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Association between modifiable-lifestyle factors and DNA methylation: a discovery of biomarkers and links to cardiovascular outcomes

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Abstract

Epigenetics is the study of mitotically heritable changes in gene expression which do not cause changes to the underlying DNA sequence. Epigenetic mechanisms, including DNA methylation at cytosine and guanine nucleotide pair (CpG) sites, have been shown to be influenced by intrinsic (such as obesity and inflammation) and extrinsic (such as diet, physical activity and smoking) exposures serving as a link between an individual's DNA and their environment. Examining DNA methylation in relation to modifiable-lifestyle risk factors may provide relevant information in several respects. As a molecular biomarker, DNA methylation could be useful in improving the detection of difficult to measure exposures or in the early detection of diseases. Additionally, as DNA methylation influences gene expression levels, it is important to examine the functional implications of these associations as they may have downstream effects on cardiometabolic health.

This dissertation project examined the association between differential DNA methylation with several modifiable-lifestyle exposures including diet quality, metabolic health and obesity. In Aim 1, we 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. We discovered that diet quality was associated with widespread differential methylation patterns, with several of the replicated sites having been previously associated with obesity, inflammation, and dysglycemia. In Aim 2, we evaluated the relationship between body mass index (BMI) and differential DNA methylation in 17,034 participants from nine population based cohort studies. We discovered 1,238 CpG sites associated with BMI. Moreover, we found a unique methylomic profile of adiposity in individuals of African descent. In Aim 3, we examined whether BMI-associated methylation is influenced by metabolic health status. We found four CpG sites which may have a differential relationship with BMI in metabolically healthy vs. unhealthy individuals. These sites are located in several genes

related to NF-kappa-B signaling suggesting that DNA methylation may differentially regulate obesity-associated inflammation by metabolic health status. Ultimately, this body of work will help to further our understanding of the molecular dysregulation caused by poor diet, metabolic abnormalities and obesity.

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Contents

List of Tables

List of Figures

CHAPTER 1

Introduction

Examining the molecular underpinnings associated with diseases can provide insight into pathogenicity and can serve as early biomarkers. Epigenetics represents one critical molecular mechanism contributing to gene expression and serves as a link between an individual's DNA and their environment. Epigenetics is the study of mitotically heritable changes in gene expression which do not change the underlying DNA sequence (1). One epigenetic mechanisms is DNA methylation, which occurs when a methyl group attaches to the 5' carbon of a cytosine, usually at a cytosine-phosphate-guanine nucleotide pair (CpG) site. DNA methylation has become an important marker to examine due to its dynamic nature. DNA methylation has been shown to be directly influenced by lifestyle exposures with consequential effects on cardiometabolic health. For example, smoking is one of the most widely characterized mechanisms to cause widespread changes in methylation, (2) which ultimately have been shown to causally influence chronic disease risk, including lung dysfunction (3) and inflammation (4). Like smoking, several lifestyle exposures have been linked to chronic diseases, including physical activity, diet, weight gain, environmental toxicants and chemicals. However, it is unclear whether DNA methylation may be mediating the relationship between these exposures and their effects on cardiometabolic disease. This dissertation examines three of these exposures, diet, metabolic health and obesity, and seeks to substantiate whether the methylome is both associated with these exposures and influencing the downstream risk of cardiometabolic diseases.

Diet has been shown to substantially contribute to chronic disease risk (5-7). However, diet is complex with protective components (diets high in fruits, vegetables, legumes, whole grains, omega-3 fatty acids) and harmful components (diets high in added sugars, trans and saturated fats, sodium) (8). While several studies have evaluated the individual effect of nutrients on the methylome, including fiber (9) and dietary fats (10-12), few studies have examined how

overall diet quality contributes to differential DNA methylation. **In chapter 4, we 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.** We found widespread differential DNA methylation associated with diet with several sites replicating in an external population. Several of the diet-related sites were also found to have previously associated with obesity. Since these sites were associated with diet when adjusted for BMI, this suggests that previous associations with BMI may represent a true relationship with diet in other populations, as diet quality tends to be upstream of changes in adiposity (13).

Poor diet quality goes hand in hand with obesity in causing non-communicable disease. In the past thirty years, the number of deaths attributable to obesity has more than doubled (14). Obesity has been found to accompany a host of underlying molecular and metabolic perturbations including impaired cell signaling leading to perturbations in adipokines, and increased inflammatory factors including interleukin (IL)-6, IL-10, interferon-γ, and tumor necrosis factor-α (TNF- α), which lends itself to hyperlipidemia and hyperglycemia (15). Ultimately, these perturbations can lead to the early onset of chronic disease.

Obesity has been shown to associate with widespread perturbations in DNA methylation (16-22). However, replication among these studies has been poor. Additionally, there have been significant differences among sites identified in the blood versus other tissues. Another important analytical element limiting our understanding of epigenetics is the large sample sizes required to conduct these analyses. Because of the significant number of tests, it is necessary to adjust for multiple comparisons using various analytic strategies including false discovery rate or Bonferroni-adjustment. Large data sets are required to maintain sufficient power to detect significant effects. DNA methylation in particular is prone to small effect sizes (23), with an average 2-10% difference between exposed and unexposed groups. With cost constraints of analyzing DNA methylation data, EWAS studies have lagged behind other genomic research

studies, such as genome-wide association studies which often include a minimum of 100K participants. Novel sites could be identified with large enough sample size. **In chapter 5, we explored the association between DNA methylation and BMI with the largest EWAS meta-analysis including 17,034 participants from 9 cohorts.** With summary data from several large cohort studies, we are also able to disentangle race-specific methylation patterns and how they can contribute to differential outcomes. With the identification of novel CpG sites, we examined whether DNA methylation can be used to predict BMI and BMI-phenotypes, such as metabolically healthy vs. unhealthy obesity.

BMI-phenotypes have come under considerable scrutiny in recent years due to the complex underlying pathophysiology and the risks associated with obesity. While obesity is meant to represent the excess of adiposity to the degree that health is adversely affected (24), it is most commonly measured by BMI (weight [kg]/height [m^2]) ≥ 30, which does not distinguish between fat mass and muscle mass. The misalignment between the disease and the diagnosis becomes apparent when considering the pathophysiological perturbations associated with the excess of adiposity which ultimately leads to the adverse health associated with obesity, including increased inflammation, hyperlipidemia, and insulin resistance, as described above. In states where obesity exists in isolation of these secondary health effects (named metabolically healthy obesity), it is unclear whether obesity poses the risk of harm to the individual.

Several studies have examined this unique phenotype, metabolically healthy obesity, to distinguish whether there is a differential risk of cardiovascular disease. Compared their healthy normal weight individuals, metabolically healthy obesity has been shown to associate with a null to moderately increased risk of cardiovascular disease. Whereas compared to unhealthy obese individuals, metabolically healthy obesity has a significantly lower risk (25). No studies have integrated these phenotypes to examine how BMI-associated methylation varies by metabolic health status. Particularly since DNA methylation has been shown to play a mediating role with obesity and cardiovascular outcomes (26), evaluating the epigenome may provide insight into

pathways contributing to the differences in outcomes. **In chapter 6, we examined whether metabolic health status differentially influences the relationship between DNA methylation and BMI**. Using two study populations, we used epigenome-wide association study of the interaction between metabolic health Z-score and BMI. In the significantly replicated sites, we examined whether DNA methylation predicted incident coronary heart disease.

The specific aims are as follows:

Aim 1 (Chapter 4): Examine the association between diet quality and DNA methylation using the Alternative Healthy Eating Index-2010 in two cohorts.

Aim 2 (Chapter 5): Conduct the largest epigenome-wide association study of BMI in 17,034 participants from 9 cohorts and evaluate the relationship between BMI-associated methylation and metabolic risk factors.

Aim 3 (Chapter 6): Examine whether metabolically healthy obesity is associated with differential DNA methylation compared to metabolically unhealthy obesity.

These findings have identified molecular biomarkers of diet and BMI, which could be useful in future studies when these phenotype data are not available. Moreover, these CpG sites may be indicative of direct pathways influenced by these exposures. For instance, we found several BMI and metabolic health-associated sites in Chapter 6 which were associated with incident myocardial infarction. This work adds to a growing body of evidence indicating the important role that epigenetics plays in disease pathophysiology.

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References

1. Smith ZD, Meissner A. DNA methylation: roles in mammalian development. Nature reviews Genetics 2013;14(3):204-20. doi: 10.1038/nrg3354.
2. Gao X, Jia M, Zhang Y, Breitling LP, Brenner H. DNA methylation changes of whole blood cells in response to active smoking exposure in adults: a systematic review of DNA methylation studies. Clinical epigenetics 2015;7(1):113. doi: 10.1186/s13148-015-0148-3.
3. Jamieson E, Korologou-Linden R, Wootton RE, Guyatt AL, Battram T, Burrows K, Gaunt TR, Tobin MD, Munafò M, Davey Smith G, et al. Smoking, DNA Methylation, and Lung Function: a Mendelian Randomization Analysis to Investigate Causal Pathways. The American Journal of Human Genetics 2020;106(3):315-26. doi: https://doi.org/10.1016/j.ajhg.2020.01.015.
4. Jhun MA, Smith JA, Ware EB, Kardia SLR, Mosley TH, Jr., Turner ST, Peyser PA, Park SK. Modeling the Causal Role of DNA Methylation in the Association Between Cigarette Smoking and Inflammation in African Americans: A 2-Step Epigenetic Mendelian Randomization Study. American journal of epidemiology 2017;186(10):1149-58. doi: 10.1093/aje/kwx181.
5. Kolb H, Martin S. Environmental/lifestyle factors in the pathogenesis and prevention of type 2 diabetes. BMC Medicine 2017;15. doi: http://dx.doi.org/10.1186/s12916-017-0901-x.
6. Shikany JM, Safford MM, Newby PK, Durant RW, Brown TM, Judd SE. Southern Dietary Pattern Is Associated With Hazard of Acute Coronary Heart Disease in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study. Circulation 2015;132(9):804-14. doi: doi:10.1161/CIRCULATIONAHA.114.014421.
7. Micha R, Peñalvo JL, Cudhea F, Imamura F, Rehm CD, Mozaffarian D. Association between dietary factors and mortality from heart disease, stroke, and type 2 diabetes in the united states. Jama 2017;317(9):912-24. doi: 10.1001/jama.2017.0947.
8. Mozaffarian D. Dietary and Policy Priorities for Cardiovascular Disease, Diabetes, and Obesity. A Comprehensive Review 2016;133(2):187-225. doi: 10.1161/circulationaha.115.018585.
9. Chen L, Dong Y, Wang X, Hao G, Huang Y, Gutin B, Zhu H. Epigenome-Wide Association Study of Dietary Fiber Intake in African American Adolescents. Molecular nutrition & food research 2018;62(12):e1800155. doi: 10.1002/mnfr.201800155.
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. European journal of human genetics : EJHG 2015;23(5):654-62. doi: 10.1038/ejhg.2014.139.
11. Arpón A, Milagro FI, Razquin C, Corella D, Estruch R, Fitó M, Marti A, Martínez-González MA, Ros E, Salas-Salvadó J, 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(1):15. doi: 10.3390/nu10010015.
12. Arpon A, Riezu-Boj JI, Milagro FI, Marti A, Razquin C, Martinez-Gonzalez MA, Corella D, Estruch R, Casas R, Fito M, et al. Adherence to Mediterranean diet is associated with methylation changes in inflammation-related genes in peripheral blood cells. Journal of physiology and biochemistry 2016;73(3):445-55. doi: 10.1007/s13105-017-0552-6.
13. Konieczna J, Romaguera D, Pereira V, Fiol M, Razquin C, Estruch R, Asensio EM, Babio N, Fitó M, Gómez-Gracia E, et al. Longitudinal association of changes in diet with changes in body weight and waist circumference in subjects at high cardiovascular risk: the PREDIMED trial. International Journal of Behavioral Nutrition and Physical Activity 2019;16(1):139. doi: 10.1186/s12966-019-0893-3.

