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Dietary intake, plasma homocysteine, and repetitive element DNA methylation in the Multi-Ethnic Study of Atherosclerosis (MESA)

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Abstract

Background and Aims—DNA methylation of repetitive elements may explain the relations among dietary intake, hyperhomocysteinemia, and cardiovascular disease risk. We investigated associations of methyl micronutrient intake and plasma total homocysteine with LINE-1 and Alu methylation in a cross-sectional study of 987 adults aged 45–84 y who participated in the Multi-Ethnic Study of Atherosclerosis (MESA) Stress Study.

Methods and Results—DNA methylation was estimated using pyrosequencing technology. A 120-item food frequency questionnaire was used to ascertain daily intake of folate, vitamin B12, vitamin B6, zinc, and methionine. Plasma total homocysteine was quantified using a fluorescence polarization immunoassay. Associations of micronutrient intake and homocysteine with LINE-1 and Alu methylation were examined using linear regression. Adjusted differences in %5-methylated cytosines (%5mC) were examined by categories of predictors using multivariable linear regression models. Intake of methyl-donor micronutrients was not associated with DNA methylation. After adjustment for covariates, each 3 $\mu\text{mol/L}$ increment of homocysteine corresponded with 0.06 (–0.01, 0.13) %5mC higher LINE-1 methylation. Additionally, BMI was positively associated with LINE-1 methylation (P trend=0.03). Participants with BMI ≥ 40 kg/m² had 0.35 (0.03, 0.67) %5mC higher LINE-1 than those with normal BMI. We also observed a 0.10

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(0.02, 0.19) %5mC difference in Alu methylation per 10 cm of height. These associations did not differ by sex.

Conclusion—Dietary intake of methyl-donor micronutrients was not associated with measures of DNA methylation in our sample. However, higher BMI was related to higher LINE-1 methylation, and height was positively associated with Alu methylation.

Introduction

DNA methylation, a modifiable epigenetic mechanism that regulates gene expression without changing the nucleotide sequence, has been implicated in the etiology of major chronic diseases such as cancer [1]. Recent evidence suggests that alterations in methylation of repetitive elements, such as long interspersed nucleotide 1 (LINE-1) and Alu, may contribute to cardiovascular disease (CVD) risk [2, 3]. However, the pathogenic mechanisms remain poorly understood.

Some small-scale studies in humans suggest that DNA methylation could play a role in CVD etiology through an influence on plasma homocysteine levels [4, 5]. Homocysteine is a non-essential amino acid produced in one-carbon metabolism, the physiologic process responsible for all mammalian DNA methylation reactions. As an intermediate product of the methionine metabolism, homocysteine is recycled back to methionine in the presence of methyl-donor micronutrients, including folate and choline, and methylation cofactors such as vitamin B12, vitamin B6, and zinc. Successful cycling of methionine from homocysteine ensures provision of the universal methyl-donor S-adenosylmethionine (SAM) for subsequent methylation reactions. Because one-carbon micronutrients are obtained from the diet, an imbalance or deficiency can lead to elevations in plasma homocysteine levels, which is an established marker of CVD risk [6].

Although the link between one-carbon micronutrient deficiencies and hyperhomocysteinemia is well-known [7], current evidence regarding their association with DNA methylation is inconsistent. For example, methyl-donor micronutrient intake was not related to LINE-1 methylation among 149 healthy adults in Texas [8], while a study of 165 cancer-free adults in New York found a positive correlation with folate intake [9]. In Colombian schoolchildren, neither erythrocyte folate nor serum vitamin B12 were associated with LINE-1 methylation [10]. Two perinatal studies examined the relations of maternal nutrient intake with LINE-1 methylation during early life [11, 12]. Prenatal intake of methyl-donor micronutrients was not related to LINE-1 methylation in either study, though Fryer et al. noted an inverse association between homocysteine and cord blood DNA methylation [12]. This was expected since elevated homocysteine may reflect reduced systemic methylation capacity. Yet, others reported no association between homocysteine and DNA methylation [13]. The conflicting literature underscores the need to elucidate the relation of methyl micronutrient intake and homocysteine levels with repetitive element methylation in a population at risk of CVD.

In this study of healthy middle-aged adults, we examined the associations of daily folate, vitamin B12, vitamin B6, methionine, and zinc intake, and plasma total homocysteine with methylation of LINE-1 and Alu repetitive elements.

Methods

Subjects

This cross-sectional investigation included participants of the MESA Stress Study, an ancillary study to the Multi-Ethnic Study of Atherosclerosis (MESA). Details on sampling and recruitment have been published [14]. The Stress Study included 1002 participants enrolled at the New York and Los Angeles sites. Participants were recruited in conjunction with the third and fourth follow-up exams of the full cohort, with approximately 500 participants enrolled at each site. All data used in these analyses were obtained from the baseline examination conducted between 2000 and 2002. At the baseline examination, anthropometry, including height and weight, was measured. Participants completed a set of subclinical CVD measurements, and a questionnaire inquiring on sociodemographic characteristics, standard CVD risk factors, and lifestyle. Physical activity was measured using a detailed, semi-quantitative questionnaire adapted from the Cross-Cultural Activity Participation Study [15].

All procedures were carried out with written consent of the subjects. The Multi-Ethnic Study of Atherosclerosis was approved by institutional review boards at all field centers: Columbia University, New York; Johns Hopkins University, Baltimore; Northwestern University, Chicago; UCLA, Los Angeles; University of Minnesota, Twin Cities; Wake Forest University, Winston-Salem.

