Huang et al. Diabetes 2015;64:3146–3154

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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Definitions

- ***HIF3A* gene** – the gene coding for Hypoxia Inducible Transcription Factor 3, Subunit Alpha, a component of Hypoxia Inducible Transcription Factor (HIF), which regulates a wide variety of cellular and vascular responses to reduced oxygen concentrations. Based on previous research it is also thought to play a role in obesity.

- **DNA Methylation** – biochemical process in which a methyl group is added to DNA nucleotides. DNA methylation levels are subject to modulation by environmental factors such as diet and lifestyle.

- **CpG Site** – region of DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5' → 3' direction. These sequences can be methylated within a gene, changing its expression.

Introduction

- The epidemic of obesity occurred during a relatively short period of time in the US between 1980-2000 paralleling a transition from traditional to obesogenic diet/lifestyle patterns
- Obesity is determined by interactions between lifestyle, environmental, and genetic factors
- DNA Methylation is one of the major epigenetic events that affects gene expression
- Growing evidence indicates that DNA methylation plays a critical role in regulating body adiposity

DNA methylation and body-mass index: a genome-wide analysis. Dick, Katherine J et al. The Lancet , Volume 383 , Issue 9933 , 1990 - 1998

Introduction

- Several B Vitamins including folate (B9), riboflavin (B2), pyridoxine (B6), and cobalamin (B12) act as important enzyme cofactors and play critical roles in DNA Methylation
- Previous studies have associated B-vitamin intake with adiposity in humans
- Previous studies have shown that DNA methylation at *HIF3A* gene is associated with BMI

B Vitamins and Methylation

Folate (B9), riboflavin (B2), pyridoxine (B6), and cobalamin (B12)

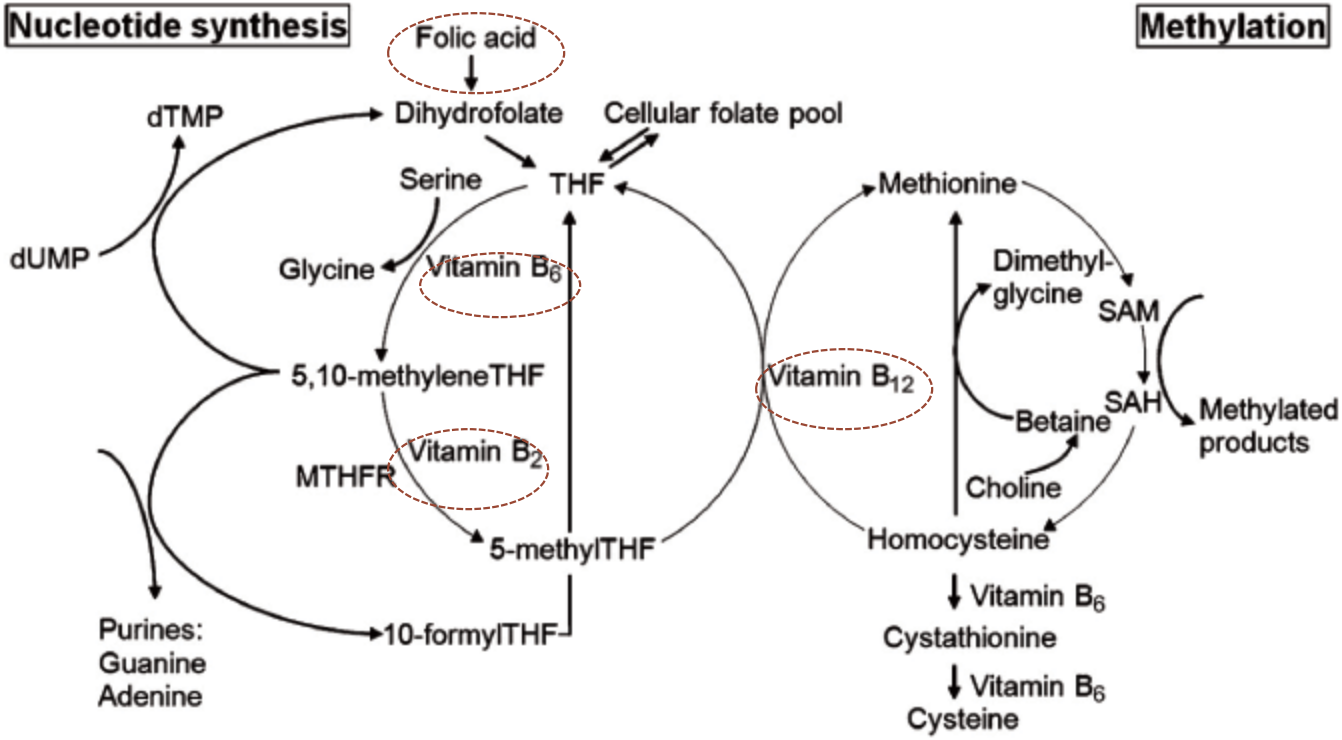
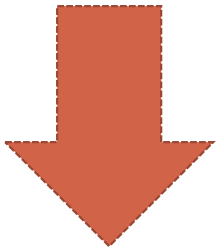


