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Dietary Carbohydrate Quality Is Associated with Epigenetic Age Acceleration: a Cross-Sectional Study of the CARDIA Cohort

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ABSTRACT

Background: Dietary intake is one lifestyle factor that is expected to impact gene expression by altering DNA methylation (DNAm), thus affecting epigenetic aging. Studies on the association between quality of carbohydrates and epigenetic age acceleration (EAA) are scarce despite the evidence that quality may be more important than amount of carbohydrates consumed.

Objectives: We aimed to identify the cross-sectional associations of carbohydrate quality and fiber-rich food score with EAA in the Coronary Artery Risk Development in Young Adults (CARDIA) study.

Methods: Trained interviewers administered the CARDIA Diet History to obtain dietary intake at examination year 20. EAA measures, PhenoAge acceleration (PhenoAA) and GrimAge acceleration (GrimAA), were generated based on epigenetic age estimates calculated using DNAm profiling data from fasting blood samples at examination years 20, 25, and 30. Linear mixed-effects regression models were used to evaluate the association of carbohydrate quality, defined using carbohydrate: fiber ratio, and fiber-rich food score with EAA measures.

Results: After adjusting for demographic and lifestyle factors, quartiles of carbohydrate quality (defined using carbohydrate: fiber ratio) were inversely associated with PhenoAA and GrimAA; the highest carbohydrate quality quartile showing a difference (standard error [SE]) of -1.19 (0.2) y for PhenoAA (P -trend < 0.001) and -1.20 (0.1) y for GrimAA (P -trend < 0.001) compared with the lowest carbohydrate quality quartile. Similarly, quartiles of fiber-rich food score (created based on daily intakes of whole grains, fruit, vegetables, nuts, and legumes) were inversely associated with PhenoAA and GrimAA; the highest quartile showing a difference (SE) of -1.06 (0.2) y for PhenoAA (P -trend = 0.002) and -1.31 (0.2) y for GrimAA (P -trend < 0.001) compared with the lowest quartile.

Conclusions: Our findings suggest that consuming a high carbohydrate quality diet and a dietary pattern composed of fiber-rich foods is cross-sectionally associated with slower biological aging.

Keywords: carbohydrates, carbohydrate quality, dietary fiber, fiber-rich foods, biological age, epigenetic age acceleration

Introduction

In efforts to identify the pathway of diseases, the evolving area of epigenetics has been proposed to uncover mechanisms that mediate the reversible effects of lifestyle and environmental factors on risks of developing chronic diseases. Among epigenetic regulators, DNA methylation (DNAm) plays a crucial role in transcriptional regulation, thereby influencing gene expression. Examples of lifestyle and environmental factors associated with DNAm include diet, smoking, physical activity, obesity, alcohol

consumption, environmental pollutants, psychological stress, and disturbances to circadian biology [1], although the mechanism of epigenetic responses to the aforementioned stimuli remains to be clarified.

In addition to DNAm itself, measures of epigenetic age are derived based on change in DNAm at certain sites. Unlike chronologic age, which is based on birthdate, epigenetic age estimates the observed risk-based age of our organ systems. There are several epigenetic age measures, such as Horvath Clock, Hannum Clock, PhenoAge, and GrimAge [2]. PhenoAge

Abbreviations: CARDIA, Coronary Artery Risk Development in Young Adults; CHO, carbohydrate; CVD, cardiovascular disease; DGA, Dietary Guidelines for Americans; DNAm, DNA methylation; EAA, epigenetic age acceleration; GrimAA, GrimAge acceleration; PhenoAA, PhenoAge acceleration; SSB, sugar-sweetened beverage.

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depends on measurements from 513 CpG sites related to 9 biomarkers, and it can conclusively predict the incidence of cardiovascular disease (CVD) using whole blood DNAm values, as well as mortality, cancer, coronary artery disease, and dementia [3]. On the other hand, GrimAge depends on measurements from 1030 CpG sites related to 12 biomarkers in addition to smoking pack-years and sex, and it predicts mortality, cancer, and coronary artery disease with high accuracy [4]. Therefore, our study focused on PhenoAge and GrimAge because these measures show better performance in association with health outcomes, especially CVD, than other measures [2–5]. The corresponding acceleration measures, PhenoAge acceleration (PhenoAA) and GrimAge acceleration (GrimAA), are measurements used to assess whether epigenetic age is older than chronologic age [6].

