, preserved vegetables, red and processed meats were identified as the major harmful food groups. In DASH, HDS, and aMED, which showed relatively stronger beneficial associations with KDM-AA, soybean products were identified as the most beneficial food group, accounting for 36%, 36.4%, and 40.3%, respectively, while salt and red and processed meats were found to be the most detrimental food groups in DASH (60.1%) and aMED (42.6%), respectively. Results from the component analysis of plant-based dietary patterns indicate that tea (34.3%) may be the significant food group contributing to the beneficial association between PDI and hPDI with KDM-AA. Additionally, a higher intake of preserved vegetables (52.3%) predominantly contributes to the detrimental association between uPDI and KDM-AA. Detailed results can be found in **Supplementary Table 4**. In the stratified analysis, the magnitude and direction of the associations between various dietary patterns and KDM-AA in different subgroups largely align with the main analysis. Further details can be found in **Supplementary Table 5**.

Mediation analysis

Supplementary Table 6 presents the baseline characteristics of the mediation analysis sample comprising 764 participants. **Figure 2.a** illustrates the joint distribution of Spearman correlation coefficients between microbial measurements and dietary patterns as well as KDM-AA. From the overall distribution trend, there were relatively stronger associations between microbial measurements and dietary patterns, while the correlation with KDM-AA were comparatively weaker. The strongest correlations between microbial measurements and dietary patterns (except DASH) were approximately 0.25, whereas the strongest correlations with KDM-AA were approximately 0.1.

In the mediation analysis sample, we found that the direction and magnitude of associations between various dietary patterns and KDM-AA remained consistent with the previous association analysis. However, we did not find significant statistical association between several dietary patterns and KDM-AA. Furthermore, we did not observe statistically significant mediation effects of α-diversity indices (**Supplementary Table 7**). The results above may be attributed to the relatively small sample size of our mediation analysis and the collection of fecal samples from specific populations in CMEC, leading to limited variation in dietary indicators and gut microbiota within the mediation sample. This makes it challenging to observe statistically significant results. Nevertheless, we identified several taxa with statistically significant mediation effects (**Figure 2.b, Supplementary Table 8**). At the phylum-level, we found that hPDI may be negatively associated with KDM-AA by reducing

the abundance of the *Synergistetes* phylum [$RD_{Indirect}$ = -0.017 (95% *CI*: -0.040, -0.001), *P* = 0.03, P_{FDR} = 0.21], with a mediating proportion of 5.61%. Within the genus constituting the *Synergistetes* phylum, the decreased abundance of the *Pyramidobacter* genus may respectively mediate 7.27% of the negative association between PDI and KDM-AA [$RD_{Indirect}$ = -0.021(95% *CI*: -0.051,0.000), *P* =0.042, P_{FDR} = 0.084], and 9.19% of the negative association between hPDI and KDM-AA [$RD_{Indirect}$ = -0.027 (95% *CI*: -0.069, 0.000), *P* =0.048, P_{FDR} =0.096].The results indicate that the decreased abundance of the *Synergistetes* phylum and its component genus *Pyramidobacter* may mediate the negative associations between plant-based diets and KDM-AA.

Sensitivity analyses

The association between various dietary patterns and KDM-AA exhibited largely robust results when excluding outliers of KDM-AA that were greater than 4 times the standard deviation, imputing the corresponding entire-population dataset, performing different classification processing (binary, ternary, quaternary) on the dietary indicators, performing a cross-sectional analysis and excluding baseline chronic disease. (**Supplementary Tables 9-15**)

Discussion

Summary of main results

Based on the 8,288 participants from the CMEC baseline and follow-up survey, we found that six dietary patterns were statistically significant associated with KDM-AA. uPDI was positively associated with KDM-AA, while the other five dietary patterns (PDI, hPDI, HDS, DASH, aMED) were negatively associated with KDM-AA, with DASH showing the strongest beneficial association. Among the food groups assessed, tea and soybean products may be significant beneficial food groups, while salt, preserved vegetables, red and processed meats were identified as the major harmful food groups. In addition, based on 764 individuals at baseline, we found that the decreased abundance of the *Synergistetes* phylum and its member, the *Pyramidobacter* genus, may mediate the negative association between plant-based diets and KDM-AA.

Dietary patterns are associated with biological age and DASH shows the strongest beneficial association

Based on our longitudinal data, healthy dietary patterns were negatively associated with biological aging measured by composite biomarkers. Our findings are consistent with previous research, although most previous studies focused on the associations of dietary patterns with telomeres[19], frailty [47], epigenetic age[8, 48, 49], composite biomarker BA based on Deep Neural Network[50], other aging metrics or aging-related outcomes [16, 18, 32, 51]. Various measures of aging may capture distinct aspects of the aging process[7]. Our study, in conjunction with previous research, comprehensively demonstrates that healthy dietary

patterns may be negatively associated with multiple dimensions of biological aging.

Furthermore, we found that DASH demonstrated the strongest beneficial association with KDM-AA among various dietary patterns. However, there is still controversy regarding the most recommended dietary pattern for coping with biological aging[8, 19, 50]. A longitudinal study based on the Melbourne Collaborative Cohort Study (MCCS) and a cross-sectional study based on the American Sister Study both preferred the Alternative Healthy Eating Index 2010 (aHEI-2010), while a cross-sectional study based on the Italian Moli-sani Study recommended MED. The different results may be attributed to the focus on various biological aging measures and variations in the food groups used to construct the dietary indices. It is evident that these studies were conducted primarily on Western populations, where dietary habits differ significantly from those in China. In particular, the DASH diet emerged as the most recommended dietary pattern in our study, likely due to the high salt intake prevalent in China, which the DASH diet specifically aims to control. The salt intake among the Chinese exceeds the World Health Organization's recommended intake by more than twice[52], and high salt intake ranks as the third leading risk factor for death and disability-adjusted life years in China[53]. Consistent with our previous research, the DASH diet was also highly recommended for attenuating cardiometabolic risks among various dietary patterns, particularly in lowering the risk of hypertension, which is closely associated with salt intake[30].