Dietary Assessment

At the baseline examination, participants completed a 120-item Block-style food-frequency questionnaire (FFQ) modified to include Chinese and Hispanic foods to accommodate the MESA population. The FFQ inquired about serving size (small, medium, large) and frequency of intake for selected foods and beverages (from “rare or never” to a maximum of “2 times/day” for foods and a maximum of “6 times/day” for beverages). The questionnaire also inquired on frequency, dosage, and duration of supplement use, allowing quantification of nutrient intake from supplements. Daily nutrient intake from foods was estimated by multiplying the reported amount consumed by its nutrient content (Nutrition Data Systems for Research; University of Minnesota; Minneapolis). Folate content from foods was converted to dietary folate equivalent (DFE) units to account for differences in absorption of naturally occurring dietary folate and the more bioavailable synthetic folic acid. All nutrients were adjusted for total energy intake using the residual method. Although FFQs do not provide estimates of absolute intake, they correctly rank people within the population according to relative intake [16]. Adjustment for total energy intake improves accuracy and reduces extraneous between-person variation in nutrient intake, and might increase precision of the estimates due to cancellation of correlated measurement errors for total energy and the nutrients of interest.

We examined total nutrient intake, intake from foods alone, and intake from supplements alone. Dietary data for participants with values of extreme nutrient intake values ($>13,000$ $\mu\text{g/d}$ folate, >7000 $\mu\text{g/d}$ vitamin B12, $>180,000$ mg/day vitamin B6, or >2000 mg/d zinc), or extreme total energy intake (>6000 or <500 kcal/d) were excluded from the analyses.

Laboratory Methods

Phlebotomists obtained approximately 80 mL of blood from all participants in the fasting state at the baseline examination. Standardized methods were used to process and ship samples to a central laboratory. Plasma was separated in an aliquot for homocysteine determinations. We measured plasma total homocysteine concentrations using a fluorescence polarization immunoassay with the IMx analyzer (Abbott Diagnostics, Abbott Park, IL). The interassay coefficient of variation (CV) range was 4.5%.

LINE-1 and Alu Methylation Determinations

High-molecular-weight DNA was extracted with PureGene Kits (Gentra Systems, Minneapolis, MN) from peripheral leukocytes. Approximately 200 ng of DNA at 10 ng/μl were bisulfite-converted using the EZ-96 DNA Methylation Kit™ (Zymo Research, Orange, CA). We used pyrosequencing-based methylation analysis to quantify methylation at four genomic LINE-1 sites and three genomic Alu sites. Repetitive element methylation was assessed through simultaneous PCR of LINE-1 and Alu elements, using primers designated towards consensus sequences to amplify a representative pool of elements. The percentage of 5-methylcytosines (%5mC) for each CpG target region was quantified using PyroQ-CpG Software. The interassay CV for LINE-1 and Alu was 2.1% and 5.7%, respectively.

Statistical Analysis

Of the 1002 participants in the Stress Study, information on LINE-1 and Alu was available for 961 and 987 persons, respectively. All participants had data on at least one exposure of interest (daily intake of folate, vitamin B12, vitamin B6, zinc, or methionine; or homocysteine).

We first evaluated %5mC at the four LINE-1 and the three Alu sites. The mean±SD %5mC for the LINE-1 sites was 79.91±2.81, 81.91±1.62, 76.93±2.48, and 84.14±2.48. For the Alu sites, mean±SD %5mC was 31.82±1.55, 26.40±1.61, and 15.07±1.03. Spearman's correlation coefficients ranged from 0.06 to 0.56 for the LINE-1 sites and from 0.44 to 0.66 for the Alu sites. Because the distributions and correlations of %5mC differed by site for both repetitive elements, we used mixed effects linear regression models to derive a single estimate of LINE-1 and Alu for each individual according to previously described methods [10].

Next, we examined the distribution of LINE-1 and Alu methylation, separately, across quartiles of homocysteine and by categories of sociodemographic, anthropometric, and lifestyle characteristics. Body mass index (BMI) was categorized according to the World Health Organization (WHO) classification of adult weight status [17]. A four-level income/wealth index was created using total family income and a 5-level wealth measure based on ownership of assets (i.e. car, home, land, investments). Daily physical activity was categorized into quartiles based on total hours per week. Cancer status was defined as "Yes" if the participant was ever informed by a physician that they had cancer. We assessed the statistical significance of differences in methylation across categories of these variables with linear regression models. For ordinal characteristics, we obtained a test of linear trend.

We also compared the distribution of LINE-1 and Alu methylation across energy-adjusted quartiles of total nutrient intake, nutrient intake from foods only, and nutrient intake from supplements only for folate, vitamin B12, vitamin B6, and zinc; for methionine, we assessed total intake only (see Supplemental Table 1 for intake category cut-points). Quartiles of micronutrient intake from foods were estimated among non-supplement users because effects of nutrients from foods may be overpowered by high intake levels from supplements.

