DNA Methylation Variants at HIF3A Locus, B-Vitamin Intake, and Long-term Weight Change: Gene-Diet Interactions in Two U.S. Cohorts

Tao Huang,[1] Yan Zheng,[1] Qibin Qi,[2] Min Xu,[3] Sylvia H. Ley,[1] Yanping Li,[1] Jae H. Kang,[4] Janey Wiggs,[5] Louis R. Pasquale,[4,5] Andrew T. Chan,[6] Eric B. Rimm,[1,4,7] David J. Hunter,[1,7] JoAnn E. Manson,[4,7,8] Walter C. Willett,[1,4,7] Frank B. Hu,[1,4,7] and Lu Qi[1,4]

DNA Methylation Variants at *HIF3A* Locus, B-Vitamin Intake, and Long-term Weight Change: Gene-Diet Interactions in Two U.S. Cohorts

Diabetes 2015;64:3146–3154 | DOI: 10.2337/db15-0264

OBESITY STUDIES

The first epigenome-wide association study of BMI identified DNA methylation at an *HIF3A* locus associated with BMI. We tested the hypothesis that DNA methylation variants are associated with BMI according to intake of B vitamins. In two large cohorts, we found significant interactions between the DNA methylation–associated *HIF3A* single nucleotide polymorphism (SNP) rs3826795 and intake of B vitamins on 10-year changes in BMI. The association between rs3826795 and BMI changes consistently increased across the tertiles of total vitamin B_2 and B_{12} intake (all *P* for interaction <0.01). The differences in the BMI changes per increment of minor allele were −0.10 (SE 0.06), −0.01 (SE 0.06), and 0.12 (SE 0.07) within subgroups defined by increasing tertiles of total vitamin B_2 intake and −0.10 (SE 0.06), −0.01 (SE 0.06), and 0.10 (SE 0.07) within subgroups defined by increasing tertiles of total vitamin B_{12} intake. In two independent cohorts, a DNA methylation variant in *HIF3A* was associated with BMI changes through interactions with total or supplemental vitamin B_2, vitamin B_{12}, and folate. These findings suggest a potential causal relation between DNA methylation and adiposity.

DNA methylation is one of the major epigenetic events that affect gene expression through a mechanism that is not induced by changes in the DNA sequence (1). Increasing evidence indicates that DNA methylation plays a pivotal role in regulating body adiposity (2–5). In a recent large-scale epigenome-wide association study, Dick et al. (4) found that DNA methylation at *HIF3A* was associated with BMI. However, genetic variants in this locus were not associated with BMI, although they were associated with *HIF3A* methylation levels in blood and adipose and skin tissues. The authors suggested that the association between *HIF3A* methylation and BMI might not be operating by the Mendelian randomization theory (4), but the study did not consider potential modifying effects of environmental factors on the genetic associations. Previous evidence supports the importance of considering interactions between genetic and diet/lifestyle factors in the development of adiposity (6–10).

It has long been acknowledged that DNA methylation levels are subject to modulation by environmental factors such as diet and lifestyle. Methylation of DNA is a biochemical process in which a methyl group is added to DNA nucleotides. Several B vitamins, including folate, riboflavin (B_2), pyridoxine (B_6), and B_{12}, act as key enzyme cofactors and play essential roles in methyl group metabolism and DNA methylation in particular (11,12). Previous studies have associated B-vitamin intake with adiposity in humans (13,14). Therefore, we hypothesized that

[1]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA
[2]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY
[3]Shanghai Clinical Center for Endocrine and Metabolic Diseases and Shanghai Institute of Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[4]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA
[5]Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA
[6]Division of Gastroenterology, Massachusetts General Hospital, Boston, MA
[7]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA
[8]Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Corresponding author: Lu Qi, nhlqi@channing.harvard.edu.

Received 25 February 2015 and accepted 14 May 2015.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0264/-/DC1.

B vitamins modify the relation between the methylation-associated genetic variants at *HIF3A* and BMI. To test this hypothesis, we examined interactions between intake of B vitamins and DNA methylation variants at the *HIF3A* locus in relation to BMI and 10-year changes in BMI in two prospective cohorts: the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS).

RESEARCH DESIGN AND METHODS

Study Population

The NHS is a prospective cohort study of 121,700 female registered nurses aged 30–55 years at study inception in 1976 (15). The HPFS is a prospective cohort study of 51,529 U.S. male health professionals aged 40–75 years at study inception in 1986 (16). The human subjects committees at the Harvard School of Public Health and Brigham and Women's Hospital approved these studies. Informed consent was obtained from all participants. In both cohorts, information about medical history, lifestyle, and health conditions has been collected by self-administered questionnaires every 2 years since inception. The current analysis baseline was set in 1980 for the NHS and 1986 for the HPFS when the first data on intake of B vitamins were collected. We included 8,109 women and 6,761 men of European ancestry. Both case and control participants with genotyping data available based on previous genome-wide association studies were included (17–22).

Assessment of Dietary Factors and Covariates

Questionnaires were sent every 2 years to update information on medical history and lifestyle factors. The cohorts had follow-up rates of >85%. Dietary intake of various nutrients at baseline, including folate and vitamins B_2, B_6, and B_{12}, as well as daily consumption of alcohol, sugar-sweetened beverages, fried food, and total meat were assessed by validated semiquantitative food frequency questionnaires every 4 years (23,24). All contributions, including those from food and supplements, were summed to derive the total intake of specific nutrients (23). The validity and reliability of the food frequency questionnaire has been established previously (23,24). Physical activity was expressed as metabolic equivalents per week by incorporating the reported time spent on various activities and the intensity level of each activity. The validity of the self-reported physical activity data has been described previously (25). The Alternate Healthy Eating Index was calculated previously in the NHS and HPFS (26).