Among dietary factors, higher diet quality has been associated with decelerated (i.e., younger) epigenetic aging measures [7,8]. Notably, the association between quality of carbohydrate (CHO) and epigenetic age acceleration (EAA) has not been studied despite evidence from nutritional epidemiology studies that quality may be more important than the amount of CHO consumed [9,10]. Therefore, we aimed to investigate the cross-sectional association between CHO quality and EAA in adults enrolled in the Coronary Artery Risk Development in Young Adults (CARDIA) study. In addition, we examined the cross-sectional associations of a fiber-rich food score with EAA. We hypothesized that higher CHO quality and fiber-rich food score would be associated with decelerated epigenetic age (slower epigenetic aging).

Methods

Details of the CARDIA study have been published elsewhere [11]. Briefly, CARDIA is a population-based, multicenter, longitudinal study that began in 1985–1986 to investigate the determinants and development of CVD and their risk factors in Black and White males and females during young adulthood.

At baseline (year 0 in 1985–1986), 5115 participants aged 18–30 y and free of overt CVD were enrolled in the study. Response rates exceeded 70% of those recruited at all follow-up examinations (from years 2–30), and 87% attended ≥ 4

examinations. The field centers are located in Birmingham, AL ($n = 1178$); Chicago, IL ($n = 1109$); Minneapolis, MN ($n = 1402$); and Oakland, CA ($n = 1426$). The local institutional review board at each field center reviewed and approved the study protocols annually, and all participants were provided with information about the study examination and asked to sign an informed consent at every clinic examination. This study is a secondary data analysis using deidentified data; therefore, it qualified for an institutional review board exemption.

Eligibility and exclusion criteria

Among 3549 participants who attended the examination at year 20, participants with missing PhenoAA and GrimAA measures or dietary data at year 20 and those with extreme energy intake (<800 kcal/d or >8000 kcal/d for males and <600 kcal/d or >6000 kcal/d for females) were excluded. A total of 2331 participants were included in this study. A flowchart of the inclusion and exclusion criteria is shown in Figure 1.

Demographic, lifestyle, and clinical factors

Standardized questionnaires were used to obtain demographic characteristics (age, sex, race, education, and family income) and lifestyle factors (physical activity, alcoholic beverage drinking habits, and cigarette smoking). Family income was categorized as $<\$50,000$, $\$50,000$ to $\$99,999$, or $\geq \$100,000$. A physical activity score was calculated based on the total time spent on activities performed during work and leisure time, weighted by estimated energy cost per minute [12]. Drinking habits were dichotomized as current drinker or not, and smoking status was dichotomized as nonsmoker or ever smoker (former and current smokers). Weight and height were measured by beam balance scale and stadiometer, respectively. BMI was calculated as weight/height (kilogram per square meter), and obesity was defined as BMI ≥ 30 kg/m². Diabetes was defined as having fasting blood glucose ≥ 7 mmol/L (126 mg/dL), non-fasting blood glucose ≥ 11.1 mmol/L (200 mg/dL), 2-h post-challenge glucose ≥ 11.1 mmol/L (200 mg/dL) from an oral glucose tolerance test, glycated hemoglobin $\geq 6.5\%$ (48 mmol/mol), and/or reported antidiabetic medication use. Blood pressure was measured 3 times while participants were sitting at rest, with the last 2 measurements being averaged.