Finally, we conducted multivariable linear regression for LINE-1 and Alu, separately, based on bivariate associations and *a priori* knowledge. In these models, the primary exposures of interest were micronutrient intake and homocysteine, as well as BMI and height as nutritional correlates of DNA methylation that are also known risk factors for CVD. The final model for LINE-1 included homocysteine, height, BMI, sex, age, race, alcohol-use, self-reported cancer, and income/wealth. We estimated the difference and 95% confidence intervals (CI) in LINE-1 methylation per standard deviation of homocysteine (3 $\mu\text{mol/L}$) and height (10 cm), as well as for each WHO BMI category versus 'normal BMI' as the referent. The final model for Alu included total vitamin B12 intake, total zinc intake, homocysteine, height, sex, age, race, physical activity, and income/wealth. We estimated differences and 95% CI in Alu methylation per 3 $\mu\text{mol/L}$ of homocysteine and per 10 cm of height. For the vitamin B12 and zinc, we estimated pairwise differences for the 2nd, 3rd, and 4th quartiles of micronutrient intake versus the 1st quartile (referent) in a separate model that did not include homocysteine since it could be on the causal pathway between diet and DNA methylation. Intake of micronutrients was assessed in quartiles rather than continuously because there was evidence of non-linear relations with DNA methylation in bivariate analyses.

Because cancer is associated with substantial changes in repetitive element methylation [1], we conducted sensitivity analyses without the 63 participants with a history of cancer. Exclusion of these persons did not change the results, thus we included them in the analyses and adjusted for cancer history in the final models.

To determine whether sex modified the associations, we tested for a statistical interaction using the likelihood ratio test. We found no evidence that relations with LINE-1 or Alu methylation differed by sex; thus, results pool men and women and are adjusted for sex.

All analyses were carried out using the Statistical Analyses System software (version 9.2; SAS Institute, Cary, NC).

Results

Mean $\pm$ SD age of the 987 participants was 61.4 $\pm$ 9.9 years; 47.5% were men. Average DNA methylation was 82.73 $\pm$ 1.07 %5mC for LINE-1 and 24.42 $\pm$ 0.85 %5mC for Alu. Spearman's correlation coefficient between LINE-1 and Alu methylation was 0.15.

In Table 1, we present bivariate associations of homocysteine and sociodemographic characteristics with LINE-1 and Alu, separately. Men had 0.40 (0.27, 0.53) %5mC higher LINE-1 than women, whereas Alu methylation did not differ by sex. Age was positively associated with LINE-1 methylation. Compared to Whites, African Americans and Hispanics had higher LINE-1 and lower Alu. Income/wealth score was negatively associated

with LINE-1 and positively related to Alu. Height was directly related to both LINE-1 and Alu. Higher BMI was associated with higher LINE-1 methylation. Plasma homocysteine levels were positively associated with LINE-1 and inversely related to Alu. Participants who never used alcohol had lower LINE-1 than current-users. Higher physical activity levels corresponded with higher Alu. Persons with a history of cancer exhibited lower LINE-1 than their counterparts.

Micronutrient intake was not related to LINE-1 (Table 2). However, total vitamin B12 and total zinc intake were both positively related to Alu methylation (Table 3).

We examined the adjusted associations of homocysteine with LINE-1 methylation using multivariable linear regression (Table 4). After accounting for sex, age, height, BMI, race, alcohol-use, cancer history, and income/wealth, each 3 $\mu\text{mol/L}$ increment in homocysteine was related to 0.06 (−0.01, 0.13) higher %5mC LINE-1, though the 95% CI included the null. We also noted that the positive association between BMI and LINE-1 remained apparent (P trend=0.03). Participants in the ‘Obese Class II’ (BMI 35.0–39.9 kg/m^2) and ‘Obese Class III’ (BMI ≥ 40 kg/m^2) categories had 0.20 (−0.05, 0.45) and 0.35 (0.03, 0.67) %5mC higher LINE-1 than normal BMI persons, respectively.

We evaluated the associations of total vitamin B12, total zinc intake, and homocysteine with Alu methylation using multivariable models that also included age, sex, height, race, income/wealth, and physical activity; the model for vitamin B12 and zinc did not include homocysteine (Table 5). The positive associations of zinc and vitamin B12 intake with Alu were attenuated after multivariable adjustment. Height remained directly related to Alu methylation (0.10 [0.02, 0.19] %5mC per 10 cm).

Discussion

In this study of healthy adults 45–84 y, methyl micronutrient intake was not related to LINE-1 or Alu methylation. However, higher plasma total homocysteine and BMI were each associated with higher LINE-1 methylation. We also found a positive relation between height and Alu methylation. Although the observed differences in LINE-1 and Alu methylation were small, these sequences comprise nearly half of genomic CpG sites [18], so the associations may reflect larger changes in the context of the entire genome.

Since methyl-donor and methylation cofactor micronutrients play a direct role in DNA methylation pathways, we anticipated that higher intake of these micronutrients would be associated with higher DNA methylation. Nevertheless, there are some potential explanations for the null associations. First, it is likely that few MESA participants were deficient in methyl micronutrients due to folic acid fortification in the U.S. A positive association between micronutrient intake and DNA methylation might be detectable in populations with a higher prevalence of methyl micronutrient deficiencies. Second, we only examined consumption of specific micronutrients, which does not account for the combinations and interactions of multiple nutrients in the human diet. Third, random measurement error in ascertaining dietary intake with the FFQ could have resulted in some misclassification of intake. Finally, we were not able to consider associations with choline.

Because the folate and choline transmethylation pathways are interrelated, it will be important to examine associations with both micronutrients in future research.

