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Huilian Zhu [*,†], Liying Zhao [†], Yemei Chen, Jiaqiu Liang, Siyu Huang, Zhaoyan Liu, Maierhaba Wusiman, Yeqin Xu, Yanxin Ye

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Phenotypic Age Mediates the Association Between Dietary Index for Gut Microbiota and Mortality: A Prospective Cohort Study Based on NHANES 1999–2018

Liying Zhao [1,†], Yemei Chen [1,†], Jiaqiu Liang [2], Siyu Huang [1,5], Zhaoyan Liu [1,5], Maierhaba Wusiman [1,5], Yeqin Xu [1], Yanxin Ye [4] and Huilian Zhu [1,5,*]

[1] Department of Nutrition, School of Public Health, Sun Yat-Sen University, 74 Zhong Shan Road 2, Guangzhou, 510080, Guangdong, China.
[2] Department of Interventional Surgery Center, Guangzhou First People's Hospital, China.
[3] Department of Clinical Nutrition, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China.
[4] Department of Neurology, Guangzhou First People's Hospital, China.
[5] Guangdong Provincial Key Laboratory of Food, Nutrition and Health, School of Public Health, Sun Yat-Sen University, Guangzhou, Guangdong, China.
* Correspondence: zhuhl@mail.sysu.edu.cn; Tel.: +86-20-8733-1811
† These authors contributed equally to this work.

Abstract: Background: The gut microbiota influences metabolism, immunity, and aging, and diet shapes microbiota composition. The Dietary Index for Gut Microbiota (DI-GM) quantifies dietary quality favoring microbial diversity; however, its relationship with mortality remains unclear. Objective: We assessed associations of DI-GM with all-cause and cardiovascular disease (CVD) mortality, and evaluated phenotypic age as a potential mediator. Methods: We included 40,314 adults aged ≥20 years from the U.S. National Health and Nutrition Examination Survey (NHANES) cycles 1999–2018, with mortality follow-up through linkage to the National Death Index up to December 31, 2019. DI-GM scores were derived from dietary recalls, and phenotypic age was computed from blood biomarkers. Cox regression and mediation analyses were performed to assess associations and mediating effects.Results: Over a median follow-up of 115 months, 6,156 all-cause and 1,933 CVD deaths occurred. Compared to participants in the lowest quartile (Q1, scores 0–2), those in the highest quartile (Q4, scores ≥5) showed reduced risks of all-cause (HR: 0.84; 95% CI: 0.73–0.97) and CVD mortality (HR: 0.76; 95% CI: 0.57–1.02). Each 1-score increment was associated with lower all-cause (HR: 0.97; 95% CI: 0.94–0.99) and CVD mortality (HR: 0.94; 95% CI: 0.90–0.99). Phenotypic age mediated 31.9% and 13.4% of these associations, respectively. Conclusions: Greater adherence to a gut microbiota–supportive dietary pattern, as measured by the DI-GM, was associated with reduced risks of all-cause and CVD mortality. These associations were partially mediated by phenotypic age, suggesting that biological aging may be an important pathway linking diet to longevity and cardiovascular health. Our findings highlight the potential of microbiota-targeted dietary strategies for promoting healthy aging in the general population.

Keywords: gut microbiota; dietary quality; phenotypic age; mortality; cardiovascular disease ; NHANES

1. Introduction

Population aging and the global surge in chronic noncommunicable diseases present significant public health challenges. According to projections from the Global Burden of Disease study, annual deaths worldwide are expected to rise from 60.1 million in 2022 to 90.7 million by 2050, driven largely

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by aging populations and lifestyle-related risk factors, particularly diet [1,2] Cardiovascular disease (CVD) remains the leading cause of death globally, strongly influenced by modifiable dietary behaviors [3]. Therefore, understanding dietary strategies that effectively mitigate aging-related mortality risks is essential.

Emerging evidence underscores the gut microbiota as a crucial intermediary linking dietary habits to chronic health outcomes [4–7]. The gut microbiota contributes to metabolic homeostasis [8], immune regulation [9], and systemic inflammation [10], thereby affecting cardiovascular [11], metabolic [12], and neurocognitive health [13]. Microbial dysbiosis, characterized by reduced microbial diversity and altered composition, has been associated with increased inflammation, impaired immune responses, and elevated risks of chronic diseases and mortality [12,14].

To quantify dietary impacts on gut microbiota health, Kase et al. recently proposed the dietary index for gut microbiota (DI-GM), a literature-derived dietary quality score incorporating foods and nutrients specifically associated with beneficial microbial diversity, such as fiber-rich foods, fermented dairy, and plant-based nutrients, along with reduced intake of red and processed meats and refined carbohydrates [15]. Higher DI-GM scores have been linked to favorable outcomes including reduced risks of depression, stroke, and type 2 diabetes [16–18]. However, whether adherence to a microbiota-supportive diet, as measured by the DI-GM, predicts long-term mortality has not yet been evaluated.

