

## Research Article

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Diet; Biological ageing; Gut microbiota; Mediation analysis; Follow-up adjusted for baseline analysis

**Abbreviations:**

aMED, alternative Mediterranean diets; CMEC, China Multi-Ethnic Cohort study; DASH, Dietary Approaches to Stop Hypertension; HDS, healthy diet score; hPDI, healthful plant-based diet index; KDM-BA, biological age based on Klemmera and Doubal's method; KDM-AA, KDM-BA acceleration; LEMR, less-developed ethnic minority regions; PDI, plant-based diet index; uPDI, unhealthful plant-based diet index

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# 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

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**Abstract**

To investigate the associations between dietary patterns and biological ageing, identify the most recommended dietary pattern for ageing 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. Anthropometric measurements and clinical biomarkers were utilised to construct biological age based on Klemmera and Doubal's method (KDM-BA) and KDM-BA acceleration (KDM-AA). Dietary information was obtained through the baseline FFQ. Six dietary patterns were constructed: plant-based diet index, healthful plant-based diet index, unhealthful plant-based diet index, healthy diet score, Dietary Approaches to Stop Hypertension (DASH), and alternative Mediterranean diets. Follow-up adjusted for baseline analysis assessed the associations between dietary patterns and KDM-AA. Additionally, quantile G-computation identified significant beneficial and harmful food groups. In the subsample of 764 participants, we used causal mediation model to explore the mediating role of gut microbiota in these associations. The results showed that all dietary patterns were associated with KDM-AA, with DASH exhibiting the strongest negative association ( $\beta = -0.91$ , 95% CI (-1.19, -0.63)). The component analyses revealed that beneficial food groups primarily included tea and soy products, whereas harmful groups mainly comprised salt and processed vegetables. In mediation analysis, the *Synergistetes* and *Pyramidobacter* 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 ageing in LEMRS, indicating that *Synergistetes* and *Pyramidobacter* may be potential mediators. Developing appropriate strategies may promote healthy ageing in LEMRS.

Ageing has emerged as a significant global challenge, with the population aged 60 years and older projected to reach 1.4 billion by 2030 and 2.1 billion by 2050<sup>(1)</sup>. 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 ageing<sup>(2)</sup>. The complex mechanisms underlying ageing are closely linked to the development of age-related diseases<sup>(3,4)</sup>. BA can be evaluated through various measures, including epigenetic clocks, telomere length, frailty and composite biomarkers, each reflecting different aspects of the ageing process<sup>(5–7)</sup>. 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 ageing<sup>(8,9)</sup>. Beyond traditional dietary patterns, such as the dietary approaches to stop hypertension (DASH)<sup>(10,11)</sup> and alternative Mediterranean diets (aMED)<sup>(12,13)</sup>, emerging dietary indices like the plant-based diet index (PDI) and healthy diet score (HDS) have demonstrated beneficial associations with healthy ageing<sup>(14–18)</sup>. Among various recommended dietary patterns, the optimal dietary pattern for coping with biological ageing 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 generalised with caution<sup>(8,13,19)</sup>. Significant disparities in dietary habits, living environments and socio-economic status 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 utilisation of limited food resources for enhanced health benefits.

Previous studies have proposed potential mechanisms linking healthy dietary patterns with biological ageing. 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<sup>(20,21)</sup>. The gut microbiome, closely associated with diet, is recognised as one of the twelve primary mechanisms of ageing<sup>(5)</sup>. 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<sup>(22–27)</sup>, 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 ageing.

The China Multi-Ethnic Cohort (CMEC) Study is a large-scale epidemiological investigation conducted in Southwest China, a region characterised by significant diversity in socio-economic status, ethnicity, habitual diet and living environments<sup>(28)</sup>. It provides an ideal opportunity to explore the associations between dietary patterns and composite biomarker BA in LEMRS. Utilising 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), unhealthy plant-based diet index(uPDI) and 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 ageing. 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<sup>(28,29)</sup>. The selected study population exhibits diverse socio-economic status, 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<sup>(28)</sup>. 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 BMI ( $n$  52) and missing covariates ( $n$  475) were excluded. Finally, we included 8288 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 online Supplementary Fig. 1.

