International Journal of Epidemiology, 2020, 1–10
doi: 10.1093/ije/dyaa215
Original Article

Original Article

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

Whitney L Do ,[1,2]* Eric A Whitsel,[3] Ricardo Costeira,[4] Olatz M Masachs,[4] Caroline I Le Roy,[4] Jordana T Bell,[4] Lisa RStaimez,[1] Aryeh D Stein,[1] Alicia K Smith,[5] Steve Horvath,[6] Themistocles L Assimes,[7] Simin Liu,[8] JoAnn E Manson,[9] Aladdin H Shadyab,[10] Yun Li,[11,12,13] Lifang Hou,[14] Parveen Bhatti ,[15,16] Kristina Jordahl,[16] KM Venkat Narayan[1] and Karen N Conneely[17]

[1]Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA, [2]Nutrition and Health Sciences Program, Laney Graduate School, Emory University, Atlanta, GA, USA, [3]Departments of Epidemiology and Medicine, University of North Carolina, Chapel Hill, NC, USA, [4]Department of Twin Research and Genetic Epidemiology, King's College London, London SE1 7EH, UK, [5]Department of Gynecology and Obstetrics, School of Medicine, Emory University, Atlanta, GA, USA, [6]Department of Human Genetics, University of California, Los Angeles, CA, USA, [7]Department of Medicine, Stanford University, Palo Alto, CA, USA, [8]Department of Epidemiology, School of Public Health, Brown University, Providence, RI, USA, [9]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA, [10]Department of Family Medicine and Public Health, University of California San Diego School of Medicine, La Jolla, CA, USA, [11]Department of Genetics, University of North Carolina, Chapel Hill, NC, USA, [12]Department of Biostatistics, University of North Carolina, Chapel Hill, NC, USA, [13]Department of Computer Science, University of North Carolina, Chapel Hill, NC, USA, [14]Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA, [15]Cancer Control Research, BC Cancer, Vancouver, BC, Canada, [16]Program in Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA and [17]Department of Human Genetics, School of Medicine, Emory University, Atlanta, GA, USA

*Corresponding author: 1518 Clifton Rd, Atlanta, GA 30322, USA. E-mail: rleet@emory.edu

Editorial decision 18 September 2020; Accepted 30 September 2020

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.[1] 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.[2] Exposure to established non-communicable disease (NCD) risk factors, such as smoking,[3] particulate matter exposure[4] and physical activity,[5] 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.[6–8] 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.[9,10] 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.[11] 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 ≥4000 kcal/day).[12] 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 time-point.[13] 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.[14] 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[15] and in other settings.[14,16]

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 β-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.[17] 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 β-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 $P = 2.7\text{x}10^{-4}$). 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 (eQTMs) found in a previous study of the Grady Trauma Project (GTP) and the Multi-Ethnic Study of Atherosclerosis (MESA).[18] The 428 CpGs identified in the discovery analysis were associated with expression of 412 genes in the eQTM database ($P < 1\text{x}10^{-5}$, Supplementary Table S6, available as Supplementary data at *IJE* online),[18] 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 ($P = 9.02 \text{ x } 10^{-6}$).

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

	n	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*-value
WHI Ancillary Study						
EMPC	1613	421	419	400	373	<0.0001
BAA23	1914	524	483	459	448	
AS311	828	155	174	204	295	
Clinical trial participant						
Yes	3536	926	918	861	831	<0.0001
No	819	163	170	228	258	
Case/control status (BAA23)						
Case	948	267	234	239	208	0.01
Control	966	254	254	225	233	
Case/control status (AS311)						
Case	416	78	84	106	148	<0.0001
Control	412	77	91	109	135	
Study year						
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
Race/Ethnicity						
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	
BMI (kg/m^2) mean (SD)	29.3 (6.1)	30.8 (6.4)	29.7 (6.2)	29.0 (5.9)	27.9 (5.5)	<0.0001
BMI categories						
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	
Smoking status						
Former and current	2108	507	508	549	544	0.15
No	2204	569	570	530	535	
Income						
<$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,[19,20] cg12458003 in the body of *NFASC*,[21] cg20954977 in the transcription start site of *B3GNT7*[22] and cg01676795 in the body of *POR*.[23] In all the above sites, methylation was 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,[24] with a positive association between methylation and C-reactive protein (CRP). CRP is another cardiometabolic risk factor playing a direct role in disease progression,[25] which has been found to associate with diet patterns,[26–29] such that poorer diets can lead to elevated CRP. cg01676795 has been found to associate with dysglycaemia in several studies.[23,30] In these studies, higher methylation was positively associated with fasting insulin[23] and haemoglobin A1c,[23,30] which corroborate our findings, as individuals with the poorest diet quality had the highest methylation.