Biological aging, distinct from chronological age, refers to the progressive decline in physiological function and resilience over time. Among the various proposed biomarkers—such as telomere length, multi-omics-based clocks, and composite scores—phenotypic age has emerged as a validated and clinically practical indicator of biological aging [19,20]. Derived from routine blood biomarkers, it has shown strong predictive value for morbidity and mortality across diverse populations [21]. Moreover, recent evidence suggests that dietary factors may influence phenotypic aging trajectories, potentially through gut microbiota-mediated mechanisms [22–25].

Given this background, we hypothesized that greater adherence to DI-GM would be associated with reduced risks of all-cause and CVD mortality, and that these associations could be partially explained by reduced biological aging as indicated by lower phenotypic age. Clarifying this association could inform dietary guidelines and interventions aimed at promoting longevity and cardiovascular health. To address this hypothesis, we analyzed data from the National Health and Nutrition Examination Survey (NHANES) 1999–2018, a large, nationally representative cohort of U.S. adults, with long-term mortality follow-up through 2019.

2. Materials and Methods

2.1. Study Design and Population

This prospective cohort study was based on data from NHANES, a nationally representative, cross-sectional survey conducted by the National Center for Health Statistics (NCHS) using a stratified, multistage sampling strategy [26,27]. Although NHANES is cross-sectional in nature, mortality outcomes were obtained via linkage to the National Death Index, enabling longitudinal follow-up. Baseline data were collected from 1999 to 2018, and mortality follow-up was available through December 31, 2019. Adults aged ≥20 years who completed the Mobile Examination Center visit and provided dietary data were eligible for inclusion. We excluded participants with missing mortality data (n = 138), missing dietary data to calculate DI-GM scores (n = 6,292), or missing covariates (n = 8,337), yielding a final analytic sample of 40,314 participants. For mediation analyses involving phenotypic age, biomarkers were only available in the 1999–2010 and 2015–2018 cycles, resulting in a subsample of 29,581 participants after similar exclusions. Participant selection is illustrated in Figure S1 and Figure S2.

This study was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [28].

2.2. Mortality Ascertainment

Mortality status and cause of death were determined through linkage to the National Death Index, with follow-up through December 31, 2019. All-cause mortality was defined as death from any cause. Cardiovascular mortality was defined based on ICD-10 codes I00–I09, I11, I13, I20–I25, I26–

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I28, I29–I51, and I60–I69 [29–31] . Follow-up time was calculated from the date of the Mobile Examination Center visit to death or the end of follow-up.

2.3. Assessment of DI-GM

Dietary intake was assessed via in-person 24-hour recalls. Although two recalls were available for NHANES cycles from 2003 onward, we used only the first-day recall for all cycles to ensure consistency. The DI-GM was calculated according to the method described by Kase et al., incorporating 14 components [15]. The 10 microbiota-beneficial components were avocado, broccoli, chickpeas, coffee, cranberries, fermented dairy products, dietary fiber, nuts, seeds, and green tea (not assessed specifically in NHANES dietary recalls). The 4 microbiota-detrimental components included red meat, processed meat, refined grains, and high-fat foods (≥40% energy from fat). For each component, participants received 1 point for beneficial foods if consumption exceeded the sex-specific median, and 1 point for detrimental foods if intake was below the median. Scores were summed to yield a total DI-GM score ranging from 0 to 13, with higher scores indicating greater adherence to a gut microbiota–supportive diet. Participants were categorized into quartiles (Q1–Q4) based on their DI-GM scores [16,18,21].

2.4. Assessment of Phenotypic Age

Phenotypic age was estimated using Levine et al.'s algorithm, which incorporates chronological age and nine blood-based biomarkers: albumin, creatinine, glucose, C-reactive protein (CRP), lymphocyte percentage, mean cell volume, red cell distribution width, alkaline phosphatase, and white blood cell count [32] . Blood samples were collected after ≥8 hours of fasting and processed under standardized protocols [21,33–35].

The formula for phenotypic age is as follows:

$$\text{Phenotypic age} = 141.50 + \left(\ln\left[-0.00553 \times \ln\left(\exp\left(-1.51714 \times \exp(xb)\right) / 0.0076927\right)\right] / 0.09165\right)$$

$$\begin{aligned} xb = -19.9067 &- 0.0336 \times \text{Albumin} + 0.0095 \times \text{Creatinine} + 0.1953 \times \text{Glucose} \\ &+ 0.0954 \times \text{Ln CRP} - 0.0120 \times \text{Lymphocyte percent} \\ &+ 0.0268 \times \text{Mean cell volume} + 0.3306 \text{ x Red cell distribution width} \\ &+ 0.00188 \times \text{Alkaline phosphatase} + 0.0554 \times \text{White blood cell count} \\ &+ 0.0804 \times \text{Chronological age} \end{aligned}$$