### Dietary measurement

We obtained self-reported information on participants' dietary habits in the year before the baseline survey through a semi-quantitative FFQ. The reproducibility and validity of the FFQ were evaluated by conducting repeated FFQ and 24-hour dietary recalls<sup>(30)</sup>. 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, soyabean products, preserved vegetables, fresh fruit, dairy products, alcohol, tea, sugar sweetened beverages, 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 and 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 online Supplementary Methods, Supplementary Tables 1. In brief, plant-based diet indices were calculated based on fifteen food groups (include tubers, fresh vegetables, soyabean 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 and animal oil), categorised into healthy plant foods, unhealthy plant foods and animal foods according to their varying health effects<sup>(18,31,32)</sup>. 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<sup>(16)</sup> was determined by evaluating five specific healthy dietary groups (fresh vegetables, soyabean 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, soyabean 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, soyabean products, fresh fruit, coarse grain, fish/seafood, MUFA: SFA (the ratio of MUFA to SFA), red and processed meats and alcohol) to assess adherence to the Mediterranean diet among non-Mediterranean populations<sup>(30)</sup>. 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 biological age measurement

We utilised clinical biomarkers, anthropometric measurements to construct BA based on Klemera and Doubal's method (KDM-BA)<sup>(33)</sup>, which has been well validated for predicting biological ageing and age-related health status<sup>(34–36)</sup>. The KDM-BA constructed based on the CMEC population has been described and validated in our previous study<sup>(29)</sup>. In summary, we selected eligible biomarkers based on the assumptions and selection criteria necessary for constructing KDM-BA. A total of fifteen indicators were included: systolic blood pressure (SBP), waist:hip ratio (WHR), peak expiratory flow, LDL-cholesterol, HDL-cholesterol, glycated Hb (HbA1C), TAG, 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 BA formula to compute the KDM-BA<sup>(33)</sup>.

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  $-80^{\circ}\text{C}$  biobank freezer prior to testing. DNA extraction from the samples was performed using the Mag-Bind<sup>®</sup> 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 electrophoresis. 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 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<sup>(37)</sup>.

### Covariates measurement

Covariates information was obtained from questionnaires. We constructed directed acyclic graphs (DAG) based on the protocol

of 'Evidence Synthesis for Constructing Directed Acyclic Graphs' (ESC-DAG) (DAG is presented in online Supplementary Fig. 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 and beverage consumption. Detailed information can be found in the online Supplementary Methods. See online 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  $\chi^2$  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 minimise potential residual confounding<sup>(38)</sup> (online Supplementary Methods). Additionally, to further elucidate the significant beneficial and detrimental food groups, we employed the quantile G-computation method<sup>(39)</sup> (online 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 quantile G-computation method evaluates the positive or negative relative contributions of each food group, and has been widely used in epidemiological studies<sup>(40,41)</sup>.

In the microbiota-related analysis, the original OTU data were rarefied to 38 000 reads<sup>(42)</sup> (the rarefaction curve is provided in online Supplementary Fig. 3). Shannon, Simpson, Chao1, ACE and Obs indices were computed using the rarefied counts to assess  $\alpha$ -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<sup>(43)</sup>. Ultimately, we incorporated 5  $\alpha$ -diversity indices, 16 phylum-level taxa and 304 genus-level taxa. Additionally, we conducted the regression-based causal mediation model<sup>(44)</sup> 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<sup>(45,46)</sup> (online Supplementary Methods, Supplementary Fig. 4). Furthermore, we utilised 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 and (5) we excluded self-reported chronic diseases at baseline (diabetes, hypertension, hyperlipidaemia, coronary heart disease and stroke) to reduce reverse causation.

Two-sided  $P$  value  $< 0.05$  were considered statistically significant. In microbiota-related analysis, we utilised the false discovery rate 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 8288 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 online Supplementary Table 3. The results showed that there were no significant differences in the above two samples.

### Associations between dietary patterns with KDM-BA acceleration

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 and 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 ( $\beta = -0.91$  (-1.19, -0.63)), while the weakest was observed for hPDI ( $\beta = -0.41$  (-0.67, -0.15)). Additionally, uPDI demonstrated a positive association with KDM-AA ( $\beta = 0.68$  (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 (Fig. 1). Overall, the results of component analysis showed that tea and soyabean 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, soyabean 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 online 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 online Supplementary Table 5.

