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Dietary Patterns Are Associated with Levels of Global Genomic DNA Methylation in a Cancer-Free Population[1,2]

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Abstract

Animal studies have provided direct evidence that dietary factors induce changes in DNA methylation patterns. In humans, studies on diet and DNA methylation have yielded inconsistent findings. Because humans tend to consume foods and nutrients that are highly interrelated, study of dietary patterns may have improved the power of detecting the effect of diet on DNA methylation. Using data collected from 149 participants aged 45–75 y in the North Texas Healthy Heart Study, we examined the relationship between dietary patterns and levels of genomic DNA methylation in peripheral blood leukocytes. Dietary data were collected from study participants using the Block FFQ. Genomic DNA methylation was measured using bisulfite conversion of DNA and real-time PCR (MethyLight) for LINE-1. Two dietary patterns were identified using factor analysis: a "prudent" dietary pattern characterized by a high intake of vegetables and fruits, and a "Western" dietary pattern characterized by a high intake of meats, grains, dairy, oils, and potatoes. The prudent dietary pattern was associated with a lower prevalence of DNA hypomethylation (Q_4 vs. Q_1; OR = 0.33, 95% CI: 0.12–0.92) and the association was dose dependent (*P*-trend = 0.04). There was no apparent association between the Western dietary pattern and global leukocyte DNA methylation (Q_4 vs. Q_1; OR = 1.28, 95% CI: 0.47–3.47; *P*-trend = 0.55). Thus, a dietary pattern characterized by a high intake of vegetables and fruits may protect against global DNA hypomethylation. Future studies with a larger sample size need to confirm that this association holds longitudinally. J. Nutr. 141: 1165–1171, 2011.

Introduction

About one-half of the human genome contains repetitive sequences that are often intensely methylated. A reduced level of methylation in repetitive elements such as long intersperses repeat sequences (LINE-1) has been associated with genome instability and chromosomal aberrations (1), leading to an increased risk of cancer. In fact, hypomethylation in repetitive elements has been reported in many cancer cells and appears to parallel overall genomic hypomethylation (1).

More recently, global DNA methylation has been examined in surrogate tissues such as leukocyte DNA. A lower level of leukocyte DNA methylation has been associated with an increased risk of head and neck squamous cell carcinoma (2), bladder cancer (3), breast cancer (4), gastric cancer (5), and colorectal adenoma and cancer (6,7) after adjusting for known risk factors. This suggests that leukocyte DNA methylation may serve as a surrogate biomarker for systemic genomic methylation and provide an independent risk factor for cancer development.

The causes of DNA hypomethylation remain elusive. Animal studies provided direct evidence that certain dietary factors such as folate deficiency and alcohol consumption can induce changes in genomic DNA methylation (8–10). However, studies on diet and DNA methylation in humans have yielded inconsistent findings. Some found dietary folate restriction or folic acid supplementation resulted in changes in DNA methylation (11–13), whereas others did not observe methylation changes in response to folate depletion or treatment (14,15). Epidemiologic studies examining the association between dietary folate intake and leukocyte DNA methylation in cancer-free controls also reported null associations (2–4).

Previous studies focused on the consumption of specific food items and nutrients and ignored the fact that humans eat diets

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characterized by different balances of food and nutrients. To explore this hypothesis, we examined the association between dietary patterns and leukocyte DNA methylation in a cancer-free population.

Materials and Methods

Study population. The North Texas Healthy Heart Study was conducted in 2006 to assess racial/ethnic differences in cardiovascular serum markers and calcium scores and has been described elsewhere (16). Briefly, 571 study participants were recruited from the general population and primary care clinics through the NorTex network through either public advertisement or a physician's referral. The inclusion criteria included: *1*) males and females over the age of 45 y; 2) being Caucasian, Hispanic, or African American; and *3*) no current or previous self-reported history of stroke, peripheral arterial disease, renal failure, heart failure or coronary heart disease, or cancer.