Tea and soybean products may be the significant beneficial food groups, while salt, preserved vegetables, red and processed meats were identified as the major harmful food groups.

The component analyses indicate that salt, preserved vegetables, red and processed meats may be significant beneficial food groups. It is acknowledged that salt and high-salt preserved vegetables [54] are the major detrimental food groups, corresponding to the significant adverse effects of high salt intake in our population. Our research indicates that salt reduction may be a crucial intervention strategy to promote healthy aging in the southwestern region of China. Salt reduction has been adopted as one of the most cost-effective public policies worldwide[55]. Additionally, China's "Healthy China 2030" plan sets a target to reduce adult daily salt intake by 20% by the year 2030. Considering the extensive geographical coverage, substantial altitude variations, and dietary habits of ethnic minorities that are challenging to modify within our study population, our research emphasizes the necessity for enhanced policy support and resource allocation in implementing salt reduction interventions, especially in the southwestern region of China.

The component analyses indicate that tea and soybean products may be primarily beneficial food groups. Tea is known to be rich in polyphenols, purine alkaloids, theanine, tea polysaccharides and caffeine bioactive compounds[29], while soybean products are abundant in polyphenols, carotenoids, phytosterols, phytic acids, alkaloids, and other phytochemicals[56]. Polyphenols, in particular, have been extensively studied and are recognized as being negatively associated with biological aging. Research has indicated that the underlying mechanism may involve polyphenols exerting anti-inflammatory effects and

modulating gut microbiota, thereby promoting healthy aging[20, 57-60].

Overall, our findings align with the fundamental principles recommended by the current Chinese dietary guidelines and may serve as a reference for their further improvement. The DASH diet, as advocated by our study, emphasizes the consumption of fresh fruits, vegetables, soybean products, dairy, and whole grains, while recommending a reduction in red and processed meats, as well as salt intake. This approach largely corresponds with the general principles outlined in the Chinese Food Guide Pagoda (2022), which serves as a crucial foundation for conducting dietary evaluations in China. Furthermore, in addition to the current recommendations of the Chinese Food Guide Pagoda, our study identifies tea as a beneficial food group and highlights preserved vegetables, red, and processed meats as harmful food groups, which may inform future enhancements to the Chinese Food Guide Pagoda.

Gut microbiota may partially mediate the association between certain dietary patterns and biological age

Our study suggested that the *Synergistetes* phylum and its member, the *Pyramidobacter* genus, may partially mediate the negative association between plant-based diets and biological aging. Previous research indicated that these two taxa may be associated with inflammation and age-related phenotypes. *Synergistetes* phylum is linked to systemic anti-inflammatory responses, infection, diabetes, cancer, and its abundance is significantly increased in patients with Parkinson's disease and heart failure[61-66]. The *Pyramidobacter* genus is associated with inflammatory factors and may contribute to pathogenic infections[67]. Furthermore, it has been recognized as a biomarker for colorectal cancer and oral squamous cell carcinoma [68, 69]. Our research is consistent with previous studies. A dietary intervention study found significantly lower abundance of Pyramidobacter among participants who consumed a plant-based diet rich in polyphenolic compounds[70]. Overall, this indicates that plant-based diets may modulate the abundance of gut microbiota through polyphenolic compounds, thereby potentially promoting healthy aging. However, considering that our microbiota-related study relies on a relatively small and specific sample, the corresponding conclusions need to be interpreted cautiously. Further research with larger and more diverse samples is essential to thoroughly explore the role of microbiota in the association between dietary patterns and biological aging.

Strength and limitations

To the best of our knowledge, this is the first study comparing the association between various dietary patterns, corresponding food groups, and biological age in LEMRs. Additionally, our study utilized longitudinal data, while most epidemiological studies focusing on the association between dietary patterns and biological aging have employed cross-sectional designs. Furthermore, by integrating metagenomic data, our study represents the first investigation into the mediating role of gut microbiota in the association between dietary patterns and biological age in LEMRs.

However, several limitations should be acknowledged. Firstly, measurement error may impact dietary measurements and distort dietary patterns. Nonetheless, our previous study showed that measurement error generally mitigated the observed association between dietary patterns and disease[71]. Therefore, we believe that this limitation is less likely to significantly influence our main conclusions. Secondly, we lacked rigorous quantification for sugar-sweetened beverages (SSBs) and lacked information on consumption of nuts. Therefore, we excluded SSBs from the DASH score, and excluded nuts from the aMED and HDS scores. This may result in the constructed dietary indices not fully reflecting true dietary patterns. However, given that consumption of SSBs and nuts is very low in the LEMRs, we believe that the impact of this deficiency on our results is limited. Thirdly, due to data availability, the biomarkers used to construct KDM-BA don't include fully biomarkers related to aging. Thus, the KDM-BA may reflect certain aspect of aging. Fourthly, although we have carefully controlled the confounding factors identified in the DAG, the impact of unmeasured confounding cannot be entirely ruled out. Finally, our study was limited to less-developed ethnic minority regions in southwestern China. The findings should be generalized to other LEMRs populations for caution.

Conclusion

Based on longitudinal data from the CMEC study, our study indicated that adherence to healthy dietary patterns (PDI, hPDI, HDS, DASH, aMED), especially DASH, was negatively associated with KDM-AA, while uPDI was positively associated with KDM-AA. Furthermore, this study identified potential beneficial food groups (tea and soybean products), as well as harmful groups (salt, red and processed meats, preserved vegetables) for coping with biological aging. It appears that gut microbiota, specifically *Synergistetes* phylum and *Pyramidobacter* genus, may mediate the negative association between plant-based diets and KDM-AA. The research provides a comprehensive exploration of the associations between various dietary patterns and biological aging, as well as potential mechanisms.