Assessment of Adiposity and 10-Year Changes in BMI

Height and body weight were assessed by questionnaire at baseline, and weight was requested on each follow-up questionnaire. Body weight at a young age (18 years old in the NHS and 21 years old in the HPFS) was also collected by questionnaire. Self-reported weights were highly correlated with directly measured values (r = 0.97 in men and women) in a validation study (27). BMI was calculated as body weight (kg)/height (m^2). Participants with a BMI ≥30 kg/m^2 were defined as obese, and those with a BMI ≥25 kg/m^2 were defined as overweight. The epidemic of obesity occurred during a relatively short period of time in the U.S. between ~1980 and 2000 (28,29), paralleling a dramatic transition from traditional to obesogenic diet/lifestyle patterns (9,10,30). Obesity traits measured at a single time point cannot capture the variance in gradual changes, whereas changes in BMI/body weight during the period of the obesity epidemic more likely reflect the long-term, dynamic response to gene-environment interactions. Furthermore, considerable interindividual variations of weight change in response to diet interventions have long been noted (10); therefore, the present study mainly focused on 10-year changes in BMI. We defined the 10-year period for changes in BMI as 1980–1990 in the NHS cohort and 1986–1996 in the HPFS cohort.

Single Nucleotide Polymorphism Selection and Genotyping

The *HIF3A* variants rs3826795 and rs8102595 were selected because they had independent associations with methylation at a specific CpG site (cg22891070) within intron 1 of *HIF3A* in adipose tissue and skin DNA associated with BMI (4). The single nucleotide polymorphisms (SNPs) were genotyped and had a high imputation quality score ($r^2 \geq 0.8$) as assessed with the use of MACH version 1.0.16 software (Center for Statistical Genetics, University of Michigan). DNA extraction methods, quality control measures, SNP genotyping, and imputations are described in detail elsewhere (17–22,31). The minor allele frequencies (MAFs) of rs3826795 and rs8102595 are 0.09 and 0.18, respectively.

Statistical Analyses

We examined the association of genetic variants with adiposity measures, baseline B-vitamin intake, and 10-year changes in BMI or body weight using general linear models. Interactions between the genetic variants and intake of baseline nutrients (e.g., intake of total B vitamins, intake of B vitamins from supplements or food sources) on body weight, BMI, and 10-year changes in body weight or BMI were tested by including a multiplicative interaction term in the models. Potential confounders considered in multivariable models were age (years, continuous), baseline physical activity (MET-h/week, continuous), baseline television watching (0–1, 2–5, 6–20, 21–40, >40 h/week), baseline smoking (never, past, current), baseline alcohol intake (0, 0.1–4.9, 5.0–9.9, 10–14.9, ≥15 g/day), baseline Alternate Healthy Eating Index (in quintiles), and baseline total energy intake (in quintiles). Sugar-sweetened beverage, fried food, and total meat consumption were further adjusted in sensitivity analyses. The Bonferroni correction was conducted for multiple comparisons (0.05/8).

In secondary analyses of incident obesity assessed in 1990 (NHS) and 1996 (HPFS), we used logistic regression models to estimate odds ratios. Results across cohorts

were pooled with inverse variance–weighted meta-analyses by fixed-effects models (if $P \geq 0.05$ for heterogeneity between studies) or random-effects models (if $P < 0.05$ for heterogeneity between studies). All reported P values are nominal and two-sided. Statistical analyses were performed with SAS 9.3 software (SAS Institute, Cary, NC).

RESULTS

Baseline Characteristics

The current study included 8,109 women from the NHS cohort and 6,761 men from the HPFS cohort. Table 1 shows the baseline characteristics for the NHS cohort (year 1980) and HPFS cohort (year 1986). At baseline, mean ± SD age was 45.8 ± 14.6 years in the NHS and 54.6 ± 8.7 years in the HPFS. The MAFs of *HIF3A* SNP rs3826795 were 0.17 in the NHS and 0.18 in the HPFS. The MAFs of *HIF3A* SNP rs8102595 were 0.10 in the NHS and 0.09 in the HPFS. The SNP rs3826795 showed only weak linkage disequilibrium with the SNP rs8102595 (r^2 = 0.006; D′ = 0.555 in the HapMap CEU [Centre d'Étude du Polymorphisme Humain from Utah] database [http://hapmap.ncbi.nlm.nih.gov]).