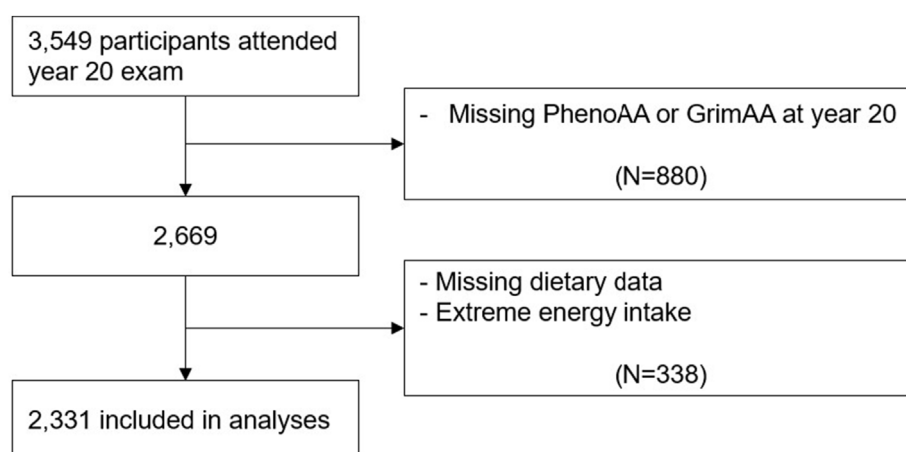


FIGURE 1. Flowchart of inclusion and exclusion criteria. Abbreviations: GrimAA, GrimAge acceleration; PhenoAA, PhenoAge acceleration.

Diet assessment

Trained interviewers conducted the CARDIA Diet History to assess the usual dietary intake of the participants at year 20. The Diet History is a dietary assessment tool to obtain what the participant consumed over the past month [13]. It includes 100 closed-ended questions (yes/no) on food and beverages such as grains, fruit, vegetables, legumes, meat, fish and seafood, dairy products, candy, sugar-sweetened beverages (SSBs), diet beverages, coffee, tea, and alcohol, to name a few. For those questions for which participants answered affirmatively, the interviewers then asked the participants to name food items eaten and to provide detailed information on them including brand name, if available, frequency (per day, week, or month), the amount consumed, how it was prepared, and whether anything was added to that food. Three-dimensional food models were used to aid the participant in recalling their intakes better. The validity and reliability of the CARDIA Diet History have been reported elsewhere [14].

Serving sizes by food groups (servings per day), total energy (kilocalories per day), and nutrient composition of foods were obtained from the Nutrition Data System for Research. Nutrition Data System for Research is a software program developed at the University of Minnesota Nutrition Coordinating Center. The primary source of food and nutrient composition is the USDA National Nutrient Database for Standard Reference. Values from other reliable databases, articles in scientific journals, and food manufacturers were utilized for food components and nutrient values not available from the USDA and brand name food products [15]. Food groups are based on USDA food groupings.

Carbohydrate quality

The CHO:fiber ratio was used to define CHO quality (greater CHO:fiber ratio representing lower CHO quality) in this study. Researchers have created indices to assess the quality of CHO in efforts to address the health impacts of CHO intake. Of those indices, the glycemic index and glycemic load, CHO quality index, and various CHO ratios, including CHO:fiber ratio, are commonly reported in the literature [16]. Among these indices, the CHO:fiber ratio is an effective and simple index to identify processed CHO-rich foods with higher nutritional quality (lower ratio indicates higher protein and minerals and lower fat, added sugar, sodium, and calories) [17]. Although the CHO:fiber ratio was first created mainly to assess the quality of grain-based foods, we decided to use the overall CHO:fiber ratio from all food groups with fiber-rich foods, including whole grains, fruit, vegetables, nuts, and legumes, which are known to have health benefits. In addition, the Dietary Guidelines for Americans (DGA) recommend including foods from the aforementioned food groups for a healthier dietary habit [18].

In addition to the CHO:fiber ratio, a fiber-rich food score was created to determine the association between intake of foods high in fiber and EAA. Food groups included in the fiber-rich food score are as follows: 1) whole grains, 2) fruit, 3) vegetables, 4) nuts, and 5) legumes [19]. To account for the rest of dietary intake, another food score, the data-driven dietary pattern score, was created using principal component analysis. It emphasizes the remaining foods and beverages including refined grains, candy, dairy, red and processed meat, poultry, eggs, fish and seafood, SSBs, and diet beverages.

Epigenetic aging measures

DNA sample collection and DNAm profiling

Overnight fasting venous blood samples were collected in EDTA tubes at years 20, 25, and 30. A PureGene DNA extraction kit (Gentra Systems) was used to extract DNA, which was stored at -70°C . Raw DNAm data were preprocessed, quality controlled, and normalized. Briefly, Illumina MethylationEPIC Beadchip was used for DNAm profiling, and the R package ENmix was used for data preprocessing and quality control [20]. Detailed criteria for low-quality DNA and DNAm data have been published elsewhere [21]. Briefly, the criteria of detection $P < 1 \times 10^{-6}$ or fewer than 3 beads were used for low-quality DNAm [21].