### Mediation analysis

Online 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 was 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  $\alpha$ -diversity indices (online 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 (Fig. 2(b), online 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 ( $RD_{\text{Indirect}} = -0.017$  (95% CI: -0.040, -0.001),  $P = 0.03$ ,  $P_{\text{FDR}} = 0.21$ ), with a mediating proportion of 5.61%. Within the genus constituting the *Synergistetes* phylum, the decreased abundance of the *Pyramidobacter* genus may, respectively, mediate

**Table 1.** Baseline characteristics of the association analysis sample according to quintiles of dietary patterns scores (*n* 8288)

Characteristic	Total		PDI				hPDI				uPDI			
			Q1		Q5		Q1		Q5		Q1		Q5	
			Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile
Number of participants	8288		1721		1396		1680		1429		1692		1624	
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
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
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Female (%)	5104	61.6	1055	61.3	782	56.0	895	53.3	910	63.7	906	53.5	1099	67.7
	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile
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, 61.0
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Married (%)	7416	89.5	1511	87.8	1257	90.0	1511	89.9	1246	87.2	1546	91.4	1416	87.2
Urban residence (%)	2957	35.7	705	41.0	357	25.6	590	35.1	485	33.9	912	53.9	342	21.1
Ethnicity (%)														
Han	5023	60.6	1026	59.6	776	55.6	996	59.3	769	53.8	1421	84.0	630	38.8
Non-Han	3265	39.4	695	40.4	620	44.4	684	40.7	660	46.2	271	16.0	994	61.2
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
High school	3267	39.4	695	40.4	488	35.0	656	39.0	481	33.7	922	54.5	411	25.3
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
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
Annual household income (CNY/year)														
< 12 000	1261	15.2	298	17.3	198	14.2	279	16.6	229	16.0	143	8.5	426	26.2
12 000–19 999	1445	17.4	262	15.2	277	19.8	293	17.4	288	20.2	181	10.7	380	23.4
20 000–59 999	3091	37.3	607	35.3	579	41.5	617	36.7	534	37.4	614	36.3	553	34.1
60 000–99 999	1255	15.1	281	16.3	165	11.8	263	15.7	179	12.5	349	20.6	162	10.0
100 000–200 000	974	11.8	207	12.0	140	10.0	174	10.4	156	10.9	322	19.0	90	5.5
> 200 000	262	3.2	66	3.8	37	2.7	54	3.2	43	3.0	83	4.9	13	0.8
Occupation (%)														

Table 1. (Continued)

Primary industry	2782	33.6	512	29.8	567	40.6	649	38.6	443	31.0	306	18.1	825	50.8
Secondary industry	527	6.4	123	7.2	87	6.2	122	7.3	62	4.3	121	7.2	85	5.2
Tertiary industry	3318	40.0	774	45.0	456	32.7	647	38.5	552	38.6	833	49.2	505	31.1
Unemployed	1661	20.0	312	18.1	286	20.5	262	15.6	372	26.0	432	25.5	209	12.9
	HDS				DASH				aMED					
	Q1		Q5		Q1		Q5		Q1		Q5			
Characteristic	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile		
Number of participants	1817		1435		2038		1155		1748		1221			
KDM-BA (years)	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)	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		
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%		
Female (%)	1131	62.2	872	60.8	1100	54.0	814	70.5	907	51.9	849	69.5		
	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile		
Age (years)	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		
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%		
Married (%)	1573	86.6	1308	91.1	1785	87.6	1038	89.9	1534	87.8	1100	90.1		
Urban residence (%)	491	27.0	758	52.8	643	31.6	616	53.3	499	28.5	626	51.3		
Ethnicity (%)														
Han	798	43.9	1155	80.5	1045	51.3	916	79.3	798	45.7	982	80.4		
Non-Han	1019	56.1	280	19.5	993	48.7	239	20.7	950	54.3	239	19.6		
Education (%)														
College or above	77	4.2	288	20.1	112	5.5	246	21.3	109	6.2	197	16.1		
High school	469	25.8	784	54.6	653	32.0	572	49.5	485	27.7	634	51.9		
Less than high school	506	27.8	235	16.4	574	28.2	195	16.9	468	26.8	236	19.3		
Never been to school	765	42.1	128	8.9	699	34.3	142	12.3	686	39.2	154	12.6		
Annual household income (CNY/year)														
< 12 000	478	26.3	107	7.5	502	24.6	88	7.6	429	24.5	114	9.3		
12 000–19 999	420	23.1	155	10.8	430	21.1	136	11.8	393	22.5	139	11.4		
20 000–59 999	610	33.6	527	36.7	684	33.6	408	35.3	601	34.4	479	39.2		
60 000–99 999	175	9.6	304	21.2	236	11.6	232	20.1	184	10.5	232	19.0		
100 000–200 000	118	6.5	267	18.6	157	7.7	220	19.0	116	6.6	197	16.1		