The 161 participants included in this study were those who returned for their second study visit in 2008 and completed a survey eliciting information on demographics, smoking, alcohol consumption, medication use, use of oral contraceptives and hormone replacement therapy for women, and perceived life stress. To record alcohol consumption, participants were asked about the frequency of alcoholic beverage consumption (never, monthly or less, 2–4 times/month, 2–3 times/wk, and ≥4 times/wk). For cigarette smoking, participants were asked whether they smoked cigarettes every day, some days, or not at all and whether they have stopped smoking for 1 d or longer during the past 12 mo because they were trying to quit smoking. During the 2008 visit, body weight, height, and waist and hip circumferences were measured using standard procedures. Daily physical activity was measured with accelerometers (Actigraph). A 25-mL sample of whole blood was collected at the 2008 visit. Informed consent was obtained from all study participants. The study protocol was approved by the Institutional Review Boards of the University of North Texas Health.

Dietary assessment. During the 2008 visit, dietary intake was assessed using a 108-item Block FFQ that asks study participants about their usual dietary intake of 110 food items during the last year. For each food item, the Block FFQ uses 8 categories to assess frequency (never or hardly ever, once per month, 2–3 times/mo, once per week, 2–3 times/wk, 4–6 times/wk, once per day, and ≥2 times/d) and 3 categories to assess portion size so that the respondent can most often describe his/her typical dietary intake. The Block FFQ administrated in this study was previously validated with three 24-h dietary recalls and generally had high correlations for most nutrients (17).

Global DNA methylation. We measured global DNA methylation using bisulfite conversion of DNA and pyrosequencing for LINE-1 in the peripheral blood as previously described by others (18). Briefly, DNA were bisulfite treated using an EZ DNA Methylation kit (Zymo Research) following the manufacturer's recommendations. The biotinylated PCR products were purified and pyrosequencing was run on a PyroMark Q24. We used non-CpG cytosine residues as internal controls to verify efficient sodium bisulfite DNA conversion, and universal unmethylated and methylated DNA were run as controls. Methylation quantification was performed using the PyroMark Q24 1.010 software. The degree of methylation was expressed for each DNA locus as percentage methylated cytosine over the sum of methylated and unmethylated cytosine.

Dietary pattern derivation. The 13 food groups derived from the Block FFQ were subjected to an exploratory factor analysis using squared multiple correlations as prior communality estimates. The principal factor method was used to extract the factors followed by an orthogonal (varimax option in SAS) rotation. Food groups with factor loadings ≥0.40 were considered as significant contributors to the factor. A positive loading indicated that the food group was positively associated with the factor, whereas a negative loading indicated an inverse association. The scree test, the proportion of variance accounted, and the interpretability criteria were evaluated to determine the number of factors (i.e. dietary patterns) to be retained. Individual factor scores were estimated for each dietary pattern (or factor) for each participant by summing standardized frequency consumption of each food group weighted by their scoring coefficients, which were computed by multiplying the matrix of factor loadings by the matrix of eigenvalues. Therefore, factor scores for a particular dietary pattern rank individuals on the basis of how well they follow that dietary pattern. For example, for each participant, a high factor score for one dietary pattern indicated a high adherence to that dietary pattern, whereas a low factor score meant a low adherence. For each dietary pattern, participants were categorized into 4 groups according to the quartiles of ranked factor scores, with higher quartiles corresponding to higher factor scores. Different from cluster analysis for which participants are separated into mutually exclusive groups based on their existing dietary behaviors, factor analysis provides estimated factor scores for each dietary pattern and for each participant. The sequential nature of factor scores makes it feasible to simultaneously evaluate 2 or more dietary patterns in regression models without affecting collinearity (19). We therefore examined quartiles of factor scores for each dietary pattern in association with levels of global DNA methylation using logistic regression models as described in detail in the following section. The factor analysis was performed using the PROC FATOR procedure in SAS (version 9.1, SAS Institute).

Statistical analyses. We compared the characteristics of the study population among different dietary patterns using chi-square test for categorical variables and ANOVA for continuous variables. A binary variable was created for the level of DNA methylation: global methylation (i.e. DNA hypomethylation) was defined as low for levels of DNA methylation less than the median and high otherwise. Median daily consumption of each of the food groups and nutrients was compared between participants with low compared with high global methylation using the nonparametric Kruskal-Wallis ANOVA test. For nutrients, density variables (intake/1000 kJ) were created to adjust for total energy intake that correspond to the effect of increasing the percentage of nutrient intake while keeping total energy intake constant (20).