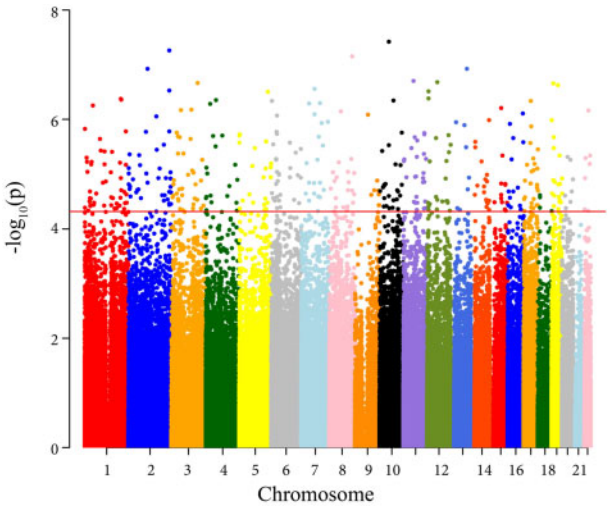

Figure 1 Manhattan Plot of the EWAS of diet quality. The *x*-axis represents chromosomal position and the *y*-axis represents *P*-values on the $-\log_{10}$ scale for each CpG site. The line denotes the threshold for significance $P = 4.8 \times 10^{-5}$.

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,[31] air pollution,[32] smoking[33] and vitamin B12 supplementation.[34] *SEL1L* has been shown to play a significant role in lipid metabolism as a regulator of lipoprotein lipase (LPL) secretion.[35,36] *SEL1L* knock-out mouse models have elevated fibroblast growth factor 21 (FGF21), a critical metabolic hormone regulating growth, nutrient metabolism and insulin,[37] and elevated levels have been associated with obesity[38] and have predicted myocardial infarction.[39,40] 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