14. Dai H, Alsalhe TA, Chalghaf N, Riccò M, Bragazzi NL, Wu J. The global burden of disease attributable to high body mass index in 195 countries and territories, 1990–2017: An analysis of the Global Burden of Disease Study. PLoS medicine 2020;17(7):e1003198. doi: 10.1371/journal.pmed.1003198.
15. O'Flanagan CH, Bowers LW, Allot EH, Hursting SD. Molecular and metabolic mechanisms underlying the obesity–cancer link. Edtion ed. Energy Balance and Obesity: IARC Working Group, 2017.
16. Chambers JC, Loh M, Lehne B, Drong A, Kriebel J, Motta V, Wahl S, Elliott HR, Rota F, Scott WR, et al. Epigenome-wide association of DNA methylation markers in peripheral blood from Indian Asians and Europeans with incident type 2 diabetes: a nested case-control study. The lancet Diabetes & endocrinology 2015;3(7):526-34. doi: 10.1016/s2213-8587(15)00127-8.
17. Dick KJ, Nelson CP, Tsaprouni L, Sandling JK, Aïssi D, Wahl S, Meduri E, Morange P-E, Gagnon F, Grallert H, et al. DNA methylation and body-mass index: a genome-wide analysis. The Lancet 2014;383(9933):1990-8. doi: https://doi.org/10.1016/S0140-6736(13)62674-4.
18. Wahl S, Drong A, Lehne B, Loh M, Scott WR, Kunze S, Tsai P-C, Ried JS, Zhang W, Yang Y, et al. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. Nature 2017;541(7635):81-6. doi: 10.1038/nature20784.
19. Aslibekyan S, Demerath EW, Mendelson M, Zhi D, Guan W, Liang L, Sha J, Pankow JS, Liu C, Irvin MR, et al. Epigenome-wide study identifies novel methylation loci associated with body mass index and waist circumference. Circulation 2014;130.
20. Demerath EW, Guan W, Grove ML, Aslibekyan S, Mendelson M, Zhou Y-H, Hedman ÅK, Sandling JK, Li L-A, Irvin MR, et al. Epigenome-wide association study (EWAS) of BMI, BMI change and waist circumference in African American adults identifies multiple replicated loci. Human Molecular Genetics 2015;24(15):4464-79. doi: 10.1093/hmg/ddv161.
21. Mendelson MM, Marioni RE, Joehanes R, Liu C, Hedman ÅK, Aslibekyan S, Demerath EW, Guan W, Zhi D, Yao C, 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 medicine 2017;14(1):e1002215. doi: 10.1371/journal.pmed.1002215.
22. Shah S, Bonder Marc J, Marioni Riccardo E, Zhu Z, McRae Allan F, Zhernakova A, Harris Sarah E, Liewald D, Henders Anjali K, Mendelson Michael M, et al. Improving Phenotypic Prediction by Combining Genetic and Epigenetic Associations. The American Journal of Human Genetics 2015;97(1):75-85. doi: https://doi.org/10.1016/j.ajhg.2015.05.014.
23. Breton CV, Marsit CJ, Faustman E, Nadeau K, Goodrich JM, Dolinoy DC, Herbstman J, Holland N, LaSalle JM, Schmidt R, 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(4):511-26. doi: 10.1289/ehp595.
24. Komaroff M. For Researchers on Obesity: Historical Review of Extra Body Weight Definitions. Journal of Obesity 2016;2016:2460285. doi: 10.1155/2016/2460285.
25. Stefan N, Häring HU, Schulze MB. Metabolically healthy obesity: the low-hanging fruit in obesity treatment? The lancet Diabetes & endocrinology 2018;6(3):249-58. doi: 10.1016/s2213-8587(17)30292-9.
26. Mendelson MM, Marioni RE, Joehanes R, Liu C, Hedman ÅK, Aslibekyan S, Demerath EW, Guan W, Zhi D, Yao C, 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(1):e1002215-e. doi: 10.1371/journal.pmed.1002215.

CHAPTER 2

Background: Epigenetics in the study of lifestyle exposures

Parts of this chapter have been adapted and is under review.

Whitney L Do, Jazib Gohar, Lauren E. McCullough, Lisa D. Travis, Karla I Galaviz, Karen N. Conneely*, K.M. Venkat Narayan*. “Examining the association between adiposity and DNA methylation: a systematic review and meta-analysis”. *Under Review.*

With increasing high-throughput technological advances, the ability to examine the molecular underpinnings associated with exposures and diseases have substantially increased. Epigenetics represents one molecular mechanism which plays a critical role in gene expression and serves as a link to both DNA and the environment. Epigenetic is the study of mitotically heritable changes in gene expression which do not change the underlying DNA sequence. There are three primary epigenetic modifications: DNA methylation, histone modification and microRNA, with DNA methylation the most widely characterized. DNA methylation occurs primarily at cytosine-guanine nucleotide (CpG) pair sites with approximately 60-80% of CpG’s in the genome methylated of the roughly 28 million CpGs in the genome, though this varies depending on physiological state and tissue type (1, 2). Functionally, DNA methylation plays an important role in gene expression. In CpG islands, methylation has been shown decrease binding of transcription factors, thus impeding transcription. DNA methylation may also contribute to heterochromatin formation by recruiting chromatin remodelers and modifiers. Gene silencing through DNA methylation is most critical in genes on the inactive X chromosome, imprinted genes and germline-specific genes. Additionally, DNA methylation is enriched in transposable elements (3). Within gene bodies, it is not entirely clear how DNA methylation functions, as they have been associated with both active genes (3) and downregulation of gene expression (4). Given all of these roles, DNA methylation has been studied with several goals in mind. It can be used as a

prognostic tool or predictor of a phenotypic state. DNA methylation may provide insight into relevant molecular pathways that are dysregulated by DNA methylation influencing gene expression. Lastly, DNA methylation may be used to predict response to a given intervention.

DNA methylation and diet and obesity have been examined in several ways. Nutrition and DNA methylation are linked as nutritional factors are critical coenzymes contributing to the generation of DNA methyltransferases (DNMT). This feature has inspired a myriad of studies examining individual nutritional factors relevant to one-carbon metabolism and DNA methylation. More recently, the role of diet and dietary factors in regulating metabolic health has motivated several epigenome-wide association studies (EWAS). Relatedly, in response to poor dietary practices and physical inactivity leading to rising rates of obesity, DNA methylation and adiposity have been examined in several contexts. Within this chapter, the relationship between DNA methylation and diet and obesity are described

2.1 Diet and DNA methylation

Nutrients and DNA methylation

The study of nutritional effects on the epigenome have largely focused on one-carbon metabolism nutrients as S-adenosylmethionine (SAM) is the primary methyl donor in DNA methylation. Several nutrients are necessary for one-carbon metabolism including folate, vitamin B12, choline, and vitamin B2. The hypothesis that higher dietary consumption of one-carbon metabolism nutrients would associate with differential DNA methylation is biologically sound as the one-carbon metabolism pathway is the generator of methyl groups for all biological molecules (5). However, in practice, the evidence is inconsistent. When examining global or gene specific methylation, several studies have demonstrated a relationship between dietary consumption of these nutrients and changes in DNA methylation. A systematic review identified a significant effect of these nutrients on global and gene specific methylation (6). However, this review did not identify significant changes at CpG level methylation. The evidence in humans using genome-wide arrays

to identify significant sites has been less conclusive. A large scale EWAS study in 5841 participants examining folate and vitamin B12 found six sites associated with folate and 29 sites associated with vitamin B12 (7). In another EWAS of 5186 participants from the Melbourne Collaborative Cohort Study (MCCS), all one-carbon metabolism nutrients were examined. No significant sites were identified at $p < 1 \times 10^{-7}$. They concluded that that these nutrients have little association with DNA methylation (8). Overall, it is clear that methylation may be affected by these nutrients. However, identifying the specific sites and genes influenced by methylation have not been determined.

Among other nutrients, two studies have examined the effect of fat intake longitudinally on changes in DNA methylation. Perfilyev et al. (2017) examined the impact of a randomized-controlled trial of high saturated fat and polyunsaturated fat intake on methylation of adipose tissue. Initially they examined the change in mean methylation of CpG sites within the annotated gene following the dietary intervention. They found 1444 genes changed. Subsequently among these genes, they examined individuals CpG site methylation finding 2961 sites significantly influenced (9). Jacobsen et al. (2012) examined the effects of high fat overfeeding on skeletal muscle DNA methylation. While they found some changes in broad methylation response (including global methylation changes), no individual changes in CpG methylation were identified (10). Both of these studies were likely underpowered to detect individual CpG site methylation changes following the dietary intervention at a genome-wide significance threshold, though a strength of these studies includes examining change in methylation following an intervention. These studies provide initial evidence that diet is likely to induce changes on the DNA methylome, even in short periods.

Two studies have examined the cross sectional relationship between dietary factors and DNA methylation. An EWAS of dietary fat quality was conducted in preadolescents. A number of sites and pathways were identified to be associated with dietary fat quality independent of BMI,

particularly in sites that appear to regulate energy metabolism and satiety (11). Another study conducted an EWAS of dietary fiber in African American adolescents, finding three differentially methylated sites independent of obesity in genes associated with adiposity and inflammation (12).

Diet and DNA methylation

While nutrients are relevant metrics of diet, nutrients are consumed as a component of the entire diet. Diet is defined by the collection of nutrients and how they influence health overall. Diet quality is a metric used to score the diet based on various dietary factors and nutrients that have been associated with favorable and unfavorable health outcomes. While indices vary in their ranking, the majority have been able to quantify risks of cardiovascular disease, cancer and mortality (13). Since one of the goals of epigenetic studies is to identify whether methylation changes may be on the pathway mediating changes in exposures and health outcomes, diet quality may then be a more informative metric to examine given its significant relationship with health outcomes.

Few studies have examined methylation and diet in the context of diet quality causal of non-communicable diseases in later life. Ma et al. (2020) examined two diet scores and how they associated with differential DNA methylation in several large population based cohorts. They examined diet quality using the Alternative Healthy Eating Index -2010 (AHEI-2010) and the Mediterranean Diet Score (MDS). They found 30 sites associated with one or both scores. Furthermore, of these 30 sites, 10 were moderately predictive of all-cause mortality. Six sites were identified to be causally associated with several cardiovascular disease risk factors through Mendelian Randomization (14).

Another study examined the change in DNA methylation following a randomized-controlled trial of the Mediterranean diet. They were underpowered to examine changes in individual CpG site methylation. Nevertheless, they examined specific genes based on change

in mean gene methylation and found some inflammatory genes to be associated with adherence to the diet (15). Another study examining a physical activity and dietary intervention compared the control group to the intervention group. Using a region based examination, they found 154 and 298 differentially methylated regions between intervention and control at 3 months and 9 months post-intervention. These regions were associated with cell cycle regulation and carcinogenesis (16).

Overall, these studies suggest that diet is likely a modifier of DNA methylation. However, none of the above studies have identified overlapping CpG sites. With many conducted in small sample sizes and even fewer replicating their findings in external populations, there is a need for further investigations of how diet interacts with the DNA methylome.

2.2 Obesity and DNA methylation

Obesity rates continue to rise worldwide with estimated global prevalence of 2.1 billion in 2013 (17). Obesity is the excess accumulation of body fat leading to a host of pathophysiological changes, including chronic low-grade inflammation, impaired sex hormone balance, hypertriglyceridemia and hyperglycemia (18). Ultimately, these metabolic disturbances contribute to the early onset of type 2 diabetes, cardiovascular disease, kidney disease, osteoarthritis, and several forms of cancer (19). Understanding the molecular perturbations associated with obesity can help to identify potential markers of disease severity and may elucidate mechanisms which contribute to obesity-associated diseases.

Increasing evidence has demonstrated significant molecular variances associated with obesity (20, 21). One of these perturbations includes differential epigenomic signatures, among which DNA methylation is the best characterized. DNAm is the addition of a methyl group on the cytosine-guanine nucleotide pair (CpG) site and can influence gene expression leading to potential downstream disease outcomes (2). Obesity has been shown to associate with widespread changes on the methylome through candidate gene (22, 23) and genome-wide

approaches (24-28), with varying replication across populations and studies. Epigenetic markers associated with obesity could provide utility as both biomarkers of disease severity, which may be useful for targeting populations for intervention, or as novel therapeutic targets potentially identifying epigenetic markers which may protect against obesity-associated chronic diseases.

Several review articles have elaborated on the association between DNAm and obesity (29-33). However, no studies have conducted a systematic review using a recommended framework (34-37), such as COCHRANE (37) or Conducting Systematic Reviews and Meta-Analyses of Observational Studies of Etiology (COSMOS-E) (36). As systematic reviews often represent the gold-standard of evidence; it is critical to utilize comprehensive, systematic methods to minimize bias and allow for replication of the findings. As epigenetic mechanisms represent important biomarkers and therapeutic targets of adiposity and given the limited overlap of findings in previous studies and the lack of previous standardized review, this study sought to systematically review the literature relating genome-wide DNAm to adult adiposity descriptively and using meta-analytic methods.