Figure 1. Methylation involves the B vitamins folate, vitamin B_2 (riboflavin), vitamin B_6 (pyridoxine), and vitamin B_{12} (cyanocobalamin).

http://www.life-enhancement.com/images/LEM1502Methylation700.gif

Gap Of Knowledge

- In a previous epigenome-wide study researchers found that DNA methylation at an *HIF3A* locus was associated with BMI, but the study did not consider potential modifying effects of environmental factors on the genetic associations.
- Previous studies have shown that B vitamin intake is associated with adiposity



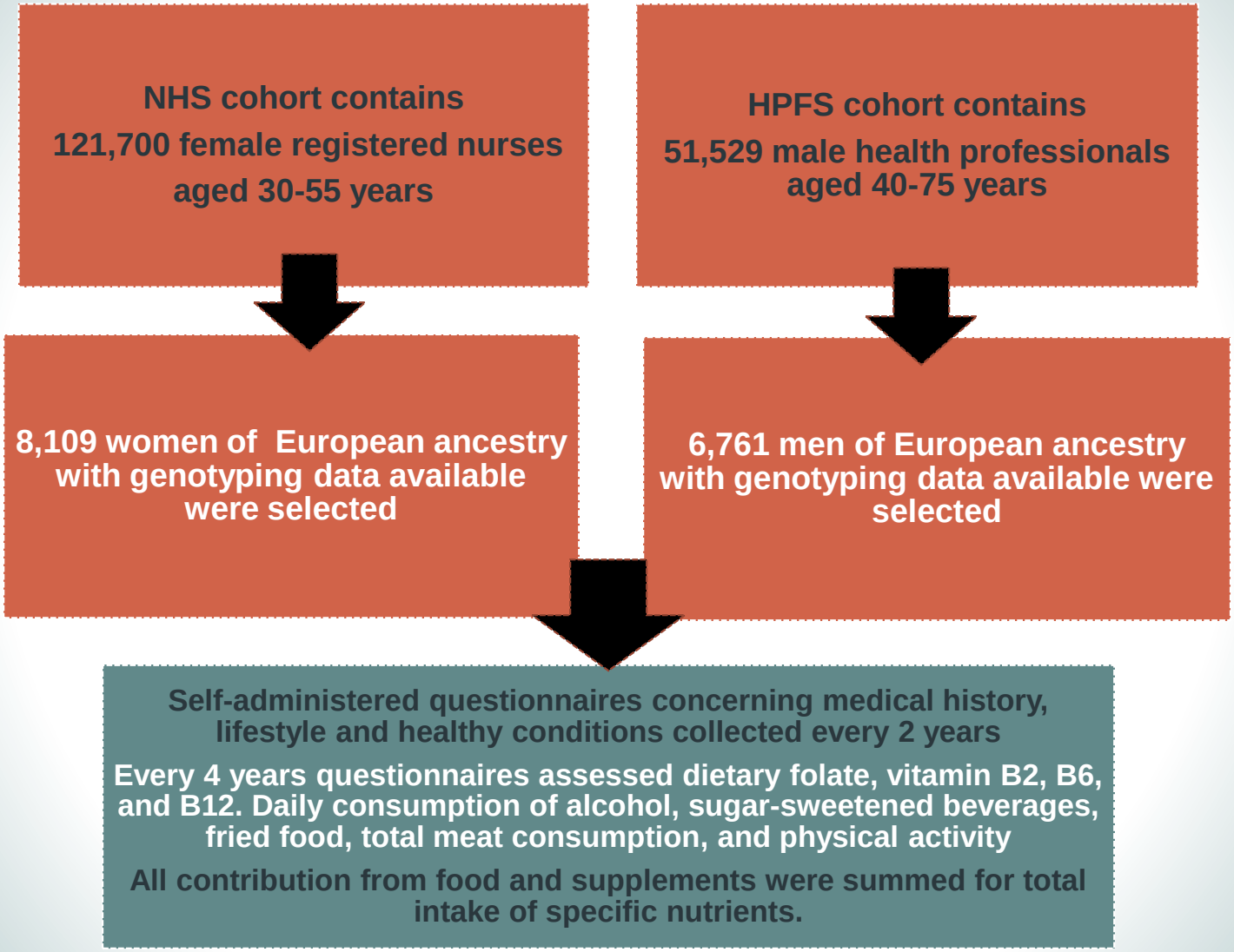
- Could B vitamin intake be the modifying environmental factor influencing changes in DNA methylation at HIF3A gene and contributing to changes in BMI?