(Continued)

Table 1. (Continued)

Characteristic	HDS			DASH			aMED					
	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5				
	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile	Median	25th, 75th percentile				
> 200 000	16	0.9	75	5.2	29	1.4	71	6.1	25	1.4	60	4.9
Occupation (%)												
Primary industry	823	45.3	280	19.5	884	43.4	214	18.5	783	44.8	253	20.7
Secondary industry	96	5.3	99	6.9	147	7.2	62	5.4	104	5.9	74	6.1
Tertiary industry	582	32.0	694	48.4	685	33.6	546	47.3	558	31.9	580	47.5
Unemployed	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).

7.27 % of the negative association between PDI and KDM-AA ( $RD_{\text{Indirect}} = -0.021$  (95 % CI:  $-0.051, 0.000$ ),  $P = 0.042$ ,  $P_{\text{FDR}} = 0.084$ ) and 9.19 % of the negative association between hPDI and KDM-AA ( $RD_{\text{Indirect}} = -0.027$  (95 % CI:  $-0.069, 0.000$ ),  $P = 0.048$ ,  $P_{\text{FDR}} = 0.096$ ). 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 four 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 (online Supplementary Tables 9–15).

## Discussion

### Summary of main results

Based on the 8288 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 and aMED) were negatively associated with KDM-AA, with DASH showing the strongest beneficial association. Among the food groups assessed, tea and soyabean 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 ageing measured by composite biomarkers. Our findings are consistent with previous research, although most previous studies focused on the associations of dietary patterns with telomeres<sup>(19)</sup>, frailty<sup>(47)</sup>, epigenetic age<sup>(8,48,49)</sup>, composite biomarker BA based on deep neural network<sup>(50)</sup>, other ageing metrics or ageing-related outcomes<sup>(16,18,32,51)</sup>. Various measures of ageing may capture distinct aspects of the ageing process<sup>(7)</sup>. Our study, in conjunction with previous research, comprehensively demonstrates that healthy dietary patterns may be negatively associated with multiple dimensions of biological ageing.

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 ageing<sup>(8,19,50)</sup>. 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.

**Table 2.** The associations of dietary patterns with KDM-AA (*n* 8288)

	PDI				hPDI				uPDI			
	Median	<i>n</i>	$\beta$	95 % CI	Median	<i>n</i>	$\beta$	95 % CI	Median	<i>n</i>	$\beta$	95 % CI
Q1	38	1721	Reference		38	1680	Reference		39	1692	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
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
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
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
Continues*	45	8288	-0.16***	-0.24, -0.08	45	8288	-0.15***	-0.23, -0.06	47	8288	0.25***	0.16, 0.35
$P_{\text{trend}}^{\dagger}$	-	-	< 0.001***		-	-	< 0.001***		-	-	< 0.001***	
	HDS				DASH				aMED			
	Median	<i>n</i>	$\beta$	95 % CI	Median	<i>n</i>	$\beta$	95 % CI	Median	<i>n</i>	$\beta$	95 % CI
Q1	10	1817	Reference		15	2038	Reference		20	1748	Reference	
Q2	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	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	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	20	1435	-0.71***	-0.99, -0.42	27	1155	-0.91***	-1.19, -0.63	31	1221	-0.53***	-0.82, -0.25
Continues*	15	8288	-0.23***	-0.32, -0.13	21	8288	-0.33***	-0.42, -0.24	25	8288	-0.19***	-0.28, -0.11
$P_{\text{trend}}^{\dagger}$	-	-	< 0.001***		-	-	< 0.001***		-	-	< 0.001***	

PDI, plant-based diet index; hPDI, healthful plant-based diet index; HDS, healthy diet score; KDM-AA, KDM-BA acceleration.