Methods

Data Sources and Searches

The methodology for this systematic review was prospectively registered at PROSPERO (ID: CRD42020162224). We followed the Conducting Systematic Reviews and Meta-Analyses of Observational Studies of Etiology (COSMOS-E) guides to conduct and report this systematic review (36). To find relevant studies, we systematically searched PubMed, Embase, Web of Science and Scopus databases for articles published until January 2020 in any language. English and MeSH terms related to adiposity and DNA methylation and the full search string is listed below**.**

(CPG OR CPGs OR cytosine phosphate guanine OR cytosine phosphate guanines OR epigenome OR epigenomic OR epigenomics OR epigenetic OR epigenetics OR EWAS OR ((DNA OR deoxyribonucleic acid OR ds-DNA) AND (methylate OR methylates OR methylated OR methylating OR methylation OR methylations))) AND (body mass index OR body mass indexes OR quetelet index OR quetelet indexes OR quetelet's index OR quetelet's indexes OR quetelets index OR quetelets indexes OR BMI OR BMIs OR obesity OR obesities OR obese OR waist circumference OR waist circumferences OR weight to height OR weight-to-height OR WTHR OR adipose OR adiposity OR adiposities)

Study selection

Study selection is defined and depicted in **Table 2.1** using the PECO guidelines (Population, Exposure, Control and Outcome) and as follows. This review included studies of non-pregnant adults aged 18-75 that examined genome-wide DNA methylation and metrics of adiposity. Adiposity was measured by body mass index (BMI, kg/m^2), body fat percentage, waist-to-height ratio, waist-to-hip ratio, android-gynoid fat ratio, waist circumference (centimeters), and weight gain/loss in cross sectional or longitudinal studies. Studies were considered genome-wide if they measured DNA methylation via a microarray (Illumina 27K, 450K or EPIC array) or bisulfite sequencing (including reduced representation bisulfite sequencing [RRBS]). Exclusion criteria included studies in animals, analyses of the epigenetic clock or epigenetic aging (38), studies examining global methylation (utilizing high-performance liquid chromatography [HPCL] tandem mass spectrometry, luminometric methylation assay [LUMA] or pyrosequencing of Alu/LINE1 repeat elements), candidate gene studies and studies examining only secondary metabolic exposures from obesity such as metabolic syndrome, type 2 diabetes or cardiovascular disease. Studies that did not conduct genome-wide associations (e.g. studies replicating previous associations) were also excluded even if they used genome-wide arrays. We additionally excluded conference abstracts due to the preliminary nature of

these findings, and dissertations due to the lack of external peer review. Two authors independently screened literature (WD and JG). Any inconsistencies were resolved by group discussion (WD, JG, and KC).

Descriptive Review

We conducted a descriptive review of the findings by extracting information on study design, study population, methylation array, statistical analyses, and main findings using a standardized form developed for this study. As the direction of effect between DNA methylation and adiposity is unknown, cross sectional studies have statistically examined DNA methylation as both an exposure and an outcome. In the systematic review, we have reported whether DNA methylation was the exposure or outcome. Among studies using similar design (same tissue and/or same exposure), we reported overlap in the significant CpG sites that were found at a genome-wide level reported in more than three studies, a threshold used in similar systematic reviews of 'omic' data (39). We also provided the direction of effect in each study when available. Significance was defined within the individual studies. We did not include sites that were identified as significant in post-hoc targeted analyses.

Assessment of Bias and Study Design Issues

All the studies reviewed were examined for bias or issues with study design by two independent reviewers (WD and JG) according to the Joanna Briggs Checklist for cross sectional studies (40) or cohort studies (41) depending on the design of the study. Criteria examined included: 1. Sample: Were the criteria for inclusion in the sample clearly defined? 2. Exposure: Were objective, standard criteria used for measurement of the condition? 3. Outcome: Was DNA methylation measured in a valid and reliable way? 4. Adjustment: Were strategies to deal with confounding factors stated? Were the confounding variables clearly defined in terms of their measurement? 5. Statistical Analysis: Was there appropriate statistical

analysis? Did they adjust for multiple comparisons? Were there procedures to correct for technical variation? For each of the five categories, we coded the studies as “Low”, “Unclear” or “High”. If there was insufficient information available within the study to determine whether the findings may be prone to bias, the category was coded as “Unclear”. Comments were provided based on the determination if the reviewers reported “Unclear” or “High”.

Statistical Analysis

Meta-analysis was conducted in studies that examined the association with BMI and waist circumference and blood DNA methylation measured by arrays. Meta-analytic techniques were not employed for other tissue types, methylation measurement types, or definitions of adiposity due to study heterogeneity. The minimum threshold for conducting a meta-analysis with a CpG site was significance found in three or more studies. If the studies included stratified summary statistics from multiple cohorts, stratified statistics were exported and analyzed for meta-analysis within the CpG sites identified in three or more studies. We used the weighted sum of Z-score meta-analysis to analyze all studies. For studies with equivalent exposure-outcome definition, we used inverse-variance random effect meta-analysis. In sub-group analyses using inverse-variance random-effect meta-analysis, three studies used the M-values instead of the β-value (Mendelson et al. [Framingham Heart Study 1 & 2], S. Li et al., and Sayols-Baixeras et al.) for DNA methylation analysis. These studies were excluded as effect sizes cannot be compared across methods.

As inclusion in the meta-analysis was determined by significance of the CpG site and null results are rarely reported in EWAS studies, we did not examine publication bias. In the meta-analysis, between-study heterogeneity was examined using I^2 statistics (low heterogeneity < 25%, moderate heterogeneity 25-70%, high heterogeneity > 70%) and Cochran’s Q test (significance defined by $p < 0.05$) (42). Additionally, we conducted outlier exclusion tests and examined the change in the I^2 and the Cochran’s Q test when outliers were excluded from the

meta-analysis. We tested for sources of heterogeneity (sex, race/ethnicity, and smoking-adjustment) using subgroup analyses (smoking) or meta-regression (sex percentage, race/ethnicity: European ancestry, African ancestry, or mixed race). We only performed subgroup analyses on CpG sites if more than 7 studies were available to be analyzed and more than three studies were included per subgroup.

Results

We identified 13,310 titles during the initial systematic search. After duplicate removal (n=2,762), 10,548 titles and abstracts were screened, with 10,414 removed. Of these, 134 were evaluated during the full text screening with 46 studies deemed eligible for inclusion (**Figure 2.1**).

Summary of Results

The full summary of results is included in **Table 2.2**. The majority of studies examined continuous BMI (48%, 22/46) as the exposure or outcome in cross sectional EWAS. Another subset of studies (46%, 21/46) compared dichotomized or categorical adiposity variables (for example obese vs non-obese). Among other adiposity measures, waist circumference was examined in 17% of studies (8/46). A smaller number of studies examined waist to hip ratio (4%, 2/46), waist to height ratio (2%, 1/46), or fat percentage (4%, 2/46). Nine percent of studies dichotomized individuals based on response to an intervention (9%, 4/46). Most studies assayed DNA methylation using microarrays including 27K (9%, 4/46), 450K (76%, 35/46), or the EPIC array (2%, 1/46), while other studies used RRBS (11%, 5/46). The majority of studies used cohort data from the United States (30%, 14/46) followed by Spain (21%, 10/46), Sweden (9%, 4/46) and the UK (7%, 3/46). Racial and ethnic diversity was limited with the majority of studies conducted exclusively in Caucasian populations of European descent (43%, 20/45) with an additional 33% not mentioning the race/ethnicity of the cohort though it can be inferred that the sample is of European descent (15/46). Only 26% (12/46) were in ethnically diverse

populations, among which 11% (5/46) included African Americans or individuals of African descent, and 2% (1/46) were conducted in Middle Eastern, Chinese, and Japanese populations, respectively.

Assessment of Bias and Study Design Issues

Several sources of heterogeneity exist in the design and analysis of the studies. Since the majority of studies employed a cross-sectional analysis to examine the relationship between obesity and methylation, exclusion and statistical adjustment for confounders were the primary mechanisms to account for bias. In the bias assessment, the most common source of bias was confounding (**Table 2.3**). Thirteen studies were identified to have a "High" risk of bias based on adjustment for confounding. Eight studies outside of the blood (in the adipose, breast, liver and endometrial tissue) did not adjust for cell composition, though other tissues may not be as heterogeneous by cell composition as the blood. Thus we did not qualify these as significant bias ("High") instead noted them as potential sources ("Unclear"). Four studies did not include definitions of obesity or BMI, though this is unlikely a significant source of bias, thus they were coded as "Unclear". Seven studies did not provide information or did not conduct probe filtering protocols to reduce the incidence of false positives. Of the studies in the blood examining BMI, none were found to have a "High" risk of bias with five studies identified as having an "Unclear" risk of bias. All of the studies examining waist circumference in the blood were found to have a "Low" risk of bias. Based on these findings, we meta-analyzed results from studies examining BMI and waist circumference in DNA methylation in the blood as measured by microarray.

Blood methylation and adiposity

Eighteen studies examining continuous BMI were conducted in blood with DNA methylation measured via a microarray. The number of significant sites varied between 0 to 7,457. Among these studies (43-60), 1,100 sites had estimates of effect and standard errors

reported in the studies. We found 77 common CpG sites significant in at least three studies. Using random effects models of studies regressing DNA methylation on BMI, we were able to meta-analyze 74 sites (44-47, 49-54, 57, 59, 60). On average, every one unit change in BMI was associated with a 0.001 change in the DNA methylation β-value (**Figure 2.2**). We also found 72 of 74 CpG sites had a consistent direction of effect in all of the meta-analyzed studies. In studies regressing BMI on DNA methylation (43, 48, 55, 56, 58), we were able to meta-analyze 69 sites. On average, every 0.01 unit change in the DNA methylation β-value was associated with a 0.21 unit change in BMI. Forty nine of the 69 sites had a consistent direction of effect. In the meta-analysis including results from all 18 studies using weighted-sum of Z-scores meta-analysis, 52 sites remained significant after FDR-adjustment (adjusted for 475K sites).

We conducted several sensitivity analyses including outlier analysis and subgroup analyses in the studies regressing DNA methylation on BMI. Of the 74 sites, 50 sites included more than three studies and were examined for outliers. Only nineteen sites identified outliers. In these analyses, 13 sites were no longer significant ($p < 0.05$). However, heterogeneity remained high. Even with exclusion of one or two outlier studies, only eight CpG sites had I^2 values below 30% indicating low heterogeneity with four CpG sites indicating high heterogeneity (I^2 values above 70% and Cochran's Q test p-value < 0.05). In the meta-regression of two sites, both were found be heterogeneous by sex. Effect sizes were found to decrease and increase with the larger percentage of females in the study population for cg00574958 and cg06500161. None of the sites could be examined for heterogeneity by race or by adjustment for smoking because no sites were found to have more than three studies per subgroup.

Seven studies examined the association between waist circumference and blood DNA methylation (24, 44-46, 51, 53, 55). Among these studies, 931 sites were reported to be significant with four overlapping CpG sites identified in three or more studies. Two were found to

associate with waist circumference in the meta-analyses. In these two sites, on average every one unit change in waist circumference was associated with a 0.0002 unit change in the DNA methylation β-value. All of the sites were found to have a consistent direction of effect.

Adipose methylation and adiposity

Several studies examined DNA methylation differences in the adipose tissue (61-68). Several metrics were used to examine adiposity across these studies including continuous BMI (61, 66, 68), dichotomized BMI categories (62, 64, 65), BMI discordance in twins (67), fat percentage change following an intervention (63), fat percentage (66), waist circumference (66) android fat mass (61), android-gynoid fat ratio (61), trunk:limb ratio (61), and waist/hip ratio (66). The number of significant sites ranged from 0-33,058. Among these studies, 741 CpG sites were reported in three or more studies with the majority having a consistent direction of effect (414 in a consistent direction). The high number of overlapping CpG sites may be due to the fact that three studies reported more than 10,000 CpG sites as differentially methylated. Given the heterogeneity in the design of these studies, we did not combine results from these studies in a meta-analysis.

