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## Epigenome-wide association study of diet quality in the Women’s Health Initiative and TwinsUK cohort

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### Abstract

**Background:** Diet quality is a risk factor for chronic disease and mortality. Differential DNA methylation across the epigenome has been associated with chronic disease risk. Whether diet quality is associated with differential methylation is unknown. This study assessed whether diet quality was associated with differential DNA methylation measured across 445 548 loci in the Women’s Health Initiative (WHI) and the TwinsUK cohort.

**Design:** The discovery cohort consisted of 4355 women from the WHI. The replication cohort consisted of 571 mono- and dizygotic twins from the TwinsUK cohort. DNA

methylation was measured in whole blood using the Illumina Infinium HumanMethylation450 Beadchip. Diet quality was assessed using the Alternative Healthy Eating Index 2010 (AHEI-2010). A meta-analysis, stratified by study cohort, was performed using generalized linear models that regressed methylation on AHEI-2010, adjusting for cell composition, chip number and location, study characteristics, principal components of genetic relatedness, age, smoking status, race/ethnicity and body mass index (BMI). Statistical significance was defined as a false discovery rate  $< 0.05$ . Significant sites were tested for replication in the TwinsUK cohort, with significant replication defined by  $P < 0.05$  and a consistent direction.

**Results:** Diet quality was significantly associated with differential DNA methylation at 428 cytosine-phosphate-guanine (CpG) sites in the discovery cohort. A total of 24 CpG sites were consistent with replication in the TwinsUK cohort, more than would be expected by chance ( $P = 2.7 \times 10^{-4}$ ), with one site replicated in both the blood and adipose tissue (cg16379999 located in the body of *SEL1L*).

**Conclusions:** Diet quality was associated with methylation at 24 CpG sites, several of which have been associated with adiposity, inflammation and dysglycaemia. These findings may provide insight into pathways through which diet influences chronic disease.

**Key words:** Epigenome, diet quality, dietary epigenetics, EWAS, Women's Health Initiative

#### Key Messages

- Given the significant role of diet quality in non-communicable disease (NCD) progression and the more recent evidence establishing the relationship between differential DNA methylation and NCDs, this study examined whether diet quality is associated with differential DNA methylation and the potential functional effects of these differentially methylated sites.
- Using genome-wide DNA methylation data, we found 24 CpG sites were associated with diet quality in both the discovery cohort (WHI) and the replication cohort (TwinsUK), with nearly all of the replicated sites (23 of 24) negatively associated with diet quality (poorer diet quality associated with higher methylation).
- In one site, cg16379999 in the body of *SEL1L*, diet quality associated with blood and adipose tissue methylation in the same direction.
- These findings may elucidate molecular pathways through which diet influences chronic disease risk.

## Background

Poor diet quality is estimated to account for nearly half of the deaths attributable to coronary heart disease (CHD) and type 2 diabetes (T2DM) in the USA.<sup>1</sup> Diet influences metabolic conditions, independent of energy balance and adiposity, through effects on glucose–insulin homeostasis, satiety, liver fat synthesis, adipocyte function and metabolic expenditure.<sup>2</sup> Exposure to established non-communicable disease (NCD) risk factors, such as smoking,<sup>3</sup> particulate matter exposure<sup>4</sup> and physical activity,<sup>5</sup> has been associated with differential DNA methylation (DNAm) patterns that contribute to regulation of gene expression. The impact of diet quality on the DNA methylome is not well understood. Given the significant influence

of diet on NCD risk, diet could plausibly induce changes to DNAm on a causal disease pathway. Assessing the relationship between diet and the methylome, particularly independent of obesity, may reveal pathways linking diet and metabolic conditions.