Hypothesis & Objectives

The researchers hypothesize that DNA methylation variants in *HIF3A* are associated with BMI according to intake of B vitamins

They will examine the interactions between intake of B vitamins and DNA methylation variants at *HIF3A* locus in relation to BMI over a 10 year period

Research Design and Methods



The Nurses' Health Study

HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH | HEALTH PROFESSIONALS FOLLOW-UP STUDY

Research Design and Methods

- Assessment of Adiposity and 10-Year Changes in BMI
  - Height and Body Weight were assessed at baseline and recorded on each follow-up questionnaire
  - Body weight at a young age (18 yrs NHS, 21 yrs HPFS) was also collected by questionnaire
  - BMI was calculated as body weight (kg)/height(m^2), and changes were studied over 10 years
  - Obese = ≥30 kg/m^2 Overweight = ≥25 kg/m^2
  - Chose to measure BMI because it would more closely reflect long-term response to gene interactions

Research Design and Methods

- Single Nucleotide Polymorphism Selection (SNP) and Genotyping
  - *HIF3A* Variants rs3826795 and rs8102595 were used due to their independent associations with methylation at a specific CpG site (cg22891070) within intron 1 of *HIF3A* in adipose tissue and skin DNA associated with BMI
  - Higher methylation at this specific CpG site in previous studies has been observed in people with a higher BMI
  - These 2 variants were used in a previous study (Dick, et al) that showed these 2 variants did not have an association with BMI however the study suggested a *confounding factor* was affecting both variables

DNA methylation and body-mass index: a genome-wide analysis. Dick, Katherine J et al. The Lancet , Volume 383 , Issue 9933 , 1990 - 1998

