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

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**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<sub>2</sub> and B<sub>12</sub> 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<sub>2</sub> 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<sub>12</sub> intake. In two independent cohorts, a DNA methylation variant in *HIF3A* was associated with BMI changes through interactions with total or supplemental vitamin B<sub>2</sub>, vitamin B<sub>12</sub>, 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<sub>2</sub>), pyridoxine (B<sub>6</sub>), and B<sub>12</sub>, 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

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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<sub>2</sub>, B<sub>6</sub>, and B<sub>12</sub>, 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<sup>2</sup>). Participants

with a BMI  $\geq 30$  kg/m<sup>2</sup> were defined as obese, and those with a BMI  $\geq 25$  kg/m<sup>2</sup> 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,  $\geq 15$  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  $\pm$  SD age was  $45.8 \pm 14.6$  years in the NHS and  $54.6 \pm 8.7$  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 $\pm$ 14.6	54.6 $\pm$ 8.7
Body weight (kg)	67.1 $\pm$ 13.4	82.2 $\pm$ 12.0
Height (cm)	163.9 $\pm$ 6.2	178.5 $\pm$ 6.6
BMI (kg/m <sup>2</sup> )	25.0 $\pm$ 4.7	25.7 $\pm$ 3.2
Alcohol intake (g/day)	6.5 $\pm$ 10.5	12.4 $\pm$ 16.2
Current smoker	2,108 (23.9)	576 (8.8)
Physical activity (MET-h/week)	14.0 $\pm$ 19.7	19.9 $\pm$ 26.3
Alternate Healthy Eating Index	29.1 $\pm$ 8.7	44.6 $\pm$ 10.9
Fried food consumption (servings/day)	0.1 $\pm$ 0.1	0.2 $\pm$ 0.2
Sugar-sweetened beverage consumption (servings/day)	0.3 $\pm$ 0.5	0.2 $\pm$ 0.4
Television watching (h/week)	13.5 $\pm$ 12.0	11.7 $\pm$ 10.9
Total energy intake (kcal/day)	1,578 $\pm$ 492	2,026 $\pm$ 612
Total vitamin B <sub>2</sub> (mg/day)	3.4 $\pm$ 6.5	5.2 $\pm$ 10.3
Total folate ( $\mu$ g/day)	368 $\pm$ 246	473 $\pm$ 260
Total vitamin B <sub>6</sub> (mg/day)	3.0 $\pm$ 8.0	8.5 $\pm$ 24.0
Total vitamin B <sub>12</sub> ( $\mu$ g/day)	8.91 $\pm$ 13.66	12.63 $\pm$ 14.55
Supplemental vitamins		
Vitamin B <sub>2</sub> (mg/day)	2.1 $\pm$ 7.8	4.5 $\pm$ 12.1
Folate ( $\mu$ g/day)	175 $\pm$ 285	189 $\pm$ 262
Vitamin B <sub>6</sub> (mg/day)	1.7 $\pm$ 9.5	8.7 $\pm$ 28.1
Vitamin B <sub>12</sub> ( $\mu$ g/day)	4.36 $\pm$ 17.13	5.31 $\pm$ 15.87
Food-sourced vitamins		
Vitamin B <sub>2</sub> (mg/day)	1.7 $\pm$ 0.5	1.9 $\pm$ 0.5
Folate ( $\mu$ g/day)	263 $\pm$ 108	351 $\pm$ 112
Vitamin B <sub>6</sub> (mg/day)	1.6 $\pm$ 0.5	2.2 $\pm$ 0.8
Vitamin B <sub>12</sub> ( $\mu$ g/day)	5.97 $\pm$ 3.09	8.88 $\pm$ 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  $\pm$  SD or  $n$  (%) unless otherwise indicated.

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

	NHS		HPFS	
	$\beta \pm SE$	<i>P</i> value	$\beta \pm SE$	<i>P</i> value
Weight at young age* (kg)	0.00 $\pm$ 0.19	0.86	−0.36 $\pm$ 0.29	0.21
Height (cm)	−0.21 $\pm$ 0.13	0.12	−0.10 $\pm$ 0.08	0.21
Weight at baseline (kg)	−0.09 $\pm$ 0.24	0.69	0.13 $\pm$ 0.33	0.69
BMI at baseline (kg/m <sup>2</sup> )	0.03 $\pm$ 0.08	0.71	0.03 $\pm$ 0.09	0.76
Weight at end point (kg)	0.06 $\pm$ 0.27	0.83	−0.01 $\pm$ 0.35	0.98
BMI at end point (kg/m <sup>2</sup> )	0.12 $\pm$ 0.11	0.18	−0.01 $\pm$ 0.10	0.93
BMI change (kg/m <sup>2</sup> )	0.04 $\pm$ 0.04	0.31	−0.05 $\pm$ 0.05	0.33
Weight change (kg)	0.07 $\pm$ 0.13	0.78	−0.13 $\pm$ 0.16	0.42
Waist circumference (cm)\$	0.11 $\pm$ 0.11	0.32	0.01 $\pm$ 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<sub>12</sub> 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<sub>12</sub> 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<sup>2</sup> in the pooled cohort. The association of vitamin B<sub>6</sub> 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<sub>2</sub> 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<sub>12</sub> and B<sub>6</sub> 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.