Acknowledgments

We thank all the team members and participants involved in the China Multi-Ethnic Cohort (CMEC).

Financial Support

The research was funded by National Natural Science Foundation of China (Grant No. 82273740). The funding had no role in study design, data collection, analysis, or interpretation; in the writing of the article; or any restrictions regarding the decision to submit the article for publication.

Conflict of Interests

All authors declare no competing interests.

Authorship

HMZ, XX, NZ, SRH designed research. HJZ, JZL, TTY, FM, LLC, MMH conducted research. HJZ provided parts of essential databases. HMZ analyzed data, wrote the paper and had primary responsibility for final content. XX, YX, JJC, YZ, NZ, FY, SRH, HXC, SCL provided technical assistance and advice for data analysis and manuscripts. All authors read and approved the final manuscript.

Reference

1. WHO. Ageing and health [cited 2022]. Available from: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health.

2. Bao H, Cao J, Chen M, Chen M, Chen W, Chen X, et al. Biomarkers of aging. Sci China Life Sci. 2023;66(5):893-1066.

3. Gonzales MM, Garbarino VR, Pollet E, Palavicini JP, Kellogg DL, Jr., Kraig E, et al. Biological aging processes underlying cognitive decline and neurodegenerative disease. J Clin Invest. 2022;132(10).

4. Fraser HC, Kuan V, Johnen R, Zwierzyna M, Hingorani AD, Beyer A, et al. Biological mechanisms of aging predict age-related disease co-occurrence in patients. Aging cell. 2022;21(4):e13524.

5. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. Cell. 2023;186(2):243-78.

6. Moqri M, Herzog C, Poganik JR, Justice J, Belsky DW, Higgins-Chen A, et al. Biomarkers of aging for the identification and evaluation of longevity interventions. Cell. 2023;186(18):3758-75.

7. Belsky DW, Moffitt TE, Cohen AA, Corcoran DL, Levine ME, Prinz JA, et al. Eleven Telomere, Epigenetic Clock, and Biomarker-Composite Quantifications of Biological Aging: Do They Measure the Same Thing? Am J Epidemiol. 2018;187(6):1220-30.

8. Kresovich JK, Park Y-MM, Keller JA, Sandler DP, Taylor JA. Healthy eating patterns and epigenetic measures of biological age. The American journal of clinical nutrition. 2022;115(1):171-9.

9. Grande de França NA, Rolland Y, Guyonnet S, de Souto Barreto P. The role of dietary strategies in the modulation of hallmarks of aging. Ageing research reviews. 2023;87:101908.

10. Barnes LL, Dhana K, Liu X, Carey VJ, Ventrelle J, Johnson K, et al. Trial of the MIND Diet for Prevention of Cognitive Decline in Older Persons. 2023;389(7):602-11.

11. Duan H, Pan J, Guo M, Li J, Yu L, Fan L. Dietary strategies with anti-aging potential: Dietary patterns and supplements. Food research international (Ottawa, Ont). 2022;158:111501.

12. Poursalehi D, Lotfi K, Saneei P. Adherence to the Mediterranean diet and risk of

frailty and pre-frailty in elderly adults: A systematic review and dose-response meta-analysis with GRADE assessment. Ageing research reviews. 2023;87.

13. Esposito S, Gialluisi A, Costanzo S, Di Castelnuovo A, Ruggiero E, De Curtis A, et al. Mediterranean diet and other dietary patterns in association with biological aging in the Moli-sani Study cohort. Clinical Nutrition. 2022;41(5):1025-33.

14. Sotos-Prieto M, Struijk EA, Fung TT, Rodríguez-Artalejo F, Willett WC, Hu FB, et al. Association between the quality of plant-based diets and risk of frailty. Journal of Cachexia, Sarcopenia and Muscle. 2022;13(6):2854-62.

15. Wang S, Li W, Li S, Tu H, Jia J, Zhao W, et al. Association between plant-based dietary pattern and biological aging trajectory in a large prospective cohort. BMC Med. 2023;21(1):310.

16. Mente A, Dehghan M, Rangarajan S, O'Donnell M, Hu W, Dagenais G, et al. Diet, cardiovascular disease, and mortality in 80 countries. European Heart Journal. 2023;44(28):2560-79.

17. Craig MF. A plant based diet benefits personal and planetary health. BMJ (Clinical research ed). 2022;379:o2651.

18. Chen H, Shen J, Xuan J, Zhu A, Ji JS, Liu X, et al. Plant-based dietary patterns in relation to mortality among older adults in China. Nat Aging. 2022;2(3):224-30.

19. Bountziouka V, Nelson CP, Wang Q, Musicha C, Codd V, Samani NJ. Dietary Patterns and Practices and Leucocyte Telomere Length: Findings from the UK Biobank. Journal of the Academy of Nutrition and Dietetics. 2023;123(6):912-22.e26.

20. Wu M, Luo Q, Nie R, Yang X, Tang Z, Chen H. Potential implications of polyphenols on aging considering oxidative stress, inflammation, autophagy, and gut microbiota. Critical reviews in food science and nutrition. 2021;61(13):2175-93.

21. Cardona F, Andrés-Lacueva C, Tulipani S, Tinahones FJ, Queipo-Ortuño MI. Benefits of polyphenols on gut microbiota and implications in human health. The Journal of nutritional biochemistry. 2013;24(8):1415-22.

22. Li W, Li H, Wang S, Han K, Liu Y, An Z, et al. Regional pattern and signatures of gut microbiota in rural residents with coronary heart disease: A metagenomic analysis. Front Cell Infect Microbiol. 2022;12:1007161.

23. Sun Y, Zuo T, Cheung CP, Gu W, Wan Y, Zhang F, et al. Population-Level Configurations of Gut Mycobiome Across 6 Ethnicities in Urban and Rural China. Gastroenterology. 2021;160(1):272-86.e11.

24. Gaulke CA, Sharpton TJ. The influence of ethnicity and geography on human gut microbiome composition. Nature medicine. 2018;24(10):1495-6.