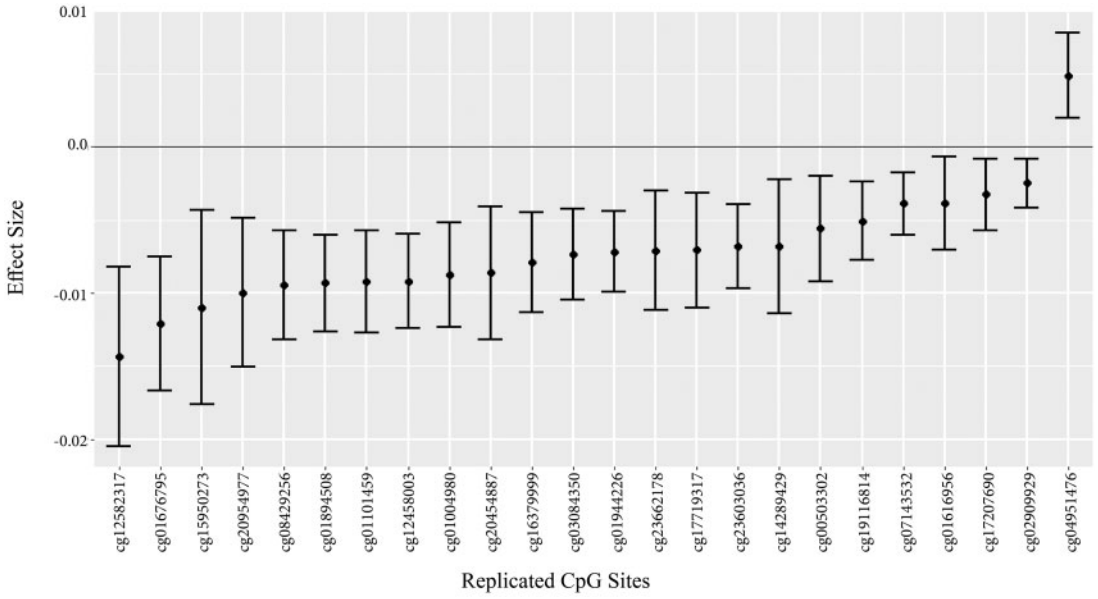

Figure 2 Difference in β-value of replicated CpG sites comparing the best diet score (AHEI-2010 > 56.7) to the worst diet score (AHEI-2010 < 42.7).

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

	WHI			TwinsUK			
CpG Site	Effect size	Standard error	*P*-value	Effect size	Standard error	*P*-value	Reference gene
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	*PRKAR2A*
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	*NMUR1*
cg01676795	−3.60E−04	6.92E−05	2.75E−07	−7.75E−03	2.46E−03	1.47E−03	*POR*
cg01894508	−2.10E−04	4.94E−05	2.76E−05	−5.48E−03	2.54E−03	3.03E−02	*ASPRV1*
cg01944226	−1.80E−04	4.11E−05	2.00E−05	−7.63E−03	3.31E−03	2.02E−02	*SLC16A3*
cg02909929	−1.10E−04	2.58E−05	4.13E−05	−5.08E−03	2.44E−03	3.53E−02	*PRF1*
cg03084350	−2.20E−04	4.56E−05	2.03E−06	−7.46E−03	2.91E−03	1.08E−02	*PLCD1*
cg04951476	2.25E−04	4.46E−05	4.59E−07	6.46E−03	2.51E−03	1.01E−02	*FAM50B*
cg07143532	−1.70E−04	3.57E−05	2.25E−06	−6.41E−03	2.96E−03	3.11E−02	*COL24A1*
cg08429256	−2.50E−04	5.49E−05	3.50E−06	−1.10E−02	3.12E−03	3.68E−04	*SLC16A3*
cg12458003	−2.50E−04	5.01E−05	4.16E−07	−1.15E−02	3.53E−03	1.10E−03	*NFASC*
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	*FAM78A*
cg15950273	−4.40E−04	1.08E−04	4.48E−05	−6.69E−03	3.24E−03	3.67E−02	*TRAF3*
cg16379999	−2.20E−04	5.07E−05	1.24E−05	−4.43E−03	2.19E−03	4.55E−02	*SEL1L*
cg17207690	−1.50E−04	3.61E−05	2.11E−05	−8.05E−03	2.57E−03	1.62E−03	*NMUR1*
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	*GPM6A*
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	*B3GNT7*
cg23603036	−2.20E−04	4.82E−05	5.92E−06	−5.75E−03	2.24E−03	9.66E−03	*DHRS3*
cg23662178	−2.60E−04	6.30E−05	2.89E−05	−5.13E−03	2.61E−03	4.79E−02	

gene expression.[41] 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,[18] 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.[42,43]

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,[44] 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 α.[28,29] Moreover, one replicated site was previously associated with CRP levels.[24]

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[6–8] and cross-sectionally.[9,10] 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.[6] 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.[7,8] 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.[10] An EWAS of dietary fiber in African-American adolescents reported three differentially methylated sites in genes associated with adiposity and inflammation.[9] 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.[45]No overlapping sites were identified in our study.