Main Genetic Associations of *HIF3A* Variants With Adiposity Measures

In both cohorts, we did not observe significant associations of *HIF3A* rs3826795 with adiposity measures, including body weight at a young age (18 years old in the NHS and 21 years old in the HPFS), baseline body weight, baseline BMI, and 10-year changes in body weight and BMI (Table 2). *HIF3A* rs3826795 was not associated

Table 1—Baseline characteristics of participants in the NHS (1980) and HPFS (1986)

	NHS (Women)	HPFS (Men)
Participants (*n*)	8,109	6,761
Age (years)	45.8 ± 14.6	54.6 ± 8.7
Body weight (kg)	67.1 ± 13.4	82.2 ± 12.0
Height (cm)	163.9 ± 6.2	178.5 ± 6.6
BMI (kg/m^2)	25.0 ± 4.7	25.7 ± 3.2
Alcohol intake (g/day)	6.5 ± 10.5	12.4 ± 16.2
Current smoker	2,108 (23.9)	576 (8.8)
Physical activity (MET-h/week)	14.0 ± 19.7	19.9 ± 26.3
Alternate Healthy Eating Index	29.1 ± 8.7	44.6 ± 10.9
Fried food consumption (servings/day)	0.1 ± 0.1	0.2 ± 0.2
Sugar-sweetened beverage consumption (servings/day)	0.3 ± 0.5	0.2 ± 0.4
Television watching (h/week)	13.5 ± 12.0	11.7 ± 10.9
Total energy intake (kcal/day)	1,578 ± 492	2,026 ± 612
Total vitamin B_2 (mg/day)	3.4 ± 6.5	5.2 ± 10.3
Total folate (μg/day)	368 ± 246	473 ± 260
Total vitamin B_6 (mg/day)	3.0 ± 8.0	8.5 ± 24.0
Total vitamin B_{12} (μg/day)	8.91 ± 13.66	12.63 ± 14.55
Supplemental vitamins		
Vitamin B_2 (mg/day)	2.1 ± 7.8	4.5 ± 12.1
Folate (μg/day)	175 ± 285	189 ± 262
Vitamin B_6 (mg/day)	1.7 ± 9.5	8.7 ± 28.1
Vitamin B_{12} (μg/day)	4.36 ± 17.13	5.31 ± 15.87
Food-sourced vitamins		
Vitamin B_2 (mg/day)	1.7 ± 0.5	1.9 ± 0.5
Folate (μg/day)	263 ± 108	351 ± 112
Vitamin B_6 (mg/day)	1.6 ± 0.5	2.2 ± 0.8
Vitamin B_{12} (μg/day)	5.97 ± 3.09	8.88 ± 5.12
rs3826795		
GG	5,928 (62.5)	4,554 (66.6)
GA	2,172 (33.4)	2,051 (30.0)
AA	389 (4.1)	229 (3.4)
rs8102595		
AA	7,720 (81.3)	5,641 (82.5)
AG	1,675 (17.7)	1,132 (16.6)
GG	94 (1.0)	59 (0.9)

Data are mean ± SD or *n* (%) unless otherwise indicated.

Table 2—Association of *HIF3A* SNP rs3826795 with measures of adiposity in the NHS and HPFS

	NHS		HPFS	
	β ± SE	*P* value	β ± SE	*P* value
Weight at young age* (kg)	0.00 ± 0.19	0.86	−0.36 ± 0.29	0.21
Height (cm)	−0.21 ± 0.13	0.12	−0.10 ± 0.08	0.21
Weight at baseline (kg)	−0.09 ± 0.24	0.69	0.13 ± 0.33	0.69
BMI at baseline (kg/m^2)	0.03 ± 0.08	0.71	0.03 ± 0.09	0.76
Weight at end point (kg)	0.06 ± 0.27	0.83	−0.01 ± 0.35	0.98
BMI at end point (kg/m^2)	0.12 ± 0.11	0.18	−0.01 ± 0.10	0.93
BMI change (kg/m^2)	0.04 ± 0.04	0.31	−0.05 ± 0.05	0.33
Weight change (kg)	0.07 ± 0.13	0.78	−0.13 ± 0.16	0.42
Waist circumference (cm)$	0.11 ± 0.11	0.32	0.01 ± 0.11	0.90

The linear regression model was used to test the association of DNA methylation variants with measures of adiposity after adjustment of age, source of genotyping data, smoking, alcohol intake, physical activity, total energy intake, television watching, and Alternate Healthy Eating Index. BMI change, changes in BMI from 1980 to 1990 in NHS and from 1986 to 1996 in HPFS; weight change, changes in body weight from 1980 to 1990 in NHS and from 1986 to 1996 in HPFS. *Young age was defined as 18 years old in NHS and 21 years old in HPFS. Data were adjusted only for age and source of genotyping data. $Waist circumference was assessed in 1986 in NHS and 1987 in HPFS.

with risk of obesity or overweight (Supplementary Table 1). The null associations were consistently observed in analyses stratified by age or lifestyle factors in the NHS and HPFS cohorts (Supplementary Table 2).

Association of Total B-Vitamin Intake With 10-Year Changes in BMI and DNA Methylation Variants

We found positive associations of baseline total vitamin B_{12} intake from food and supplemental sources with 10-year BMI change in the NHS and HPFS cohorts (both *P* for trend <0.01) after adjustment for age, source of genotyping data, smoking, alcohol intake, physical activity, total energy intake, television watching, and Alternate Healthy Eating Index (Supplementary Table 3). Across tertiles of total vitamin B_{12} intake (T1, T2, and T3), the changes in BMI were 0.74 (SD 0.68), 0.83 (SD 0.76), and 0.88 (SD 0.82) kg/m^2 in the pooled cohort. The association of vitamin B_6 with BMI changes over a 10-year period was significant in NHS (*P* = 0.03) but borderline significant in HPFS (*P* = 0.07). Vitamin B_2 was significantly associated with 10-year BMI changes in HPFS but not in NHS. However, folate intake was not related to BMI changes. Further analyses indicated that food sources of vitamins B_{12} and B_6 but not supplemental B vitamins were associated with BMI changes. In addition, we did not observe significant associations of total, supplemental, and food sources of B vitamins with the DNA methylation variants.