Few studies have evaluated the association between diet and the methylome among adults, particularly in the context of diet quality and dietary factors causally associated with NCDs. Three studies have examined methylation changes in dietary clinical trials, including a high fat overfeeding trial and the Mediterranean diet.<sup>6–8</sup> These studies found some differences in either mean gene methylation or cytosine-phosphate-guanine (CpG) site-specific methylation. Two cross-sectional studies conducted epigenome-

wide association studies (EWAS) of dietary fat and fiber. These studies found differential methylation among genes potentially related to metabolism, though neither study validated findings in independent samples.<sup>9,10</sup> While all of these studies report associations between dietary factors and the methylome, limitations in sample size and lack of replication support further investigation into the association of diet with the adult methylome. This study therefore evaluated the association between diet quality as measured by the Alternative Healthy Eating Index-2010 (AHEI-2010) and the methylome using cross-sectional data from the Women's Health Initiative (WHI) and the TwinsUK cohort.

## Methods

### Study populations

The discovery cohort derives from the WHI, a large, US-based cohort study of post-menopausal women, aged 50–79 years at the time of enrollment, consisting of two study arms: the clinical trial (CT) and the observational study (OS). DNAm data from three ancillary studies in WHI were included: *Epigenetic Mechanisms of Particulate Matter-Mediated Cardiovascular Disease* (EMPC,  $n=2200$ ), the *Integrative Genomics for Risk of Coronary Heart Disease and Related Phenotypes in the WHI cohort* (BAA23,  $n=2107$ ), and *Bladder Cancer and Leukocyte Methylation* (AS311,  $n=882$ ). The replication cohort was derived from the TwinsUK cohort, a large registry of male and female twins between the ages of 19 and 82 years in the UK.<sup>11</sup> DNAm derives from a sub-study of female twins ( $n=571$ ). Further description of both discovery and replication cohorts have been included in the [Supplementary Methods and Results](#), available as [Supplementary data](#) at *IJE* online.

### Inclusion/exclusion criteria

Participants from the WHI cohorts were included if they completed their food frequency questionnaire (FFQ) in the same year as the blood draw on which DNAm was measured. Participants were excluded if they did not have dietary information or if they reported implausible dietary intake ( $<600$  kcal/day or  $\geq 4000$  kcal/day).<sup>12</sup> These criteria were only used in the discovery cohort. In the replication cohort, TwinsUK, some of the DNAm and diet quality measurements were not obtained from the same timepoint.<sup>13</sup> The replication analyses included female monozygotic (MZ) and dizygotic (DZ) twins from the TwinsUK from all years of blood sampling for DNAm profiling. In sensitivity analyses, we restricted the replication sample

further to those individuals with diet measured within 2 years and 1 year of 2007 (the year of methylation measurement).

### Methylation data

Methylation was measured in DNA derived from whole blood samples using the Illumina Infinium HumanMethylation450 Beadchip. The methylation protocols and quality control (QC) procedures are described in the [Supplementary Methods and Results](#), available as [Supplementary data](#) at *IJE* online. After QC, 445 548 CpG sites were available for analysis in the discovery cohort.

### Dietary quality assessment

Diet quality was assessed on a scale of 0–100 (lower score indicates poor diet) using the AHEI-2010, which evaluates foods and nutrients strongly predictive of chronic disease.<sup>14</sup> AHEI-2010 was assessed through participant FFQ and is composed of dietary and nutritional factors including: linolenic:linoleic fatty acid ratio, vegetable servings, fruit servings, whole grain servings, nuts and legumes servings, sugar-sweetened beverage servings, red/processed meat servings, sodium intake, *trans* fat intake and alcohol servings. The AHEI-2010 has been extensively evaluated and shown to associate prospectively with CHD and T2DM within the WHI<sup>15</sup> and in other settings.<sup>14,16</sup>