We also found an unexpected positive association between homocysteine and LINE-1 methylation. During the DNA methylation reaction, SAM is de-methylated to S-adenosylhomocysteine (SAH), which is subsequently hydrolyzed to homocysteine. Under optimal conditions, homocysteine is re-methylated to methionine, which is converted to SAM to provide the methyl group for subsequent reactions. A deficiency in methyl-donor micronutrients leads to an increase in homocysteine and reversal of the SAH hydrolase reaction, resulting in accumulation of intracellular SAH and decreased DNA methylation [19]. Interestingly, two human cases of SAH hydrolase deficiency exhibited hypermethylated leukocyte DNA despite high plasma SAH [20, 21]. Although the mechanism through which elevated SAH leads to higher methylation is unknown, it is important to note that the magnitude of the association we observed is small, and that only 3.7% of the study population was hyperhomocysteinemic according to the American Heart Association [22]. Perhaps the expected inverse association between homocysteine and DNA methylation would be observed in populations with higher homocysteine levels.

Our finding of a positive relation between BMI and LINE-1 methylation contributes to ongoing discussions regarding the role of DNA methylation in obesity-related disease etiology. Studies from the Dutch Winter Famine Cohort reported that periconceptional famine exposure was related to alterations in methylation of genes involved in cardiometabolic diseases and higher BMI in middle-age [23], suggesting that aberrant DNA methylation is related to excess weight. However, current evidence from cross-sectional studies is mixed [2, 24]. A cohort study of Singaporean-Chinese adults found a positive association between satellite-2 (AS) repetitive element methylation and BMI at baseline [3]. Additionally, AS hypermethylation predicted incident CVD during follow-up among men only. Yet, DNA methylation did not differ by intake of folate or B-vitamins, plasma folate, or folate-metabolizing genotypes, leading the investigators to postulate that the unexpected positive relations were driven by a mechanism independent of one-carbon metabolic pathways, such as systemic inflammation. While we cannot rule out this possibility, it remains important to consider that repetitive element methylation could be a biomarker of systemic changes related to weight. An intervention study of obese postmenopausal women revealed differential methylation at 35 loci associated with weight-loss responsiveness to dieting, with differences in gene-expression profiles after dieting as well [25]. The extent to which gene-specific perturbations are reflected in repetitive elements is largely unexplored. Further research is warranted to confirm the direction of the association between BMI and repetitive element methylation, and longitudinal investigations are required to disentangle whether differences in DNA methylation are a cause or consequence of weight gain.

We also found a positive relation between height and Alu methylation. A recent study that used data from two prospective birth cohorts reported that every 1 %5mC increment in *ALPL* methylation from cord blood was related to 0.15% lower height in offspring at 9 y [26]. Because the *ALPL* gene plays a critical role in bone mineralization, methylation silencing could hinder skeletal development. Although this finding sheds light on a distinct pathway, changes in methylation of repetitive elements are also important to understand as

their activity can lead to genomic instability [1], which has health implications beyond the function of specific genes. Considering that adult stature is inversely related to CVD risk [27], understanding mechanisms underlying linear growth could enhance knowledge of disease etiology and identify avenues for intervention.

The discrepancies in associations with LINE-1 and Alu methylation are noteworthy. A detailed study that compared LINE-1 and Alu methylation in placental chorionic villi at three time-points during pregnancy, as well as in four somatic tissues demonstrated that LINE-1 and Alu followed distinctly different methylation patterns [28]. These findings could be due to differences in structure and function of the two elements. LINE-1 is an autonomous element capable of transposition using its own transcriptional machinery, whereas Alu is non-autonomous and relies on LINE-1 for its activity [18]. Additionally, LINE-1 sequences are sites of *de novo* methylation often concentrated in low guanine-cytosine (GC) content regions. Methylation silencing of genes spreads from these regions to transcription start sites (TSS) [29], a phenomenon thought to be buffered by Alu elements which typically cluster around TSS [30]. Taken together, the evidence suggests that LINE-1 and Alu are functionally different, and accordingly, their respective methylation profiles likely have different biological implications. There is need to characterize shared and independent predictors of LINE-1 and Alu methylation, how modification of these predictors affects methylation, and the degree to which these changes influence disease risk.

Our study had several strengths. We were able to examine LINE-1 and Alu methylation from circulating leukocytes in a large and ethnically-diverse population of healthy adults using a highly reproducible pyrosequencing-based technology. We used a culturally-tailored FFQ to ascertain nutrient intake, which is the most appropriate measure of long-term dietary habits. Additionally, because the FFQ ranks persons within a population by their usual intake, it is useful for micronutrients which exhibit substantial day-to-day variation. This study also has some limitations. First, the cross-sectional design hampers causal inference on the predictors of DNA methylation. Second, use of the FFQ to determine dietary intake does not preclude measurement error. Third, we did not account for the proportion of white blood cell subtypes from the buffy coat in the analyses. There is some evidence that DNA methylation is inversely related to the proportion of lymphocytes [24]; whether micronutrient intake, homocysteine, and anthropometry are associated with differential leukocyte count merits further investigation.

In summary, higher plasma total homocysteine and BMI were each associated with higher LINE-1 methylation, whereas being taller was related to higher Alu methylation. The value of LINE-1 and Alu methylation as biomarkers of health outcomes requires further examination in prospective studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

LINE-1	long interspersed nucleotide element-1
BMI	body mass index
CVD	cardiovascular disease
MESA	Multi-Ethnic Study of Atherosclerosis
SAM	S-adenosylmethionine
FFQ	food frequency questionnaire
PCR	polymerase chain reaction
SAH	S-adenosylhomocysteine
DNMT	DNA methyltransferase