We used unconditional logistic regression to separately evaluate the association between dietary patterns and global DNA methylation for each dietary pattern. Factor scores of each dietary pattern were categorized into quartiles. Participants categorized into a higher quartile of a particular dietary pattern (e.g. Q_4) are more likely to follow that dietary pattern than participants categorized into a lower quartile (e.g. Q1). A prior list of confounders was specified in the model subject to stepwise selection, including age (continuous), gender (male vs. female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic), education (high school or less vs. some college/college graduate), BMI (continuous), smoking status (nonsmokers, former smokers, current smokers), alcohol consumption (never, monthly or less, ≥2–4 times/mo), and levels of physical activity (physically active vs. physically inactive). Participants were considered to be physically active if they had moderate physical activity ≥ 2.5 h/wk or vigorous physical activity ≥ 1.25 h/wk and were otherwise physically inactive. The significance level for stepwise selection (α) was set at 0.05. After stepwise selection, only gender was retained in the final model with dietary patterns. The logistic regression model fully adjusted for the above confounders yielded similar OR and 95% CI as the stepwise selection model. Therefore, only results from the stepwise selection model were presented. All analyses were performed using SAS (version 9.1; SAS Institute). Values in the text are median (IQR) unless otherwise indicated.

Results

Among the 161 participants who completed the baseline survey, 12 (7.4%) did not return the Block FFQ. Participants who returned the dietary questionnaire (mean ± SD, 57.2 ± 7.9 y; n = 149) tended to be younger than those who did not return the questionnaire (mean ± SD, 62.1 ± 6.3 y; P for ANOVA = 0.04). However, they did not differ in gender, race/ethnicity, education, BMI, cigarette smoking status, alcoholic beverage consumption, or levels of physical activity (data not shown).

Two dietary patterns were identified by exploratory factor analysis (**Table 1**). The "prudent" dietary pattern (factor 1) loaded heavily on vegetables (dark green vegetables, orange vegetables, and other vegetables including tomatoes but not including legumes and potatoes) and fruits and the "Western" dietary pattern (factor 2) loaded heavily on grains, meats, potatoes, oils, dairy, nuts, and seeds.

We compared population characteristics for the quartiles of each of the 2 dietary patterns (**Table 2**). Participants in the highest quartile of the Western dietary pattern had the lowest percentage of being Hispanic (15.1%) and the highest percentage of receiving a college or higher education (30.6%) ($P \leq 0.02$), and they also had the highest BMI ($P = 0.04$). Participants in the highest quartile of the prudent dietary patterns also had the highest percentage of receiving a college or higher education (30.6%) ($P = 0.03$). Otherwise, participants did not differ in age, gender, smoking status, drinking status, obesity, or physical activity for the quartiles of each of the 2 dietary patterns.

For all participants, the median level of leukocyte DNA methylation was 73.7%. When the 13 food items were individually examined by levels of DNA methylation, only the consumption of dark green vegetables was positively associated with global DNA methylation (**Table 3**). Participants with low DNA methylation or DNA hypomethylation (i.e. $<$ median) consumed fewer dark green vegetables [0.17 (0.36) cup/d] (1 cup = 150 g) compared with those with high DNA methylation (i.e. $\geq$ median) who consumed more [0.25 (0.39) cup/d] ($P = 0.04$). Participants with high and low levels of DNA methylation did not differ in their daily intake of those nutrients involved in 1-carbon metabolism, such as dietary folate, vitamin B-6, riboflavin, vitamin B-12, and methionine. However, participants with low DNA methylation consumed more saturated fat [2.88 (0.86) g/1000 kJ] than participants with high DNA methylation [2.57 (0.66) g/1000 kJ] ($P = 0.02$) (Table 3).

The results of the regression analysis for the association between the 2 dietary patterns and levels of global DNA methylation indicated that participants in the highest quartile of the prudent dietary pattern had an ~70% reduced risk of having DNA hypomethylation compared with those in the lowest quartile (Q4 vs. Q1: OR = 0.33, 95% CI: 0.12–0.92) and the association showed a dose-response relationship (P-trend = 0.04) (**Table 4**). The Western dietary pattern and DNA hypomethylation were not associated (Q4 vs. Q1; OR = 1.28, 95%CI: 0.47–3.47; P-trend = 0.55).