### Data analysis

We used R software to conduct all analyses. The discovery analysis flow chart is included as [Supplementary Figure S1](#), available as [Supplementary data](#) at *IJE* online. Overall, 834 women were excluded due to missing dietary intake, implausible dietary intake or overlapping samples. The final discovery cohort included 4355 women. EWAS meta-analysis was conducted by separately regressing methylation  $\beta$ -values for each CpG site on continuous AHEI-2010 score for each ancillary study and combining through inverse-variance weighted meta-analysis. Models were adjusted for study-specific covariates including case/control status (BAA23 and AS311), study year (EMPC), randomization arm (OS vs CT) and CT participant type and randomization assignment (dietary modification, calcium/vitamin D trial or hormone replacement therapy trial). Covariates in all analyses included chip location, estimated cell type proportions, the top three principal components of genetic relatedness (when available), body mass index (BMI), smoking status, age and race/ethnicity, with a random effect for chip number. Significant sites were tested for replication in the TwinsUK cohort using generalized

linear regression adjusting for cell composition, age, smoking and BMI as fixed effects, with random effects for chip number and location, genetic relatedness and zygosity. Significant sites were also explored for association between AHEI-2010 and adipose tissue DNAm in 400 female twins from the TwinsUK cohort.<sup>17</sup> In the discovery analysis, significance was defined as a false discovery rate (FDR) < 0.05. In replication analyses, significance was defined as a  $P < 0.05$  and a consistent direction of effect. Further description of these analyses is included in the [Supplementary Methods and Results](#), available as [Supplementary data](#) at *IJE* online.

### Additional *post hoc* analyses

We conducted additional *post hoc* analyses, including evaluation of methylation associated with gene expression in two external cohorts, enrichment testing, and several sensitivity analyses. Further description of these analyses is included in the [Supplementary Methods and Results](#), available as [Supplementary data](#) at *IJE* online.

## Results

Demographic characteristics are described by quartile of AHEI-2010 ([Table 1](#)). Older women had a higher AHEI-2010 score (indicating a healthier diet) compared with younger women. Those with higher BMI and obesity had a lower AHEI-2010 score. White women had a higher AHEI-2010 score compared with African-American and Hispanic women. Smoking status did not differ by quartile of diet quality.

In the discovery analysis ( $n = 4355$ ), AHEI-2010 was significantly associated with methylation levels of 428 CpG sites ([Figure 1](#), [Supplementary Table S1](#), available as [Supplementary data](#) at *IJE* online). On average, for every 1 SD increase in AHEI-2010 (9.9 units), the  $\beta$ -values (estimated methylation proportions) decreased by 0.0003 at the significant sites. In the WHI population, women in quartile 4 (best diet quality) had AHEI-2010 scores >56.7 and women in quartile 1 (worst diet quality) had AHEI-2010 scores <42.7. Women consuming the best diet had an average difference in methylation of 0.001 at the significant sites compared with those consuming the worst diet ([Figure 2](#), [Supplementary Table S2](#), available as [Supplementary data](#) at *IJE* online). Results of sensitivity analyses are included in the [Supplementary Methods and Results](#), available as [Supplementary data](#) at *IJE* online.

### Replication in whole blood

A total of 419 of the 428 significant sites passed QC in the TwinsUK cohort and were tested for replication in whole blood samples from 571 women ([Supplementary Table S3](#), available as [Supplementary data](#) at *IJE* online). AHEI-2010 score was significantly associated with methylation at 24 sites with a  $P < 0.05$  and a consistent direction of effect ([Table 2](#)), more sites than would be expected by chance (binomial test  $P = 2.7 \times 10^{-4}$ ). None of the sites was significant after FDR adjustment.

### Replication in adipose tissue

A total of 421 of 428 CpG sites were examined in the adipose tissue of 400 female twins in the TwinsUK cohort ([Supplementary Table S4](#), available as [Supplementary data](#) at *IJE* online). Diet quality was associated with 4 sites with a  $P < 0.05$  and a consistent direction of effect, one of which was also replicated in the blood: cg16379999 ([Supplementary Table S5](#), available as [Supplementary data](#) at *IJE* online). None of the sites was significant after FDR adjustment.