References

1. Woo HD, Kim J. Global DNA hypomethylation in peripheral blood leukocytes as a biomarker for cancer risk: a meta-analysis. *PLoS One*. 2012; 7(4):e34615. [PubMed: 22509334]
2. Cash HL, et al. Cardiovascular disease risk factors and DNA methylation at the LINE-1 repeat region in peripheral blood from Samoan Islanders. *Epigenetics*. 2011; 6(10)
3. Kim M, et al. DNA methylation as a biomarker for cardiovascular disease risk. *PLoS One*. 2010; 5(3):e9692. [PubMed: 20300621]
4. Jacob RA, et al. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. *J Nutr*. 1998; 128(7):1204–12. [PubMed: 9649607]
5. Castro R, et al. Increased homocysteine and S-adenosylhomocysteine concentrations and DNA hypomethylation in vascular disease. *Clin Chem*. 2003; 49(8):1292–6. [PubMed: 12881445]
6. Selhub J. The many facets of hyperhomocysteinemia: studies from the Framingham cohorts. *J Nutr*. 2006; 136(6 Suppl):1726S–1730S. [PubMed: 16702347]
7. Mason JB. Biomarkers of nutrient exposure and status in one-carbon (methyl) metabolism. *J Nutr*. 2003; 133(Suppl 3):941s–947s. [PubMed: 12612180]
8. Zhang FF, et al. Dietary patterns are associated with levels of global genomic DNA methylation in a cancer-free population. *J Nutr*. 2011; 141(6):1165–71. [PubMed: 21525250]
9. Zhang FF, et al. White blood cell global methylation and IL-6 promoter methylation in association with diet and lifestyle risk factors in a cancer-free population. *Epigenetics*. 2012; 7(6)
10. Perng W, et al. Micronutrient status and global DNA methylation in school-age children. *Epigenetics*. 2012; 7(10)
11. Boeke CE, et al. Gestational intake of methyl donors and global LINE-1 DNA methylation in maternal and cord blood: prospective results from a folate-replete population. *Epigenetics*. 2012; 7(3):253–60. [PubMed: 22430801]

12. Fryer AA, et al. LINE-1 DNA methylation is inversely correlated with cord plasma homocysteine in man: a preliminary study. *Epigenetics*. 2009; 4(6):394–8. [PubMed: 19755846]
13. Bromberg A, et al. No association between global leukocyte DNA methylation and homocysteine levels in schizophrenia patients. *Schizophr Res*. 2008; 101(1–3):50–7. [PubMed: 18276118]
14. Bild DE, et al. Multi-ethnic study of atherosclerosis: objectives and design. *Am J Epidemiol*. 2002; 156(9):871–81. [PubMed: 12397006]
15. Ainsworth BE, et al. Moderate physical activity patterns of minority women: the Cross-Cultural Activity Participation Study. *J Womens Health Gend Based Med*. 1999; 8(6):805–13. [PubMed: 10495261]
16. Willett, WC. *Nutritional Epidemiology*. Oxford University Press; New York: 1998. Implications of total energy intake for epidemiologic analyses; p. 296–297.
17. World Health Organization. BMI Classification. Global Database on Body Mass Index. May 21, 2012 [cited 2012 5/21]; Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html
18. Cordaux R, Batzer MA. The impact of retrotransposons on human genome evolution. *Nat Rev Genet*. 2009; 10(10):691–703. [PubMed: 19763152]
19. Yi P, et al. Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation. *J Biol Chem*. 2000; 275(38):29318–23. [PubMed: 10884384]
20. Baric I, et al. S-adenosylhomocysteine hydrolase deficiency in a human: a genetic disorder of methionine metabolism. *Proc Natl Acad Sci U S A*. 2004; 101(12):4234–9. [PubMed: 15024124]
21. Buist NR, et al. S-adenosylhomocysteine hydrolase deficiency in a 26-year-old man. *J Inherit Metab Dis*. 2006; 29(4):538–45. [PubMed: 16736098]
22. Malinow MR, Bostom AG, Krauss RM. Homocyst(e)ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. *Circulation*. 1999; 99(1):178–82. [PubMed: 9884399]
23. Heijmans BT, et al. The epigenome: archive of the prenatal environment. *Epigenetics*. 2009; 4(8):526–31. [PubMed: 19923908]
24. Zhu ZZ, et al. Predictors of global methylation levels in blood DNA of healthy subjects: a combined analysis. *Int J Epidemiol*. 2010
25. Bouchard L, et al. Differential epigenomic and transcriptomic responses in subcutaneous adipose tissue between low and high responders to caloric restriction. *Am J Clin Nutr*. 2010; 91(2):309–20. [PubMed: 19939982]
26. Relton CL, et al. DNA methylation patterns in cord blood DNA and body size in childhood. *PLoS One*. 2012; 7(3):e31821. [PubMed: 22431966]
27. Rich-Edwards JW, et al. Height and the risk of cardiovascular disease in women. *Am J Epidemiol*. 1995; 142(9):909–17. [PubMed: 7572971]
28. Price EM, et al. Different measures of “genome-wide” DNA methylation exhibit unique properties in placental and somatic tissues. *Epigenetics*. 2012; 7(6)
29. Turker MS. Gene silencing in mammalian cells and the spread of DNA methylation. *Oncogene*. 2002; 21(35):5388–93. [PubMed: 12154401]
30. Kang MI, et al. The length of CpG islands is associated with the distribution of Alu and L1 retroelements. *Genomics*. 2006; 87(5):580–90. [PubMed: 16488573]

Table 1

Mean LINE-1 and Alu DNA methylation according to characteristics of the MESA Stress Study participants