25. Deschasaux M, Bouter KE, Prodan A, Levin E, Groen AK, Herrema H, et al. Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. Nature medicine. 2018;24(10):1526-31.

26. Sheng Y, Wang J, Gao Y, Peng Y, Li X, Huang W, et al. Combined analysis of cross-population healthy adult human microbiome reveals consistent differences in gut microbial characteristics between Western and non-Western countries. Comput Struct Biotechnol J. 2024;23:87-95.

27. He Y, Wu W, Zheng HM, Li P, McDonald D, Sheng HF, et al. Regional variation limits applications of healthy gut microbiome reference ranges and disease models. Nature medicine. 2018;24(10):1532-5.

28. Zhao X, Hong F, Yin J, Tang W, Zhang G, Liang X, et al. Cohort Profile: the China Multi-Ethnic Cohort (CMEC) study. International journal of epidemiology. 2021;50(3):721-l.

29. Xiang Y, Xu H, Chen H, Tang D, Huang Z, Zhang Y, et al. Tea consumption and attenuation of biological aging: a longitudinal analysis from two cohort studies. Lancet Reg Health West Pac. 2024;42:100955.

30. Xiao X, Qin Z, Lv X, Dai Y, Ciren Z, Yangla Y, et al. Dietary patterns and cardiometabolic risks in diverse less-developed ethnic minority regions: results from the China Multi-Ethnic Cohort (CMEC) Study. Lancet Reg Health West Pac. 2021;15:100252.

31. Satija A, Bhupathiraju SN, Rimm EB, Spiegelman D, Chiuve SE, Borgi L, et al. Plant-Based Dietary Patterns and Incidence of Type 2 Diabetes in US Men and Women: Results from Three Prospective Cohort Studies. PLoS Med. 2016;13(6):e1002039.

32. Shan Z, Wang F, Li Y, Baden MY, Bhupathiraju SN, Wang DD, et al. Healthy Eating Patterns and Risk of Total and Cause-Specific Mortality. JAMA internal medicine. 2023;183(2):142-53.

33. Klemera P, Doubal S. A new approach to the concept and computation of biological age. Mech Ageing Dev. 2006;127(3):240-8.

34. Jee H, Park J. Selection of an optimal set of biomarkers and comparative analyses of biological age estimation models in Korean females. Arch Gerontol Geriatr. 2017;70:84-91.

35. Levine ME. Modeling the rate of senescence: can estimated biological age predict mortality more accurately than chronological age? The journals of gerontology Series A, Biological sciences and medical sciences. 2013;68(6):667-74.

36. Gao X, Wang Y, Song Z, Jiang M, Huang T, Baccarelli AA. Early-life risk factors, accelerated biological aging, and the late-life risk of mortality and morbidity. Qjm. 2023.

37. Zuo H, Zheng T, Wu K, Yang T, Wang L, Nima Q, et al. High-altitude exposure decreases bone mineral density and its relationship with gut microbiota: Results from the China multi-ethnic cohort (CMEC) study. Environmental Research. 2022;215.

38. Tennant PWG, Arnold KF, Ellison GTH, Gilthorpe MS. Analyses of 'change scores' do not estimate causal effects in observational data. International journal of epidemiology. 2022;51(5):1604-15.

39. Keil AP, Buckley JP, O'Brien KM, Ferguson KK, Zhao S, White AJ. A Quantile-Based g-Computation Approach to Addressing the Effects of Exposure Mixtures. Environ Health Perspect. 2020;128(4):47004.

40. Zhang N, Liu X, Wang L, Zhang Y, Xiang Y, Cai J, et al. Lifestyle factors and their relative contributions to longitudinal progression of cardio-renal-metabolic multimorbidity: a prospective cohort study. Cardiovascular diabetology. 2024;23(1):265.

41. Li S, Guo B, Jiang Y, Wang X, Chen L, Wang X, et al. Long-term Exposure to Ambient PM2.5 and Its Components Associated With Diabetes: Evidence From a Large Population-Based Cohort From China. Diabetes care. 2023;46(1):111-9.

42. Li S, Guo B, Dong K, Huang S, Wu J, Zhou H, et al. Association of long-term exposure to ambient PM2.5 and its constituents with gut microbiota: Evidence from a China cohort. Science of The Total Environment. 2023;884.

43. Rinott E, Meir AY, Tsaban G, Zelicha H, Kaplan A, Knights D, et al. The effects of the Green-Mediterranean diet on cardiometabolic health are linked to gut microbiome modifications: a randomized controlled trial. Genome Med. 2022;14(1):29.

44. VanderWeele TJ. Mediation Analysis: A Practitioner's Guide. Annu Rev Public Health. 2016;37:17-32.

45. Ye D, Huang J, Wu J, Xie K, Gao X, Yan K, et al. Integrative metagenomic and metabolomic analyses reveal gut microbiota-derived multiple hits connected to development of gestational diabetes mellitus in humans. Gut microbes. 2023;15(1):2154552.

46. Ryan CP, Lee NR, Carba DB, MacIsaac JL, Lin DTS, Atashzay P, et al. Pregnancy is linked to faster epigenetic aging in young women. Proceedings of the National Academy of Sciences of the United States of America. 2024;121(16):e2317290121.

47. Ghosh TS, Rampelli S, Jeffery IB, Santoro A, Neto M, Capri M, et al. Mediterranean diet intervention alters the gut microbiome in older people reducing frailty and improving health status: the NU-AGE 1-year dietary intervention across five European countries. Gut. 2020;69(7):1218-28.

48. Kim Y, Huan T, Joehanes R, McKeown NM, Horvath S, Levy D, et al. Higher diet quality relates to decelerated epigenetic aging. The American journal of clinical nutrition. 2022;115(1):163-70.

49. Li DL, Hodge AM, Cribb L, Southey MC, Giles GG, Milne RL, et al. Body Size, Diet Quality, and Epigenetic Aging: Cross-Sectional and Longitudinal Analyses. The journals of gerontology Series A, Biological sciences and medical sciences. 2024;79(4).