Genetic Association With 10-Year Changes in BMI According to B-Vitamin Intake From Supplemental or Food Sources

We examined whether B vitamins from supplemental or food sources modified the genetic association with 10-year changes in BMI differently. We observed significant interactions for vitamins B_2 and B_{12} intakes from supplements. The genetic association with 10-year changes in BMI consistently increased across the three categories of vitamins B_2 and B_{12} intakes from supplemental use in the NHS and HPFS (all *P* for interaction <0.01). Differences in 10-year changes of BMI (kg/m^2) were −0.19 (SE 0.07), −0.08 (SE 0.06), and 0.09 (SE 0.07) across the tertiles of vitamin B_{12} supplement intake and −0.08 (SE 0.07), −0.06 (SE 0.07), and 0.11 (SE 0.06) across the tertiles of vitamin B_2 supplement intake in the pooled cohorts. We also found that supplemental folate intake modified the genetic association with 10-year changes in BMI in the NHS, HPFS, and pooled results (*P* for interaction = 0.08, 0.01, and 0.007, respectively) (Fig. 1). Across tertiles of supplemental folate intake, differences in 10-year changes in BMI were −0.06 (SE 0.07), −0.06 (SE 0.07), and 0.08 (SE 0.07) in the pooled cohort. No significant heterogeneity in the interaction effects was observed between the two cohorts (both *P* for heterogeneity >0.15). Among the four nutrients, vitamin B_2 (*P* for interaction = 0.002), vitamin B_{12} (*P* for interaction = 0.0004), and folate (*P* for interaction = 0.007) remained significant in the pooled data at $P < 0.01$ (0.05/4) after correction for multiple testing. We did not observe a significant interaction between *HIF3A* variants and B vitamins from food sources on BMI changes (Supplementary Table 4).

Genetic Association With 10-Year Changes in BMI According to Total B-Vitamin Intake

We further tested whether total B-vitamin intake modified the genetic association with BMI changes. We found significant interactions of *HIF3A* rs3826795 with total vitamins B_2 and B_{12} intakes in relation to 10-year BMI changes in the NHS and HPFS after adjustment for age, source of genotyping data, smoking, alcohol intake, physical activity, total energy intake, Alternate Healthy Eating

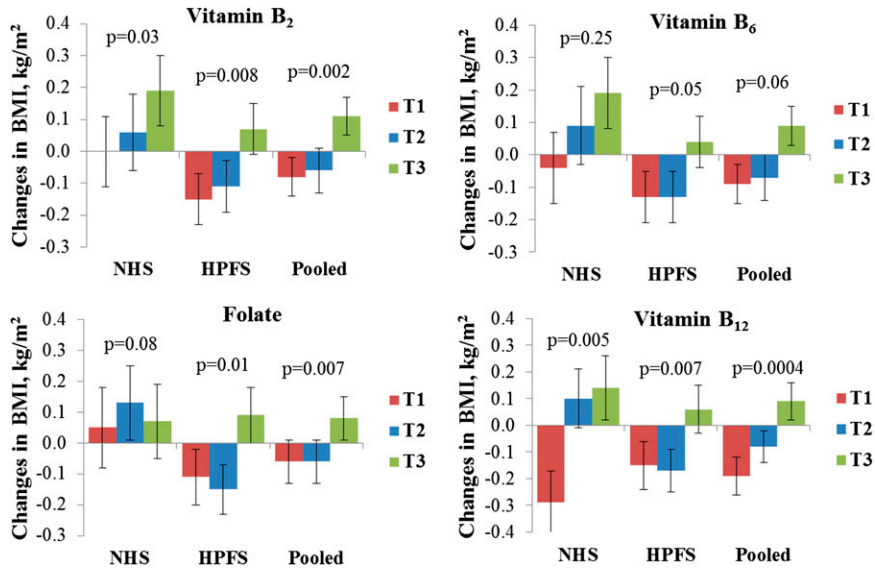

Figure 1—Differences in 10-year changes in BMI per minor allele of rs3826795 according to baseline intake of B vitamins from supplemental use among participants in the NHS (1980–1990) and HPFS (1986–1996). Data are β-coefficients ± SE. Data on baseline intake of B vitamins from supplemental use were assessed in 1980 (NHS) and 1986 (HPFS). Data on BMI were assessed in 1980 and 1990 in NHS and 1986 and 1996 in HPFS. The general linear model was used to test the genetic association of baseline intake of B vitamins from supplemental use with 10-year changes in BMI after adjustment for age, source of genotyping data, smoking, alcohol intake, physical activity, total energy intake, Alternate Healthy Eating Index, television watching, baseline BMI, and other B vitamins (mutually adjusted). Results for the two cohorts were pooled by means of inverse variance–weighted fixed-effects meta-analyses.