Reduced Representation Bisulfite Sequencing

Five studies in three distinct tissue types examined methylation using RRBS (66, 69-72). We only examined overlapping genes, given the differential reporting of results. 12,968 unique genes were found to associate with adiposity in at least one study. Of these, 1,047 genes were reported to be significant in three or more studies with the majority (734) showing inconsistent direction of effect across studies.

Other tissues and adiposity exposures

Twenty studies examined methylation differences between categorical adiposity measurements across 23 tissue types (various blood cells, adipose, periprostatic adipose,

breast, colorectal, muscle, liver and endometrial tissue and tumor cells from several tissue types) (62, 64, 65, 67, 69-71, 73-85). Among the studies examined in the blood, significant sites ranged from 0-2,102 CpG sites including 3,310 unique CpG sites. No overlapping CpG sites were reported in three or more studies.

Several studies sought to examine how weight change associated with DNA methylation either through an intervention with repeated measures design or based on response to a dietary intervention (46, 63, 72, 78, 84, 86, 87). The design varied significantly among these studies including in tissue type (adipose, leucocytes, whole blood, white blood cells, dried blood spot and mononuclear cells) and exposure (fat percentage change, BMI change, and response to weight loss intervention). Of the studies in the blood, 2,139 unique CpG sites were found significant in one study, though no overlapping CpG sites were found. When weight change studies in the adipose were included, no CpG sites overlapped.

Given the prior association between several forms of cancer and obesity, two studies focused on normal breast tissue or breast tumor tissue (74, 88). Among these studies, one site overlapped in two studies, cg24527008.

Discussion

This study provides a synthesis of the evidence associating DNA methyation and adiposity. Overall, 46 studies were included in this systematic review. We identified a number of CpG sites which have been associated with some metric of adiposity, however significant heterogeneity in study design and analysis have resulted in limited overlap in many of the findings. Among the CpG sites identified in three or more studies, there was no overlap between the CpG sites in the adipose (742 sites) and the blood associated with waist circumference (4 sites) or BMI (77 sites). This may be due in large part to heterogeneity in the studies conducted within the adipose tissue.

Of the CpG sites found significant in three or more studies, 52 CpG sites were significantly associated with BMI when meta-analyzed using a genome-wide threshold for significance. These sites have been found to associate with several metabolic health parameters including depression, atherosclerosis, kidney disease, blood lipids (triglycerides, high-density lipoproteins), hepatic disease, type 2 diabetes, insulin resistance, metabolic syndrome, hemoglobin A1c and Crohn's disease/inflammatory bowel syndrome (**Figure 2.3**). These associations may represent both upstream cardiometabolic risk factors associated with obesity (blood lipids, blood pressure, glomerular filtration rate, insulin) and downstream diseases caused by obesity (atherosclerosis, hypertension, type 2 diabetes, metabolic syndrome and non-alcoholic fatty liver disease).

Whether obesity is the cause of differential DNA methylation in these sites, with methylation mediating the outcomes discussed above, is unknown. Using Mendelian randomization (MR), Mendelson et al. found that of the 83 replicated CpG sites within their study, 16 were causally influenced by obesity, five of which were significant in our study (cg06500161, cg13708645, cg24678869, cg25649826 and cg26950531) (49). These results suggest that these CpG sites are likely influenced by obesity, but may have downstream effects on other diseases. cg06500161 has also been shown to be causally impacted by triglycerides and HDL-cholesterol, leading to reduced expression levels (89). None of the five sites have been identified in MR studies examining cardiovascular disease (90) or type 2 diabetes (91, 92). Another site identified in our study (cg00574958) has been shown to be both causally (93) and consequentially (89, 93) associated with blood lipids. cg00574958 was causally influenced by a polygenic risk score for triglycerides and blood triglycerides were also causally associated with cis methylation quantitative trait loci for cg00574958 (93). Given these findings, we can conclude that DNA methylation in these six sites are causally influenced either directly or indirectly by excess adiposity. Methylation may also influence changes in adiposity. Mendelson

et al. found cg11024682 was causally associated with BMI via two-step MR (methylation → gene expression → BMI), as well as several adiposity related traits (49).

Several of the BMI-associated CpG sites were found to previously associate with alcohol consumption, diet quality and smoking (three upstream exposures associated with obesity). Eleven CpG sites of the 52 significant CpG sites have been associated with alcohol consumption (94-96). The link between BMI and alcohol is not linear as heavy drinking is positively associated with weight gain, however light to moderate consumption has been shown to protect against weight gain (97). The CpG sites associated with alcohol may represent confounding by alcohol consumption, as a majority of BMI EWAS studies did not adjust for alcohol consumption. Two sites have been found to associate with diet quality (98). As these sites were previously associated with diet quality in BMI-adjusted models, these also likely represent the upstream effects of differential diet quality associated with obesity. Fifteen CpG sites have previously been associated with smoking (99-113). This may represent a significant confounder in the BMI-methylation association in these sites. Of the EWAS examined in our meta-analysis, less than half included smoking as a covariate in the EWAS models. We were unable to examine heterogeneity by smoking statistically.

The fact that many of these sites are associated with one or more chronic disease is anticipated. BMI is not universally included as a confounder in the literature. These methylation sites may provide utility as early predictors of obesity-associated chronic diseases. Future research efforts utilizing longitudinal methylation data may also help to provide a clearer picture of the relationship between obesity, DNA methylation and chronic disease.

There are several limitations in our study. While we did not restrict our study by language, we may not have identified studies without a title and abstract in English. Nevertheless, we did identify and extract information for at least one study in Spanish. Our quantitative results are prone to publication bias since they were extracted exclusively from sites

that were found to be significant in three studies. Few studies provide summary statistics on null associations. Of the 18 studies included in the meta-analysis, only one made full summary statistics of all the results publicly available. Thus, we are not able to include the results from these sites in the meta-analysis. Nevertheless, only two of 77 sites meta-analyzed had an opposite direction of effect in one of the individual study effect sizes. This gives us confidence in these findings. However, future research efforts should focus on quantitative meta-analysis of results encompassing all CpG sites studied to more comprehensively understand this relationship.

In summary, this study found widespread differences in DNA methylation in multiple tissue associated with adiposity. Significant heterogeneity in study design, tissue type, and statistical methods have ultimately yielded limited overlap of significant sites. Nevertheless, 52 CpG sites were significantly associated with BMI using a genome-wide threshold for significance. These CpG sites have been shown to associate with a myriad of other obesity-associated outcomes, suggesting their relevance as either causes or consequences of obesity.

References

1. Singer BD. A Practical Guide to the Measurement and Analysis of DNA Methylation. American journal of respiratory cell and molecular biology 2019;61(4):417-28. doi: 10.1165/rcmb.2019-0150TR.
2. Smith ZD, Meissner A. DNA methylation: roles in mammalian development. Nature reviews Genetics 2013;14(3):204-20. doi: 10.1038/nrg3354.
3. Greenberg MVC, Bourc'his D. The diverse roles of DNA methylation in mammalian development and disease. Nature Reviews Molecular Cell Biology 2019;20(10):590-607. doi: 10.1038/s41580-019-0159-6.
4. Kennedy EM, Goehring GN, Nichols MH, Robins C, Mehta D, Klengel T, Eskin E, Smith AK, Conneely KN. An integrated -omics analysis of the epigenetic landscape of gene expression in human blood cells. BMC genomics 2018;19(1):476. doi: 10.1186/s12864-018-4842-3.
5. Zeisel S. Choline, Other Methyl-Donors and Epigenetics. Nutrients 2017;9(5):445.
6. Amenyah SD, Hughes CF, Ward M, Rosborough S, Deane J, Thursby SJ, Walsh CP, Kok DE, Strain JJ, McNulty H, et al. Influence of nutrients involved in one-carbon metabolism on DNA methylation in adults-a systematic review and meta-analysis. Nutrition reviews 2020;78(8):647-66. doi: 10.1093/nutrit/nuz094.
7. Mandaviya PR, Joehanes R, Brody J, Castillo-Fernandez JE, Dekkers KF, Do AN, Graff M, Hanninen IK, Tanaka T, de Jonge EAL, et al. Association of dietary folate and vitamin B-12 intake with genome-wide DNA methylation in blood: a large-scale epigenome-wide association analysis in 5841 individuals. The American journal of clinical nutrition 2019;110(2):437-50. doi: 10.1093/ajcn/nqz031.
8. Chamberlain JA, Dugué P-A, Bassett JK, Hodge AM, Brinkman MT, Joo JE, Jung C-H, Makalic E, Schmidt DF, Hopper JL, et al. Dietary intake of one-carbon metabolism nutrients and DNA methylation in peripheral blood. The American journal of clinical nutrition 2018;108(3):611-21. doi: 10.1093/ajcn/nqy119.
9. Perfilyev A, Dahlman I, Gillberg L, Rosqvist F, Iggman D, Volkov P, Nilsson E, Riserus U, Ling C. Impact of polyunsaturated and saturated fat overfeeding on the DNA-methylation pattern in human adipose tissue: a randomized controlled trial. The American journal of clinical nutrition 2017. doi: 10.3945/ajcn.116.143164.
10. Jacobsen SC, Brons C, Bork-Jensen J, Ribel-Madsen R, Yang B, Lara E, Hall E, Calvanese V, Nilsson E, Jorgensen SW, et al. Effects of short-term high-fat overfeeding on genome-wide DNA methylation in the skeletal muscle of healthy young men. Diabetologia 2012;55(12):3341-9. doi: 10.1007/s00125-012-2717-8.
11. 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. European journal of human genetics : EJHG 2015;23(5):654-62. doi: 10.1038/ejhg.2014.139.
12. Chen L, Dong Y, Wang X, Hao G, Huang Y, Gutin B, Zhu H. Epigenome-Wide Association Study of Dietary Fiber Intake in African American Adolescents. Molecular nutrition & food research 2018;62(12):e1800155. doi: 10.1002/mnfr.201800155.
13. Wirt A, Collins CE. Diet quality – what is it and does it matter? Public health nutrition 2009;12(12):2473-92. doi: 10.1017/S136898000900531X.
14. Ma J, Liu C, Joehanes R, Mendelson MM, Liang L, Lichtenstein AH, Hu FB, Levy D. Whole Blood DNA Methylation Signatures of a Mediterranean-style Dietary Pattern. Circulation 2017;135.
15. Nicoletti CF, Cortes-Oliveira C, Noronha NY, Pinhel MAS, Dantas WS, Jácome A, Marchini JS, Gualano B, Crujeiras AB, Nonino CB. DNA methylation pattern changes following a short-term