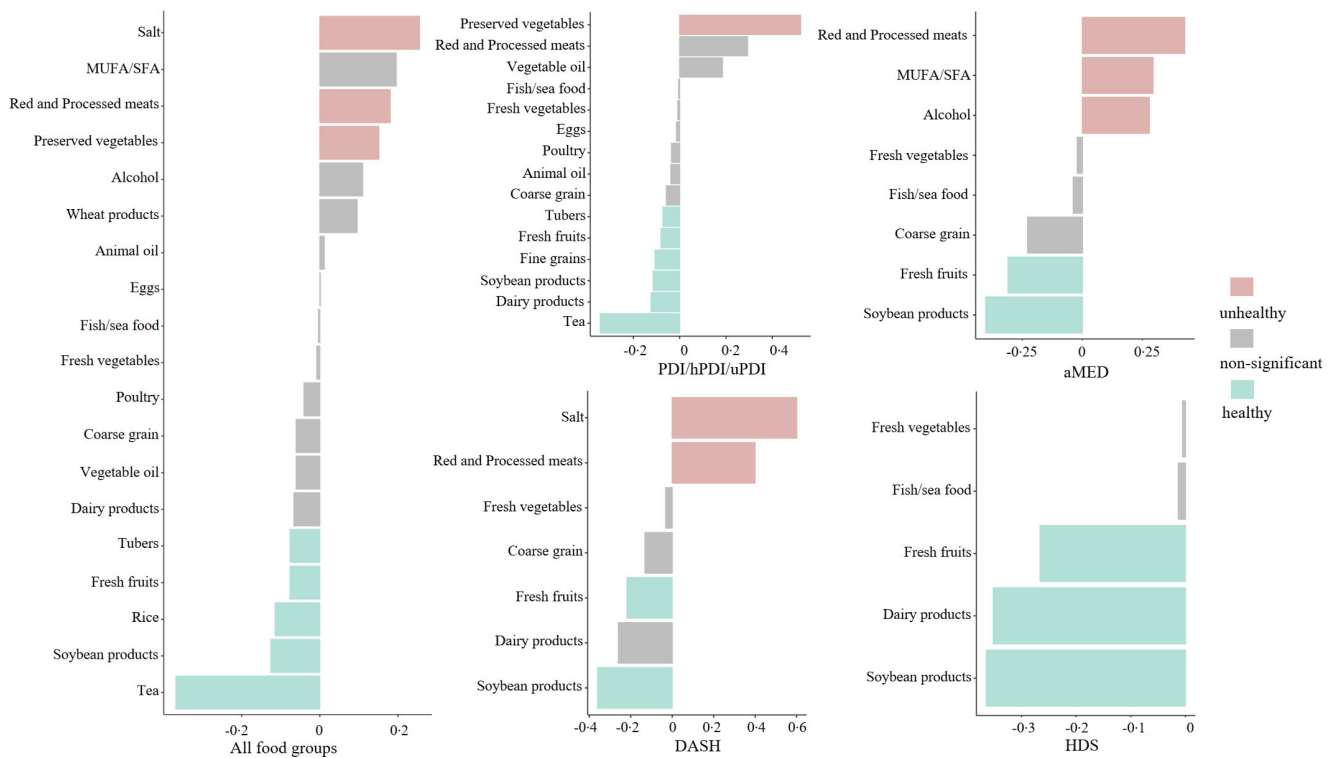
Results were adjusted for covariates: the baseline KDM-AA, age, sex, ethnicity, marital status, education, annual household income, occupation, family history, urbanicity, smoking status, physical activity, total energy intake, BMI, dietary supplement, insomnia symptom, depressive symptom, anxiety symptom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

\*\*\*Presented *P* value < 0.001. \*\*presented *P* value  $\geq$  0.001 & < 0.01. \*presented *P* value  $\geq$  0.01 & < 0.05.