2.5. Assessment of Covariates

Covariates were selected based on prior literature and included demographic, socioeconomic, behavioral, and clinical factors [36–43]. Age was treated continuously and categorized (20–39, 40–64, ≥65 years) for stratified analyses. Race/ethnicity was dichotomized into Non-Hispanic White versus others. Marital status was grouped into living with a partner (married/cohabitating) and living alone (single/divorced/widowed) [30]. Education level was classified as less than high school, high school or equivalent, and above high school. PIR, indicating household income relative to poverty thresholds, was categorized into low (≤1.30), medium (1.31–3.50), and high (>3.50) [17]. Smoking status was defined as never, former, or current based on lifetime smoking of ≥100 cigarettes [29,44]. Alcohol intake was similarly categorized as never, former, and current drinkers based on historical drinking patterns [45]. Physical activity was assessed by weekly minutes spent on walking and physical tasks [46,47]. BMI was classified as normal (<25 kg/m²), overweight (25–29.9 kg/m²), or obese (≥30 kg/m²) [41]. Self-reported history of CVD included physician diagnosis of congestive heart failure, coronary heart disease, angina, myocardial infarction, or stroke. Metabolic syndrome diagnosis followed the 2005 Adult Treatment Panel III criteria [36,37].

2.6. Statistical Analysis

All analyses accounted for the complex sampling design using NHANES survey weights and design variables [48]. Cox proportional hazards models estimated hazard ratios (HRs) and 95% confidence intervals (CIs) for mortality outcomes. DI-GM scores were analyzed continuously and categorically, with linear trends assessed using median scores. Three models progressively adjusted for demographic, behavioral, and clinical covariates. Restricted cubic spline (RCS) models (knots at 10th, 50th, 90th percentiles) evaluated potential nonlinearity. Subgroup analyses assessed interactions stratified by demographic, behavioral and clinical factors. Mediation analyses quantified the indirect

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effect of phenotypic age using quasi-Bayesian Monte Carlo simulations (1,000 iterations) [33]. Sensitivity analyses included: (1) excluding deaths within 2 years of follow-up, (2) excluding participants with baseline cancer, and (3) applying multiple imputation (5 datasets) for missing covariates using chained equations. Analyses were performed using R (v4.2.3; packages: "survey," "mediation," "mice") and Free Statistics software (v1.9.2). Two-sided $P < 0.05$ indicated statistical significance.

2.7. Ethics Approval

NHANES protocols were approved by the NCHS Research Ethics Review Board. Written informed consent was obtained from all participants. This analysis used publicly available, anonymized data, exempting it from additional ethical review.

3. Results

3.1. Participant Characteristics

Baseline characteristics of the final analytic sample (n = 40,314) are summarized by DI-GM categories in Table 1. Participants with higher DI-GM scores were generally older, female, non-Hispanic White, married or cohabiting, better educated, and had higher income levels. They also exhibited lower BMI, greater physical activity, a higher likelihood of current alcohol use and being never smokers, and a lower prevalence of metabolic syndrome.

Table 1. Baseline characteristics of participants by DI-GM score categories in NHANES 1999–2018[1].

Characteristics	Total	DI-GM[2]				*P* value
		Q1	Q2	Q3	Q4	
N	184,946,720	14,969,328	30,112,913	46,481,402	93,383,078	
Mean (SE)	4.57 (0.02)	1.81 (0.01)	3.00 (0.00)	4.00 (0.00)	5.81 (0.01)	<0.001
Age (year)	46.90 (0.21)	42.46 (0.48)	43.26 (0.30)	45.47 (0.25)	49.49 (0.26)	<0.001
Sex, n (%)						<0.001
male	19,694 (48.49%)	1,718 (50.04%)	3,560 (51.61%)	5,287 (50.61%)	9,129 (46.18%)	
female	20,620 (51.51%)	1,771 (49.96%)	3,440 (48.39%)	5,149 (49.39%)	10,260 (53.82%)	
Race, n (%)						<0.001
Non-Hispanic White	18,881 (70.29%)	1,413 (63.17%)	2,909 (63.89%)	4,696 (67.94%)	9,863 (74.67%)	
Other	21,433 (29.71%)	2,076 (36.83%)	4,091 (36.11%)	5,740 (32.06%)	9,526 (25.33%)	
Marital status, n (%)						<0.001
Married or living with a partner	24,571 (63.14%)	2,015 (60.40%)	4,034 (58.94%)	6,289 (61.73%)	12,233 (65.63%)	
Living alone	15,743 (36.86%)	1,474 (39.60%)	2,966 (41.06%)	4,147 (38.27%)	7,156 (34.37%)	
PIR, n (%)						<0.001
≤1.30	12,109 (21.31%)	1,246 (27.12%)	2,475 (27.28%)	3,411 (24.45%)	4,977 (16.90%)	
1.30-3.50	15,358 (35.25%)	1,355 (37.15%)	2,762 (37.08%)	4,030 (36.78%)	7,211 (33.60%)	
>3.50	12,847 (43.43%)	888 (35.73%)	1,763 (35.63%)	2,995 (38.77%)	7,201 (49.51%)	
Educational level, n (%)						<0.001
Less than high school	10,173 (15.89%)	970 (20.13%)	1,998 (19.76%)	2,858 (18.24%)	4,347 (12.80%)	