hypocaloric diet in women with obesity. European journal of clinical nutrition 2020;74(9):1345-53. doi: 10.1038/s41430-020-0660-1.
16. Hibler E, Huang L, Andrade J, Spring B. Impact of a diet and activity health promotion intervention on regional patterns of DNA methylation. Clinical epigenetics 2019;11(1):133. doi: 10.1186/s13148-019-0707-0.
17. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet (London, England) 2014;384(9945):766-81. doi: 10.1016/S0140-6736(14)60460-8.
18. Asghar A, Sheikh N. Role of immune cells in obesity induced low grade inflammation and insulin resistance. Cell Immunol 2017;315:18-26. doi: 10.1016/j.cellimm.2017.03.001.
19. Bray GA, Kim KK, Wilding JPH, Federation obotWO. Obesity: a chronic relapsing progressive disease process. A position statement of the World Obesity Federation. Obesity Reviews 2017;18(7):715-23. doi: 10.1111/obr.12551.
20. Homuth G, Wahl S, Müller C, Schurmann C, Mäder U, Blankenberg S, Carstensen M, Dörr M, Endlich K, Englbrecht C, et al. Extensive alterations of the whole-blood transcriptome are associated with body mass index: results of an mRNA profiling study involving two large population-based cohorts. BMC Medical Genomics 2015;8(1):65. doi: 10.1186/s12920-015-0141-x.
21. Cirulli ET, Guo L, Leon Swisher C, Shah N, Huang L, Napier LA, Kirkness EF, Spector TD, Caskey CT, Thorens B, et al. Profound Perturbation of the Metabolome in Obesity Is Associated with Health Risk. Cell Metabolism 2019;29(2):488-500.e2. doi: https://doi.org/10.1016/j.cmet.2018.09.022.
22. Milagro FI, Gómez-Abellán P, Campión J, Martínez JA, Ordovás JM, Garaulet M. CLOCK, PER2 and BMAL1 DNA Methylation: Association with Obesity and Metabolic Syndrome Characteristics and Monounsaturated Fat Intake. Chronobiology International 2012;29(9):1180-94. doi: 10.3109/07420528.2012.719967.
23. Voisin S, Almén MS, Zheleznyakova GY, Lundberg L, Zarei S, Castillo S, Eriksson FE, Nilsson EK, Blüher M, Böttcher Y, et al. Many obesity-associated SNPs strongly associate with DNA methylation changes at proximal promoters and enhancers. Genome Medicine 2015;7(1):103. doi: 10.1186/s13073-015-0225-4.
24. Arpon A, Milagro FI, Ramos-Lopez O, Mansego ML, Riezu-Boj JI, Martinez JA. Methylome-Wide Association Study in Peripheral White Blood Cells Focusing on Central Obesity and Inflammation. Genes 2019;10(6). doi: 10.3390/genes10060444.
25. Demerath EW, Guan W, Grove ML, Aslibekyan S, Mendelson M, Zhou Y-H, Hedman ÅK, Sandling JK, Li L-A, Irvin MR, et al. Epigenome-wide association study (EWAS) of BMI, BMI change and waist circumference in African American adults identifies multiple replicated loci. Human Molecular Genetics 2015;24(15):4464-79. doi: 10.1093/hmg/ddv161.
26. Mendelson MM, Marioni RE, Joehanes R, Liu C, Hedman ÅK, Aslibekyan S, Demerath EW, Guan W, Zhi D, Yao C, 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(1):e1002215-e. doi: 10.1371/journal.pmed.1002215.
27. Wang X, Zhu H, Snieder H, Su S, Munn D, Harshfield G, Maria BL, Dong Y, Treiber F, Gutin B, et al. Obesity related methylation changes in DNA of peripheral blood leukocytes. BMC Medicine 2010;8(1):87. doi: 10.1186/1741-7015-8-87.
28. Almén MS, Nilsson EK, Jacobsson JA, Kalnina I, Klovins J, Fredriksson R, Schiöth HB. Genome-wide analysis reveals DNA methylation markers that vary with both age and obesity. Gene 2014;548(1):61-7. doi: 10.1016/j.gene.2014.07.009.

29. Campion J, Milagro F, Martinez JA. Epigenetics and obesity. Progress in molecular biology and translational science 2010;94:291-347. doi: 10.1016/s1877-1173(10)94011-8

10.1016/b978-0-12-375003-7.00011-x.
30. de Mello VDF, Pulkkinen L, Lalli M, Kolehmainen M, Pihlajamäki J, Uusitupa M. DNA methylation in obesity and type 2 diabetes. Annals of Medicine 2014;46(3):103-13. doi: 10.3109/07853890.2013.857259.
31. Martinez JA, Milagro FI, Claycombe KJ, Schalinske KL. Epigenetics in adipose tissue, obesity, weight loss, and diabetes. Advances in nutrition (Bethesda, Md) 2014;5(1):71-81. doi: 10.3945/an.113.004705.
32. van Dijk SJ, Molloy PL, Varinli H, Morrison JL, Muhlhausler BS, Buckley M, Clark SJ, McMillen IC, Noakes M, Samaras K, et al. Epigenetics and human obesity. International Journal of Obesity 2015;39(1):85-97. doi: 10.1038/ijo.2014.34.
33. van Dijk SJ, Tellam RL, Morrison JL, Muhlhausler BS, Molloy PL. Recent developments on the role of epigenetics in obesity and metabolic disease. Clinical epigenetics 2015;7:66. doi: 10.1186/s13148-015-0101-5.
34. Moher D, Liberati A, Tetzlaff J, Altman DG, Group atP. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. Annals of internal medicine 2009;151(4):264-9. doi: 10.7326/0003-4819-151-4-200908180-00135.
35. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB, et al. Meta-analysis of Observational Studies in EpidemiologyA Proposal for Reporting. JAMA 2000;283(15):2008-12. doi: 10.1001/jama.283.15.2008.
36. Dekkers OM, Vandenbroucke JP, Cevallos M, Renehan AG, Altman DG, Egger M. COSMOS-E: Guidance on conducting systematic reviews and meta-analyses of observational studies of etiology. PLoS medicine 2019;16(2):e1002742-e. doi: 10.1371/journal.pmed.1002742.
37. Higgins J, Thomas J, Chandler J, Cumpston M, Li T, Page M, Welch V. Cochrane Handbook for Systematic Reviews of Interventions, 2020.
38. Ryan J, Wrigglesworth J, Loong J, Fransquet PD, Woods RL. A Systematic Review and Meta-analysis of Environmental, Lifestyle, and Health Factors Associated With DNA Methylation Age. The Journals of Gerontology: Series A 2019. doi: 10.1093/gerona/glz099.
39. Guasch-Ferré M, Hruby A, Toledo E, Clish CB, Martínez-González MA, Salas-Salvadó J, Hu FB. Metabolomics in Prediabetes and Diabetes: A Systematic Review and Meta-analysis. Diabetes care 2016;39(5):833-46. doi: 10.2337/dc15-2251.
40. The Joanna Briggs Institute Critical Appraisal tools for use in JBI Systematic Reviews Checklist for Analytical Cross Sectional Studies. Joanna Briggs Institute, 2017.
41. The Joanna Briggs Institute Critical Appraisal tools for use in JBI Systematic Reviews Checklist for Analytical Cohort Studies. Joanna Briggs Institute, 2017.
42. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ (Clinical research ed) 2003;327(7414):557-60. doi: 10.1136/bmj.327.7414.557.
43. Al Muftah WA, Al-Shafai M, Zaghlool SB, Visconti A, Tsai PC, Kumar P, Spector T, Bell J, Falchi M, Suhre K. Epigenetic associations of type 2 diabetes and BMI in an Arab population. Clinical epigenetics 2016;8:13. doi: 10.1186/s13148-016-0177-6.
44. Aslibekyan S, Demerath EW, Mendelson M, Zhi D, Guan W, Liang L, Sha J, Pankow JS, Liu C, Irvin MR, et al. Epigenome-wide study identifies novel methylation loci associated with body mass index and waist circumference. Obesity (Silver Spring, Md) 2015;23(7):1493-501. doi: 10.1002/oby.21111.
45. Campanella G, Gunter MJ, Polidoro S, Krogh V, Palli D, Panico S, Sacerdote C, Tumino R, Fiorito G, Guarrera S, et al. Epigenome-wide association study of adiposity and future risk of obesity-

related diseases. International journal of obesity (2005) 2018;42(12):2022-35. doi: 10.1038/s41366-018-0064-7.
46. Demerath EW, Guan W, Grove ML, Aslibekyan S, Mendelson M, Zhou YH, Hedman AK, Sandling JK, Li LA, Irvin MR, 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(15):4464-79. doi: 10.1093/hmg/ddv161.
47. Geurts YM, Dugue PA, Joo JE, Makalic E, Jung CH, Guan W, Nguyen S, Grove ML, Wong EM, Hodge AM, et al. Novel associations between blood DNA methylation and body mass index in middle-aged and older adults. International journal of obesity (2005) 2018;42(4):887-96. doi: 10.1038/ijo.2017.269.
48. Dick KJ, Nelson CP, Tsaprouni L, Sandling JK, Aïssi D, Wahl S, Meduri E, Morange P-E, Gagnon F, Grallert H, et al. DNA methylation and body-mass index: a genome-wide analysis. The Lancet 2014;383(9933):1990-8. doi: http://dx.doi.org/10.1016/S0140-6736(13)62674-4.
49. Mendelson MM, Marioni RE, Joehanes R, Liu C, Hedman ÅK, Aslibekyan S, Demerath EW, Guan W, Zhi D, Yao C, 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 medicine 2017;14(1):e1002215. doi: 10.1371/journal.pmed.1002215.
50. Agha G, Houseman EA, Kelsey KT, Eaton CB, Buka SL, Loucks EB. Adiposity is associated with DNA methylation profile in adipose tissue. International journal of epidemiology 2015;44(4):1277-87. doi: 10.1093/ije/dyu236.
51. Dhana K, Braun KVE, Nano J, Voortman T, Demerath EW, Guan W, Fornage M, van Meurs JBJ, Uitterlinden AG, Hofman A, et al. An Epigenome-Wide Association Study of Obesity-Related Traits. American journal of epidemiology 2018;187(8):1662-9. doi: 10.1093/aje/kwy025.
52. Li S, Dugue PA, Baglietto L, Severi G, Wong EM, Nguyen TL, Stone J, English DR, Southey MC, Giles GG, et al. Genome-wide association study of peripheral blood DNA methylation and conventional mammographic density measures. Int J Cancer 2019;145(7):1768-73. doi: 10.1002/ijc.32171.
53. Meeks KAC, Henneman P, Venema A, Burr T, Galbete C, Danquah I, Schulze MB, Mockenhaupt FP, Owusu-Dabo E, Rotimi CN, et al. An epigenome-wide association study in whole blood of measures of adiposity among Ghanaians: the RODAM study. Clinical epigenetics 2017;9:103. doi: 10.1186/s13148-017-0403-x.
54. Ramos-Lopez O, Arpón A, Riezu-Boj JI, Milagro FI, Mansego ML, Martinez JA. DNA methylation patterns at sweet taste transducing genes are associated with BMI and carbohydrate intake in an adult population. Appetite 2018;120:230-9. doi: 10.1016/j.appet.2017.09.004.
55. Sayols-Baixeras S, Subirana I, Fernandez-Sanles A, Senti M, Lluis-Ganella C, Marrugat J, Elosua R. DNA methylation and obesity traits: An epigenome-wide association study. The REGICOR study. Epigenetics 2017;12(10):909-16. doi: 10.1080/15592294.2017.1363951.
56. Shah S, Bonder Marc J, Marioni Riccardo E, Zhu Z, McRae Allan F, Zhernakova A, Harris Sarah E, Liewald D, Henders Anjali K, Mendelson Michael M, et al. Improving Phenotypic Prediction by Combining Genetic and Epigenetic Associations. The American Journal of Human Genetics 2015;97(1):75-85. doi: https://doi.org/10.1016/j.ajhg.2015.05.014.
57. Sun D, Zhang T, Su S, Hao G, Chen T, Li QZ, Bazzano L, He J, Wang X, Li S, et al. Body Mass Index Drives Changes in DNA Methylation: A Longitudinal Study. Circ Res 2019;125(9):824-33. doi: 10.1161/circresaha.119.315397.
58. Wahl S, Drong A, Lehne B, Loh M, Scott WR, Kunze S, Tsai PC, Ried JS, Zhang W, Yang Y, et al. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. Nature 2017;541(7635):81-6. doi: 10.1038/nature20784.