TABLE 1 Factor loadings of 2 dietary patterns for 149 participants in the North Texas Healthy Heart Study, 2008

	Dietary patterns	
Food group intake,[1] *unit/d*	Prudent	Western
Fruits, total including juice, *cup*	0.42	0.17
Vegetables not including legumes/potatoes, *cup*	0.97	0.19
Dark green vegetables, *cup*	0.91	−0.03
Orange vegetables, *cup*	0.80	0.07
Legumes and soy, *1-cup equivalent*	0.19	0.26
Potato, *cup*	0.31	0.58
Other vegetables including tomatoes, *cup*	0.88	0.28
Total grains, *oz. equivalent*	−0.06	0.75
Whole grains, *1-oz. equivalent*	0.14	0.43
Meat, *oz.*	0.06	0.65
Nuts and seeds, *1-oz. equivalent*	0.22	0.43
Dairy, *1-cup equivalent*	0.01	0.47
Oils, *tsp.*	0.40	0.56

[1] 1 cup or 1-cup equivalent (chopped vegetables and fruits) = 150 g; 1 oz. or 1-oz. equivalent (grains, meat, nuts, and seeds) = 28.3 g; 1-cup equivalent (dairy) = 237 mL; 1 tsp. (oils) = 4.93 mL.

Discussion

Two dietary patterns were identified in a cancer-free population using factor analysis. The prudent dietary pattern was characterized by a high intake of vegetables and fruits and the Western dietary pattern was characterized by a high intake of energy-dense foods such as grains, meats, potato, oil, and dairy. These 2 dietary patterns also have been identified in other U.S. populations, including the Health Professionals Follow-Up Study (21) and the Nurses' Health Study (22), and consistently across 4 cohorts of 4 European countries (23).

Various health outcomes have been examined in association with these 2 dietary patterns. The risk of coronary heart disease appeared to be reduced by the prudent dietary pattern in a cohort of 44,875 middle-aged men (24) and increased by the Western dietary pattern in a cohort of 69,017 middle-aged women with no history of cardiovascular disease at baseline (25). A follow-up on 72,113 women in the Nurses' Health Study confirmed that the prudent dietary pattern was associated with a 28% lower cardiovascular mortality and a 17% lower all-cause mortality, whereas the Western dietary pattern was associated with a higher mortality from cardiovascular disease and all causes (26). Although the report from the World Cancer Research Fund and the American Institute for Cancer Research in 2007 concluded that there was insufficient evidence to reach a conclusion about dietary patterns and the risk of cancer (27), a more recent systematic review that included newer studies found a significantly decreased trend of breast cancer associated with the prudent dietary pattern (28). In the Nurses' Health Study, the Western dietary pattern was associated with 16% increased cancer mortality (26).

Dietary factors may exert their effects on carcinogenesis through DNA methylation. Studies have shown that insufficient amounts of methyl-group donors such as folate, vitamin B-12, vitamin B-6, and methionine can cause DNA hypomethylation, leading to an increased risk of cancer (25). A reduced methylation in leukocytes may indicate systematic genomic hypomethylation and reflect the cumulative environmental impact on carcinogenesis (29). In this cancer-free population, the median level of LINE-1 methylation was 73.7%. This was similar to the median LINE-1 methylation level (73.5%) in leukocytes reported among 18 cancer-free controls from a breast cancer case-control study in Buffalo, NY (4) and among 526 controls (74.7%) of a case-control study of head and neck squamous cell carcinoma in the Greater Boston area (2). In our study, participants with low DNA methylation had a lower dietary folate intake than those with high DNA methylation, but the association was not significant. Dietary intake of other one carbon nutrients such as methionine, riboflavin, vitamin B-6, and vitamin B-12 did not differ by levels of DNA methylation. Our findings are in line with previous findings from the Greater Boston area case-control study on head and neck cancer (2) and the Spanish bladder cancer study (3), both of which reported that dietary folate intake and global DNA methylation were not associated among cancer-free controls. The Spanish bladder cancer study further examined other nutrients in 1-carbon metabolism and also observed no associations (3).