### Enrichment

We examined whether the sites identified in the primary analysis were expression quantitative trait methylation loci (eQTLs) found in a previous study of the Grady Trauma Project (GTP) and the Multi-Ethnic Study of Atherosclerosis (MESA).<sup>18</sup> The 428 CpGs identified in the discovery analysis were associated with expression of 412 genes in the eQTL database ( $P < 1 \times 10^{-5}$ , [Supplementary Table S6](#), available as [Supplementary data](#) at *IJE* online),<sup>18</sup> for a total of 1842 CpG-transcript associations. Gene ontology analysis of these 412 genes revealed enrichment for 342 ontologies (FDR < 0.05, [Supplementary Table S7](#), available as [Supplementary data](#) at *IJE* online), which were primarily immune response pathways with several pathways related to metabolism, including regulation of proteins and protein transport, response to fatty acid and cellular response to low-density lipoprotein particle stimulus. We next examined whether cg16379999 associated with expression of specific genes. In the MESA study, cg16379999 (on chromosome 14) positively associated with increased expression in *ABHD3* gene (on chromosome 18) representing a *trans* association between methylation and expression ( $P = 9.02 \times 10^{-6}$ ).

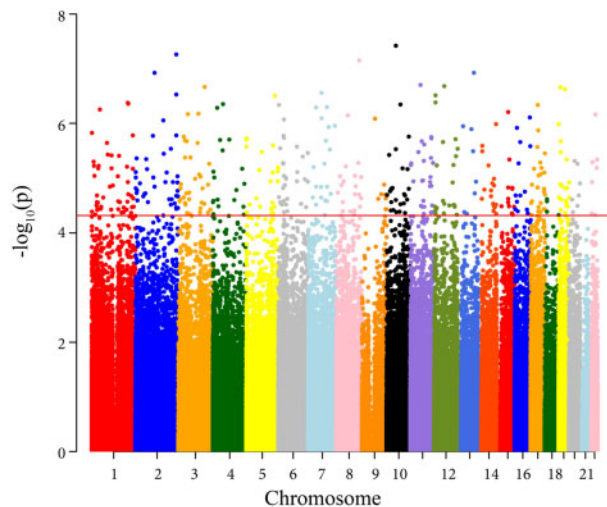
**Table 1.** Demographic and study characteristics by quartile of the AHEI-2010. Counts and means (SD) are presented for categorical and continuous variables, respectively. T-test and chi-square tests were used to examine differences by AHEI-2010 quartile. Quartiles defined as follows: Q1 is <42.7, Q2 is 42.7–49.2, Q3 is 49.3–56.7 and Q4 is >56.7

	<i>n</i>	Quartile 1	Quartile 2	Quartile 3	Quartile 4	<i>P</i> -value
<b>WHI Ancillary</b>						
<b>Study</b>						
EMPC	1613	421	419	400	373	<0.0001
BAA23	1914	524	483	459	448	
AS311	828	155	174	204	295	
<b>Clinical trial participant</b>						
Yes	3536	926	918	861	831	<0.0001
No	819	163	170	228	258	
<b>Case/control status (BAA23)</b>						
Case	948	267	234	239	208	0.01
Control	966	254	254	225	233	
<b>Case/control status (AS311)</b>						
Case	416	78	84	106	148	<0.0001
Control	412	77	91	109	135	
<b>Study year</b>						
Baseline	4097	1039	1028	1019	1011	0.29
3 years	163	31	37	46	49	
6 years	95	19	23	24	29	
Age (years), mean (SD)	64.0 (7.11)	62.4 (7.1)	64.1 (7.1)	64.3 (7.0)	65.1 (7.0)	<0.0001
<b>Race/Ethnicity</b>						
White	2495	501	639	628	727	<0.0001
African-American	1076	369	261	252	194	
Hispanic/Latino	610	190	153	157	110	
Asian or Pacific Islander	105	8	18	27	52	
American Indian or Alaskan Native	38	13	11	12	2	
Other	30	8	6	12	4	
<b>BMI (kg/m<sup>2</sup>) mean (SD)</b>	29.3 (6.1)	30.8 (6.4)	29.7 (6.2)	29.0 (5.9)	27.9 (5.5)	<0.0001
<b>BMI categories</b>						
Underweight	23	2	5	10	6	<0.0001
Normal	1060	177	246	281	356	
Overweight	1506	356	380	379	391	
Obese	1735	544	449	411	331	
<b>Smoking status</b>						
Former and current	2108	507	508	549	544	0.15
No	2204	569	570	530	535	
<b>Income</b>						
<\$20 000	1007	326	276	222	183	<0.0001
\$20 000–\$49 999	1888	481	464	488	455	
>\$50 000	1196	215	275	310	396	

## Discussion

Diet quality was associated with 428 CpG sites in the discovery cohort of post-menopausal women from the WHI, with 24 sites consistent with replication, one of which was associated with blood and adipose tissue in a consistent direction.