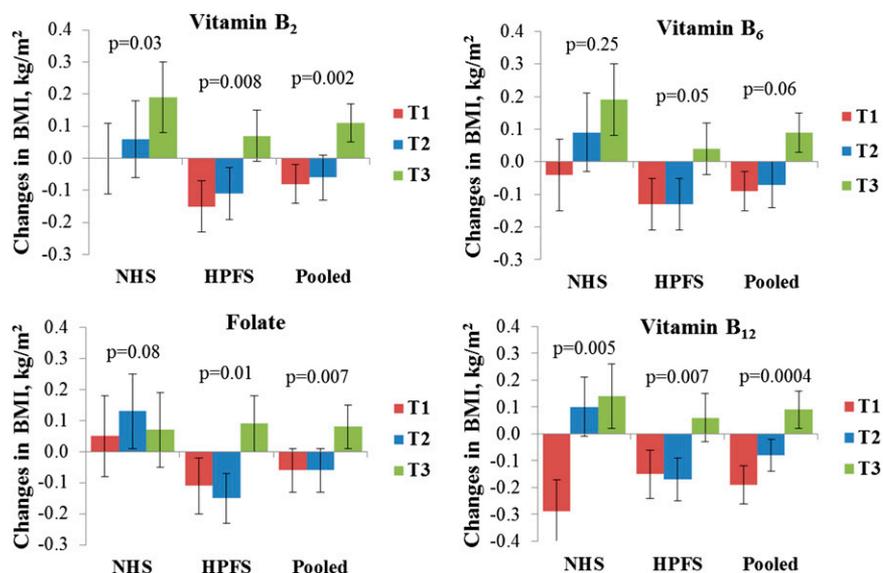
#### Genetic Association With 10-Year Changes in BMI According to B-Vitamin Intake From Supplemental or Food Sources

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<sub>2</sub> and B<sub>12</sub> intakes from supplements.

The genetic association with 10-year changes in BMI consistently increased across the three categories of vitamins B<sub>2</sub> and B<sub>12</sub> intakes from supplemental use in the NHS and HPFS (all *P* for interaction <0.01). Differences in 10-year changes of BMI (kg/m<sup>2</sup>) were −0.19 (SE 0.07), −0.08 (SE 0.06), and 0.09 (SE 0.07) across the tertiles of vitamin B<sub>12</sub> 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<sub>2</sub> 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<sub>2</sub> (*P* for interaction = 0.002), vitamin B<sub>12</sub> (*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<sub>2</sub> and B<sub>12</sub> 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  $\beta$ -coefficients  $\pm$  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<sub>2</sub> 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<sup>2</sup> in the NHS;  $-0.10$  (SE 0.09),  $-0.08$  (SE 0.08), and  $0.06$  (SE 0.09) kg/m<sup>2</sup> in the HPFS; and  $-0.10$  (SE 0.06),  $-0.01$  (SE 0.06), and  $0.12$  (SE 0.07) kg/m<sup>2</sup> in the pooled cohort. We observed similar interaction patterns for 10-year changes in body weight. For the tertiles of total B<sub>12</sub> 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<sup>2</sup> in the NHS;  $-0.20$  (SE 0.09),  $-0.06$  (SE 0.08), and  $0.01$  (SE 0.09) kg/m<sup>2</sup> in the HPFS; and  $-0.10$  (SE 0.06),  $-0.01$  (SE 0.06), and  $0.10$  (SE 0.07) kg/m<sup>2</sup> 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<sub>2</sub>, and 0.7–1.6% of the 10-year change in BMI was explained by an interaction between *HIF3A* variant and vitamin B<sub>12</sub> 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<sub>2</sub> (*P* for interaction = 0.004) and B<sub>12</sub> (*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<sub>2</sub>, vitamin B<sub>12</sub>, 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<sub>2</sub>, vitamin B<sub>12</sub>, and folate, whereas the association with a greater increase in BMI was observed in the subgroup with higher intake of vitamin B<sub>2</sub>, vitamin B<sub>12</sub>, 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)**

Cohort\$	Tertiles of total intake of B vitamins			P value for interaction
	T1	T2	T3	
<b>Vitamin B<sub>2</sub> (mg)</b>				
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
<b>Vitamin B<sub>6</sub> (mg)</b>				
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
<b>Folate (μg)</b>				
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
<b>Vitamin B<sub>12</sub> (μg)</b>				
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  $\beta$ -coefficients  $\pm$  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<sub>2</sub>, vitamin B<sub>12</sub>, 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<sub>2</sub>, vitamin B<sub>12</sub>, 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<sub>12</sub>) 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<sub>12</sub> with a genetic variant might modulate gene expression through DNA methylation (45). DNA methylation changes caused by

folate and vitamin B<sub>12</sub> 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<sub>12</sub>, 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<sub>12</sub> deficiency is common among people with a low intake of animal food sources and among elderly people owing to malabsorption of B<sub>12</sub> from food (50). Vitamin B<sub>12</sub> 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<sub>2</sub> and B<sub>12</sub> 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.

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**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.

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