Research Design and Methods

- Statistical Analysis
  - General linear models examined the association of genetic variants with adiposity measures, B vitamin intake, and 10 year changes in BMI or body weight
  - Interactions between genetic variants and intake of baseline nutrients (total B Vitamins, intake of B vitamins from supplements, and B vitamins from foods) 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 were age (years), as well as baseline measures of physical activity (MET-h/week), television watching, smoking, alcohol intake, Alternate Healthy Eating Index, and total energy intake
  - Bonferroni correction was conducted for multiple comparisons
  - Logistic regression models were used to estimate odds ratios for obesity incidence in the NHS and HPFS groups
  - Results across cohorts were pooled with inverse variance-weighted meta-analyses by: Fixed-effects models (if $P \geq 0.05$ for heterogeneity between studies) and Random-effects models (if $P < 0.05$ for heterogeneity between studies)
  - Statistical analyses were performed using SAS 9.3 software

Results

- Table 1:Baseline characteristics of participants in the NHS (1980) and HPFS (1986)
- Table 2: Association of *HIF3A* SNP rs3826795 with measures of adiposity in the NHS and HPFS
- 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)
- 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)

HIF3A SNPs independently associated with methylation at cg22891070

- A specific CpG site within intron 1 of *HIF3A*
- Methylation level is associated with BMI

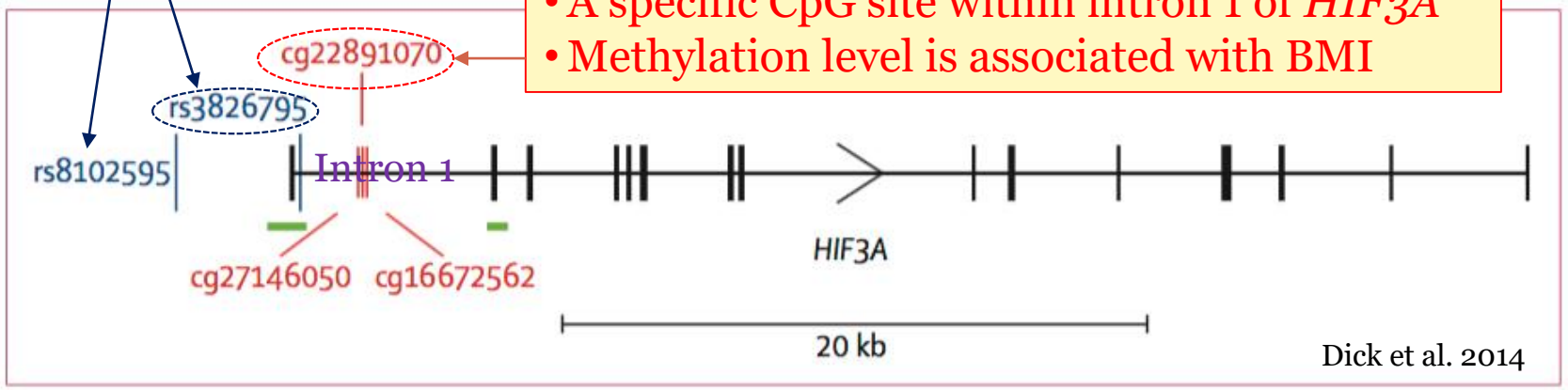


Figure 2: **Location of methylation probes associated with body-mass index and SNPs affecting methylation levels of these probes in the *HIF3A* locus**

- ✓ Methylation at cg22891070 is associated with BMI
- ✓ *HIF3A* SNP rs3826795 is associated with methylation at cg22891070

But…

- ✓ *HIF3A* SNP rs3826795 is not associated with BMI

<u>The Question:</u> Will intake of B vitamins modify the association of *HIF3A* SNP rs3826795 with BMI ?

Table 1. Baseline Characteristic of Participants

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)	1.3mg	3.4 ± 6.5		5.2 ± 10.3	
Total folate (μg/day)	400 mcg	368 ± 246		473 ± 260	
Total vitamin B_6 (mg/day)	1.3mg	3.0 ± 8.0		8.5 ± 24.0	
Total vitamin B_{12} (μg/day)	2.4mcg	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			**MAF**		**MAF**
GG		5,928 (62.5)		4,554 (66.6)	
GA		2,172 (33.4)		2,051 (30.0)	
AA		389 (4.1)	0.17	229 (3.4)	0.18
rs8102595					
AA		7,720 (81.3)		5,641 (82.5)	
AG		1,675 (17.7)		1,132 (16.6)	
GG		94 (1.0)	0.10	59 (0.9)	0.09

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

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

	NHS (1980-1990)		HPFS (1986-1996)	
	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.