Index, television watching, baseline BMI, and other B vitamins (Table 3). Across the tertiles of total vitamin B_2 intake, the differences in BMI changes per increment of minor allele were −0.10 (SE 0.09), 0.07 (SE 0.09), and 0.22 (SE 0.12) kg/m^2 in the NHS; −0.10 (SE 0.09), −0.08 (SE 0.08), and 0.06 (SE 0.09) kg/m^2 in the HPFS; and −0.10 (SE 0.06), −0.01 (SE 0.06), and 0.12 (SE 0.07) kg/m^2 in the pooled cohort. We observed similar interaction patterns for 10-year changes in body weight. For the tertiles of total B_{12} intake, the differences in BMI changes per increment of minor allele were 0.00 (SE 0.09), −0.10 (SE 0.11), and 0.20 (SE 0.10) kg/m^2 in the NHS; −0.20 (SE 0.09), −0.06 (SE 0.08), and 0.01 (SE 0.09) kg/m^2 in the HPFS; and −0.10 (SE 0.06), −0.01 (SE 0.06), and 0.10 (SE 0.07) kg/m^2 in the pooled cohort. The results show that 0.9–1.2% of the 10-y change in BMI was explained by an interaction between *HIF3A* variant and vitamin B_2, and 0.7–1.6% of the 10-year change in BMI was explained by an interaction between *HIF3A* variant and vitamin B_{12} in the two cohorts. The amount of baseline vitamin B intake in corresponding tertiles in NHS and HPFS cohorts is shown in Supplementary Table 5. Further adjustment for sugar-sweetened beverages, fried food, and total meat consumption did not materially change the results. No significant heterogeneity in the interaction effects was observed between the two cohorts (both P for heterogeneity >0.05).

In addition, total folate intake showed a significant gene-diet interaction on 10-year changes in BMI in the pooled analyses (P for interaction = 0.02). Among the four nutrients, interactions with vitamins B_2 (P for interaction = 0.004) and B_{12} (P for interaction = 0.002) in the pooled data remained significant at $P < 0.006$ (0.05/8) after correction for multiple testing. We did not observe a significant interaction between *HIF3A* rs8102595 and total B-vitamin intake on BMI or BMI change.

DISCUSSION

In two large prospective cohorts of U.S. women and men, we found significant interactions of DNA methylation variant *HIF3A* rs3826795 with total or supplemental vitamin B_2, vitamin B_{12}, and folate intake on 10-year changes in BMI. A genetic association with a smaller increase in BMI was observed in the subgroup with lower intake of vitamin B_2, vitamin B_{12}, and folate, whereas the association with a greater increase in BMI was observed in the subgroup with higher intake of vitamin B_2, vitamin B_{12}, and folate. The findings support the hypothesis that DNA methylation may causally affect body adiposity. However, we did not measure DNA methylation at the *HIF3A* locus; with such data, we would have performed Mendelian randomization analysis to provide stronger evidence to support or dispute causality, although results from genetic associations could also provide evidence for a potential causal relation between the genetic variants–associated exposure and outcomes, as suggested in previous studies (32).

Growing evidence indicates that epigenetic factors contribute to the development of obesity (2–5). DNA methylation, the major epigenetic regulator in mammalian

Table 3—Differences in 10-year changes in BMI per minor allele of rs3826795 according to baseline total intake of B vitamins among participants in NHS (1980–1990) and HPFS (1986–1996)

	Tertiles of total intake of B vitamins			
Cohort$	T1	T2	T3	*P* value for interaction
Vitamin B_2 (mg)				
NHS	−0.10 ± 0.09	0.07 ± 0.09	0.22 ± 0.12	0.02
HPFS	−0.10 ± 0.09	−0.08 ± 0.08	0.06 ± 0.09	0.02
Pooled#	−0.10 ± 0.06	−0.01 ± 0.06	0.12 ± 0.07	0.004
Vitamin B_6 (mg)				
NHS	−0.01 ± 0.10	−0.01 ± 0.09	0.16 ± 0.11	0.14
HPFS	−0.08 ± 0.09	−0.09 ± 0.08	0.05 ± 0.09	0.18
Pooled	−0.05 ± 0.07	−0.06 ± 0.06	0.09 ± 0.07	0.18
Folate (μg)				
NHS	0.11 ± 0.09	−0.08 ± 0.10	0.07 ± 0.10	0.11
HPFS	−0.10 ± 0.09	−0.12 ± 0.08	0.09 ± 0.09	0.03
Pooled	0.01 ± 0.06	−0.10 ± 0.06	0.08 ± 0.07	0.02
Vitamin B_{12} (μg)				
NHS	0.00 ± 0.09	−0.10 ± 0.11	0.20 ± 0.10	0.003
HPFS	−0.20 ± 0.09	0.06 ± 0.08	0.01 ± 0.09	0.07
Pooled	−0.10 ± 0.06	0.01 ± 0.06	0.10 ± 0.07	0.002

Data are β-coefficients ± SE. Data on baseline total intake of B vitamins were assessed in 1980 (NHS) and 1986 (HPFS). Data on BMI were assessed in 1980 and 1990 in NHS and 1986 and 1996 in HPFS. $The general linear model was used to test the genetic association of baseline total intake of B vitamins with 10-year changes in BMI after adjustment for age, source of genotyping data, smoking, alcohol intake, physical activity, total energy intake, Alternate Healthy Eating Index, television watching, baseline BMI, and other B vitamins (mutually adjusted). #Results for the two cohorts were pooled by means of inverse variance–weighted fixed-effects meta-analyses.

cells, has been associated with BMI or other indices of obesity (33–35). A recent large-scale epigenome-wide study demonstrated that increased DNA methylation at the *HIF3A* locus in blood cells and adipose tissue was associated with increased BMI in adults of European origin (4). Nevertheless, genetic variants in the *HIF3A* locus, which has been associated with DNA methylation, were not related to BMI in the whole study population (4). These findings are consistent with the current results that DNA methylation variants were not associated with measures of adiposity in the NHS and HPFS. Taken together, these data appear to support the previous postulation that the association between *HIF3A* methylation and BMI may be not causal (4). However, our further subgroup analyses accounting for B-vitamin intake refute such a conclusion.