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High school or equivalent	9,354 (24.01%)	965 (30.12%)	1,870 (29.23%)	2,601 (26.37%)	3,918 (20.17%)	
Above high school	20,787 (60.10%)	1,554 (49.75%)	3,132 (51.01%)	4,977 (55.40%)	11,124 (67.03%)	
Smoking status, n (%)						<0.001
never	21,646 (53.33%)	1,874 (52.53%)	3,644 (52.31%)	5,487 (51.91%)	10,641 (54.50%)	
former	10,187 (25.09%)	736 (20.82%)	1,541 (20.95%)	2,452 (22.97%)	5,458 (28.17%)	
current	8,481 (21.58%)	879 (26.65%)	1,815 (26.74%)	2,497 (25.12%)	3,290 (17.33%)	
Alcohol use, n (%)						0.036
never	5,648 (11.12%)	484 (12.38%)	930 (11.39%)	1,508 (11.65%)	2,726 (10.57%)	
former	7,144 (14.37%)	648 (15.12%)	1,249 (15.13%)	1,846 (14.39%)	3,401 (14.00%)	
current	27,522 (74.50%)	2,357 (72.50%)	4,821 (73.48%)	7,082 (73.96%)	13,262 (75.43%)	
Physical activity, minutes per week	180.00 (15.75, 660.00)	126.00 (0.00, 600.00)	141.75 (0.00, 660.00)	157.50 (7.88, 708.75)	200.00 (31.50, 660.00)	<0.001
BMI (kg/m2)	28.81 (0.07)	30.26 (0.20)	29.65 (0.13)	28.95 (0.10)	28.25 (0.08)	<0.001
CVD, n (%)						0.035
No	35,927 (91.27%)	3,132 (90.67%)	6,325 (91.92%)	9,320 (91.84%)	17,150 (90.86%)	
Yes	4,387 (8.73%)	357 (9.33%)	675 (8.08%)	1,116 (8.16%)	2,239 (9.14%)	
Metabolic syndrome, n (%)						0.400
No	25,302 (66.17%)	2,229 (65.32%)	4,543 (67.12%)	6,668 (66.69%)	11,862 (65.75%)	
Yes	15,012 (33.83%)	1,260 (34.68%)	2,457 (32.88%)	3,768 (33.31%)	7,527 (34.25%)	

Abbreviations: DI-GM, dietary Index for gut microbiota; NHANES, National Health and Nutrition Examination Survey; PIR, poverty income ratio; BMI, body mass index; CVD, cardiovascular disease; SE, standard error; Q, quartile. [1]Continuous variables are presented as weighted mean (standard error) or median (interquartile range), whereas categorical variables are presented as actual frequency (weighted percentage). N represents weighted counts to reflect the population distribution, while n represents unweighted counts from the actual sample size. [2]Range of DI-GM scores: Q1, 0-2 score; Q2, 3 score; Q3, 4 score; Q4, 5-11 score.

3.2. DI-GM and Risk of Mortality

Over a median follow-up of 9.58 years, there were 6,156 all-cause deaths and 1,933 deaths attributed to CVD. When DI-GM was modeled as a continuous variable, each 1-score increase in DI-GM was associated with a 3% reduction in all-cause mortality risk (HR: 0.97; 95% CI: 0.94–0.99; p = 0.01) and a 6% reduction in CVD mortality risk (HR: 0.94; 95% CI: 0.90–0.99; p = 0.014) after full adjustment. In categorical analyses, participants in the highest quartile of DI-GM (Q4) experienced a 16% lower risk of all-cause mortality compared to those in the lowest quartile (Q1) (HR: 0.84; 95% CI: 0.73–0.97; p-trend = 0.024). The highest quartile (Q4) also showed a lower risk of CVD mortality compared to the lowest quartile (Q1), although the confidence interval included unity (HR: 0.76; 95% CI: 0.57–1.02; p-trend = 0.060) (Table 2).

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Table 2. Association between DI-GM and all-cause and CVD mortality among U.S. adults, NHANES 1999–2018.