	LINE-1 ^a			Alu ^a		
	N ^b	N = 961	P ^c	N ²	N = 987	P ^c
Sex						
M	457	80.94 (1.03)	<0.0001	469	24.45 (0.81)	0.74
F	504	80.54 (1.06)		518	24.42 (0.89)	
Age, years						
45 – 54	290	80.66 (1.07)	0.07	298	24.43 (0.84)	0.34
55 – 64	263	80.68 (1.09)		272	24.44 (0.86)	
65 – 74	292	80.81 (1.01)		299	24.31 (0.83)	
75 – 84	116	80.80 (1.15)		118	24.69 (0.83)	
Race						
White, Caucasian	180	80.50 (1.21)	0.008	185	24.61 (0.96)	0.01
Black, African-American	271	80.84 (0.98)		277	24.40 (0.79)	
Hispanic	510	80.75 (1.05)		525	24.38 (0.83)	
Education						
Less than high school	257	80.72 (1.09)	0.41	268	24.34 (0.85)	0.29
High school	196	80.87 (1.07)		200	24.50 (0.83)	
Some college	287	80.65 (0.98)		293	24.46 (0.85)	
Bachelor's degree or higher	221	80.71 (1.13)		226	24.43 (0.86)	
Income/wealth index						
0–2 (lowest)	311	80.82 (1.14)	0.04	324	24.33 (0.86)	0.002
3–4	319	80.71 (0.96)		323	24.42 (0.81)	
5–6	202	80.71 (1.09)		207	24.51 (0.88)	
7–8 (highest)	126	80.57 (1.09)		130	24.56 (0.83)	
Height, cm						
Q1: <162	239	80.57 (1.03)	0.001	243	24.41 (0.90)	0.12
Q2: 162–165	241	80.65 (0.97)		250	24.34 (0.89)	
Q3: 165–172	240	80.85 (1.12)		246	24.45 (0.83)	
Q4: 172	241	80.84 (1.12)		248	24.50 (0.77)	

	LINE-1 ^a			Alu ^a		
	N ^b	N = 961	P ^c	N ²	N = 987	P ^c
BMI, kg/m ²						
Normal (< 25.0)	220	80.67 (1.13)		231	24.37 (0.84)	
Overweight (25.0–29.0)	382	80.71 (1.07)		392	24.42 (0.82)	
Obese class I (30.0–34.9)	234	80.72 (1.05)	0.06	237	24.45 (0.90)	0.19
Obese class II (35.0–39.9)	79	80.86 (0.94)		81	24.62 (0.96)	
Obese class III (40.0)	46	80.96 (1.01)		46	24.35 (0.67)	
Homocysteine, μmol/L						
Q1: < 7.2	231	80.53 (1.09)		240	24.53 (0.93)	
Q2: 7.2 – 8.5	231	80.75 (1.09)	0.0009	235	24.40 (0.82)	0.15
Q3: 8.6 – 10.2	256	80.75 (0.97)		263	24.39 (0.84)	
Q4: 10.3	242	80.88 (1.10)		248	24.41 (0.79)	
History of cancer						
No	895	80.74 (1.08)	0.09	920	24.43 (0.85)	0.36
Yes	63	80.54 (0.91)		64	24.34 (0.79)	
Cigarette Use						
Never	504	80.66 (1.06)		520	24.45 (0.91)	
Current	346	80.81 (1.05)	0.13	354	24.43 (0.78)	0.31
Former	111	80.75 (1.11)		112	24.33 (0.76)	
Alcohol Use						
Never	201	80.60 (0.99)		191	24.44 (0.91)	
Current	242	80.81 (1.12)	0.08	225	24.38 (0.81)	0.52
Former	517	80.74 (1.06)		465	24.45 (0.84)	
Daily physical activity level						
1 (lowest)	238	80.85 (1.08)		244	24.36 (0.83)	
2	231	80.69 (1.05)	0.19	239	24.36 (0.84)	0.06
3	250	80.66 (0.99)		256	24.53 (0.85)	
4 (highest)	241	80.72 (1.13)		247	24.47 (0.88)	

^aFrom mixed effects linear regression models where site was treated as a random effect.

^bTotals may be < 961 for LINE-1 and < 987 for Alu due to missing values.

^cRepresents a test for linear trend for all variables except for sex, race, self-reported cancer, alcohol use, and cigarette use (ANOVA).

Table 2

Percent LINE-1 DNA methylation of leukocyte DNA by quartiles of micronutrient intake