50. Esposito S, Gialluisi A, Costanzo S, Di Castelnuovo A, Ruggiero E, De Curtis A, et al. Mediterranean diet and other dietary patterns in association with biological aging in the Moli-sani Study cohort. Clin Nutr. 2022;41(5):1025-33.

51. Mi MY, Gajjar P, Walker ME, Miller P, Xanthakis V, Murthy VL, et al. Association of healthy dietary patterns and cardiorespiratory fitness in the community. Eur J Prev Cardiol. 2023;30(14):1450-61.

52. Tan M, He FJ, Wang C, MacGregor GA. Twenty-Four-Hour Urinary Sodium and Potassium Excretion in China: A Systematic Review and Meta-Analysis. Journal of the American Heart Association. 2019;8(14):e012923.

53. Zhou M, Wang H, Zeng X, Yin P, Zhu J, Chen W, et al. Mortality, morbidity, and risk factors in China and its provinces, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2019;394(10204):1145-58.

54. Zhuang P, Wu F, Liu X, Zhu F, Li Y, Jiao J, et al. Preserved vegetable consumption and its association with mortality among 440,415 people in the China Kadoorie Biobank. BMC Med. 2023;21(1):135.

55. Zhang X, Zhang P, Shen D, Li Y, He FJ, Ma J, et al. Effect of home cook interventions for salt reduction in China: cluster randomised controlled trial. BMJ (Clinical research ed). 2023;382:e074258.

56. Tor-Roca A, Garcia-Aloy M, Mattivi F, Llorach R, Andres-Lacueva C, Urpi-Sarda M. Phytochemicals in Legumes: A Qualitative Reviewed Analysis. J

Agric Food Chem. 2020;68(47):13486-96.

57. Zhu F, Du B, Xu B. Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. Critical reviews in food science and nutrition. 2018;58(8):1260-70.

58. Messaoudene M, Pidgeon R, Richard C, Ponce M, Diop K, Benlaifaoui M, et al. A Natural Polyphenol Exerts Antitumor Activity and Circumvents Anti-PD-1 Resistance through Effects on the Gut Microbiota. Cancer Discov. 2022;12(4):1070-87.

59. Esposito S, Gialluisi A, Costanzo S, Di Castelnuovo A, Ruggiero E, De Curtis A, et al. Dietary Polyphenol Intake Is Associated with Biological Aging, a Novel Predictor of Cardiovascular Disease: Cross-Sectional Findings from the Moli-Sani Study. Nutrients. 2021;13(5).

60. Xing W, Gao W, Zhao Z, Xu X, Bu H, Su H, et al. Dietary flavonoids intake contributes to delay biological aging process: analysis from NHANES dataset. J Transl Med. 2023;21(1):492.

61. Cao Y, Zheng X, Hu Y, Li J, Huang B, Zhao N, et al. Levels of systemic inflammation response index are correlated with tumor-associated bacteria in colorectal cancer. Cell Death Dis. 2023;14(1):69.

62. Marchandin H, Damay A, Roudière L, Teyssier C, Zorgniotti I, Dechaud H, et al. Phylogeny, diversity and host specialization in the phylum Synergistetes with emphasis on strains and clones of human origin. Res Microbiol. 2010;161(2):91-100.

63. Woodall CA, Hammond A, Cleary D, Preston A, Muir P, Pascoe B, et al. Oral and gut microbial biomarkers of susceptibility to respiratory tract infection in adults: A feasibility study. Heliyon. 2023;9(8):e18610.

64. Kenna JE, Chua EG, Bakeberg M, Tay A, McGregor S, Gorecki A, et al. Changes in the Gut Microbiome and Predicted Functional Metabolic Effects in an Australian Parkinson's Disease Cohort. Front Neurosci. 2021;15:756951.

65. Maskarinec G, Raquinio P, Kristal BS, Setiawan VW, Wilkens LR, Franke AA, et al. The gut microbiome and type 2 diabetes status in the Multiethnic Cohort. PLoS One. 2021;16(6):e0250855.

66. Huang Z, Mei X, Jiang Y, Chen T, Zhou Y. Gut Microbiota in Heart Failure Patients With Preserved Ejection Fraction (GUMPTION Study). Front Cardiovasc Med. 2021;8:803744.

67. Xu Y, Wang Y, Li H, Dai Y, Chen D, Wang M, et al. Altered Fecal Microbiota

Composition in Older Adults With Frailty. Front Cell Infect Microbiol. 2021;11:696186.

68. Huo RX, Wang YJ, Hou SB, Wang W, Zhang CZ, Wan XH. Gut mucosal microbiota profiles linked to colorectal cancer recurrence. World J Gastroenterol. 2022;28(18):1946-64.

69. Nie F, Wang L, Huang Y, Yang P, Gong P, Feng Q, et al. Characteristics of Microbial Distribution in Different Oral Niches of Oral Squamous Cell Carcinoma. Front Cell Infect Microbiol. 2022;12:905653.

70. McCann SE, Hullar MAJ, Tritchler DL, Cortes-Gomez E, Yao S, Davis W, et al. Enterolignan Production in a Flaxseed Intervention Study in Postmenopausal US Women of African Ancestry and European Ancestry. Nutrients. 2021;13(3).

71. Hu Y, Tang D, Yang F, Dai S, Xiao X, Zhao X. The impacts of measurement errors on a dietary pattern analyses:a simulation study based on dietary data from the China Multi-Ethnic Cohort (CMEC) study. The American journal of clinical nutrition. 2022;116(2):523-30.

Tables

Table1. Baseline characteristics of the association analysis sample according to quintiles of dietary patterns scores (N = 8288).