	DI-GM				DI-GM group[1]				
					Q1	Q2	Q3	Q4	
	Deaths, no./ total no.	Person-year	HR(95%CI)	P value	Reference	HR(95%CI)	HR(95%CI)	HR(95%CI)	Trend test
CVD mortality									
Model 1[2]	1933/40314	396,995.167	0.92(0.88, 0.96)	<0.001	1.00	0.81(0.59, 1.11)	0.82(0.63, 1.07)	0.71(0.53,0.94)	0.008
Model 2[3]	1933/40314	396,995.167	0.94(0.89, 0.98)	0.004	1.00	0.80(0.58, 1.10)	0.83(0.64, 1.08)	0.73(0.55,0.98)	0.032
Model 3[4]	1933/40314	396,995.167	0.94(0.90, 0.99)	0.014	1.00	0.83(0.61, 1.13)	0.86(0.66, 1.12)	0.76(0.57,1.02)	0.060
All-cause mortality									
Model 1[2]	6156/40314	396,995.167	0.95(0.92, 0.97)	<0.001	1.00	0.88(0.74,1.03)	0.90(0.78,1.04)	0.79(0.69,0.92)	<0.001
Model 2[3]	6156/40314	396,995.167	0.96(0.94, 0.99)	<0.003	1.00	0.86(0.73,1.02)	0.90(0.78,1.04)	0.82(0.71,0.95)	0.011
Model 3[4]	6156/40314	396,995.167	0.97(0.94, 0.99)	0.01	1.00	0.87(0.74,1.03)	0.92(0.79,1.06)	0.84(0.73,0.97)	0.024

Abbreviations: DI-GM, dietary index for gut microbiota; CVD, cardiovascular disease; NHANES, National Health and Nutrition Examination Survey; HR, hazard ratio; CI, confidence interval; PIR, poverty income ratio; BMI, body mass index; Q, quartile. [1]Range of DI-GM scores: Q1, 0-2 score; Q2, 3 score; Q3, 4 score; Q4, 5-11 score. [2]Model 1: adjusted for age, sex, marital status, race/ethnicity, education, PIR, and survey cycle. [3]Model 2: additionally adjusted for smoking status, alcohol use, and physical activity. [4]Model 3: further adjusted for BMI, history of CVD, and metabolic syndrome.

RCS analyses indicated linear inverse dose–response relationships between DI-GM and both outcomes (p for non-linearity = 0.739 for all-cause mortality; p = 0.893 for CVD mortality), supporting the robustness of these associations across the full range of DI-GM scores (Figure S3).

Findings remained consistent in sensitivity analyses. Excluding participants who died within the first 2 years of follow-up or those with cancer at baseline yielded similar results. Multiple imputation for missing data also confirmed the observed associations (Table S1 and Table S2).

3.3. Effect Modification and Subgroup Analyses

Subgroup analyses identified several significant interactions (Figures 1 and 2).

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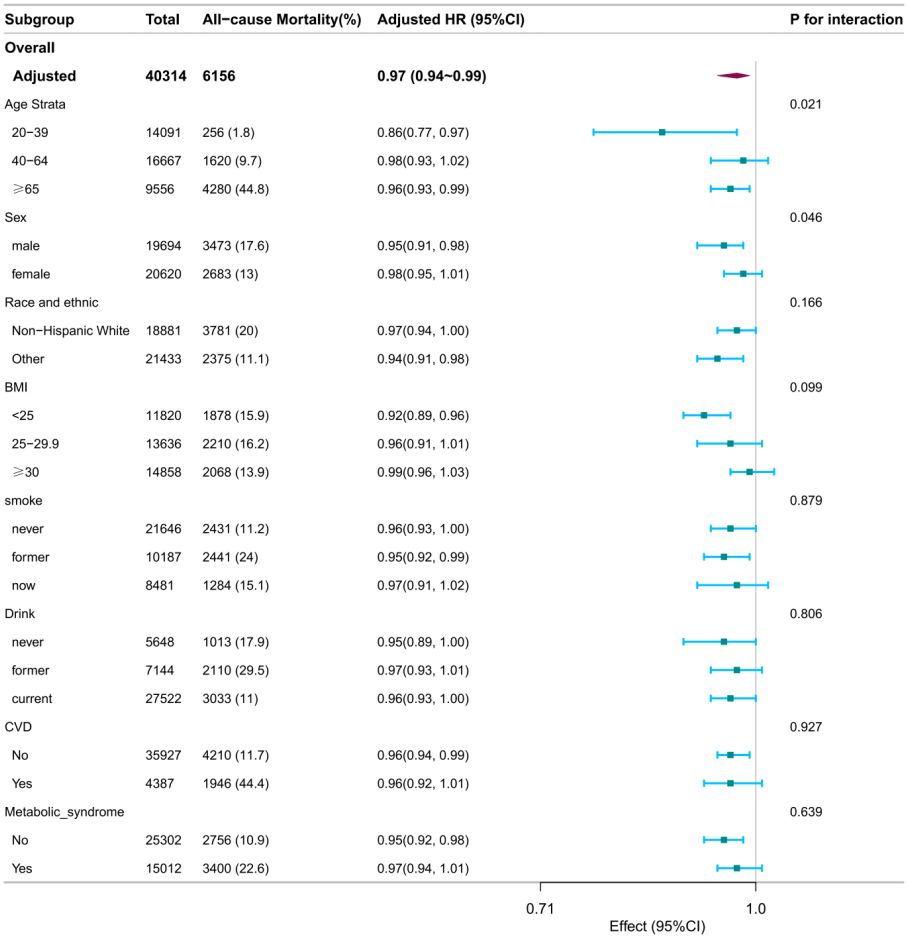