	LINE-1 DNA Methylation by Quartiles of Micronutrient Intake ¹				
	Q1	Q2	Q3	Q4	P ²
Folate					
Total Intake	80.78 ± 1.13	80.57 ± 1.00	80.90 ± 1.06	80.74 ± 1.05	
β (95% CI)	Reference	-0.21 (-0.42, -0.01)	0.12 (-0.09, 0.33)	-0.04 (-0.25, 0.17)	0.53
From foods only³	81.80 ± 1.12	8.68 ± 1.04	80.69 ± 1.10	80.79 ± 1.04	
β (95% CI)	Reference	-0.12 (-0.38, 0.13)	-0.11 (-0.37, 0.15)	-0.01 (-0.26, 0.24)	0.97
From supplements only	80.74 ± 1.07	80.75 ± 1.06	80.84 ± 1.03	80.69 ± 1.09	
β (95% CI)	Reference	0.02 (-0.21, 0.26)	0.12 (-0.11, 0.34)	-0.04 (-0.27, 0.20)	0.95
Vitamin B12					
Total	80.70 ± 1.04	80.81 ± 0.92	80.74 ± 1.15	80.74 ± 1.15	
β (95% CI)	Reference	0.10 (-0.09, 0.29)	0.04 (-0.17, 0.25)	0.04 (-0.18, 0.25)	0.90
From foods only³	80.70 ± 0.93	80.80 ± 0.97	80.82 ± 1.01	80.80 ± 1.19	
β (95% CI)	Reference	0.09 (-0.14, 0.32)	0.11 (-0.12, 0.35)	0.09 (-0.16, 0.35)	0.46
From supplements only	80.78 ± 1.03	80.60 ± 1.08	80.89 ± 1.02	80.59 ± 1.25	
β (95% CI)	Reference	-0.16 (-0.39, 0.07)	0.13 (-0.09, 0.34)	-0.18 (-0.43, 0.08)	0.33
Vitamin B6					
Total	80.70 ± 1.07	80.73 ± 1.05	80.79 ± 1.10	80.78 ± 1.06	
β (95% CI)	Reference	0.03 (-0.17, 0.23)	0.09 (-0.12, 0.30)	0.08 (-0.13, 0.28)	0.38
From foods only³	80.75 ± 0.98	80.68 ± 1.07	80.75 ± 1.07	80.84 ± 1.04	
β (95% CI)	Reference	-0.07 (-0.32, 0.18)	-0.01 (-0.16, 0.24)	0.09 (-0.16, 0.33)	0.41
From supplements only	80.76 ± 1.04	80.64 ± 1.21	80.91 ± 1.04	80.63 ± 1.06	
β (95% CI)	Reference	-0.10 (-0.35, 0.15)	0.17 (-0.05, 0.39)	-0.11 (-0.33, 0.12)	0.74
Zinc					
Total	80.74 ± 0.99	80.65 ± 1.07	80.89 ± 1.09	80.71 ± 1.10	
β (95% CI)	Reference	-0.09 (-0.29, 0.11)	0.14 (-0.06, 0.34)	-0.04 (-0.24, 0.16)	0.71
From foods only³	80.80 ± 1.02	80.70 ± 0.92	80.73 ± 1.13	80.77 ± 1.12	
β (95% CI)	Reference	-0.09 (-0.32, 0.14)	-0.06 (-0.32, 0.19)	-0.03 (-0.28, 0.23)	0.91

LINE-1 DNA Methylation by Quartiles of Micronutrient Intake ¹					
	Q1	Q2	Q3	Q4	P ²
Mean ± SD %5mC					
From supplements only	80.75 ± 1.05	80.75 ± 1.03	80.75 ± 1.11	80.71 ± 1.17	
β (95% CI)	Reference	0.01 (−0.21, 0.24)	0.01 (−0.23, 0.25)	−0.02 (−0.27, 0.22)	0.79
<i>Methionine</i>					
Total Intake ⁴	80.72 ± 1.09	80.75 ± 1.05	80.71 ± 1.05	80.81 ± 1.08	
β (95% CI)	Reference	0.04 (−0.17, 0.24)	−0.01 (−0.21, 0.20)	0.09 (−0.12, 0.30)	0.49

¹ Adjusted for total energy intake using the residual method.

² Represents a test for linear trend from univariate linear regression models where an ordinal variable for quartiles of the micronutrient was entered into the model as a continuous variable.

³ From non-supplement users; *n* = 570 for folate, *n* = 539 for vitamin B12, *n* = 542 for vitamin B6, *n* = 560 for zinc

⁴ From foods only.

Table 3

Alu DNA methylation by quartiles of micronutrient intake

	Alu DNA Methylation by Quartiles of Micronutrient Intake ¹				
	Q1	Q2	Q3	Q4	P ²
Folate					
Total Intake	24.48 ± 0.87	24.42 ± 0.92	24.42 ± 0.77	24.48 ± 0.83	
β (95% CI)	Reference	-0.06 (-0.23, 0.11)	-0.05 (-0.21, 0.10)	0.00 (-0.16, 0.16)	0.98
From foods only³	24.41 ± 0.89	24.46 ± 0.90	24.53 ± 0.84	24.36 ± 0.82	
β (95% CI)	Reference	0.05 (-0.16, 0.26)	0.12 (-0.08, 0.32)	-0.05 (-0.25, 0.15)	0.81
From supplements only	24.41 ± 0.86	24.58 ± 0.86	24.50 ± 0.81	24.32 ± 0.77	
β (95% CI)	Reference	0.16 (-0.02, 0.35)	0.09 (-0.09, 0.26)	-0.10 (-0.27, 0.07)	0.86
Vitamin B12					
Total Intake	24.33 ± 0.80	24.49 ± 0.89	24.44 ± 0.88	24.53 ± 0.81	
β (95% CI)	Reference	0.16 (0.00, 0.33)	0.12 (-0.04, 0.28)	0.21 (0.05, 0.36)	0.02
From foods only³	24.31 ± 0.82	24.50 ± 0.81	24.35 ± 0.85	24.49 ± 0.93	
β (95% CI)	Reference	0.19 (-0.01, 0.38)	0.04 (-0.16, 0.24)	0.17 (-0.04, 0.38)	0.27
From supplements only	24.39 ± 0.85	24.46 ± 0.87	24.58 ± 0.79	24.47 ± 0.85	
β (95% CI)	Reference	0.06 (-0.11, 0.24)	0.19 (0.02, 0.35)	0.07 (-0.10, 0.25)	0.09
Vitamin B6					
Total Intake	24.50 ± 0.88	24.36 ± 0.87	24.46 ± 0.83	24.47 ± 0.81	
β (95% CI)	Reference	-0.14 (-0.30, 0.03)	-0.04 (-0.20, 0.13)	-0.02 (-0.18, 0.14)	0.91
From foods only³	24.54 ± 0.97	24.31 ± 0.84	24.43 ± 0.80	24.43 ± 0.81	
β (95% CI)	Reference	-0.23 (-0.45, -0.02)	-0.11 (-0.33, 0.10)	-0.11 (-0.32, 0.11)	0.56
From supplements only	24.40 ± 0.85	24.50 ± 0.92	24.53 ± 0.78	24.41 ± 0.80	
β (95% CI)	Reference	0.09 (-0.09, 0.28)	0.13 (-0.04, 0.29)	0.01 (-0.16, 0.17)	0.38
Zinc					
Total Intake	24.37 ± 0.84	24.44 ± 0.90	24.44 ± 0.81	24.54 ± 0.85	
β (95% CI)	Reference	0.07 (-0.10, 0.23)	0.07 (-0.09, 0.23)	0.16 (0.00, 0.32)	0.06
From foods only³	24.40 ± 0.88	24.37 ± 0.85	24.42 ± 0.85	24.44 ± 0.84	
β (95% CI)	Reference	-0.02 (-0.23, 0.18)	0.02 (-0.18, 0.22)	0.04 (-0.16, 0.24)	0.61