Epigenetic age acceleration

Epigenetic age estimates were calculated online at <https://dnamage.genetics.ucla.edu/new> [22]. PhenoAge [3] and GrimAge [4] were estimated from 513 and 1030 CpG sites, respectively. The corresponding acceleration measures, PhenoAA and GrimAA were generated. These measures are independent of chronologic age because they were defined as the residuals of a linear model of the corresponding epigenetic age regressed on chronologic age [22].

Statistical methods

We used SAS software (version 9.4, SAS Institute Inc.) for all data analyses in the study, and an alpha level of 0.05 was used to determine statistical significance. For those with missing covariates at examination year 20, values reported at examination year 15 were used given the assumption that values did not change over 5 y. Mixed-effects regression models were used to evaluate the association between CHO quality at year 20 and EAA measures repeated at years 20, 25, and 30. Random intercepts for individuals were included to account for correlations between measurements from the same individual over time, and CHO quality was treated as a fixed effect. The compound symmetry structure was used in the models. The CHO:fiber ratio was calculated by dividing the intakes of total CHO (grams per day) by total dietary fiber (grams per day). The CHO:fiber ratio was ranked inversely to create quartiles such that higher quartiles represent higher CHO quality. The fiber-rich food score was created based on the intake of each fiber-rich food group (whole grains, fruit, vegetables, nuts, and legumes) [19] ranked in quintiles. Scores from each food group were added together to form the fiber-rich food score (score range: 0–20). The linear trend across quartiles was evaluated to estimate P -trend. Pearson correlation coefficients between EAA measures at years 20, 25, and 30 are reported in Supplemental Table 1.

The mixed-effects models included a set of potential confounders. Model 1 was adjusted for time, sex, race, field center, education, family income, and energy intake. Model 2 was adjusted for model 1 plus total fat intake, physical activity, current drinker, and ever smoker. The same procedures were performed for fiber-rich food score analyses, except that model 2 was adjusted for a principal component analysis-derived dietary pattern score including the remaining food and beverage intake instead of total fat intake. Model 3 was further adjusted for BMI, diabetes, and systolic blood pressure. We examined effect modification by including cross-product terms in the models and verified that there was no effect modification by time, race, sex, or obesity ($P > 0.10$).

Additional sensitivity analyses were conducted to support the validity of the planned analyses. First, we additionally adjusted the models for age in separate models. Second, we removed energy intake from the models to see if the diet and EAA associations were affected if not adjusted for energy intake. Third, we replaced the covariate ever smoker with current smoker. Lastly, individual nutrients (CHO and fiber) and food groups included in the fiber-rich food score were analyzed in separate models.

Results

At examination year 20, the study participants were aged 38–43 y (mean 45.2 y), 57.8% female, and 56.7% White. The mean CHO:fiber ratio and the fiber-rich food score were 14.0 and 9.8, respectively. PhenoAA ranged from –23.7 to 23.9 y (SD: 5.9), and GrimAA ranged from –11.3 to 17.8 y (SD: 4.4) at examination year 20. Study participants who had a higher CHO quality diet were more likely to be older, female, White, educated, physically active, current drinkers, former smokers or noncurrent smokers, and have lower BMI and systolic blood pressure than those who had a lower CHO quality diet (Table 1).

Table 2 reports adjusted dietary intake (nutrients and food groups) stratified by quartiles of CHO quality. Briefly, participants with a higher CHO quality diet consumed less energy, total CHO, added sugar, saturated fat, SSBs, candy, and dairy products and consumed more fiber, protein, total fat, fiber-rich foods (whole grains, fruit, vegetables, nuts, and legumes), poultry, eggs, fish and seafood, and diet beverages than those with a lower CHO quality diet.

The adjusted mean EAA values across quartiles of CHO quality and fiber-rich food score are reported in Tables 3 and 4. As hypothesized, higher CHO quality showed a linear inverse association with PhenoAA and GrimAA; the highest CHO quality

group having a difference (SE) of –1.19 (0.2) y for PhenoAA (P -trend < 0.001) and –1.20 (0.1) y for GrimAA (P -trend < 0.001) compared with the lowest CHO quality group after adjusting for demographic and lifestyle factors (model 2). Similarly, the fiber-rich food score showed a linear inverse association with PhenoAA and GrimAA; the highest quartile having a difference (SE) of –1.06 (0.2) y for PhenoAA (P -trend = 0.002) and –1.31 (0.2) y for GrimAA (P -trend < 0.001) compared with the lowest quartile after adjusting for demographic and lifestyle factors (model 2). The associations were statistically significant after further adjusting for cardiometabolic factors (model 3).