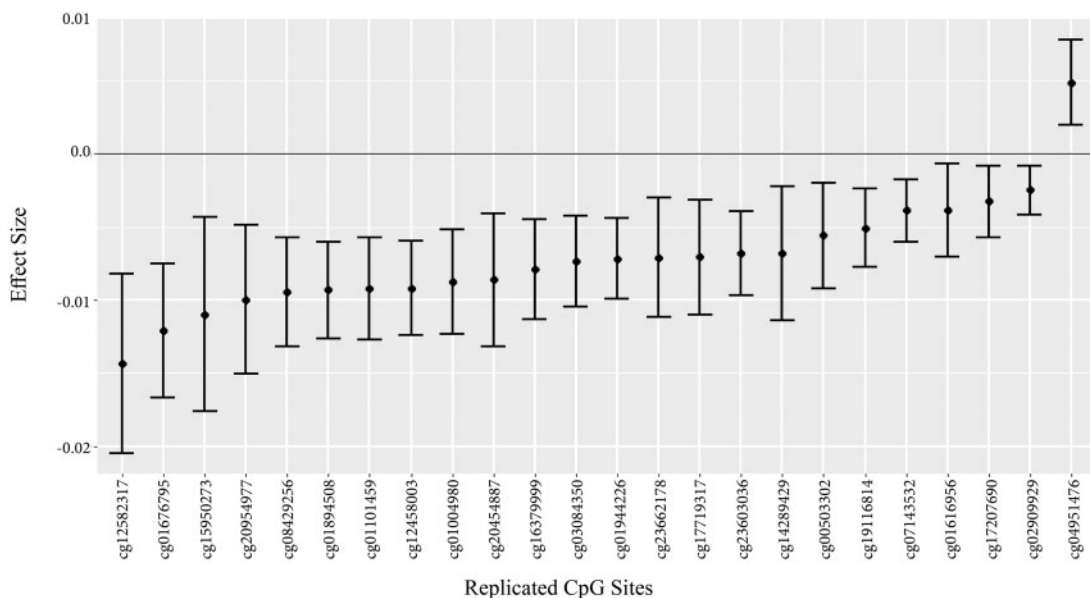
Among the 24 sites, several have been previously associated with diet-related outcomes. BMI has been associated with cg01101459 in an unannotated gene,<sup>19,20</sup> cg12458003 in the body of *NFASC*,<sup>21</sup> cg20954977 in the transcription start site of *B3GNT7*<sup>22</sup> and cg01676795 in the body of *POR*.<sup>23</sup> In all the above sites, methylation was



**Figure 1** Manhattan Plot of the EWAS of diet quality. The x-axis represents chromosomal position and the y-axis represents  $P$ -values on the  $-\log_{10}$  scale for each CpG site. The line denotes the threshold for significance  $P = 4.8 \times 10^{-5}$ .

negatively associated with diet quality (poorer diets had the highest methylation), and in previous studies these sites were positively associated with BMI. These findings align with our study since poor diet is associated with higher BMI. cg01101459 has also been associated with chronic low-grade inflammation,<sup>24</sup> with a positive association between methylation and C-reactive protein (CRP). CRP is another cardiometabolic risk factor playing a direct role in disease progression,<sup>25</sup> which has been found to associate with diet patterns,<sup>26–29</sup> such that poorer diets can lead to elevated CRP. cg01676795 has been found to associate with dysglycaemia in several studies.<sup>23,30</sup> In these studies, higher methylation was positively associated with fasting insulin<sup>23</sup> and haemoglobin A1c,<sup>23,30</sup> which corroborate our findings, as individuals with the poorest diet quality had the highest methylation.