- **No genetic association of *HIF3A* rs3826795 with adiposity**, 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.

Supplemental Table 5: The amount of baseline B vitamins in corresponding tertiles in NHS & HPFS

Cohorts	RDA	Tertiles of B vitamin intake*		
		T1	T2	T3
NHS				
Total vitamins intake				
Riboflavin (B_2), milligrams/day	1.3mg	1.4	2.3	6.6
Folate, micrograms/day	400mcg	186.5	325.6	649.9
Pyridoxine (B_6), milligrams/day	1.3mg	1.3	2	5.3
Vitamin B_{12}, micrograms/day	2.4mcg	4	7.9	16.4
Supplemental vitamins				
Riboflavin (B_2), milligrams/day	1.3mg	0	0.4	6.2
Folate, micrograms/day	400mcg	0.1	1.5	476.2
Pyridoxine (B_6), milligrams/day	1.3mg	0	0.3	4.9
Vitamin B_{12}, micrograms/day	2.4mcg	0.2	1.5	11.5
Food sourced vitamins				
Riboflavin (B_2), milligrams/day	1.3mg	1.2	1.6	2.3
Folate, micrograms/day	400 mcg	167.9	245.4	377.1
Pyridoxine (B_6), milligrams/day	1.3mg	1.1	1.5	2.2
Vitamin B_{12}, micrograms/day	2.4mcg	3.5	5.3	9.2
HPFS				
Total vitamins intake				
Riboflavin (B_2), milligrams/day	1.3mg	1.6	2.5	11.4
Folate, micrograms/day	400mcg	265.9	397.7	763
Pyridoxine (B_6), milligrams/day	1.3mg	1.8	2.7	20.9
Vitamin B_{12}, micrograms/day	2.4mcg	5.6	9.7	22.5
Supplemental vitamins				
Riboflavin (B_2), milligrams/day	1.3mg	0.1	1	12.7
Folate, micrograms/day	400mcg	5	66.3	497.9
Pyridoxine (B_6), milligrams/day	1.3mg	0.1	1.1	25.1
Vitamin B_{12}, micrograms/day	2.4mcg	0.2	1.7	13.9
Food sourced vitamins				
Riboflavin (B_2), milligrams/day	1.3mg	1.5	1.9	2.5
Folate, micrograms/day	400mcg	248.3	337.2	470.1
Pyridoxine (B_6), milligrams/day	1.3mg	1.7	2.1	2.8
Vitamin B_{12}, micrograms/day	2.4mcg	5	7.7	14.1

Data was presented as median

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).