Figure 1. Stratified associations between DI-GM and all-cause mortality. Stratified Cox models were adjusted for all covariates (age, sex, race/ethnicity, marital status, education, income, survey cycle, smoking, alcohol use, physical activity, BMI, CVD history, and metabolic syndrome), except for the stratification variable. Squares represent HRs; horizontal lines, 95% CIs; diamonds, overall estimates. Abbreviations: BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; DI-GM, dietary index for gut microbiota; HR, hazard ratio.

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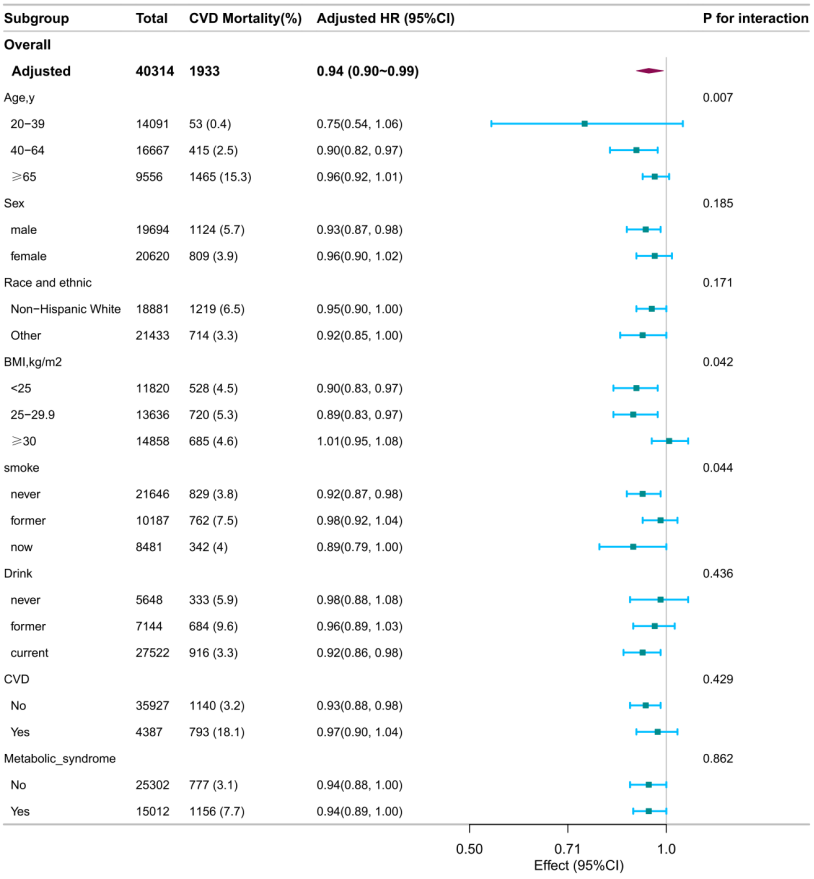

Figure 2. Stratified associations between DI-GM and CVD mortality. Stratified Cox models were adjusted for all covariates (age, sex, race/ethnicity, marital status, education, income, survey cycle, smoking, alcohol use, physical activity, BMI, CVD history, and metabolic syndrome), except for the stratification variable. Squares represent HRs; horizontal lines, 95% CIs; diamonds, overall estimates. Abbreviations: BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; DI-GM, dietary index for gut microbiota; HR, hazard ratio.

Age significantly modified associations with all-cause mortality (interaction $P = 0.021$) and CVD mortality (interaction $P = 0.007$). Specifically, inverse associations per 1-score increase in DI-GM were observed for all-cause mortality among younger (20–39 years; HR: 0.86; 95% CI: 0.77–0.97) and older adults (≥65 years; HR: 0.96; 95% CI: 0.93–0.99), but not among middle-aged participants (40–64 years; HR: 0.98; 95% CI: 0.93–1.02). For CVD mortality, an inverse association was significant only among participants aged 40–64 years (HR: 0.90; 95% CI: 0.82–0.97).

Sex interaction was significant for all-cause mortality (Pinteraction = 0.046), with associations stronger in males (HR: 0.95; 95% CI: 0.91–0.98) compared to females (HR: 0.98; 95% CI: 0.95–1.01).