59. Wilson LE, Harlid S, Xu Z, Sandler DP, Taylor JA. An epigenome-wide study of body mass index and DNA methylation in blood using participants from the Sister Study cohort. Int J Obes (Lond) 2017;41(1):194-9. doi: 10.1038/ijo.2016.184.
60. Xu K, Zhang X, Wang Z, Hu Y, Sinha R. Epigenome-wide association analysis revealed that SOCS3 methylation influences the effect of cumulative stress on obesity. Biol Psychol 2018;131:63-71. doi: 10.1016/j.biopsycho.2016.11.001.
61. Agha G, A EH, Kelsey KT, Eaton CB, Buka SL, Loucks EB. Adiposity is associated with DNA methylation profile in adipose tissue. Int J Epidemiol 2015;44(4):1277-87. doi: 10.1093/ije/dyu236.
62. Arner P, Sinha I, Thorell A, Ryden M, Dahlman-Wright K, Dahlman I. The epigenetic signature of subcutaneous fat cells is linked to altered expression of genes implicated in lipid metabolism in obese women. Clinical epigenetics 2015;7:93. doi: 10.1186/s13148-015-0126-9.
63. Bollepalli S, Kaye S, Heinonen S, Kaprio J, Rissanen A, Virtanen KA, Pietilainen KH, Ollikainen M. Subcutaneous adipose tissue gene expression and DNA methylation respond to both short- and long-term weight loss. International journal of obesity (2005) 2018;42(3):412-23. doi: 10.1038/ijo.2017.245.
64. Cheng Y, Monteiro C, Matos A, You J, Fraga A, Pereira C, Catalan V, Rodriguez A, Gomez-Ambrosi J, Fruhbeck G, et al. Epigenome-wide DNA methylation profiling of periprostatic adipose tissue in prostate cancer patients with excess adiposity-a pilot study. Clinical epigenetics 2018;10:54. doi: 10.1186/s13148-018-0490-3.
65. Crujeiras AB, Diaz-Lagares A, Sandoval J, Milagro FI, Navas-Carretero S, Carreira MC, Gomez A, Hervas D, Monteiro MP, Casanueva FF, et al. DNA methylation map in circulating leukocytes mirrors subcutaneous adipose tissue methylation pattern: a genome-wide analysis from non-obese and obese patients. Sci Rep 2017;7:41903. doi: 10.1038/srep41903.
66. Orozco LD, Farrell C, Hale C, Rubbi L, Rinaldi A, Civelek M, Pan C, Lam L, Montoya D, Edillor C, et al. Epigenome-wide association in adipose tissue from the METSIM cohort. Hum Mol Genet 2018;27(10):1830-46. doi: 10.1093/hmg/ddy093.
67. Pietilaeinen KH, Ismail K, Jaervinen E, Heinonen S, Tummers M, Bollepalli S, Lyle R, Muniandy M, Moilanen E, Hakkarainen A, et al. DNA methylation and gene expression patterns in adipose tissue differ significantly within young adult monozygotic BMI-discordant twin pairs. Int J Obes 2016;40(4):654-61. doi: http://dx.doi.org/10.1038/ijo.2015.221.
68. Ronn T, Volkov P, Gillberg L, Kokosar M, Perfilyev A, Jacobsen AL, Jorgensen SW, Brons C, Jansson PA, Eriksson KF, et al. Impact of age, BMI and HbA1c levels on the genome-wide DNA methylation and mRNA expression patterns in human adipose tissue and identification of epigenetic biomarkers in blood. Hum Mol Genet 2015;24(13):3792-813. doi: 10.1093/hmg/ddv124.
69. Day SE, Coletta RL, Kim JY, Campbell LE, Benjamin TR, Roust LR, De Filippis EA, Dinu V, Shaibi GQ, Mandarino LJ, et al. Next-generation sequencing methylation profiling of subjects with obesity identifies novel gene changes. Clinical epigenetics 2016;8:77. doi: 10.1186/s13148-016-0246-x.
70. Day SE, Coletta RL, Kim JY, Garcia LA, Campbell LE, Benjamin TR, Roust LR, De Filippis EA, Mandarino LJ, Coletta DK. Potential epigenetic biomarkers of obesity-related insulin resistance in human whole-blood. Epigenetics 2017;12(4):254-63. doi: 10.1080/15592294.2017.1281501.
71. Dong L, Ma L, Ma GH, Ren H. Genome-wide Analysis Reveals DNA Methylation Alterations in Obesity Associated with High Risk of Colorectal Cancer. Sci Rep 2019;9(1):5100. doi: 10.1038/s41598-019-41616-0.
72. Li W, Zhang D, Wang W, Wu Y, Mohammadnejad A, Lund J, Baumbach J, Christiansen L, Tan Q. DNA methylome profiling in identical twin pairs discordant for body mass index. International journal of obesity (2005) 2019;43(12):2491-9. doi: 10.1038/s41366-019-0382-4.

73. Almen MS, Klovins J, Kalnina I, Nilsson E, Fredriksson R, Schioth HB, Jacobsson JA. Genome-wide analysis reveals DNA methylation markers that varies with both age and obesity. ArrayExpress Archive 2014.
74. Crujeiras AB, Diaz-Lagares A, Stefansson OA, Macias-Gonzalez M, Sandoval J, Cueva J, Lopez-Lopez R, Moran S, Jonasson JG, Tryggvadottir L, et al. Obesity and menopause modify the epigenomic profile of breast cancer. 2017;24(7):351. doi: 10.1530/erc-16-0565.
75. Crujeiras AB, Morcillo S, Diaz-Lagares A, Sandoval J, Castellano-Castillo D, Torres E, Hervas D, Moran S, Esteller M, Macias-Gonzalez M, et al. Identification of an episignature of human colorectal cancer associated with obesity by genome-wide DNA methylation analysis. Int J Obes 2019;43(1):176-88. doi: http://dx.doi.org/10.1038/s41366-018-0065-6.
76. Gomez-Uriz AM, Milagro FI, Mansego ML, Cordero P, Abete I, De Arce A, Goyenechea E, Blazquez V, Martinez-Zabaleta M, Martinez JA, et al. Obesity and ischemic stroke modulate the methylation levels of KCNQ1 in white blood cells. Hum Mol Genet 2015;24(5):1432-40. doi: 10.1093/hmg/ddu559.
77. Hohos NM, Smith AK, Kilaru V, Park HJ, Hausman DB, Bailey LB, Lewis RD, Phillips BG, Meagher RB. CD4(+) and CD8(+) T-Cell-Specific DNA Cytosine Methylation Differences Associated With Obesity. Obesity (Silver Spring, Md) 2018;26(8):1312-21. doi: 10.1002/oby.22225.
78. Huang YT, Maccani JZJ, Hawley NL, Wing RR, Kelsey KT, McCaffery JM. Epigenetic patterns in successful weight loss maintainers: a pilot study. International journal of obesity (2005) 2015;39(5):865-8. doi: 10.1038/ijo.2014.213.
79. Kirchner H, Sinha I, Gao H, Ruby MA, Schonke M, Lindvall JM, Barres R, Krook A, Naslund E, Dahlman-Wright K, et al. Altered DNA methylation of glycolytic and lipogenic genes in liver from obese and type 2 diabetic patients. Molecular metabolism 2016;5(3):171-83. doi: 10.1016/j.molmet.2015.12.004.
80. Kvaloy K, Page CM, Holmen TL. Epigenome-wide methylation differences in a group of lean and obese women - A HUNT Study. Sci Rep 2018;8(1):16330. doi: 10.1038/s41598-018-34003-8.
81. Mansego ML, Milagro FI, Zulet MA, Moreno-Aliaga MJ, Martinez JA. Differential DNA Methylation in Relation to Age and Health Risks of Obesity. International journal of molecular sciences 2015;16(8):16816-32. doi: 10.3390/ijms160816816.
82. Nagashima M, Miwa N, Hirasawa H, Katagiri Y, Takamatsu K, Morita M. Genome-wide DNA methylation analysis in obese women predicts an epigenetic signature for future endometrial cancer. Sci Rep 2019;9(1):6469. doi: 10.1038/s41598-019-42840-4.
83. Ollikainen M, Ismail K, Gervin K, Kyllönen A, Hakkarainen A, Lundbom J, Järvinen EA, Harris JR, Lundbom N, Rissanen A, et al. Genome-wide blood DNA methylation alterations at regulatory elements and heterochromatic regions in monozygotic twins discordant for obesity and liver fat. Clinical epigenetics 2015;7(1):39. doi: 10.1186/s13148-015-0073-5.
84. Samblas M, Mansego ML, Zulet MA, Milagro FI, Martinez JA. An integrated transcriptomic and epigenomic analysis identifies CD44 gene as a potential biomarker for weight loss within an energy-restricted program. European journal of nutrition 2019;58(5):1971-80. doi: 10.1007/s00394-018-1750-x.
85. Gu Y, Zhang CW, Wang L, Zhao Y, Wang H, Ye Q, Gao S. Association analysis between body mass index and genomic DNA methylation across 15 major cancer types. Journal of Cancer 2018;9(14):2532-42. doi: 10.7150/jca.23535.
86. Luisa Mansego M, Zulet M, Campion J J, Milagro F F, Alfredo Martinez J. Epigenomic association study in weight loss after a nutritional intervention: RESMENA Study. Anales de la Real Academia Nacional de Farmacia 2014;80(3):614-23.
87. Milagro FI, Campion J, Cordero P, Goyenechea E, Gomez-Uriz AM, Abete I, Zulet MA, Martinez JA. A dual epigenomic approach for the search of obesity biomarkers: DNA methylation in

relation to diet-induced weight loss. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2011;25(4):1378-89. doi: 10.1096/fj.10-170365.
88. Hair BY, Xu Z, Kirk EL, Harlid S, Sandhu R, Robinson WR, Wu MC, Olshan AF, Conway K, Taylor JA, et al. Body mass index associated with genome-wide methylation in breast tissue. Breast Cancer Res Treat 2015;151(2):453-63. doi: http://dx.doi.org/10.1007/s10549-015-3401-8.
89. Dekkers KF, van Iterson M, Slieker RC, Moed MH, Bonder MJ, van Galen M, Mei H, Zhernakova DV, van den Berg LH, Deelen J, et al. Blood lipids influence DNA methylation in circulating cells. Genome Biology 2016;17(1):138. doi: 10.1186/s13059-016-1000-6.
90. Richardson TG, Zheng J, Davey Smith G, Timpson NJ, Gaunt TR, Relton CL, Hemani G. Mendelian Randomization Analysis Identifies CpG Sites as Putative Mediators for Genetic Influences on Cardiovascular Disease Risk. The American Journal of Human Genetics 2017;101(4):590-602. doi: https://doi.org/10.1016/j.ajhg.2017.09.003.
91. Elliott HR, Shihab HA, Lockett GA, Holloway JW, McRae AF, Smith GD, Ring SM, Gaunt TR, Relton CL. Role of DNA Methylation in Type 2 Diabetes Etiology: Using Genotype as a Causal Anchor. Diabetes 2017;66(6):1713-22. doi: 10.2337/db16-0874.
92. Richardson TG, Haycock PC, Zheng J, Timpson NJ, Gaunt TR, Davey Smith G, Relton CL, Hemani G. Systematic Mendelian randomization framework elucidates hundreds of CpG sites which may mediate the influence of genetic variants on disease. Hum Mol Genet 2018;27(18):3293-304. doi: 10.1093/hmg/ddy210.
93. Sayols-Baixeras S, Tiwari HK, Aslibekyan SW. Disentangling associations between DNA methylation and blood lipids: a Mendelian randomization approach. BMC Proceedings 2018;12(9):23. doi: 10.1186/s12919-018-0119-8.
94. Dugué P-A, Wilson R, Lehne B, Jayasekara H, Wang X, Jung C-H, Joo JE, Makalic E, Schmidt DF, Baglietto L, et al. Alcohol consumption is associated with widespread changes in blood DNA methylation: Analysis of cross-sectional and longitudinal data. Addiction Biology;n/a(n/a):e12855. doi: 10.1111/adb.12855.
95. Wilson LE, Xu Z, Harlid S, White AJ, Troester MA, Sandler DP, Taylor JA. Alcohol and DNA Methylation: An Epigenome-Wide Association Study in Blood and Normal Breast Tissue. American journal of epidemiology 2019;188(6):1055-65. doi: 10.1093/aje/kwz032.
96. Liu C, Marioni RE, Hedman ÅK, Pfeiffer L, Tsai PC, Reynolds LM, Just AC, Duan Q, Boer CG, Tanaka T, et al. A DNA methylation biomarker of alcohol consumption. Molecular Psychiatry 2018;23(2):422-33. doi: 10.1038/mp.2016.192.
97. Sayon-Orea C, Martinez-Gonzalez MA, Bes-Rastrollo M. Alcohol consumption and body weight: a systematic review. Nutrition reviews 2011;69(8):419-31. doi: 10.1111/j.1753-4887.2011.00403.x.
98. Do WL, Whitsel EA, Costeira R, Masachs OM, Le Roy CI, Bell JT, L RS, Stein AD, Smith AK, Horvath S, et al. Epigenome-wide association study of diet quality in the Women's Health Initiative and TwinsUK cohort. International journal of epidemiology 2020. doi: 10.1093/ije/dyaa215.
99. Lee J-E, Kim H-R, Lee M-h, Kim N-H, Wang K-M, Lee S-h, Park O, Hong E-J, Youn J-W, Kim Y-Y. Smoking-Related DNA Methylation is Differentially Associated with Cadmium Concentration in Blood. Biochemical Genetics 2020;58(4):617-30. doi: 10.1007/s10528-020-09965-y.
100. Wilson R, Wahl S, Pfeiffer L, Ward-Caviness CK, Kunze S, Kretschmer A, Reischl E, Peters A, Gieger C, Waldenberger M. The dynamics of smoking-related disturbed methylation: a two time-point study of methylation change in smokers, non-smokers and former smokers. BMC genomics 2017;18(1):805. doi: 10.1186/s12864-017-4198-0.
101. Joehanes R, Just AC, Marioni RE, Pilling LC, Reynolds LM, Mandaviya PR, Guan W, Xu T, Elks CE, Aslibekyan S, et al. Epigenetic Signatures of Cigarette Smoking. Circulation Cardiovascular genetics 2016;9(5):436-47. doi: 10.1161/circgenetics.116.001506.