The regression coefficients (β) for CHO:fiber ratio and fiber-rich food score as continuous variables for describing EAA are reported in Supplemental Table 2, and the results stratified by sex and race are reported in Supplemental Table 3.

Sensitivity analyses

The significant associations of CHO quality and fiber-rich food score with EAA did not change when the models were additionally adjusted for age or when ever smoker was replaced with current smoker. Not adjusting for energy intake did not change the associations of CHO quality and fiber-rich food score with EAA. In the individual nutrient and food groups analyses, intakes of dietary fiber, whole grains, and fruit showed linear inverse associations with EAA, whereas intakes of total CHO and other food groups included in the fiber-rich food score (vegetables, nuts, and legumes) were not associated with EAA (Supplemental Table 4).

Discussion

Higher CHO quality (represented by lower CHO:fiber ratio) and the fiber-rich food score were associated with slower DNAm-

TABLE 1
Mean (SD) of unadjusted participant characteristics stratified by quartiles of CHO quality (N = 2331).

	CHO quality			
	Q1 (low quality) (n = 583)	Q2 (n = 583)	Q3 (n = 583)	Q4 (high quality) (n = 582)
CHO:fiber ratio, ¹ median (range)	19.3 (16.2–129.9)	14.3 (12.9–16.1)	11.5 (10.2–12.8)	8.5 (3.4–10.1)
Demographic				
Age, y	44.3 (3.8)	45.0 (3.6)	45.5 (3.5)	45.9 (3.3)
Female, n (%)	284 (48.7)	315 (54.0)	335 (57.5)	414 (71.1)
White, n (%)	217 (37.2)	314 (53.9)	357 (61.2)	434 (74.6)
Education, y	14.0 (2.5)	15.1 (2.5)	15.5 (2.5)	15.9 (2.4)
Family income, n (%)				
<\$50,000	295 (50.6)	197 (33.8)	152 (26.1)	128 (22.0)
\$50,000–\$99,999	177 (30.4)	221 (37.9)	206 (35.3)	207 (35.5)
≥\$100,000	111 (19.0)	165 (28.3)	225 (38.6)	247 (42.5)
Lifestyle				
Physical activity	287 (269)	320 (269)	357 (280)	400 (285)
Current drinker, n (%)	431 (73.9)	447 (76.7)	473 (81.1)	487 (82.1)
Smoking, n (%)				
Never	350 (60.1)	361 (61.9)	377 (64.7)	374 (64.2)
Former	73 (12.5)	122 (20.9)	133 (22.8)	151 (26.0)
Current	160 (27.4)	100 (17.2)	73 (12.5)	57 (9.8)
Clinical				
BMI, kg/m ²	30.3 (6.8)	29.9 (7.0)	29.2 (6.7)	28.0 (6.3)
Obesity, n (%)	264 (45.3)	231 (39.6)	207 (35.5)	172 (30.0)
Diabetes, n (%)	43 (7.4)	71 (12.2)	56 (9.6)	68 (11.7)
SBP, mmHg	118 (15.6)	116 (15.6)	114 (14.3)	110 (14.1)

Abbreviations: BMI, body mass index; CHO, carbohydrate; SBP, systolic blood pressure; SD, standard deviation.

¹ The CHO:fiber ratio was calculated by dividing the intakes of total CHO (grams per day) by total dietary fiber (grams per day).

TABLE 2
Adjusted dietary intake¹ stratified by quartiles of CHO quality (N = 2331).