cg16379999 was found to negatively associate with diet quality in both the blood and adipose tissue. cg16379999 is located in the body of *SEL1L*. This site has been previously found to associate with obesity,<sup>31</sup> air pollution,<sup>32</sup> smoking<sup>33</sup> and vitamin B12 supplementation.<sup>34</sup> *SEL1L* has been shown to play a significant role in lipid metabolism as a regulator of lipoprotein lipase (LPL) secretion.<sup>35,36</sup> *SEL1L* knock-out mouse models have elevated fibroblast growth factor 21 (FGF21), a critical metabolic hormone regulating growth, nutrient metabolism and insulin,<sup>37</sup> and elevated levels have been associated with obesity<sup>38</sup> and have predicted myocardial infarction.<sup>39,40</sup> In our study, diet quality was negatively associated with methylation at this site. As this site is located in the gene body, the implications may be difficult to infer as mixed evidence has been reported on the effects of gene body methylation on



**Figure 2** Difference in  $\beta$ -value of replicated CpG sites comparing the best diet score (AHEI-2010 > 56.7) to the worst diet score (AHEI-2010 < 42.7).

**Table 2.** Replicated CpG sites associated with diet quality in the WHI and TwinsUK. Models were adjusted for age, ethnicity (WHI), smoking status, BMI, cell composition, top three principal components of genetic relatedness, study specific covariates (WHI), zygosity (TwinsUK) and batch effects

CpG Site	WHI			TwinsUK			Reference gene
	Effect size	Standard error	P-value	Effect size	Standard error	P-value	
cg00503302	-2.60E-04	6.12E-05	1.82E-05	-4.58E-03	2.34E-03	4.78E-02	
cg01004980	-2.60E-04	5.46E-05	2.10E-06	-6.26E-03	2.53E-03	1.38E-02	<i>PRKAR2A</i>
cg01101459	-2.50E-04	5.50E-05	6.78E-06	-7.16E-03	2.63E-03	6.62E-03	
cg01616956	-2.50E-04	5.14E-05	1.64E-06	-5.90E-03	2.26E-03	8.56E-03	<i>NMUR1</i>
cg01676795	-3.60E-04	6.92E-05	2.75E-07	-7.75E-03	2.46E-03	1.47E-03	<i>POR</i>
cg01894508	-2.10E-04	4.94E-05	2.76E-05	-5.48E-03	2.54E-03	3.03E-02	<i>ASPRV1</i>
cg01944226	-1.80E-04	4.11E-05	2.00E-05	-7.63E-03	3.31E-03	2.02E-02	<i>SLC16A3</i>
cg02909929	-1.10E-04	2.58E-05	4.13E-05	-5.08E-03	2.44E-03	3.53E-02	<i>PRF1</i>
cg03084350	-2.20E-04	4.56E-05	2.03E-06	-7.46E-03	2.91E-03	1.08E-02	<i>PLCD1</i>
cg04951476	2.25E-04	4.46E-05	4.59E-07	6.46E-03	2.51E-03	1.01E-02	<i>FAM50B</i>
cg07143532	-1.70E-04	3.57E-05	2.25E-06	-6.41E-03	2.96E-03	3.11E-02	<i>COL24A1</i>
cg08429256	-2.50E-04	5.49E-05	3.50E-06	-1.10E-02	3.12E-03	3.68E-04	<i>SLC16A3</i>
cg12458003	-2.50E-04	5.01E-05	4.16E-07	-1.15E-02	3.53E-03	1.10E-03	<i>NFASC</i>
cg12582317	-4.10E-04	1.01E-04	4.22E-05	-7.36E-03	2.76E-03	8.66E-03	
cg14289429	-3.00E-04	7.09E-05	2.01E-05	-5.15E-03	2.17E-03	1.74E-02	<i>FAM78A</i>
cg15950273	-4.40E-04	1.08E-04	4.48E-05	-6.69E-03	3.24E-03	3.67E-02	<i>TRAF3</i>
cg16379999	-2.20E-04	5.07E-05	1.24E-05	-4.43E-03	2.19E-03	4.55E-02	<i>SEL1L</i>
cg17207690	-1.50E-04	3.61E-05	2.11E-05	-8.05E-03	2.57E-03	1.62E-03	<i>NMUR1</i>
cg17719317	-2.90E-04	6.59E-05	9.76E-06	-5.18E-03	2.64E-03	4.94E-02	
cg19116814	-1.80E-04	4.52E-05	4.65E-05	-9.47E-03	3.95E-03	1.68E-02	<i>GPM6A</i>
cg20454887	-3.30E-04	7.79E-05	1.90E-05	-5.18E-03	2.55E-03	4.07E-02	
cg20954977	-3.40E-04	8.16E-05	3.64E-05	-6.99E-03	2.85E-03	1.43E-02	<i>B3GNT7</i>
cg23603036	-2.20E-04	4.82E-05	5.92E-06	-5.75E-03	2.24E-03	9.66E-03	<i>DHRS3</i>
cg23662178	-2.60E-04	6.30E-05	2.89E-05	-5.13E-03	2.61E-03	4.79E-02	