102. Ringh MV, Hagemann-Jensen M, Needhamsen M, Kular L, Breeze CE, Sjöholm LK, Slavec L, Kullberg S, Wahlström J, Grunewald J, et al. Tobacco smoking induces changes in true DNA methylation, hydroxymethylation and gene expression in bronchoalveolar lavage cells. EBioMedicine 2019;46:290-304. doi: 10.1016/j.ebiom.2019.07.006.
103. Zhu X, Li J, Deng S, Yu K, Liu X, Deng Q, Sun H, Zhang X, He M, Guo H, et al. Genome-Wide Analysis of DNA Methylation and Cigarette Smoking in a Chinese Population. Environmental Health Perspectives 2016;124(7):966-73. doi: doi:10.1289/ehp.1509834.
104. Dugué PA, Jung CH, Joo JE, Wang X, Wong EM, Makalic E, Schmidt DF, Baglietto L, Severi G, Southey MC, et al. Smoking and blood DNA methylation: an epigenome-wide association study and assessment of reversibility. Epigenetics 2020;15(4):358-68. doi: 10.1080/15592294.2019.1668739.
105. Qiu W, Wan E, Morrow J, Cho MH, Crapo JD, Silverman EK, DeMeo DL. The impact of genetic variation and cigarette smoke on DNA methylation in current and former smokers from the COPDGene study. Epigenetics 2015;10(11):1064-73. doi: 10.1080/15592294.2015.1106672.
106. Sayols-Baixeras S, Lluís-Ganella C, Subirana I, Salas LA, Vilahur N, Corella D, Muñoz D, Segura A, Jimenez-Conde J, Moran S, et al. Identification of a new locus and validation of previously reported loci showing differential methylation associated with smoking. The REGICOR study. Epigenetics 2015;10(12):1156-65. doi: 10.1080/15592294.2015.1115175.
107. Ambatipudi S, Cuenin C, Hernandez-Vargas H, Ghantous A, Calvez-Kelm FL, Kaaks R, Barrdahl M, Boeing H, Aleksandrova K, Trichopoulou A, et al. Tobacco smoking-associated genome-wide DNA methylation changes in the EPIC study. Epigenomics 2016;8(5):599-618. doi: 10.2217/epi-2016-0001.
108. Sikdar S, Joehanes R, Joubert BR, Xu C-J, Vives-Usano M, Rezwan FI, Felix JF, Ward JM, Guan W, Richmond RC, et al. Comparison of smoking-related DNA methylation between newborns from prenatal exposure and adults from personal smoking. Epigenomics 2019;11(13):1487-500. doi: 10.2217/epi-2019-0066.
109. Guida F, Sandanger TM, Castagné R, Campanella G, Polidoro S, Palli D, Krogh V, Tumino R, Sacerdote C, Panico S, et al. Dynamics of smoking-induced genome-wide methylation changes with time since smoking cessation. Hum Mol Genet 2015;24(8):2349-59. doi: 10.1093/hmg/ddu751.
110. Zeilinger S, Kühnel B, Klopp N, Baurecht H, Kleinschmidt A, Gieger C, Weidinger S, Lattka E, Adamski J, Peters A, et al. Tobacco Smoking Leads to Extensive Genome-Wide Changes in DNA Methylation. PLoS ONE 2013;8(5):e63812. doi: 10.1371/journal.pone.0063812.
111. Marabita F, Almgren M, Sjöholm LK, Kular L, Liu Y, James T, Kiss NB, Feinberg AP, Olsson T, Kockum I, et al. Smoking induces DNA methylation changes in Multiple Sclerosis patients with exposure-response relationship. Sci Rep 2017;7(1):14589. doi: 10.1038/s41598-017-14788-w.
112. Marzi C, Holdt LM, Fiorito G, Pei-Chien T, Kretschmer A, Wahl S, Guarrera S, Teupser D, Spector TD, Iacoviello L, et al. Epigenetic Signatures at AQP3 and SOCS3 Engage in Low-Grade Inflammation across Different Tissues. PLoS ONE 2016;11(11). doi: http://dx.doi.org/10.1371/journal.pone.0166015.
113. Sun Y, Smith A, Conneely KN, Chang Q, Li W, Lazarus A, Smith JA, Almli L, Binder EB, Klengel T, et al. Epigenomic association analysis identifies smoking-related DNA methylation sites in African Americans. Human genetics 2013;132(9):1027-37. doi: doi:10.1007/s00439-013-1311-6.

Tables and Figures

Table 2.1. *PECO Guidelines of inclusion criteria.*

Population	Non-pregnant adults aged 18-75
Exposure/Control	Adiposity measured dichotomously or continuously via body mass index (BMI), body fat percentage, waist-to-height ratio, waist-to-hip ratio, android-gynoid fat ratio, waist circumference or weight gain/loss in cross sectional or longitudinal trial
Outcome	DNA methylation as measured using genome-wide approaches, including array based measurement (27K, 450K and EPIC array) or bisulfite sequencing

Table 2.2. *Studies identified examining genome-wide DNA methylation and adiposity*

Reference	N	Exposure	Outcome	Array	Biological Sample	Key Findings
Agha, et al., 2015, Int J Epidemiol	106	Android fat mass, android-ganoid fat ratio, trunk:limb ratio, and body mass index (BMI)	DNA methylation M-value	450K	Adipose tissue & peripheral blood leucocytes	Adipose tissue DNA methylation was associated with all four adiposity phenotypes. When adjusted for adipose cell composition, associations remained. In peripheral blood, DNA methylation not associated with any adiposity phenotype.
Al Muftah, et al., 2016, Clin Epigenetics	123 in Qatari Family Study, 810 in TwinsUK	DNA methylation β-value	Standardized BMI	450K	Whole Blood	No sites associated with BMI in full EWAS. The study replicated previous associations in 39 sites. 8 sites replicated at a Bonferroni-corrected p-value.
Almen et al., 2014, Gene	46 (24 obese and 22 lean)	Obese (BMI ≥ 30) vs. Normal Weight (BMI <25)	DNA methylation β-value	27K	Peripheral Blood	No sites associated independently with obesity. Examined interaction between age and obesity and found 8 associations in age-associated CpG sites.
Arner, et al., 2015, Clin Epigenetics	29	Obese (BMI ≥ 30) vs. Never-obese (BMI <30)	DNA methylation M-value	450K	Subcutaneous white adipose tissue	Identified 32,724 differentially methylated sites. Among those sites linked to genes (excluding unannotated sites), found that 3,878 genes were differentially expressed in white adipose tissue. Of those genes, 5529 CpG sites were associated with 2223 genes differentially expressed between obese cases and non-obese controls.
Arpon, et al., 2019, Genes	474	Waist Circumference	DNA methylation β-value	450K	Peripheral white blood cells	Found 669 sites associated with waist circumference. Among these sites when stratified by sex and examining differences dichotomized waist circumference, significant differences identified in 375 CpG sites in females and 95 CpG sites in males.
Aslibekyan, et al., 2015, Obesity	GOLDN n=991, FHS n=2377, ARIC n=2106	BMI and waist circumference	DNA methylation β-value	450K	CD4+ T-cells	In GOLDN, 8 sites associated with BMI and 5 sites associated with waist circumference. In replication analyses, 4 out of 8 replicated in FHS case-control with 2 of the 4 replicating in

						larger FHS cohort (CPT1A and PHGDH).
Bollepalli, et al., 2018, Int J Obes	19 participants in weight loss intervention, validation in 26 BMI-discordant twins from the Finnish Twin Cohort Study	Fat percentage	DNA methylation	450K	Subcutaneous Adipose Tissue	No genome-wide significant sites were identified when examining change in methylation from any time point in the weight loss intervention. Using a targeted approach examining only sites in the 69 genes where expression changed in the first 5 months, 28 CpG sites differentially correlated. Of the 5 genes differentially expressed between 5 to 12 months, 3 CpG sites showed differential correlations. Between baseline and 12 months, among 35 differentially expressed genes, 23 CpG sites were differentially correlated.
Campanella, et al., 2018, Int J Obes	EPIC-Netherlands n=148, EPIC-Italy n=1274, NOWAC n=192, EnviroGenoMarkers n=641	BMI, waist circumference, waist to hip ratio and waist to height ratio	DNA methylation β-value	450K	Peripheral blood leukocytes	In discovery analysis, 40 CpG sites associated with one or more adiposity measure. 26 sites associated with BMI, 12 associated with waist circumference, 9 waist-to-hip ratio, and 12 for waist-to-height ratio. cg06500161 associated with all four adiposity measures. Examining replication by pyrosequencing, associations yielded a linear relationship.
Cheng, et al., 2018, Clin Epigenetics	10 (5 normal weight, 5 overweight/obese)	Overweight and Obese (BMI ≥ 25) vs. Normal Weight (BMI < 25)	DNA methylation M-value	450K	Periprostatic adipose tissue	Differential methylation was associated with 5526 after FDR control (adjusted P < 0.25).
Crujeiras, et al., 2017, Endocr Relat Cancer	Discovery: 64, Validation: 99	Overweight and Obese (BMI ≥25) vs. Normal Weight (BMI < 25)	DNA methylation β-value	450K	Breast tumor tissue	No sites significant in EWAS. Subsequently examined high risk group (pre-menopausal normal weight and post-menopausal obese) vs low risk group (pre-menopausal obese and post-menopausal normal weight) finding 1,287 differentially methylated CpG sites.
Crujeiras, et al., 2017, Sci Rep	55 (8 adipose/10 leucocytes non-obese and 45 paired	Overweight and Obese (BMI ≥ 25) vs. Normal Weight (BMI ≤ 25)	DNA methylation β-value	450K	Subcutaneous adipose tissue/Leukocytes	Found 12,043 CpG sites were differentially methylated in subcutaneous adipose tissue of obese vs. non-obese participants. In leukocytes, 4,815 CpG sites were

	adipose/leucocytes obese)					differentially methylated. 176 sites were common among the two analyses.
Crujeiras, et al., 2019, Int J Obes	28	Overweight and Obese (BMI ≥ 25) vs. Normal Weight (BMI < 25)	DNA methylation β-value	450K	Colorectal tumor tissue	Identified 299 differentially methylated CpG sites between non-obese and obese patients.
Day, et al., 2016, Clin Epigenetics	22	Obese vs. Lean	Reduced Representation Bisulfite Sequencing	Reduced Representation Bisulfite Sequencing	Vastus lateralis muscle tissue	Found 13,130 differentially methylated cytosines (unadjusted p <0.05). After FDR adjustment, no site were associated with the obesity.
Day, et al., 2017, Epigenetics	20	Obese (BMI ≥ 30) vs. Normal Weight (BMI <25)	Reduced Representation Bisulfite Sequencing	Reduced Representation Bisulfite Sequencing	Whole Blood	Of the 5,227,488 CpG sites examined, 52,995 were significantly different between groups (unadjusted p-value < 0.05). 49 CpG sites remained significantly associated with obesity after FDR correction.
Demerath, et al., 2015, Hum Mol Genet	2097	BMI, waist circumference and BMI change	DNA methylation β-value	450K	Leucocytes	In discovery analysis, BMI, waist circumference and BMI change were associated with 76, 164 and 8 CpG sites, respectively. In replication, 37/76 BMI-associated sites and 8/164 waist circumference associated sites replicated in FHS and GOLDN. Additionally, among replicated sites, 18 BMI-associated sites and 1 waist circumference associated site replicated in adipose tissue.
Dhana, et al., 2018, Am J Epidemiol	1450	BMI and waist circumference	DNA methylation β-value	450K	Peripheral whole blood	In discovery analysis, 14 sites associated with BMI and 26 associated with WC. In ARIC, 12 successfully replicated for BMI and 13 of 26 replicated for WC.
Dick et al., 2014, Lancet	459 (20 excluded)	DNA methylation β-value	Log-transformed BMI	450K	Whole Blood	Five sites associated with log-transformed BMI. In replication analyses in MARTHA, three sites in HIF3A were significant after Bonferroni-adjustment. In further replication in KORA, all three HIF3A probes replicated.
Dong, et al., 2019, Sci Rep	15 CRC patients, 10 obese subjects and 15 lean controls	DNA methylation	Obese vs. Lean	Reduced Representation Bisulfite Sequencing	Whole Blood	Study examining overlap between differential methylation associated with obesity and differential methylation associated with colorectal cancer.