Because the discovery analysis found small effect sizes (±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.[46] In our study, the best diet had as much as a ~2% difference in β-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.[47–49]

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.[19–24,30,31] 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

Funding

The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201600018C, HHSN268201600001C, HHSN268201600002C, HHSN268201600003C, and HHSN268201600004C. The TwinsUK study was funded by the Wellcome Trust; European Community's Seventh Framework Programme (FP7/2007–2013); National Institute for Health Research (NIHR)-funded BioResource, Clinical Research Facility and Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London. The TwinsUK methylation study received support from the ESRC (ES/N000404/1 to J.T.B.) and the Joint Programming Initiative HDHL DIMENSION (administered by the BBSRC UK, BB/

S020845/1 to J.T.B.). K.M.V.N., L.S. and W.L.D. were supported in part by the National Institute of Diabetes And Digestive And Kidney Diseases of the National Institutes of Health under award number P30DK111024 and the Hubert Foundation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. W.L.D. was supported in part by the Nalini and Ravi Saligram Scholarship. This work was supported in part by National Institute of Environmental Health Sciences grant R01-ES020836 (L.H., E.A.W.) and National Heart, Lung, and Blood Institute contract HHSN268201100046C (K.N.C.). P.B. and K.J. were supported by the American Cancer Society (125299-RSG-13–100-01-CCE) with additional support for K.J. through National Cancer Institute training grants (R25 CA094880 and T32 CA094880). S.H. acknowledges support by U01 AG060908/AG/NIA NIH HHS/US. Y.L. was supported through R01 HL129132.

Acknowledgements

The authors thank the WHI investigators and staff for their dedication, and the study participants for making the programme possible. A full listing of WHI investigators can be found at: http://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf. The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. The data used for the analyses described in this manuscript were obtained from the GTEx Portal on 10/15/2019.

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 statement

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.

References

1. GBD 2015 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016;**388**:1659–724.
2. Mozaffarian D. Dietary and policy priorities for cardiovascular disease, diabetes, and obesity: a comprehensive review. *Circulation* 2016;**133**:187–225.
3. Prince C, Hammerton G, Taylor AE *et al.* Investigating the impact of cigarette smoking behaviours on DNA methylation patterns in adolescence. *Hum Mol Genet* 2019;**28**:155–65.
4. Madrigano J, Baccarelli A, Mittleman Murray A *et al.* Prolonged exposure to particulate pollution, genes associated with glutathione pathways, and DNA methylation in a cohort of older men. *Environ Health Perspect* 2011;**119**:977–82.
5. Nitert MD, Dayeh T, Volkov P *et al.* Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. *Diabetes* 2012;**61**:3322–32.
6. Perfilyev A, Dahlman I, Gillberg L *et al.* Impact of polyunsaturated and saturated fat overfeeding on the DNA-methylation pattern in human adipose tissue: a randomized controlled trial. *Am J Clin Nutr* 2017;**105**:991–1000.
7. Arpón A, Milagro FI, Razquin C *et al.* Impact of consuming extra-virgin olive oil or nuts within a Mediterranean diet on DNA methylation in peripheral white blood cells within the PREDIMED-Navarra randomized controlled trial: a role for dietary lipids. *Nutrients* 2017;**10**:15.
8. Arpon A, Riezu-Boj JI, Milagro FI *et al.* Adherence to Mediterranean diet is associated with methylation changes in inflammation-related genes in peripheral blood cells. *J Physiol Biochem* 2016;**73**:445–55.
9. Chen L, Dong Y, Wang X *et al.* Epigenome-wide association study of dietary fiber intake in African American Adolescents. *Mol Nutr Food Res* 2018;**62**:e1800155.
10. Voisin S, Almén MS, Moschonis G, Chrousos GP, Manios Y, Schiöth HB. Dietary fat quality impacts genome-wide DNA methylation patterns in a cross-sectional study of Greek preadolescents. *Eur J Hum Genet* 2015;**23**:654–62.
11. Moayyeri A, Hammond CJ, Valdes AM, Spector TD. Cohort Profile: TwinsUK and healthy ageing twin study. *Int J Epidemiol* 2013;**42**:76–85.
12. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann Epidemiol* 1999;**9**:178–87.
13. Bowyer RCE, Jackson MA, Pallister T *et al.* Use of dietary indices to control for diet in human gut microbiota studies. *Microbiome* 2018;**6**:77.
14. Chiuve SE, Fung TT, Rimm EB *et al.* Alternative dietary indices both strongly predict risk of chronic disease. *J Nutr* 2012;**142**:1009–18.
15. George SM, Ballard-Barbash R, Manson JE *et al.* Comparing indices of diet quality with chronic disease mortality risk in postmenopausal women in the Women's Health Initiative Observational Study: Evidence to Inform National Dietary Guidance. *Am J Epidemiol* 2014;**180**:616–25.
16. Schwingshackl L, Bogensberger B, Hoffmann G. Diet quality as assessed by the healthy eating index, alternate healthy eating index, dietary approaches to stop hypertension score, and health outcomes: an updated systematic review and meta-analysis of cohort studies. *J Acad Nutr Diet* 2018;**118**:74–100.e11.