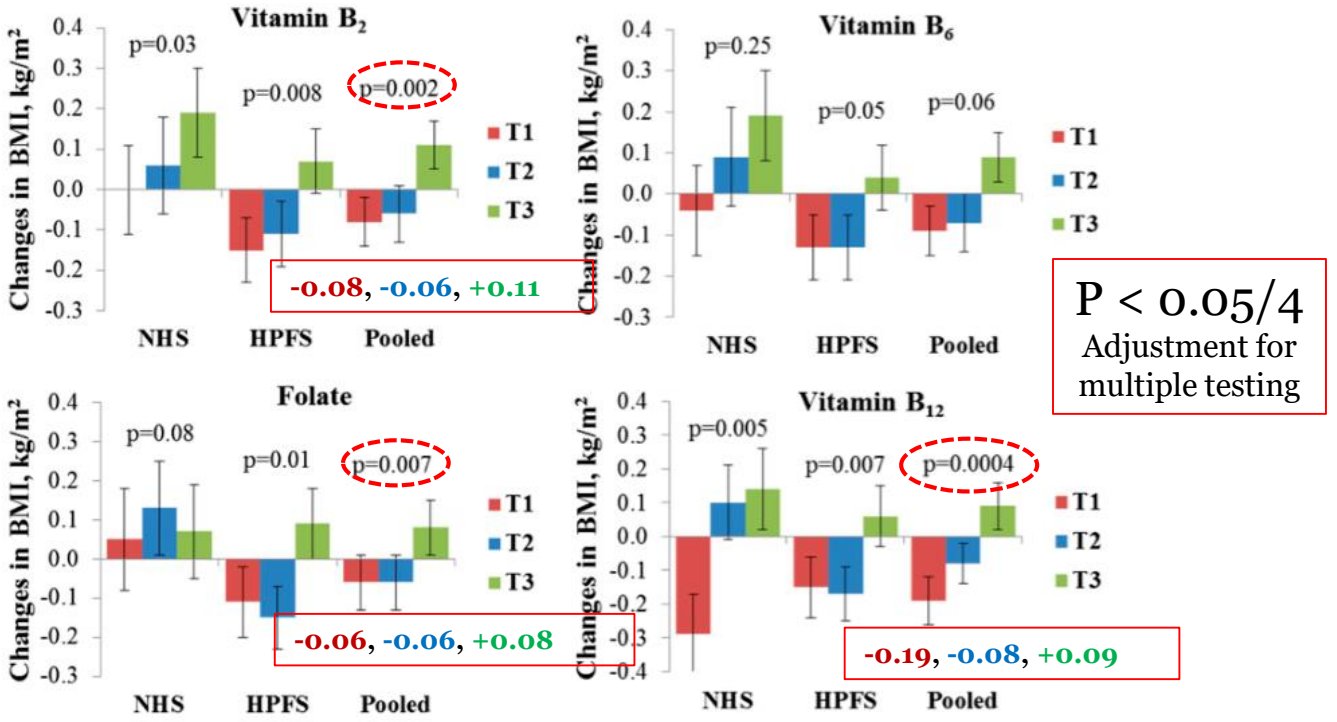


Figure 1—Differences in 10-year changes in BMI per minor allele of rs3826795 according to baseline intake of B vitamins from supplemental use among participants in the NHS (1980–1990) and HPFS (1986–1996). Data are β-coefficients ± SE. Data on baseline intake of B vitamins from supplemental use were 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.

- Signification Association of rs3826795 with 10-Year Change in BMI According to B2 and B12 intakes **from Supplements**.

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)

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 > 0.05/4
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.

Conclusion

- In two independent cohorts, a DNA methylation variant in *HIF3A* (rs3826795) was associated with 10-year BMI changes through interactions with total or supplemental vitamin B2, vitamin B12, and folate.
- The findings support the hypothesis that B vitamins modify the relation between the methylation-associated genetic variant at *HIF3A* (rs3826795) and BMI, and suggest a potential causal relation between DNA methylation and adiposity.

Strengths

- 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.
- Findings support their hypothesis that *HIF3A* SNP rs3826795 is associated with BMI according to intake of B vitamins.
- Highlight the importance in considering effect modifications by environmental factors when assessing genetic associations.

Limitations/Weakness (1)

- Dietary B vitamins and adiposity measures were self-reported, and errors in these measurement are inevitable.
- 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.
- Whether the genetic markers are functional variants or simply correlated markers is unclear.

Limitations/Weaknesses (2)

- Two *HIF3A* variants independently associated with methylation at cg22891070 were examined, but no interaction for rs8102595 was found.
- Did not measure DNA methylation at *HIF3A* locus to support causality. Instead, used genetic variants to act as a proxy of DNA methylation levels.
- The mechanism for the observed interactions between B vitamins and DNA methylation variant in relation to BMI changes remain unknown.

Future Directions

- 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.
- To investigate the differential interactions of BMI-associated variants on the association of DNA methylation with adiposity and their diverse interactions with diets.