\*Continuous dietary indicators were standardised.

†Two-sided  $P_{\text{trends}}$  were obtained by assigning median values to each quintile and then incorporating it into the model as a continuous variable.





**Figure 1.** 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 and 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 grey bars represent food groups with no statistically significant association with KDM-AA in the model. KDM-AA, KDM-BA acceleration.

The different results may be attributed to the focus on various biological ageing 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 WHO's recommended intake by more than twice<sup>(52)</sup>, and high-salt intake ranks as the third leading risk factor for death and disability-adjusted life years in China<sup>(53)</sup>. 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<sup>(30)</sup>.

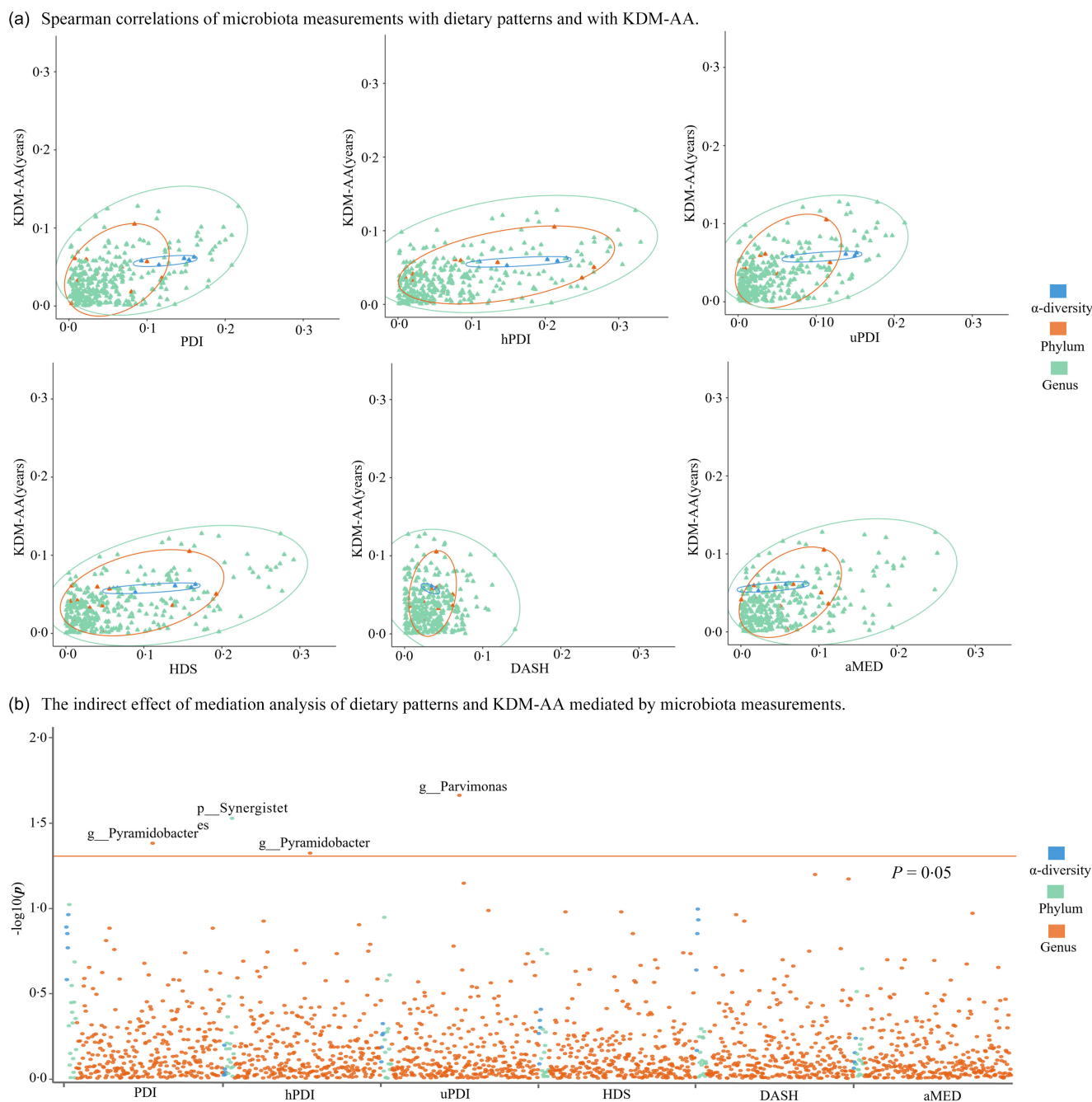
*Tea and soyabean products may be the significant beneficial food groups, while salt, preserved vegetables, red and processed meats were identified as the major harmful food groups*