Although no significant difference was found for dietary intake of folate and other 1-carbon nutrients, consumption of dark green vegetables was positively correlated with global DNA methyla-

TABLE 2 Characteristics of the study population for quartiles of the 2 dietary patterns in the North Texas Healthy Heart Study, 2008[1]

	Dietary patterns	Q1	Q2	Q3	Q4	*P*-value
n		37	37	37	38	
Age, *y*	Prudent	56.1 ± 6.6	57.8 ± 8.6	57.8 ± 8.2	57.3 ± 8.3	0.76
	Western	56.1 ± 7.4	56.6 ± 7.9	57.5 ± 7.1	58.6 ± 9.2	0.54
Male, *n* (%)	Prudent	13 (22.8)	17 (29.8)	15 (26.3)	12 (21.1)	0.60
	Western	10 (17.5)	12 (21.1)	16 (28.1)	19 (33.3)	0.16
Hispanic, *n* (%)	Prudent	10 (18.9)	14 (26.4)	17 (32.1)	12 (22.6)	0.34
	Western	18 (34.0)	17 (32.1)	10 (18.9)	8 (15.1)	0.02
Some college/college graduate, *n* (%)	Prudent	26 (26.5)	25 (25.5)	17 (17.4)	30 (30.6)	0.03
	Western	17 (17.4)	20 (20.4)	31 (31.6)	30 (30.6)	<0.001
BMI,[1] kg/m^2	Prudent	31.5 ± 6.3	31.1 ± 4.6	30.5 ± 8.0	31.3 ± 6.9	0.93
	Western	30.7 ± 6.3	30.3 ± 5.2	29.7 ± 5.4	33.6 ± 8.2	0.04
Smoker, *n* (%)	Prudent	12 (21.4)	11 (19.6)	15 (26.8)	18 (32.1)	0.41
	Western	13 (23.2)	15 (26.8)	13 (23.2)	15 (26.8)	0.92
Drinker, *n* (%)	Prudent	22 (24.4)	21 (23.3)	19 (21.1)	28 (31.1)	0.23
	Western	21 (23.3)	20 (22.2)	27 (30.0)	22 (24.4)	0.34
Physically inactive, *n* (%)	Prudent	31 (27.4)	27 (23.9)	30 (26.6)	25 (22.1)	0.25
	Western	29 (25.7)	24 (21.2)	32 (28.3)	28 (24.8)	0.18

[1] Values are mean ± SD or *n* (%).

tion. Results for nutrients and for food groups may be not directly comparable. Nutrients were adjusted for total energy intake, whereas food groups were not. The differences for both nutrients and food groups were small, but nutrients other than folate may account for the positive association between dark green vegetables and global DNA methylation.

This study found that saturated fat intake was higher in participants with low global DNA methylation compared with those with high methylation. The biological mechanism underlying this association is still not understood. In control-fed rats, promoter methylation of the leptin gene in adipose tissue is associated with a high-fat diet (30). Dyslipidemia in ApoE $^{-/-}$ mice characterized by an increased ratio of LDL:HDL has been correlated with DNA hypomethylation (31). Although 1-carbon nutrients are the focus of most previous studies of nutritional influences on epigenetic regulation, other nutritional factors such as dietary fatty acids have been suggested to affect DNA methylation patterns by epigenetic mechanisms independent of 1-carbon nutrients (32,33). Further studies are needed to confirm the association between dietary fat intake and DNA methylation and to further examine how and when it occurs.

When evaluated individually, none of the 13 food groups except for dark green vegetables was significantly associated with DNA methylation. However, a broader view of dietary patterns revealed an inverse association between the prudent dietary pattern and global DNA hypomethylation in a dose-response fashion. Study of dietary patterns may have improved the power of detecting the effect of diet on DNA methylation than examining individual nutrients or individual food groups. The consumption of multiple nutrients from the prudent dietary pattern, such as 1-carbon nutrients, antioxidants, and others may work interactively to protect against DNA hypomethylation in a complex network. As a result, intervention on the overall dietary pattern rather than on single nutrients or food groups may be a more effective way to protect against cancer risk through epigenetic regulation.

Beyond diet, leukocyte DNA hypomethylation can be modulated by chemical exposures. For example, Bollati et al. (18) reported a significant reduction in leukocyte methylation measured in LINE-1 among gas station attendants and traffic police officers who were chronically exposed to low-dose benzene. Pilsner et al. (34) used the methyl acceptance assay that measures methylation at all CpG sites throughout the genome and observed a dose-response relationship between arsenic exposure and increased level of leukocyte DNA methylation among adults exposed to a wide range of water arsenic concentrations in Bangladesh. These findings provide additional support to the hypothesis that DNA methylation can be influenced by the external environment, including diet.