	CHO:fiber ratio			
	Q1 (low quality) (n = 583)	Q2 (n = 583)	Q3 (n = 583)	Q4 (high quality) (n = 582)
CHO:fiber ratio, ² median (range)	19.3 (16.2–129.9)	14.3 (12.9–16.1)	11.5 (10.2–12.8)	8.5 (3.4–10.1)
Nutrients				
Energy, kcal	2534 (41)	2377 (39)	2298 (40)	2322 (43)
Total CHO, g	292 (2.5)	273 (2.4)	268 (2.4)	250 (2.6)
Fiber, g	14 (0.3)	19 (0.2)	23 (0.2)	30 (0.3)
Added sugar, g	116 (1.8)	76 (1.7)	62 (1.7)	47 (1.8)
Protein, g	79 (0.9)	87 (0.9)	92 (0.9)	98 (0.9)
Total fat, g	90 (1.0)	95 (1.0)	95 (1.0)	102 (1.1)
Saturated fat, g	30 (0.4)	31 (0.4)	30 (0.4)	29 (0.4)
Food groups (servings per day)				
Whole grains	0.08 (0.03)	0.21 (0.02)	0.31 (0.02)	0.51 (0.03)
Fruit	2.19 (0.10)	2.66 (0.09)	3.04 (0.10)	3.40 (0.10)
Vegetables	2.48 (0.09)	3.36 (0.08)	4.21 (0.08)	5.89 (0.09)
Nuts	0.37 (0.07)	0.81 (0.07)	1.13 (0.07)	2.19 (0.08)
Legumes	0.13 (0.02)	0.26 (0.02)	0.37 (0.02)	0.66 (0.03)
Desserts with refined grains	0.22 (0.03)	0.26 (0.03)	0.25 (0.03)	0.19 (0.03)
Desserts without refined grains	6.14 (0.09)	6.52 (0.09)	6.23 (0.09)	5.16 (0.09)
SSBs	3.38 (0.08)	1.90 (0.08)	1.56 (0.08)	1.20 (0.08)
Candy	0.39 (0.02)	0.33 (0.02)	0.23 (0.02)	0.19 (0.02)
Dairy products	3.49 (0.15)	3.22 (0.15)	2.73 (0.15)	2.47 (0.16)
Red/processed meat	3.00 (0.08)	3.05 (0.08)	3.00 (0.08)	2.61 (0.08)
Poultry	1.18 (0.06)	1.34 (0.06)	1.50 (0.06)	1.78 (0.06)
Eggs	0.50 (0.03)	0.59 (0.03)	0.59 (0.03)	0.65 (0.03)
Fish/seafood	0.76 (0.06)	0.98 (0.05)	1.16 (0.05)	1.38 (0.06)
Diet beverages	0.58 (0.08)	0.85 (0.08)	0.84 (0.08)	0.92 (0.08)

Abbreviations: CHO, carbohydrate; SE, standard error; SSB, sugar-sweetened beverage.

Multiple linear regression models were used to estimate the adjusted mean and SE.

¹ Adjusted for age, sex, race, education, field center, and energy intake.

² The CHO:fiber ratio was calculated by dividing the intakes of total CHO (grams per day) by total dietary fiber (grams per day).

TABLE 3
Crude and adjusted mean (SE) epigenetic age acceleration measures stratified by quartiles of CHO quality (N = 2331)¹.

	CHO quality				P-trend
	Q1 (low quality) (n = 583)	Q2 (n = 583)	Q3 (n = 583)	Q4 (high quality) (n = 582)	
CHO:fiber ratio, ² median (range)	19.3 (16.2–129.9)	14.3 (12.9–16.1)	11.5 (10.2–12.8)	8.5 (3.4–10.1)	
PhenoAA					
Crude	1.07 (0.2)	0.13 (0.2)	−0.12 (0.2)	−0.79 (0.2)	<0.001
Model 1	0.74 (0.2)	0.10 (0.2)	0.03 (0.2)	−0.56 (0.2)	<0.001
Model 2	0.70 (0.2)	0.06 (0.2)	0.04 (0.2)	−0.49 (0.2)	<0.001
Model 3	0.66 (0.2)	−0.02 (0.2)	0.02 (0.2)	−0.38 (0.2)	0.003
GrimAA					
Crude	1.58 (0.2)	0.28 (0.2)	−0.62 (0.2)	−1.33 (0.2)	<0.001
Model 1	0.59 (0.2)	0.19 (0.2)	−0.29 (0.2)	−0.61 (0.2)	<0.001
Model 2	0.62 (0.1)	0.14 (0.1)	−0.29 (0.1)	−0.58 (0.1)	<0.001
Model 3	0.62 (0.1)	0.10 (0.1)	−0.30 (0.1)	−0.52 (0.1)	<0.001

Abbreviations: CHO, carbohydrate; GrimAA, GrimAge acceleration; PhenoAA, PhenoAge acceleration; SE, standard error.