Alu DNA Methylation by Quartiles of Micronutrient Intake ¹					
	Q1	Q2	Q3	Q4	P ²
Mean ± SD %5mC					
From supplements only	24.39 ± 0.85	24.43 ± 0.81	24.61 ± 0.82	24.54 ± 0.88	
β (95% CI)	Reference	0.05 (−0.13, 0.22)	0.22 (0.04, 0.39)	0.16 (−0.03, 0.34)	0.01
<i>Methionine</i>					
Total Intake ⁴	24.44 ± 0.80	24.42 ± 0.87	24.50 ± 0.84	24.43 ± 0.89	
β (95% CI)	Reference	−0.02 (−0.18, 0.14)	0.06 (−0.10, 0.22)	−0.01 (−0.17, 0.15)	0.88

¹ Adjusted for total energy intake using the residual method.

² Represents a test for linear trend from univariate linear regression models where an ordinal variable for quartiles of the micronutrient was entered into the model as a continuous variable.

³ From non-supplement users; *n* = 586 for folate, *n* = 556 for vitamin B12, *n* = 557 for vitamin B6, *n* = 576 for zinc.

⁴ From foods only.

Table 4

Nutritional correlates of LINE-1 DNA methylation

	<u>Mean %5mC difference (95% CI)</u>	
	All	
	<u>Unadjusted</u>	<u>Adjusted¹</u>
Homocysteine, per 3 μ mol/L	0.13 (0.07, 0.20)	0.06 (−0.01, 0.13)
Height, per 10 cm	0.13 (0.06, 0.20)	0.02 (−0.08, 0.12)
BMI, kg/m ²		
Normal (< 25.0)	Reference	Reference
Overweight (25.0–29.0)	0.04 (−0.14, 0.23)	−0.02 (−0.20, 0.16)
Obese class I (30.0–34.9)	0.05 (−0.15, 0.25)	0.02 (−0.17, 0.22)
Obese class II (35.0–39.9)	0.19 (−0.06, 0.44)	0.20 (−0.05, 0.45)
Obese class III (40.0)	0.30 (−0.02, 0.63)	0.35 (0.03, 0.67)
<i>p</i> ²	0.06	0.03

¹ Multivariable model included sex, age, race, height, BMI, plasma total homocysteine, alcohol use, self-report cancer, and income/wealth index.

² Represents a test for linear trend from a linear regression model where the outcome was LINE-1 methylation and an ordinal indicator for the BMI categories was entered as a continuous variable.

Table 5

Mean differences in Alu DNA methylation associated with nutritional correlates

	Mean %5mC difference (95% CI)	
	All	
	Unadjusted	Adjusted^a
Height, per 10 cm	0.04 (−0.02, 0.10)	0.10 (0.02, 0.19)
Homocysteine, per 3 μmol/L	−0.04 (−0.09, 0.01)	−0.04 (−0.09, 0.02)
Total vitamin B12 intake, μg ^c		
Q1: < 2.40	Reference	Reference
Q2: 2.40 – 4.02	0.16 (0.00, 0.33)	0.14 (−0.02, 0.31)
Q3: 4.03 – 12.77	0.12 (−0.04, 0.28)	0.04 (−0.14, 0.22)
Q4: 12.78	0.21 (0.05, 0.36)	0.10 (−0.10, 0.30)
<i>p^b</i>	0.02	0.44
Total zinc intake, mg ^c		
Q1: < 6.89	Reference	Reference
Q2: 6.89 – 8.67	0.07 (−0.10, 0.23)	0.03 (−0.14, 0.20)
Q3: 8.68 – 19.30	0.07 (−0.09, 0.23)	0.04 (−0.14, 0.21)
Q4: 19.30	0.16 (0.00, 0.32)	0.09 (−0.12, 0.29)
<i>p^b</i>	0.06	0.42

^a Multivariable model includes sex, age, height, plasma total homocysteine, total vitamin B12 intake, total zinc intake, race, physical activity level, and income/wealth index. Model for vitamin B12 and zinc intake does not include homocysteine.

^b Represents a test for linear trend from a linear regression model where the outcome was Alu methylation and an ordinal indicator for the variable was entered as a continuous variable.

^c Total micronutrient intake (foods + supplements); adjusted for total energy intake using the residuals method.