Our study has limitations. The cross-sectional design of the present study in which diet and DNA methylation were simultaneously assessed limits the study's ability to draw strong conclusions about a causal relationship between dietary patterns and genomic DNA methylation. Moreover, we did not assess prenatal and early postnatal dietary exposure, which may represent a critical window for environmental influences on DNA methylation (35). Nevertheless, in support of our findings, studies of monozygotic twins found remarkable differences in global DNA methylation in the peripheral blood of older than of younger twins, likely to reflect the cumulative impact of the environmental exposure on DNA methylation over the lifetime (36). Significant changes in global DNA methylation have also been identified in the peripheral blood of the same adult population followed for >10 y (37).

In addition, the dietary patterns reported in this study were identified using factor analysis that involves some subjectivity in choosing and grouping the food items and determining the methods of rotation and the number of factors to be retained. However, we used a standard approach to identify dietary patterns and the patterns identified in our study were also reported in other U.S. populations. The association between dietary patterns and DNA methylation may be confounded by other lifestyle factors such as smoking, drinking, physical activity, and BMI. Although we adjusted for these variables in the analyses, residual confounding may still exist. The high BMI of our study population may also affect the generalizability of this study. Women had a significantly lower level of global DNA methylation than the men in our study population; we also found racial/

TABLE 3 My Pyramid food groups and nutrients daily intake by levels of global leukocyte DNA methylation in the North Texas Healthy Heart Study population, 2008[1]

	Global leukocyte DNA methylation		
	Low	High	*P*-value
n	72	77	
Food groups[2]			
Fruits total including juice, *cup*	0.72 (0.80)	0.63 (0.82)	0.74
Vegetables not including legumes/potatoes, *cup*	0.89 (0.97)	0.99 (1.14)	0.12
Dark green vegetables, *cup*	0.17 (0.36)	0.25 (0.39)	0.04
Orange vegetables, *cup*	0.07 (0.11)	0.08 (0.12)	0.33
Legumes and soy, *1-cup equivalent*	0.08 (0.19)	0.11 (0.27)	0.16
Potato, *cup*	0.14 (0.18)	0.12 (0.14)	0.44
Other vegetables including tomatoes, *cup*	0.62 (0.65)	0.63 (0.68)	0.22
Total grains, *1-oz. equivalent*	4.13 (3.31)	4.73 (3.36)	0.55
Whole grains, *1-oz. equivalent*	0.77 (0.68)	0.91 (0.88)	0.58
Meat, *oz.*	2.89 (2.68)	2.95 (2.03)	0.50
Nuts and seeds, *1-oz. equivalent*	0.21 (0.24)	0.24 (0.28)	0.74
Dairy, *1-cup equivalent*	0.68 (0.88)	0.55 (0.72)	0.29
Oils, *tsp.*	1.67 (1.96)	2.11 (2.22)	0.44
Nutrients			
Protein, *g/1000 kJ*	9.34 (1.99)	9.00 (2.52)	0.86
Carbohydrate, *g/1000 kJ*	28.5 (5.83)	29.6 (5.95)	0.39
Fat, *g/1000 kJ*	9.55 (2.05)	9.13 (2.33)	0.59
Saturated fat, *g/1000 kJ*	2.88 (0.86)	2.57 (0.66)	0.02
Monounsaturated fat, *g/1000 kJ*	3.67 (0.87)	3.59 (1.23)	0.69
Polyunsaturated fat, *g/1000 kJ*	2.08 (0.68)	2.14 (0.72)	0.21
Dietary folate equivalents, *μg/1000 kJ*	64.8 (26.4)	66.2 (22.8)	0.74
Dietary folate, *μg/1000 kJ*	33.9 (23.2)	39.0 (16.9)	0.26
Supplemental folic acid, *μg/1000 kJ*	37.7 (68.2)	43.3 (78.2)	0.45
Vitamin B-6, *mg/1000 kJ*	0.22 (0.08)	0.25 (0.10)	0.38
Riboflavin, *mg/1000 kJ*	0.26 (0.09)	0.24 (0.10)	0.08
Vitamin B-12, *μg/1000 kJ*	0.64 (0.25)	0.51 (0.27)	0.08
Methionine, *mg/1000 kJ*	0.18 (0.06)	0.19 (0.06)	0.44

[1] Values are median (IQR) of daily intake of food groups or nutrients.
[2] 1 cup or 1-cup equivalent (chopped vegetables and fruits) = 150 g; 1 oz. or 1-oz. equivalent (grains, meat, nuts, and seeds) = 28.3 g; 1-cup equivalent (dairy) = 237 mL; 1 tsp. (oils) = 4.93 mL.

ethnic differences in levels of global DNA methylation (38). Gender and race/ethnicity were adjusted in models evaluating the effect of dietary patterns on global DNA methylation, but because of the limitation of the sample size, we were unable to stratify the association by gender and race/ethnicity, which may modify the association between the diet and DNA methylation.