Multiple linear regression models were used to estimate the adjusted mean and SE. The linear trend across quartiles was evaluated to estimate P-trend.

¹ Model 1 was adjusted for sex, race, education, family income, field center, and energy intake. Model 2 was adjusted for sex, race, education, family income, field center, energy intake, total fat intake, current drinker, ever smoking, and physical activity. Model 3 was adjusted for sex, race, education, family income, field center, energy intake, total fat intake, current drinker, ever smoking, and physical activity, body mass index, diabetes, and systolic blood pressure.

² The CHO:fiber ratio was calculated by dividing the intakes of total CHO (grams per day) by total dietary fiber (grams per day).

based epigenetic aging after adjusting for demographic and lifestyle factors. Sensitivity analyses further demonstrated that intakes of the scores' separate constituents, namely total CHO and individual fiber-rich foods vegetables, nuts, and legumes,

were not associated with EAA, which emphasizes the importance of the quality of CHO and a dietary pattern rather than only considering the amount of an individual nutrient or food group alone. Given that PhenoAge was associated with incident CVD in

TABLE 4
Crude and adjusted mean (SE) epigenetic age acceleration measures stratified by quartiles of fiber-rich food score (N = 2331)¹.

	Fiber-rich food score				P-trend
	Q1 (low fiber) (n = 590)	Q2 (n = 561)	Q3 (n = 635)	Q4 (high fiber) (n = 545)	
Fiber-rich food score, ² median (range)	4 (0–6)	7 (7–9)	11 (10–13)	16 (14–20)	
PhenoAA					
Crude	0.78 (0.2)	0.24 (0.2)	−0.16 (0.2)	−0.58 (0.2)	<0.001
Model 1	0.67 (0.2)	0.27 (0.2)	−0.19 (0.2)	−0.49 (0.2)	<0.001
Model 2	0.64 (0.2)	0.22 (0.2)	−0.17 (0.2)	−0.42 (0.2)	0.002
Model 3	0.40 (0.2)	0.21 (0.2)	−0.15 (0.2)	−0.23 (0.2)	0.04
GrimAA					
Crude	0.89 (0.2)	0.27 (0.2)	−0.48 (0.2)	−0.77 (0.2)	<0.001
Model 1	0.69 (0.2)	0.32 (0.2)	−0.47 (0.2)	−0.67 (0.2)	<0.001
Model 2	0.68 (0.2)	0.22 (0.2)	−0.47 (0.2)	−0.63 (0.2)	<0.001
Model 3	0.28 (0.1)	0.11 (0.1)	−0.36 (0.1)	−0.29 (0.1)	0.001

Abbreviations: GrimAA, GrimAge acceleration; PhenoAA, PhenoAge acceleration; SE, standard error. Multiple linear regression models were used to estimate the adjusted mean and SE. The linear trend across quartiles was evaluated to estimate P-trend.

¹ Model 1 was adjusted for sex, race, education, family income, field center, and energy intake. Model 2 was adjusted for sex, race, education, family income, field center, energy intake, principal component analysis-derived dietary pattern score, current drinker, ever smoker, and physical activity. Model 3 was adjusted for sex, race, education, family income, field center, energy intake, principal component analysis-derived dietary pattern score, current drinker, ever smoker, and physical activity, body mass index, diabetes, and systolic blood pressure.

² The fiber-rich food score was calculated based on daily intakes of whole grains, fruit, vegetables, nuts, and legumes.

this cohort [23], CHO quality may subsequently be associated with the risk of developing CVD.