Characteristic	Total	PDI		hPDI		uPDI		HDS		DASH		aMED	
		Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5
Number of participants	8288	1721	1396	1680	1429	1692	1624	1817	1435	2038	1155	1748	1221
KDM-BA (years)	53.7 (45.0 ,62.7)	53.3 (43.0, 63.0)	55.1 (46.6, 63.7)	54.0 (44.6, 63.0)	54.3 (45.6, 63.6)	51.5 (42.8, 60.7)	56.8 (48.6, 65.2)	57.1 (48.6, 65.7)	50.7 (41.7, 59.6)	57.0 (48.3, 65.5)	50.8 (41.9, 60.6)	56.6 (47.6, 65.4)	52.3 (44.3 ,61.2)
KDM-AA (years)	0.2 (-2.9, 3.4)	0.1 (-3.0, 3.3)	0.3 (-3.0, 3.5)	0.2 (-2.6, 3.3)	0.3 (-3.0, 3.4)	-0.4 (-3.6, 2.7)	1.1 (-2.0, 4.2)	1.1 (-2.2 ,4.3)	-0.7 (-3.6, 2.1)	0.8 (-2.2, 4.0)	-1.0 (-3.9, 2.3)	0.8 (-2.2, 3.8)	-0.5 (-3.6, 2.7)
Female (%)	5104 (61.6)	1055 (61.3)	782(56.0)	895(53.3)	910(63.7)	906(53.5)	1099 (67.7)	1131 (62.2)	872 (60.8)	1100 (54.0)	814 (70.5)	907 (51.9)	849 (69.5)
Age (years)	51.0 (44.0, 59.0)	50.0 (42.0, 60.0)	52.0 (45.0, 61.0)	51.0 (44.0, 60.0)	51.0 (44.0, 61.0)	49.0 (43.0, 57.0)	53.0 (46.0, 61.)	53.0 (46.0, 62.0)	49.0 (42.0, 56.0)	53.0 (46.0, 61.8)	49.0 (42.0, 57.0)	53.0 (46.0, 62.0)	50.0 (44.0, 58.0)
Married (%)	7416 (89.5	1511 (87.	1257 (90.	1511	1246 (87.	1546 (91.	1416 (87.	1573	1308	1785	1038	1534 (87.	1100 (90.1

	)	8)	0)	(89.9)	2)	4)	2)	(86.6)	(91.1)	(87.6)	(89.9)	8)	)
Urban residence (%)	2957 (35.7)	705 (41.0)	357 (25.6)	590 (35.1)	485 (33.9)	912 (53.9)	342 (21.1)	491 (27.0)	758 (52.8)	643 (31.6)	616 (53.3)	499 (28.5)	626 (51.3)
Ethnicity (%)													
Han	5023 (60.6)	1026 (59.6)	776 (55.6)	996 (59.3)	769 (53.8)	1421 (84.0)	630 (38.8)	798 (43.9)	1155 (80.5)	1045 (51.3)	916 (79.3)	798 (45.7)	982 (80.4)
Non-Han	3265 (39.4)	695 (40.4)	620 (44.4)	684 (40.7)	660 (46.2)	271 (16.0)	994 (61.2)	1019 (56.1)	280 (19.5)	993 (48.7)	239 (20.7)	950 (54.3)	239 (19.6)
Education (%)													
College or above	924 (11.2)	252 (14.6)	83(5.9)	170 (10.1)	134 (9.4)	323 (19.1)	73 (4.5)	77 (4.2)	288 (20.1)	112 (5.5)	246 (21.3)	109 (6.2)	197 (16.1)
High school	3267 (39.4)	695 (40.4)	488 (35.0)	656 (39.0)	481 (33.7)	922 (54.5)	411 (25.3)	469 (25.8)	784 (54.6)	653 (32.0)	572 (49.5)	485 (27.7)	634 (51.9)
Less than high school	1994 (24.1)	395 (23.0)	404 (28.9)	455 (27.1)	316 (22.1)	291 (17.2)	436 (26.8)	506 (27.8)	235 (16.4)	574 (28.2)	195 (16.9)	468 (26.8)	236 (19.3)
Never been to school	2103 (25.4)	379 (22.0)	421 (30.2)	399 (23.8)	498 (34.8)	156 (9.2)	704 (43.3)	765 (42.1)	128 (8.9)	699 (34.3)	142 (12.3)	686 (39.2)	154 (12.6)

Annual household income (CNY/ year)													
<12000	1261 (15.2)	298(17.3)	198(14.2)	279(16.6)	229(16.0)	143 (8.5)	426(26.2)	478 (26. 3)	10 7 (7. 5)	50 2 (2 4. 6)	88(7.6)	429 (24. 5)	114 (9.3)
12000-19999	1445 (17.4)	262(15.2)	277(19.8)	293 (17. 4)	288 (20. 2)	181(10.7)	380(23.4)	420 (23. 1)	15 5 (1 0. 8)	43 0 (2 1. 1)	13 6 (11 .8)	393 (22. 5)	139 (11.4)
20000-59999	3091 (37.3)	607(35.3)	579(41.5)	617(36.7)	534(37.4)	614(36.3)	553 (34. 1)	610 (33. 6)	52 7 (3 6. 7)	68 4 (3 3. 6)	40 8 (35 .3)	601 (34. 4)	479 (39.2)
60000-99999	1255 (15.1)	281(16.3)	165(11.8)	263(15.7)	179(12.5)	349(20.6)	162 (10. 0)	175 (9.6)	30 4 (2 1. 2)	23 6 (1 1. 6)	23 2 (20 .1)	184 (10. 5)	232 (19.0)
100000-200000	974(11.8)	207(12.0)	140(10.0)	174(10.4)	156(10.9)	322(19.0)	90(5 .5)	118 (6.5)	26 7 (1 8. 6)	15 7 (7. 7)	22 0 (19 .0)	116 (6.6)	197 (16.1)
>200000	262(3.2)	66(3 .8)	37 (2.7)	54(3 .2)	43(3 .0)	83 (4.9)	13(0 .8)	16 (0.9)	75 (5. 2)	29 (1. 4)	71 (6. 1)	25 (1.4)	60 (4.9)
Occupation (%)													
Primary industry	2782 (33.6)	512(29.8)	567(40.6)	649(38.6)	443(31.0)	306(18.1)	825 (50. 8)	823 (45. 3)	28 0 (1 9. 5)	88 4 (4 3. 4)	21 4 (18 .5)	783 (44. 8)	253 (20.7)
Second	527(	123(	87	122(	62(4	121(	85	96	99	14	62	104	74