17. Grundberg E, Meduri E, Sandling JK *et al.* Global analysis of DNA methylation variation in adipose tissue from twins reveals links to disease-associated variants in distal regulatory elements. *Am J Hum Genet* 2013;**93**:876–90.
18. Kennedy EM, Goehring GN, Nichols MH *et al.* An integrated -omics analysis of the epigenetic landscape of gene expression in human blood cells. *BMC Genomics* 2018;**19**:476.
19. Hannon E, Knox O, Sugden K *et al.* Characterizing genetic and environmental influences on variable DNA methylation using monozygotic and dizygotic twins. *PLoS Genet* 2018;**14**: e1007544.
20. Wahl S, Drong A, Lehne B *et al.* Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. *Nature* 2017;**541**:81–86.
21. Mendelson MM, Marioni RE, Joehanes R *et al.* Association of body mass index with DNA methylation and gene expression in blood cells and relations to cardiometabolic disease: A Mendelian randomization approach. *PLoS Med* 2017;**14**: e1002215-e.
22. Demerath EW, Guan W, Grove ML *et al.* Epigenome-wide association study (EWAS) of BMI, BMI change and waist circumference in African American adults identifies multiple replicated loci. *Hum Mol Genet* 2015;**24**:4464–79.
23. Cardona A, Day FR, Perry JRB *et al.* Epigenome-wide association study of incident type 2 diabetes in a British population: EPIC-Norfolk Study. *Diabetes* 2019;**68**:2315–26.
24. Ligthart S, Marzi C, Aslibekyan S *et al.* DNA methylation signatures of chronic low-grade inflammation are associated with complex diseases. *Genome Biol* 2016;**17**:255.
25. Minihane AM, Vinoy S, Russell WR *et al.* Low-grade inflammation, diet composition and health: current research evidence and its translation. *Br J Nutr* 2015;**114**:999–1012.
26. Dias JA, Wirfält E, Drake I *et al.* A high quality diet is associated with reduced systemic inflammation in middle-aged individuals. *Atherosclerosis* 2015;**238**:38–44.
27. Barbaresko J, Koch M, Schulze MB, Nöthlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. *Nutr Rev* 2013;**71**:511–27.
28. Arnold K, Weinhold KR, Andridge R, Johnson K, Orchard TS. Improving diet quality is associated with decreased inflammation: findings from a pilot intervention in postmenopausal women with obesity. *J Acad Nutr Diet* 2018;**118**:2135–43.
29. Piccand E, Vollenweider P, Guessous I, Marques-Vidal P. Association between dietary intake and inflammatory markers: results from the CoLaus study. *Public Health Nutr* 2019;**22**: 498–505.
30. Soriano-Tárraga C, Jiménez-Conde J, Giralt-Steinhauer E *et al.* Epigenome-wide association study identifies TXNIP gene associated with type 2 diabetes mellitus and sustained hyperglycemia. *Hum Mol Genet* 2016;**25**:609–19.
31. Cheng Y, Monteiro C, Matos A *et al.* Epigenome-wide DNA methylation profiling of periprostatic adipose tissue in prostate cancer patients with excess adiposity-a pilot study. *Clin Epigenet* 2018;**10**:54.
32. de F C Lichtenfels AJ, van der Plaat DA, de Jong K *et al.* Long-term air pollution exposure, genome-wide DNA methylation and lung function in the LifeLines Cohort Study. *Environ Health Perspect* 2018;**126**:027004.