Although studies on the quality of CHO relative to EAA are scarce, other dietary factors were reported to be associated with EAA. Data from NHANES showed that greater intakes of fiber, high-quality CHO foods (cumulative intakes of whole grains, whole fruits, nonstarchy vegetables, and legumes), plant protein, and PUFAs were associated with decelerated PhenoAge, whereas greater intakes of low-quality CHO foods (cumulative intakes of refined grains, fruit juices, starchy vegetables, and added sugar) and saturated fatty acids were associated with accelerated PhenoAA [24]. Higher diet quality, as represented by Dietary Approaches to Stop Hypertension, Mediterranean dietary patterns, adherence to the American Heart Association recommendations, healthy eating index-2015, and the alternate healthy eating index, was associated with decelerated PhenoAge and GrimAge among participants in the Framingham Offspring Study [7], Sister Study [8], Kailuan study [25], and NHANES cohort [25] after adjusting for potential confounders. Among midlife Black and White females, higher diet quality and lower intake of added sugar were associated with lower epigenetic age [26]. PhenoAA was positively correlated with red meat intake and negatively correlated with nut intake among adults in the Women’s Health Initiative [3]. GrimAA was positively correlated with intakes of total fat and red meat, but was negatively correlated with intakes of total CHO and food groups including whole grains, fruit, vegetables, and dairy; however, intakes of total energy, total protein, poultry, fish, and nuts were not correlated with GrimAA in the Framingham Heart Study cohort [4]. In a recent study using a data-driven approach, peaches, nuts, poultry, butter, and discretionary oil and fat were associated with decelerated PhenoAge, whereas starchy vegetables, legumes, lunch meat, organ meat, sausages, eggs, cheese, added sugar, and fat added after cooking were associated with accelerated PhenoAge [27].

The favorable associations of higher CHO quality and diet high in fiber-rich foods with slower epigenetic aging may be

explained by the effects of various nutrients in plant-based diets that are involved in reducing oxidative and inflammatory stress [28]. In the Nurses’ Health Study, higher CHO:fiber ratio (lower CHO quality) was associated with lower adiponectin among diabetes-free females [29]. Adiponectin increases insulin sensitivity and anti-inflammatory effects [30], which may influence DNAm. In addition, Yamashita et al. [31] reported that many CpG sites were hypermethylated by inflammation along with aging, and interestingly, methylation in certain CpG sites was induced specifically by inflammation, which highlights the role of inflammation in the epigenetics of aging.

Epigenetic age measures were developed based on epigenetic correlates of protein and smoking and have been shown to be highly correlated with mortality and other health outcomes [3, 4]. The other health outcomes include epigenetic score correlations with other interesting biomarkers, such as serum carotenoids [32] and cytokines [33] that were not included as criterion measures in the development of the epigenetic age measures. Although it is clear that the epigenome helps to regulate genomic action, how the epigenome forms and is maintained is not clear [34]. Thus, epigenetic age is a complex measure. Therefore, further studies identifying the causal relationships of biomarkers and DNAm sites used to calculate the epigenetic age measures are warranted to better understand the mechanism of epigenetic responses to dietary intake.

There are several limitations of this study. First, self-reported dietary intake is prone to measurement error; however, the CARDIA Diet History is comprehensive and in-depth, capturing usual dietary intake better than most dietary assessment tools. For example, it captures more details about dietary intake than a simple food frequency questionnaire because it includes brand name information, food preparation, and additions to the food or beverage before consumption and is not limited by the somewhat arbitrary grouping of foods (e.g., apples and pears) that occurs by necessity given the nature of a food frequency questionnaire. Second, the causal relationship cannot be established due to the

nature of the study design. However, causal hypotheses were stated in advance, and potential confounders were included in the models, which are informative for causal inference. Third, there may be residual confounding by environmental factors or any other factors that were not part of the CARDIA study, and it lacks generalizability as CARDIA only enrolled Black and White participants who were relatively young. Unlike other traditional cross-sectional studies, this study used mixed-effects models utilizing EAA measures assessed across 3 examinations over a 10-y period, with the measures being highly correlated.

In conclusion, our findings are consistent with the idea that consuming a diet including higher CHO quality food sources, such as more fiber-rich foods, may provide a potential strategy to slow down biological aging. In addition, these findings support the DGA that recommend intakes of whole grains, fruit, vegetables, nuts, and legumes.

Author contributions

The authors' responsibilities were as follows – S-YY: analyzed the data and drafted the paper; S-YY, LMS, DRJ, BJ: interpreted the results; LMS, DRJ, BJ, WG, DD, KL, YZ, LH: critically reviewed and edited the manuscript; S-YY: had primary responsibility for final content; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Data availability

Data described in the manuscript, code book, and analytic code will be made available upon request. CARDIA complies with data sharing requirements of the National Institutes of Health by providing limited-access data sets from various CARDIA examinations to the National Heart, Lung and Blood Institute BioLINCC. Data are available through BioLINCC (<https://biolincc.nhlbi.nih.gov/>).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tjnnt.2025.01.022>.

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