ary industry	6.4)	7.2)	(6.2)	7.3)	.3)	7.2)	(5.2)	(5.3)	(6.9)	7 (7.2)	(5.4)	(5.9)	(6.1)
Tertiary industry	3318 (40.0)	774(45.0)	456(32.7)	647(38.5)	552(38.6)	833(49.2)	505(31.1)	582 (32.0)	694 (48.4)	685 (33.6)	546 (47.3)	558 (31.9)	580 (47.5)
Unemployed	1661 (20.0)	312(18.1)	286(20.5)	262 (15.6)	372 (26.0)	432(25.5)	209(12.9)	316 (17.4)	362 (25.2)	322 (15.8)	333 (28.8)	303 (17.3)	314 (25.7)

In order to simplify the presentation of results, only the descriptive results of each dietary indicator Q1(the lowest percentile) and Q5(the highest percentile) are shown in the table.

Data are presented as median (25th, 75th percentile) or numbers (percentages).

Table2. The associations of dietary patterns with KDM-AA (N = 8288).

	PDI			hPDI			uPDI			HDS			DASH			aMED		
	Median	N	β(95% CI)	Median	N	β(95% CI)	Median	N	β(95% CI)	Median	N	β(95% CI)	Median	N	β(95% CI)	Median	N	β(95% CI)
Q1	38	1721	Reference	38	1680	Reference	39	1692	Reference	10	1817	Reference	15	2038	Reference	20	1748	Reference
Q2	43	2049	-0.06 (-0.3,0.17)	43	2076	-0.06 (-0.29, 0.18)	44	1627	0.12 (-0.13, 0.38)	13	2120	-0.2 (-0.43, 0.04)	19	1927	-0.31** (-0.54,-0.08)	23	1969	-0.18 (-0.42, 0.05)
Q3	46	1753	-0.27* (-0.52,-0.03)	46	1727	-0.1 (-0.35, 0.15)	47	1826	0.27* (0.02, 0.53)	16	1621	-0.36** (-0.61,-0.1)	22	1421	-0.51*** (-0.77,-0.26)	25	1457	-0.38** (-0.64,-0.12)
Q4	49	1369	-0.22 (-0.48, 0.05)	49	1376	-0.3* (-0.56,-0.04)	51	1519	0.18 (-0.1,0.46)	17	1295	-0.32* (-0.6,-0.04)	24	1747	-0.57*** (-0.81,-0.33)	28	1893	-0.4** (-0.65,-0.15)
Q5	53	1396	-0.46*** (-0.73,-0.19)	53	1429	-0.41** (-0.67,-0.15)	56	1624	0.68*** (0.39, 0.97)	20	1435	-0.71*** (-0.99,-0.42)	27	1155	-0.91*** (-1.19,-0.63)	31	1221	-0.53*** (-0.82,-0.25)

Continuous[1]	458	288	-0.16*** (-0.24,-0.08)	458	288	-0.15*** (-0.23,-0.06)	478	288	0.25*** (0.16, 0.35)	158	288	-0.23*** (-0.32,-0.13)	218	288	-0.33*** (-0.42,-0.24)	258	288	-0.19*** (-0.28,-0.11)
P trend[2]	-	-	<0.001***	-	-	<0.001***	-	-	<0.001***	-	-	<0.001***	-	-	<0.001***	-	-	<0.001***

1. Continuous dietary indicators were standardized.

2. Two-sided *P* trends were obtained by assigning median values to each quintile, and then incorporating it into the model as a continuous variable.

*** presented *P* value < 0.001. ** presented *P* value >=0.001 & <0.01. * presented *P* value >=0.01 & <0.05.

Results were adjusted for covariates: the baseline KDM-AA, age, sex, ethnicity, marital status, education, annual household income, occupation, family history, urbanicity, smoking status, physical activity, total energy intake, BMI, dietary supplement, insomnia symptom, depressive symptom, anxiety symptom, beverage consumption.