33. Joehanes R, Just AC, Marioni RE *et al.* Epigenetic signatures of cigarette smoking. *Circ Cardiovasc Genet* 2016;**9**:436–47.
34. Yadav DK, Shrestha S, Lillycrop KA *et al.* Vitamin B12 supplementation influences methylation of genes associated with Type 2 diabetes and its intermediate traits. *Epigenomics* 2018;**10**: 71–90.
35. SEL1L Gene. [cited; Available from: https://www.genecards.org/cgi-bin/carddisp.pl? gene=SEL1L.
36. Sha H, Sun S, Francisco AB *et al.* The ER-associated degradation adaptor protein Sel1L regulates LPL secretion and lipid metabolism. *Cell Metab* 2014;**20**:458–70.
37. Bhattacharya A, Sun S, Wang H *et al.* Hepatic Sel1L-Hrd1 ER-associated degradation (ERAD) manages FGF21 levels and systemic metabolism via CREBH. *Embo J* 2018;**37**:e99277.
38. Zhang X, Yeung DCY, Karpisek M *et al.* Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes* 2008;**57**:1246–53.
39. Zhang W, Chu S, Ding W, Wang F. Serum level of fibroblast growth factor 21 Is independently associated with acute myocardial infarction. *PloS One* 2015;**10**:e0129791.
40. Shen Y, Zhang X, Xu Y *et al.* Serum FGF21 is associated with future cardiovascular events in patients with coronary artery disease. *Cardiology* 2018;**139**:212–28.
41. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet* 2012;**13**:484–92.
42. Long JZ, Cisar JS, Milliken D *et al.* Metabolomics annotates ABHD3 as a physiologic regulator of medium-chain phospholipids. *Nat Chem Biol* 2011;**7**:763–65.
43. Lord CC, Thomas G, Brown JM. Mammalian alpha beta hydrolase domain (ABHD) proteins: lipid metabolizing enzymes at the interface of cell signaling and energy metabolism. *Biochim Biophys Acta* 2013;**1831**:792–802.
44. Houseman EA, Molitor J, Marsit CJ. Reference-free cell mixture adjustments in analysis of DNA methylation data. *Bioinformatics* 2014;**30**:1431–39.
45. Ma J, Rebholz CM, Braun K et al. Whole Blood DNA Methylation Signatures of Diet Are Associated With Cardiovascular Disease Risk Factors and All-Cause Mortality. Circ Genom Precis Med 2020;**13**:e002766
46. Breton CV, Marsit CJ, Faustman E *et al.* Small-magnitude effect sizes in epigenetic end points are important in children's environmental health studies: The Children's Environmental Health and Disease Prevention Research Center's Epigenetics Working Group. *Environ Health Perspect* 2017;**125**:511–26.
47. Argos M, Chen L, Jasmine F *et al.* Gene-specific differential DNA methylation and chronic arsenic exposure in an epigenome-wide association study of adults in Bangladesh. *Environ Health Perspect* 2015;**123**:64–71.
48. Everson TM, Punshon T, Jackson BP *et al.* Cadmium-associated differential methylation throughout the placental genome: epigenome-wide association study of Two U.S. Birth Cohorts. *Environ Health Perspect* 2018;**126**:017010.
49. Lee MK, Hong Y, Kim S-Y, London SJ, Kim WJ. DNA methylation and smoking in Korean adults: epigenome-wide association study. *Clin Epigenet* 2016;**8**:103.