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<sup>(54)</sup> 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 ageing in the southwestern region of China. Salt reduction has been adopted as one of the most cost-effective public policies worldwide<sup>(55)</sup>. 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 emphasises 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 soyabean products may be primarily beneficial food groups. Tea is known to be rich in polyphenols, purine alkaloids, theanine, tea polysaccharides and caffeine bioactive compounds<sup>(29)</sup>, while soybean products are abundant in polyphenols, carotenoids, phytosterols, phytic acids, alkaloids and other phytochemicals<sup>(56)</sup>. Polyphenols, in particular, have been extensively studied and are recognised as being negatively associated with biological ageing. Research has indicated that the underlying mechanism may involve polyphenols exerting anti-inflammatory effects and modulating gut microbiota, thereby promoting healthy ageing<sup>(20,57–60)</sup>.

Overall, our findings align with the fundamental principles recommended by the current Chinese dietary guidelines and may



**Figure 2.** Interrelation of various dietary patterns, gut microbiome measurements and KDM-AA ( $n = 764$ ). (a) 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  $\alpha$ -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  $\alpha$ -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. (b) 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  $-\log_{10}(P)$  values of the indirect effect of mediation analysis. A higher  $-\log_{10}(P)$  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  $\alpha$ -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. KDM-AA, KDM-BA acceleration.

serve as a reference for their further improvement. The DASH diet, as advocated by our study, emphasises 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 ageing. 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 and cancer, and its abundance is significantly increased in patients with Parkinson's disease and heart failure<sup>(61–66)</sup>. The *Pyramidobacter* genus is associated with inflammatory factors and may contribute to pathogenic infections<sup>(67)</sup>. Furthermore, it has been recognised as a biomarker for colorectal cancer and oral squamous cell carcinoma<sup>(68,69)</sup>. 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<sup>(70)</sup>. Overall, this indicates that plant-based diets may modulate the abundance of gut microbiota through polyphenolic compounds, thereby potentially promoting healthy ageing. 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 ageing.

#### *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 BA in LEMRs. Additionally, our study utilised longitudinal data, while most epidemiological studies focusing on the association between dietary patterns and biological ageing 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 BA in LEMRs.

However, several limitations should be acknowledged. First, 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<sup>(71)</sup>. Therefore, we believe that this limitation is less likely to significantly influence our main conclusions. Second, we lacked rigorous quantification for sugar-sweetened beverages (sugar sweetened beverages) and lacked information on consumption of nuts. Therefore, we excluded sugar sweetened beverages 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 sugar sweetened beverages and nuts is very low in the LEMRs, we believe that the impact of this deficiency on our results is limited. Third, due to data availability, the biomarkers used to construct KDM-BA do not include fully biomarkers related to ageing. Thus, the KDM-BA may reflect certain aspect of ageing. Fourth, 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 generalised 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 and 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 and preserved vegetables) for coping with biological ageing. 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 ageing, as well as potential mechanisms.

**Supplementary material.** For supplementary material/s referred to in this article, please visit <https://doi.org/10.1017/S0007114524002733>

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H. M. Z., N. Z. and S. R. H. designed research. H. J. Z., J. Z. L., T. T. Y., F. M., L. L. C. and M. M. H. conducted research. H. J. Z. provided parts of essential databases. HMZ analysed data, wrote the paper and had primary responsibility for final content. X. X., Y. X., J. J. C., Y. Z., N. Z., F. Y., S. R. H., H. X. C. and S. C. L. provided technical assistance and advice for data analysis and manuscripts. All authors read and approved the final manuscript.

All authors declare no competing interests.

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