gene expression.<sup>41</sup> However, a large EWAS of mRNA transcripts from the MESA and GTP cohorts found that gene body methylation correlated with reduced gene expression 61% and 72% of the time, respectively,<sup>18</sup> which would align with our study. As this gene may play a protective role against metabolic disturbances, the higher methylation patterns associated with poor diet would be deleterious. Methylation was also shown to associate with expression in the *ABHD3* gene in the MESA study. *ABHD3* has been shown to play a catabolic role in medium-chain and oxidatively-truncated phospholipids.<sup>42,43</sup>

The 428 CpG sites identified in the discovery cohort were also found to associate with differential expression of 412 genes in the blood. According to gene ontology analysis, this set of genes was enriched for primarily immune response pathways. This finding supports the role of diet quality in the immune response and potentially in an upstream effect of diet on cardiometabolic diseases. Although we adjusted for differences in cell composition,<sup>44</sup> there are potentially systemic differences in rarer cell types that would not be captured using this method. Thus the

methylation differences we identified may be due to differential inflammatory profiles associated with poor diet. Indeed diet quality was shown to be significantly correlated with natural killer cells, granulocytes and CD8 lymphocytes, even when adjusted for BMI (Supplementary Table S8, available as Supplementary data at *IJE* online). Improving diet quality has been shown to improve inflammatory profiles and decrease inflammatory markers such as CRP and tumor necrosis factor  $\alpha$ .<sup>28,29</sup> Moreover, one replicated site was previously associated with CRP levels.<sup>24</sup>

We conducted several sensitivity analyses in the discovery analysis (exclusion of individual ancillary studies, exclusion of bladder cancer cases, and additional adjustments for BMI and socio-economic status). Although all of these analyses resulted in a change in the number of significant sites (ranging from 0 to 1851 CpG sites), any change in significance was likely due to a change in power as there was very little variation in the effect size (correlation of effect sizes between analyses was  $>0.98$  for all analyses, see Supplementary Methods and Results, available as Supplementary data at *IJE* online).



Several studies have evaluated the association between various aspects of diet quality and the methylome longitudinally<sup>6–8</sup> and cross-sectionally.<sup>9,10</sup> One study evaluated adipose methylation following overfeeding of saturated or polyunsaturated fats in 31 participants, finding increased and decreased methylation at 4795 and 138 CpG sites, respectively, and changes in gene expression with saturated fat overfeeding.<sup>6</sup> Two studies examined methylation changes following a long-term Mediterranean diet in 40 participants. As neither study observed significant differences when applying a genome-wide significance level, they subsequently filtered CpG sites based on change in methylation for an ingenuity pathway analysis, and reported enrichment in inflammatory pathways.<sup>7,8</sup> Two cross-sectional studies have examined metrics of diet quality via EWAS. An EWAS of dietary fat quality conducted in pre-adolescents identified a number of CpG sites and pathways associated with dietary fat quality.<sup>10</sup> An EWAS of dietary fiber in African-American adolescents reported three differentially methylated sites in genes associated with adiposity and inflammation.<sup>9</sup> However results from these studies have not been replicated, and these CpGs were not significant in our study. Finally, a recent EWAS examined the AHEI-2010 score and the Mediterranean-style diet score (MDS) in 5 population based cohorts. They found significant associations with DNAm and diet quality in 30 sites.<sup>45</sup> No overlapping sites were identified in our study.