In the current study, we found that DNA methylation variant *HIF3A* rs3826795 was significantly associated with BMI changes among participants with either high or low intakes of vitamin B_2, vitamin B_{12}, and folate. Although we did not conduct a Mendelian randomization analysis owing to lack of DNA methylation data, the results at least partly support a potential causal relation between *HIF3A* methylation and adiposity according to Mendelian randomization theory (36). According to the theory, the associations between the genetic variants, which act as a proxy of DNA methylation levels (4), and adiposity are free of risk for reverse causation and less likely to be affected by confounding, therefore providing evidence for causality (37,38). The current findings provide consistent evidence from two cohorts showing robust associations between the DNA methylation variants and BMI changes. Of note, the methylation variant exhibited opposite effects on weight change in response to low and high B-vitamin intakes. These findings are in line with the hypothesis of differential susceptibility (39,40), which suggests that vulnerability genes may function like plasticity genes; thus, genetic risk is either attenuated by a favorable environment or amplified by an adverse environment (39,40). The current results highlight the importance in considering effect modifications by environmental factors when assessing genetic associations.

The mechanism for the observed interactions between B vitamins and DNA methylation variant in relation to BMI changes remains unknown. However, the present findings are biologically plausible because B vitamins play an important role in DNA methylation. Vitamin B_2, vitamin B_{12}, and folate are well established as the major determinants of one-carbon metabolism in which a methyl group donor is formed (41). In the DNA methylation process, a methyl group is added to the cytosine or adenine DNA nucleotide that typically occurs in a CpG dinucleotide context. Animal studies have shown that maternal methyl supplements (e.g., folic acid, vitamin B_{12}) increase offspring DNA methylation (42,43). A randomized trial demonstrated that folic acid supplementation increases DNA methylation in leukocytes and colonic mucosa (44). In addition, previous studies have shown that the interaction of dietary folate and vitamin B_{12} with a genetic variant might modulate gene expression through DNA methylation (45). DNA methylation changes caused by

folate and vitamin B_{12} can have downstream effects by changing gene expression (46) and can modulate disease risk associated with genetic variants (47). The current findings underscore the need to assess the effect of DNA methylation on *HIF3A* function in experimental studies and the effect of diet on epigenetic changes in the *HIF3A* genomic region through in vivo studies.

In the current study, we separately analyzed B-vitamin intake from supplemental and food sources and observed stronger interactions on BMI changes for B vitamins from supplements. The differences in these findings for B vitamins from food sources compared with supplemental sources could be explained by differences in the bioavailability of these vitamins (48). This may be most pertinent for folate and vitamin B_{12}, which are more bioavailable in supplemental forms (43). A substantial body of evidence shows that the bioavailability of folic acid (both from supplements and from fortified foods), which is in large part governed by the extent of intestinal absorption, is almost always substantially higher than the net bioavailability of naturally occurring dietary folate from food sources (49). In addition, vitamin B_{12} deficiency is common among people with a low intake of animal food sources and among elderly people owing to malabsorption of B_{12} from food (50). Vitamin B_{12} or folate supplement users have higher plasma values of these nutrients (51). Therefore, it is not surprising that stronger interactions for B vitamins from supplements were observed in the current study. In further analyses, we did not find a significant interaction between B-vitamin–rich foods (e.g., fish, poultry, meat, vegetables, dairy, legumes, whole grains) and methylation variants; the possible reason is that the associations/interactions are likely to be contaminated by many other components in the foods.

The major strengths of the current study include consistent findings from two well-established prospective cohorts, detailed assessments of nutrient and food intakes and measures of adiposity, and minimal population stratification. Use of longitudinal data minimized a random measurement error and enhanced the robustness of the findings. However, several limitations need to be acknowledged. First, dietary B vitamins and adiposity measures were self-reported, and errors in these measurement are inevitable; however, the food frequency questionnaires (23,24) and adiposity measurement data (27) have been well validated. Second, confounding by other unmeasured or unknown factors might exist, although we carefully adjusted for multiple dietary and lifestyle factors. Third, similar to other genetic studies, the current analyses to detect gene-diet interaction might have suffered from a multiple testing burden that could have hampered detection and interpretation (52). However, the interactions observed in the current study for vitamins B_2 and B_{12} remained significant at $P < 0.006$ (0.05/8) after Bonferroni correction for multiple testing. Indeed, Bonferroni correction is usually too conservative, and overadjustment for multiple comparisons may increase the risk for type II error and reduce power to detect significant differences (53). Fourth, whether the genetic markers are functional variants or simply correlated markers is unclear. The two methylation variants may tag for a functional epigenetic marker; therefore, it is not surprising that the two SNPs showed distinct interactions, which do not imply interaction at the causal variant (54). Fifth, the participants were middle-aged and older adults of European ancestry recruited in the U.S., and it remains to be examined whether the results could be generalized to other demographic or ethnic groups. Finally, we examined two *HIF3A* variants independently associated with methylation at a specific CpG site within intron 1 of *HIF3A*, but we did not find any interaction for rs8102595. Our previous study may provide a possible reason (8). Not all SNPs are sensitive to environmental modifications, and the current study of 32 BMI variants showed diverse interactions with fried food consumption in relation to adiposity. Further studies are required to investigate the differential interactions of these variants on the association of DNA methylation with adiposity.