Promoter CpG island hypermethylation of tumor-suppressor genes and global DNA hypomethylation are 2 hallmarks of

TABLE 4 The association between dietary patterns and levels of global leukocyte DNA methylation in the North Texas Healthy Heart Study population, 2008

	Global DNA methylation			
	Low (< median)	High (≥ median)	OR (95%CI) (crude)	OR (95%CI) (step-wise selection)
Prudent dietary pattern	*n (%)*			
Q1 (<−0.73)	25 (34.7)	12 (15.6)	1.00	1.00
Q2 (−0.73, −0.26)	17 (23.6)	20 (26.0)	0.41 (0.16–1.05)	0.38 (0.41–1.04)
Q3 (−0.26, 0.40)	15 (20.8)	22 (28.6)	0.33 (0.13–0.85)	0.38 (0.14–1.04)
Q4 (≥0.40)	15 (20.8)	23 (29.9)	0.31 (0.12–0.81)	0.33 (0.12–0.92)
			P-trend = 0.02	*P*-trend = 0.04
Western dietary pattern				
Q1 (<−0.66)	18 (25.0)	19 (24.7)	1.00	1.00
Q2 (−0.66, −0.16)	16 (22.2)	21 (29.2)	0.80 (0.32–2.01)	1.10 (0.40–3.01)
Q3 (−0.16, 0.48)	19 (86.4)	18 (23.4)	1.14 (0.45–2.77)	1.39 (0.52–3.73)
Q4 (≥0.48)	19 (86.4)	19 (24.7)	1.06 (0.43–2.31)	1.28 (0.47–3.47)
			P-trend = 0.74	*P*-trend = 0.55

methylation changes in carcinogenesis. Promoter hypermethylation is associated with gene silencing. One study investigating gene-specific hypermethylation and diet found that leafy green vegetables and dietary folate intake protected against promoter methylation of tumor-suppressor genes in exfoliated airway epithelium cells of smokers (39). Global DNA hypomethylation is associated with genome instability and chromosomal aberrations. We measured LINE-1 methylation as a surrogate for global DNA methylation by pyrosequencing, because methylation of repetitive elements such as LINE-1 has been shown to be a major contributor to total genomic DNA methylation in the human genome. Pyrosequencing has been extensively used to measure global DNA methylation. Although variation in measuring global methylation can come from the accuracy of measuring the area under the curve for different peaks, this variation is generally very small. Previous studies and our own data suggest that pyrosequencing is a reproducible assay with a SD $\leq$ 2% (40).

In addition, we evaluated global DNA methylation in peripheral blood. Christensen et al. (41) analyzed normal human tissues from 10 anatomic sites and found that age-related changes in global DNA methylation had similar patterns irrespective of tissue types including peripheral blood, suggesting common mechanisms may underlie methylation changes and the use of blood may be relevant for epidemiologic studies. Finally, a subtle source of bias may result from the diet modifying the proportions of leukocyte cell types that have been shown to be differentially methylated (42). We cannot rule out the existence of such a mechanism that would affect leukocyte DNA methylation not by absolute loss or gain but by a change in the density of leukocyte populations.

To the best of our knowledge, our study is the first to evaluate the association between dietary patterns and leukocyte global DNA methylation in a cancer-free population. We found an inverse association between a prudent dietary pattern and DNA hypomethylation, suggesting a dietary pattern characterized by a high intake of vegetables and fruits may protect against DNA hypomethylation. These findings highlight the need for future large-scale studies to evaluate whether the association between dietary pattern and DNA methylation holds longitudinally.

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F.F.Z. designed and conducted research, performed statistical analyses, and wrote the paper; R.M.S. and K.G. performed the laboratory analyses; M.K. assisted in statistical analyses; R.C., J.C., and K.F. designed and conducted the North Texas Healthy Heart Study; and A.M., R.M.S., R.C., J.C., K.F., and J.K.V. played important roles in manuscript preparation and interpretation of the results. All authors read and approved the final manuscript.

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