BMI interaction was observed for CVD mortality (Pinteraction = 0.042), with inverse associations among normal-weight (BMI <25 kg/m²; HR: 0.90; 95% CI: 0.83–0.97) and overweight individuals (BMI 25–29.9 kg/m²; HR: 0.89; 95% CI: 0.83–0.97), but not among those with obesity (BMI ≥30 kg/m²; HR: 1.01; 95% CI: 0.95–1.08).

Smoking status interaction was significant for CVD mortality (Pinteraction = 0.044), with a significant inverse association observed only in never smokers (HR: 0.92; 95% CI: 0.87–0.98). The associations in former (HR: 0.98; 95% CI: 0.92–1.04) and current smokers (HR: 0.89; 95% CI: 0.79–1.00) were not statistically significant.

No significant interactions were found for race/ethnicity, alcohol consumption, CVD history, or metabolic syndrome with either mortality outcome.

3.4. Phenotypic Age as a Mediating Pathway

Phenotypic age partially mediated the associations between DI-GM and both all-cause and CVD mortality. Specifically, phenotypic age mediated approximately 32% (95% CI: 21–61%; P < 0.001) of

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the association between DI-GM and all-cause mortality, and about 13% (95% CI: 7–44%; P = 0.006) of the association with CVD mortality (Figure 3).

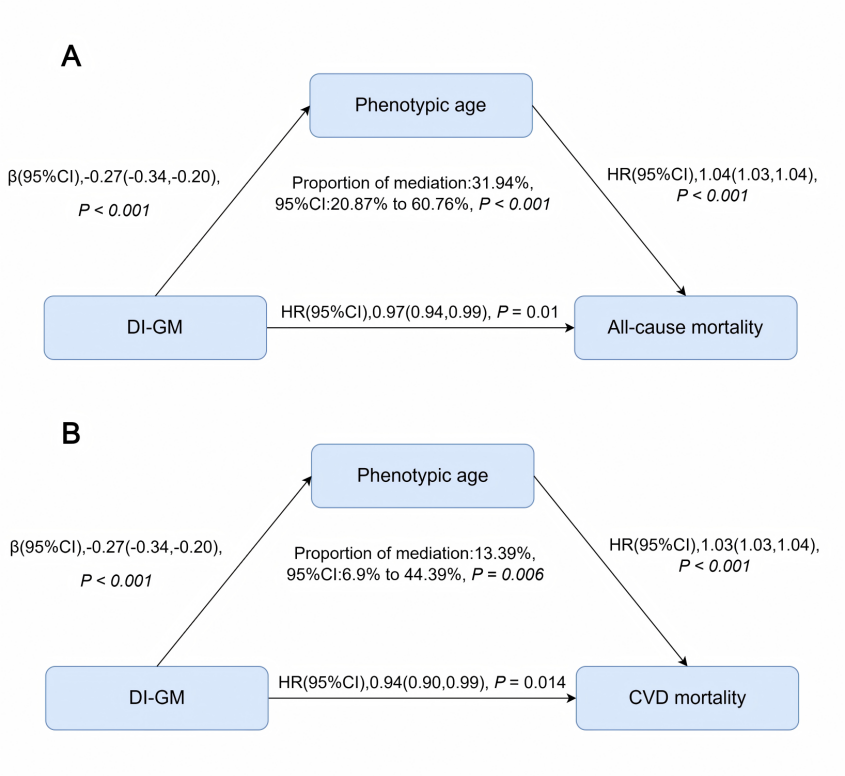

Figure 3. Mediation effect of phenotypic age on the association between DI-GM and mortality outcomes. Adjusted for all covariates (age, sex, race/ethnicity, marital status, education, income, survey cycle, smoking, alcohol use, physical activity, BMI, CVD history, and metabolic syndrome). Arrows represent direct and indirect paths in the mediation framework. Abbreviations: CI, confidence interval; DI-GM, dietary index for gut microbiota; HR, hazard ratio.

Descriptive characteristics of the mediation analysis subsample (n = 29,581) are presented in Table S3, with baseline patterns broadly consistent with the main analytic sample.

4. Discussion

In this nationally representative cohort of U.S. adults, we found that greater adherence to DI-GM was significantly associated with reduced risks of both all-cause and CVD mortality. This inverse association persisted across multiple analytic approaches, including subgroup and sensitivity analyses, and was further supported by mediation analyses highlighting phenotypic age as a potential biological pathway linking DI-GM to mortality outcomes. Our findings suggest a gut microbiota–supportive dietary pattern could be important for promoting longevity and cardiovascular health.

4.1. DI-GM and Risk of Mortality

To our knowledge, this is the first study investigating the association between DI-GM adherence and long-term mortality in a nationally representative U.S. population. Consistent with previous research linking gut microbiota diversity and composition to chronic disease outcomes and longevity [14,49], our study demonstrated that higher DI-GM scores were independently associated with lower risks of mortality. These results extend earlier evidence, such as associations reported for specific gut microbial taxa and fermented dairy intake with cardiovascular health outcomes [50], by emphasizing the holistic dietary approach targeting gut microbiota health.