Because the discovery analysis found small effect sizes ( $\pm 0.0003$  per 1 SD diet quality), the biological implications are difficult to infer. A recent review found that most environmental studies resulted in a 2–8% difference in methylation between exposed and unexposed.<sup>46</sup> In our study, the best diet had as much as a  $\sim 2\%$  difference in  $\beta$ -value compared with the worst diet (Supplementary Table S2, available as Supplementary data at *IJE* online, Figure 2). Thus our findings are slightly below the average effect. In terms of functional implications, we do not know what impact this may have on gene expression. However, studies have found differences in expression associated with methylation effect sizes as low as 0.02.<sup>47–49</sup>

Some limitations in our study are also important to note. There may be epigenetic differences that we were unable to discover due to a narrow distribution of diet quality in the discovery study population and competing effects of nutrients on the epigenome. In the replication analysis, we had the power to detect associations explaining  $>1\%$  of variation in methylation; however, the partial  $r^2$  contribution of diet observed in our discovery analysis was only above this in 76 of the 428 sites in more than one individual ancillary study model (Supplementary Table S1, available as Supplementary data at *IJE* online). We included women from the TwinsUK cohort with methylation

measured within 3 years of diet quality, which may have influenced our replication results. However, the direction of association did not differ in the replicated sites when we restricted the analysis to individuals with methylation and diet quality measured within 2 years or 1 year. The TwinsUK cohort also differed from the WHI cohorts as they were younger and racially homogenous (Supplementary Table S9, available as Supplementary data at *IJE* online), nevertheless we were able to replicate 24 sites. Additionally, given that the WHI was conducted in post-menopausal women and the TwinsUK cohort was only in women, generalizability to other populations may be limited.

Another potential limitation is the use of blood-based methylation in the context of diet quality. To examine the biological impact of diet on the methylome, the diet-associated blood methylation would correlate with the tissue of interest that is most impacted by diet. We examined adipose tissue methylation and were able to replicate one significant site. Other relevant tissues might include the liver and gastrointestinal cells. However, few studies have examined methylation in these tissues.

In summary, diet quality was significantly associated with methylation at 24 CpG sites in the blood and one site in the adipose tissue among adult women. These sites may mark molecular pathways underlying diet and chronic disease, especially given the previous identification of associations between several of these sites and cardiometabolic risk factors in previous studies.<sup>19–24,30,31</sup> Future research should utilize more precise and unbiased estimates of diet quality through use of dietary biomarkers and metabolomic indices to fully elucidate the effect of diet quality on the epigenome.

## Supplementary data

Supplementary data are available at *IJE* online

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## Author contributions

W.L.D., K.M.V.N. and K.N.C. conceived of the study. The methodology was developed by W.L.D., K.M.V.N., E.A.W. and K.N.C. The data for WHI were curated by E.A.W., S.H., T.L.A., Y.L., L.H., P.B. and K.J. The TwinsUK data were curated by J.T.B. The formal analysis was completed by W.L.D. with support from K.N.C. Replication analyses were completed by R.C., O.M.M., C.I.L.R. and J.T.B. The manuscript was written by W.L.D. Editorial and content expertise was provided by all authors.

## Data availability

The WHI methylation and clinical data are available through the Women's Health Initiative website. The majority of the TwinsUK methylation datasets analysed in the current study are available through GEO GSE62992 and GSE121633 (blood methylation) and ArrayExpress E-MTAB-1866 (adipose methylation).

## Conflict of interest

None declared.

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