Figure legends

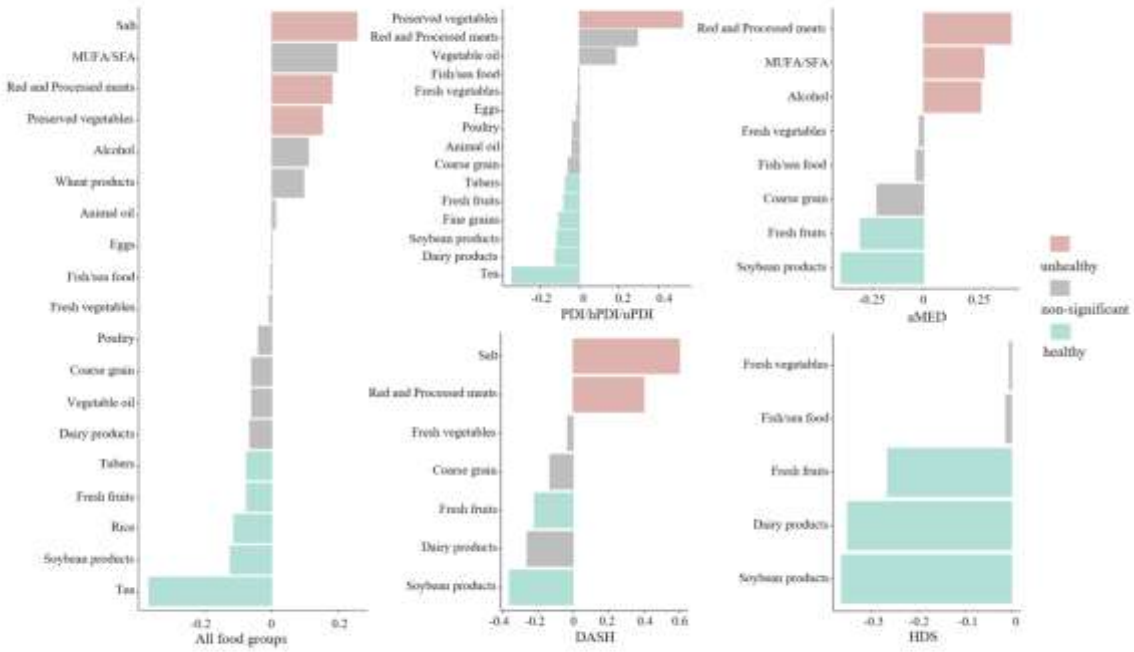

Figure1. Relative weight of each food group in the dietary patterns associated with KDM-AA (N = 8288). All models were adjusted for the baseline KDM-AA, age, sex, ethnicity, marital status, education, annual household income, occupation, family history, urbanicity, smoking status, physical activity, total energy intake, BMI, dietary supplement, insomnia symptom, depressive symptom, anxiety symptom, beverage consumption. The x-axis represents the relative weight size (positive and negative weights) of each food group in association with KDM-AA, and the y-axis represents food groups. The red bars represent food groups with a positive coefficient in the model and statistically significant associations, while the green bars represent food groups with a negative coefficient in the model and statistically significant associations. The gray bars represent food groups with no statistically significant association with KDM-AA in the model.

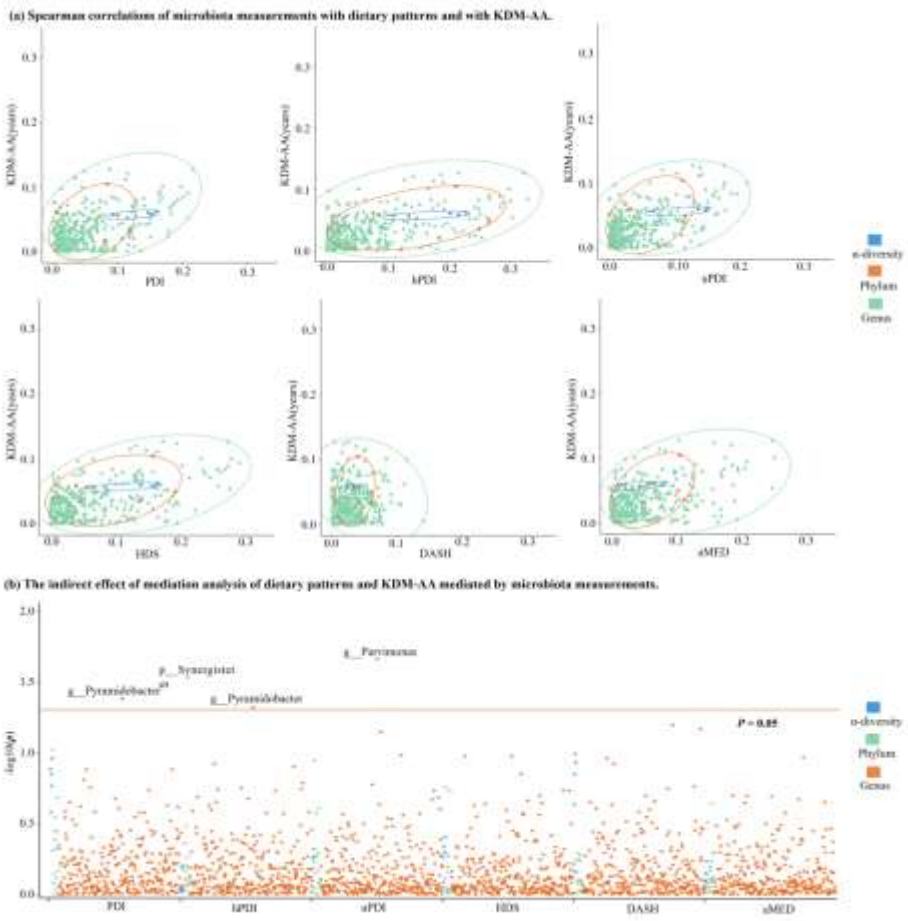

Figure2. Interrelation of various dietary patterns, gut microbiome measurements and KDM-AA (N = 764). Figure2a. Spearman correlations of microbiota measurements with dietary indicators and with KDM-AA. The X-axis indicates the correlation coefficients between dietary patterns and microbiota measurements, while the Y-axis represents the correlation coefficients between KDM-AA and microbiota measurements (both absolute values). Triangles on the axes represent 325 microbiota measurements: blue for α-diversity indices, orange for phylum-level taxa, and green for genus-level taxa. The blue, orange, and green ellipses on the axes encompass the distribution range of α-diversity indices, phylum-level taxa, and genus-level taxa, respectively. Some ellipses have incomplete shapes because parts that extend beyond the axis range are not displayed. Figure2b. The indirect effect of mediation analysis of dietary patterns and KDM-AA mediated by microbiota measurements. The X-axis represents six dietary pattern indicators, and the Y-axis represents the -log10 transformed *P* values of the indirect effect of mediation analysis. A higher -log10(*P*) value indicates a smaller *P* value. Each dietary pattern corresponds to points in the upper area of the coordinate axis, representing 325 microbiota measurements. Blue points represent α-diversity indices, green points represent phylum-level taxa, and orange points represent genus-level taxa. The orange horizontal line represents the reference line for *P* = 0.05. All models were adjusted for age, sex, ethnicity, marital status, urbanicity, physical activity, total energy intake, BMI, insomnia symptoms, and alcohol intake.

Supplementary material

Supplementary material.docx