Our age-stratified analysis revealed nuanced associations by age group. Specifically, DI-GM showed significant inverse associations with all-cause mortality in both younger (20–39 years) and older adults (≥65 years), but a less pronounced relationship in middle-aged adults (40–64 years). Conversely, the protective association for CVD mortality was most evident among middle-aged

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individuals. These differential patterns might reflect varying sensitivity to dietary influences on aging-related processes at distinct life stages: dietary patterns may shape early-life trajectories in young adults and modulate cumulative aging-related risk among older adults. The notable association with CVD mortality observed in middle-aged individuals might represent a critical intervention window for cardiovascular prevention.

4.2. Biological Aging as a Potential Mechanism

Our mediation analyses suggest that phenotypic age, a validated biomarker of biological aging, partially mediates the association between DI-GM and mortality outcomes. Specifically, phenotypic age explained approximately 32% and 13% of the associations with all-cause and CVD mortality, respectively. These findings indicate that dietary influences on biological aging pathways may be a critical mechanism underlying the observed health benefits of gut microbiota–supportive diets.

The stronger mediating role observed for all-cause mortality underscores that biological aging may impact multiple health domains beyond cardiovascular pathways. This aligns with previous findings showing gut microbiota composition changes with age and metabolic health, influencing aging-related processes such as systemic inflammation and immune function [22,25,51,52]. Given the established predictive ability of phenotypic age for adverse health outcomes independently of chronological age [21,53], our results provide further evidence supporting the hypothesis that diet and gut microbiota interactions significantly affect aging trajectories.

4.3. Strengths and Limitations

Key strengths of our study include a nationally representative sample of U.S. adults with extended follow-up, allowing broad generalizability of our findings. The robust analytical framework we employed, including sensitivity tests, subgroup analyses, and mediation analyses, further enhances the reliability and interpretability of our results. Additionally, our investigation into phenotypic age provides valuable insights into potential biological pathways linking diet to health outcomes, enriching current nutritional epidemiology literature.

Nevertheless, certain limitations exist. First, due to the observational nature of the study, we cannot establish causality. Second, dietary intake was assessed via a single 24-hour recall, possibly not fully capturing participants' habitual dietary patterns. Finally, although we adjusted extensively for multiple confounders, residual confounding remains possible. Future longitudinal studies with repeated dietary assessments and microbiome analyses are warranted to confirm and expand upon our findings.

4.4. Public Health Implications and Future Research

Our findings have important implications for dietary recommendations and public health strategies aimed at reducing mortality risk and promoting longevity. Encouraging dietary patterns that emphasize intake of fiber-rich foods (e.g., broccoli, chickpeas), fermented dairy, nuts, and reduced intake of processed meats and refined grains may represent practical strategies for chronic disease prevention and longevity through gut microbiota modulation.

Future research should validate these observational findings in different demographic and clinical populations, preferably through prospective cohort studies with repeated dietary assessments to better capture habitual dietary patterns. Further investigation using longitudinal gut microbiome assessments and mechanistic studies could elucidate specific microbial pathways linking diet to aging and mortality. Randomized controlled trials investigating interventions designed to modulate the gut microbiome and biological aging could provide causal evidence and practical dietary guidance. Additionally, refining the DI-GM and examining individual dietary components may enhance its application in precision nutrition and targeted dietary interventions.

5. Conclusions

In this nationally representative cohort of U.S. adults, greater adherence to DI-GM was associated with significantly reduced risks of all-cause and cardiovascular mortality. Phenotypic age partially mediated these relationships, indicating biological aging as a potential underlying mechanism. These findings highlight the importance of dietary modulation of gut microbiota for promoting

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longevity and cardiovascular health. Future intervention trials and mechanistic studies are warranted to establish causality and optimize dietary strategies for healthy aging.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: Participant flowchart for the main analytic sample; Figure S2: Participant flowchart for mediation analysis subsample; Figure S3: Dose–response association between DI-GM and mortality; Table S1: Sensitivity analyses between DI-GM and all-cause mortality; Table S2: Sensitivity analyses between DI-GM and CVD mortality; Table S3: Baseline characteristics of participants by DI-GM score categories for mediation analysis subsample.

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Abbreviations

The following abbreviations are used in this manuscript:

BMI: body mass index; CI: confidence interval; CRP: C-reactive protein; CVD: cardiovascular disease; DI-GM: dietary Index for gut microbiota; HR: hazard ratio; NCHS: National Center for Health Statistics; NHANES: National Health and Nutrition Examination Survey; PIR: poverty-income ratio; RCS: restricted cubic spline; SE: standard error

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