In conclusion, the data provide consistent evidence from two cohorts that a DNA methylation variant interacts with total or supplemental B vitamins in relation to 10-year BMI changes. These results, which need to be replicated, support a potential causal relation between DNA methylation and adiposity. In addition, these findings emphasize the importance of considering gene-environment interactions in assessing genetic associations.

Acknowledgments. The authors thank Gary C. Curhan (Harvard T.H. Chan School of Public Health) for contributions to the genetic data.

Funding. This study was supported by grants HL-126024, DK-100383, DK-091718, HL-071981, HL-073168, CA-87969, CA-49449, CA-055075, HL-34594, HL-088521, U01-HG-004399, DK-080140, P30-DK-46200, U01-CA-137088, U54-CA-155626, DK-58845, DK-098311, U01-HG-004728, EY-015473, CA-134958, DK-70756, and DK-46200 from the National Institutes of Health. L.R.P. is supported by the Arthur Ashley Williams Foundation and a Harvard Medical School Ophthalmology Scholar Award from the Glaucoma Center of Excellence. A.T.C. is a Damon Runyon Clinical Investigator. L.Q. is a recipient of an American Heart Association Scientist Development Grant (0730094N).

The funding sources had no role in the design or conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.

Duality of Interest. Additional support for genotyping was received from Merck Research Laboratories (North Wales, PA). No other conflicts of interest relevant to this article were reported.

Author Contributions. T.H. contributed to the study design; data analysis; interpretation of the results; and writing, critical revision for important intellectual content, and final approval of the manuscript. Y.Z., Q.Q., M.X., and Y.L. contributed statistical expertise and to the interpretation of the results and critical revision for important intellectual content and final approval of the manuscript. S.H.L. contributed to the interpretation of the results and critical revision for important intellectual content and final approval of the manuscript. J.H.K., J.W., L.R.P., A.T.C., E.B.R., D.J.H., J.E.M., W.C.W., and F.B.H. contributed to the data collection, interpretation of the results, and critical revision for important intellectual content and final approval of the manuscript. L.Q. contributed to the study design; data collection; interpretation of the results; and writing, critical revision for important intellectual content, and final approval of the manuscript. T.H. and L.Q. are the guarantors of this work and, as such, had full access to all

the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Bird A. Perceptions of epigenetics. Nature 2007;447:396–398
2. Gluckman PD, Hanson MA. Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond) 2008; 32(Suppl. 7):S62–S71
3. Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. Science 2001;293:1068–1070
4. Dick KJ, Nelson CP, Tsaprouni L, et al. DNA methylation and body-mass index: a genome-wide analysis. Lancet 2014;383:1990–1998
5. Martínez JA, Milagro FI, Claycombe KJ, Schalinske KL. Epigenetics in adipose tissue, obesity, weight loss, and diabetes. Adv Nutr 2014;5:71–81
6. Qi Q, Chu AY, Kang JH, et al. Sugar-sweetened beverages and genetic risk of obesity. N Engl J Med 2012;367:1387–1396
7. Qi Q, Li Y, Chomistek AK, et al. Television watching, leisure time physical activity, and the genetic predisposition in relation to body mass index in women and men. Circulation 2012;126:1821–1827
8. Qi Q, Chu AY, Kang JH, et al. Fried food consumption, genetic risk, and body mass index: gene-diet interaction analysis in three US cohort studies. BMJ 2014; 348:g1610
9. Qi L, Cho YA. Gene-environment interaction and obesity. Nutr Rev 2008;66: 684–694
10. Qi L. Gene-diet interaction and weight loss. Curr Opin Lipidol 2014;25: 27–34
11. Dominguez-Salas P, Cox SE, Prentice AM, Hennig BJ, Moore SE. Maternal nutritional status, C(1) metabolism and offspring DNA methylation: a review of current evidence in human subjects. Proc Nutr Soc 2012;71:154–165
12. Brosnan JT, Brosnan ME. The sulfur-containing amino acids: an overview. J Nutr 2006;136(Suppl.):1636S–1640S
13. Mahabir S, Ettinger S, Johnson L, et al. Measures of adiposity and body fat distribution in relation to serum folate levels in postmenopausal women in a feeding study. Eur J Clin Nutr 2008;62:644–650
14. Krishnaveni GV, Hill JC, Veena SR, et al. Low plasma vitamin B12 in pregnancy is associated with gestational 'diabesity' and later diabetes. Diabetologia 2009;52:2350–2358
15. Colditz GA, Manson JE, Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. J Womens Health 1997;6:49–62
16. Rimm EB, Giovannucci EL, Willett WC, et al. Prospective study of alcohol consumption and risk of coronary disease in men. Lancet 1991;338:464–468
17. Cornelis MC, Monda KL, Yu K, et al. Genome-wide meta-analysis identifies regions on 7p21 (AHR) and 15q24 (CYP1A2) as determinants of habitual caffeine consumption. PLoS Genet 2011;7:e1002033
18. Jensen MK, Pers TH, Dworzynski P, Girman CJ, Brunak S, Rimm EB. Protein interaction-based genome-wide analysis of incident coronary heart disease. Circ Cardiovasc Genet 2011;4:549–556
19. Wiggs JL, Kang JH, Yaspan BL, et al.; GENEVA Consortium. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma in Caucasians from the USA. Hum Mol Genet 2011;20:4707–4713
20. Hunter DJ, Kraft P, Jacobs KB, et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet 2007;39:870–874
21. Qi L, Cornelis MC, Kraft P, et al.; Meta-Analysis of Glucose and Insulin-related traits Consortium (MAGIC); Diabetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium. Genetic variants at 2q24 are associated with susceptibility to type 2 diabetes. Hum Mol Genet 2010;19:2706–2715
22. Köttgen A, Albrecht E, Teumer A, et al.; LifeLines Cohort Study; CARDIoGRAM Consortium; DIAGRAM Consortium; ICBP Consortium; MAGIC Consortium. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. Nat Genet 2013;45:145–154
23. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122: 51–65
24. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114–1126; discussion 1127–1136
25. Wolf AM, Hunter DJ, Colditz GA, et al. Reproducibility and validity of a self-administered physical activity questionnaire. Int J Epidemiol 1994;23: 991–999
26. Chiuve SE, Fung TT, Rimm EB, et al. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr 2012;142:1009–1018
27. Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB, Willett WC. Validity of self-reported waist and hip circumferences in men and women. Epidemiology 1990;1:466–473
28. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. JAMA 2002;288:1723–1727
29. Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999-2000. JAMA 2002;288: 1728–1732
30. Winkleby MA, Cubbin C. Changing patterns in health behaviors and risk factors related to chronic diseases, 1990-2000. Am J Health Promot 2004;19: 19–27
31. Peters U, Jiao S, Schumacher FR, et al. Identification of genetic susceptibility loci for colorectal tumors in a genome-wide meta-analysis. Gastroenterology 2013;144:799–807
32. Qi Q, Forman JP, Jensen MK, et al. Genetic predisposition to high blood pressure associates with cardiovascular complications among patients with type 2 diabetes: two independent studies. Diabetes 2012;61: 3026–3032
33. Relton CL, Groom A, St Pourcain B, et al. DNA methylation patterns in cord blood DNA and body size in childhood. PLoS One 2012;7:e31821
34. Wang X, Zhu H, Snieder H, et al. Obesity related methylation changes in DNA of peripheral blood leukocytes. BMC Med 2010;8:87
35. Almén MS, Jacobsson JA, Moschonis G, et al. Genome wide analysis reveals association of a FTO gene variant with epigenetic changes. Genomics 2012; 99:132–137
36. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet 2014;23(R1):R89–R98
37. Qi L. Mendelian randomization in nutritional epidemiology. Nutr Rev 2009; 67:439–450
38. Burgess S, Butterworth A, Malarstig A, Thompson SG. Use of Mendelian randomisation to assess potential benefit of clinical intervention. BMJ 2012;345: e7325
39. Belsky J, Pluess M. Beyond diathesis stress: differential susceptibility to environmental influences. Psychol Bull 2009;135:885–908
40. Belsky J, Jonassaint C, Pluess M, Stanton M, Brummett B, Williams R. Vulnerability genes or plasticity genes? Mol Psychiatry 2009;14:746–754
41. Selhub J. Homocysteine metabolism. Annu Rev Nutr 1999;19:217–246
42. Waterland RA, Dolinoy DC, Lin JR, Smith CA, Shi X, Tahiliani KG. Maternal methyl supplements increase offspring DNA methylation at Axin Fused. Genesis 2006;44:401–406
43. Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr 2002;132(Suppl.): 2393S–2400S
44. Pufulete M, Al-Ghnaniem R, Khushal A, et al. Effect of folic acid supplementation on genomic DNA methylation in patients with colorectal adenoma. Gut 2005;54:648–653
45. Friso S, Choi SW. Gene-nutrient interactions and DNA methylation. J Nutr 2002;132(Suppl.):2382S–2387S

46. Schadt EE. Molecular networks as sensors and drivers of common human diseases. Nature 2009;461:218–223
47. Relton CL, Davey Smith G. Epigenetic epidemiology of common complex disease: prospects for prediction, prevention, and treatment. PLoS Med 2010;7: e1000356
48. Bailey LB. Folate and vitamin B12 recommended intakes and status in the United States. Nutr Rev 2004;62:S14–S20; discussion S21
49. Sanderson P, McNulty H, Mastroiacovo P, et al.; UK Food Standards Agency. Folate bioavailability: UK Food Standards Agency workshop report. Br J Nutr 2003;90:473–479
50. Stabler SP, Allen RH. Vitamin B12 deficiency as a worldwide problem. Annu Rev Nutr 2004;24:299–326
51. Malinow MR, Duell PB, Hess DL, et al. Reduction of plasma homocyst(e)ine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease. N Engl J Med 1998;338:1009–1015
52. Manco M, Dallapiccola B. Genetics of pediatric obesity. Pediatrics 2012; 130:123–133
53. Zhang X, Qi Q, Zhang C, et al. FTO genotype and 2-year change in body composition and fat distribution in response to weight-loss diets: the POUNDS LOST Trial [published correction appears in Diabetes 2013;62:662]. Diabetes 2012;61:3005–3011
54. Dudbridge F, Fletcher O. Gene-environment dependence creates spurious gene-environment interaction. Am J Hum Genet 2014;95:301–307