						91,809 differentially methylated sites associated with obesity.
Geurts, et al., 2018, Int J Obes	5361 (2775 control, 2586 cases)	Log-transformed BMI	DNA methylation M-value	450K	Peripheral blood mononuclear cells, dried blood spots and buffy coats	Study identified novel associations in 225 replicated sites. Results were consistent in 34 sites in repeated measures analyses of BMI change and DNA methylation change.
Gomez-Uriz, et al., 2015, Hum Mol Genet	24 (6 non-stroke non-obese, 6 stroke non-obese, 6 obese non-stroke, 6 obese stroke patients)	Obesity (BMI >30) vs Normal weight (BMI <25)	DNA methylation β-value	27K	White Blood Cells	Between obese and control non-stroke patients, 96 CpG sites were differentially methylated ($p<0.05$ and methylation difference > 5%). Further examined differences in stroke patients.
Gu, et al., 2018, J Cancer	2415 (883 non-obese, 1532 obese)	Obesity (BMI >30) vs Normal weight (BMI <25) & BMI	DNA methylation M-value or β-value (unknown which)	450K	Tumor tissue of 15 cancer types	Between obese and normal weight, 3, 1169, and 394 sites were differentially methylated in cholangiocarcinoma, colon adenocarcinoma and uterine corpus endometrial carcinoma, respectively. In BMI models, 1 site was differentially methylated in cholangiocarcinoma and colon adenocarcinoma.
Hair, et al., 2015, Breast Cancer Res Treat	81	BMI	DNA methylation β-value	450K	Breast tissue	2,573 CpG sites were associated with BMI after adjustment for age, race, and alcohol use.
Hohos, et al., 2018, Obesity	81	Obesity (BMI >30) vs Normal weight (BMI <25) and visceral adipose tissue	DNA methylation M-value	450K	CD4+ Tcells, CD8+ Tcells and CD16+ neutrophils	In models examining categorical BMI, 19 sites were differentially methylated in CD4+ T-cells. 16 sites were differentially methylated in CD8+ T-cells. No sites were differentially methylated in CD16+ neutrophils. In models examining visceral adipose tissue, 79 sites were differentially methylated in CD4+ T cells.
Huang, et al., 2015, Int J Obes	48 (16 in each group)	Normal weight never obese, weight loss maintainers, and obese	DNA methylation M-value	450K	Peripheral blood mononuclear cells	No sites were associated following false discovery rate adjustment. Subsequently examined top 20 loci.
Krichner, et al., 2016, Mol Metab	22	Non-obese non-diabetic, obese non-diabetic, and	DNA methylation M-value	450K	Liver tissue	Found 5,834 sites that were differentially methylated in one of the t-test analyses.

		obese type 2 diabetic				
Kvaloy, et al., 2018, Sci Rep	120	Obese (largest BMI in cohort) vs. Lean (lowest BMI in cohort)	DNA methylation M-value	450K	Whole Blood	In fully adjusted model, 10 sites were differentially methylated between two groups. Of the 10, 3 sites replicated in FDR-adjusted models.
S. Li, et al., 2019, Int J Obes	66 monozygotic (MZ) twins, 66 dizygotic (DZ) twins and 215 of their sisters from 130 families	Self-reported current BMI, BMI at 18-21 years and change between the two time points	DNA methylation M-value	450K	Dried blood spot	Current BMI, BMI at age 18-21 and change in BMI was associated with nine, six and 12 CpG sites, respectively. Two sites associated with both current BMI and BMI change.
W. Li, et al., 2019, Int J Obes	30 MZ twin pairs (15 male, 15 female pairs)	BMI	DNA methylation M-value	Reduced Representation Bisulfite Sequencing	Whole Blood	No sites reached genome-wide significance. Subsequently conducted functional analyses of sites with p-value < 0.05.
Mansego, et al., 2014, Anales de la Real Academica Nacional de Farmacia	46 obese subjects (28 positive response to weight loss diet [>= 5% weight loss in initial 24 weeks], 20 poor response to weight loss diet [< 5% weight loss])	Positive response to weight loss diet (≥5% weight loss in initial 24 weeks of intervention) vs. poor response to weight loss (<5% weight loss)	DNA methylation β-value	450K	Whole Blood	No sites reached genome-wide significance, 90 sites associated with p-value < 0.01. Further analyzed top ten sites associated with response to dietary intervention.
Mansego, et al., 2015, Int J Mol Sci	73	Low risk obesity (overweight or class I obesity; BMI 25-35, [LRO]) and High risk obesity (class 2 and 3 obesity; BMI ≥35, [HRO])	DNA methylation β-value	450K	White Blood Cells	85 sites were differentially methylated between low HRO and high HRO. However, none were significant after FDR adjustment.
Meeks, et al., 2017, Clin Epigenetics	547	BMI and waist circumference	DNA methylation M-value	450K	Whole blood	18 sites found to associate with BMI and 23 sites associated with waist circumference.
Mendelson, et al., 2017, PLoS Med	Discovery: 3724, Replication: 4055	BMI	Inverse-normal DNA methylation	450K	Whole blood	In meta-analysis, 135 CpG sites significantly associated with BMI. In replication analyses, 83 sites replicated with at least one cohort.
Milagro, et al., 2011, Faseb j	12 (6 high responders, 6 low responders)	Positive response to weight loss diet (>=5% weight loss	DNA methylation	27K	Peripheral blood mononuclear cells	At baseline, 1,034 sites were differentially methylated between groups. At endpoint, 15 sites were

		in initial 24 weeks of intervention) vs. poor response to weight loss (<5% weight loss)				differentially methylated. When pooled, 170 sites were differentially methylated as a result of the energy-restricted dietary intervention.
Nagashima, et al., 2019, Sci Rep	8	Normal weight vs obese	DNA methylation β-value	EPIC	Endometrial epithelial cells	10,601 sites identified as differentially methylated with p<0.05 and slope > 0.2.
Ollikainen, et al., 2015, Clin Epigenetics	80 (40 twin pairs)	BMI-discordance in twins	DNA methylation M-value	450K	Whole Blood	After correcting for multiple comparisons and applying a relevance cutoff (mean within-pair difference >=5%), no sites were differentially methylated among the 30 BMI-discordant twins. However, when comparing discordant BMI twins with differing levels of liver fat (n=13 twins), 1,236 sites were differentially methylated.
Orozco, et al., 2018, Hum Mol Genet	201	DNA methylation	Inverse-normal clinical traits	Reduced Representation Bisulfite Sequencing	Subcutaneous Adipose Tissue	51 associations corresponding to 21 sites were associated with 15 unique phenotypes.
Pietilaeinen, et al., 2016, Int J Obes	37 twin pairs (26 BMI-discordant twins and 11 BMI-concordant twin pairs)	BMI-discordance in twins	DNA methylation M-value	450K	Subcutaneous Adipose Tissue	In BMI-discordant twins, 22 CpGs were differentially methylated.
Ramos-Lopez, et al., 2018, Appetite	474	BMI	DNA methylation β-value	450K	Peripheral white blood cells	7,457 CpG sites correlated with BMI (adjusted FDR <0.0001)
Rönn, 2015, Hum Mol Genet	96	BMI	DNA methylation M-value	450K	Subcutaneous Adipose Tissue	In male discovery cohort, 33,058 CpG sites significantly associated with BMI. In female cohort, 39,533 CpG sties associated with BMI. 4,979 sites overlapped between male and female cohort.
Samblas, et al., 2019, Eur J Nutr	47 (LR, n=31; HR, n=16), validation n=47 (LR, n=26; HR, n=21)	Low responders (weight loss < 8% of initial weight) vs high responders (weight loss > 8% initial weight)	DNA methylation β-value	450K	White Blood Cells	2,102 sites were differentially methylated between HR and LR groups. No sites remained significant after FDR adjustment.

Sayols-Baixeras, et al., 2017, Epigenetics	641 (REGICOR), 2515 (FOS)	DNA methylation M-value	BMI and waist circumference	450K	Whole Blood	40 sites were significantly associated with BMI and 7 with waist circumference in Model 1. In model 2, 214 sites associated with BMI and 36 associated with waist circumference. In meta-analyses, 52 sites associated with BMI and 26 associated waist circumference in model 1. In model 2, 94 sites associated with BMI and 49 sites associated with waist circumference.
Shah, et al., 2015, Am J Hum Genet	LBC 1921: 446, LBC 1936: 920, LifeLines DEEP: 750	DNA methylation M-value	BMI Z-score	450K	Whole Blood	Nine CpG sites in LBC dataset and five sites in LifeLines DEEP cohort associated with BMI. Two sites overlapped in each (cg06500161 and cg11024682).
Sun, et al., 2019, Circulation	1485 (995 white and 490 black), Replication 480 (252 white and 228 black)	BMI	DNA methylation β-value	450K	Whole blood	In discovery analysis, 3966 and 85 CpG sites were significantly associated with BMI in white and black participants, respectively. Among these, 349 and 36 sites replicated in the GSHS cohort.
Wahl, et al., 2017, Nature	KORA F4: 1709, KORA F3: 485, LOLIPOP: 2680, EPICOR: 584; Replication: ALSPAC: 701, BIOS: 4000, EGCUT: 269, LOLIPOP: 656, RS: , TwinsUK: 355, LifeLines Deep: , LLS	DNA methylation β-value	BMI	450K	Whole Blood	278 sites associated with BMI. In replication analyses, 187 out of 207 replicated with directional consistency and epigenome-wide significance in meta-analysis.
Wilson, et al., 2017, Int J Obes	871, replication 187	BMI	DNA methylation β-value	27K	Blood	In the analysis excluding women who later developed breast cancer (n=571), two sites associated BMI. When additionally including these cases (adjusting for case-status), four sites associated with BMI, including the original two sites identified.
Xu, et al., 2018, Biol Psychol	510	BMI	DNA methylation β-value	450K	Whole Blood	In the BMI EWAS, 20 sites were associated with BMI following FDR-adjustment.

Table 2.3. *Assessment of potential bias and other study design issues*

Reference	Sample	Exposure	Outcomes	Adjustment	Statistical Analysis
Agha, 2015, IJE	Low	Low	Low	Low	Low
Al Muftah, 2016, Clinical Epigenetics	Low	Unclear	Low	Unclear	Low
Almen, 2014, Gene	Low	Low	Low	Unclear	Unclear
Arner, 2015, Clinical Epigenetics	Low	Low	Low	High	Low
Arpon, et al., 2019, Genes	Unclear	Low	Low	Unclear	Low
Aslibekyan, 2015, Obesity	Low	Low	Low	Low	Low
Bollepalli, 2018, International Journal of Obesity	Low	Low	Low	Unclear	Unclear
Campanella, 2018, IJO	Low	Low	Low	Unclear	Low
Cheng, 2018, Clinical Epigenetics	Low	Low	Low	Unclear	Low
Crujeiras, 2017, Endocrine-Related Cancer	Low	Low	Low	High	Low
Crujeiras, 2017, Scientific Reports	Low	Low	Low	High	Low
Crujeiras, 2019, International Journal of Obesity	Low	Low	Low	High	Low
Day, 2016, Clinical Epigenetics	Low	Unclear	Low	Unclear	Low
Day, 2017, Epigenetics	Low	Low	Low	Unclear	Low
Demerath, 2015, Human Molecular Genetics	Low	Low	Low	Low	Low
Dhana, 2018, AJE	Low	Low	Low	Low	Low
Dick, 2014, Lancet	Low	Low	Unclear	Unclear	Low
Dong, 2019, Scientific Reports	Low	Unclear	Low	High	Low
Geurts, 2018, Int J Obes	Low	Low	Low	Low	Low
Gomez-Uriz, 2015, Human Molecular Genetics	Low	Low	Low	High	Unclear
Gu, 2018, J Cancer	Low	Low	Low	High	Unclear
Hair, 2015, Breast Cancer Res Treat	Low	Low	Low	Unclear	Low
Hohos, 2018, Obesity	Low	Low	Low	Unclear	Low

Huang, 2015, IJO	Low	Low	Low	Unclear	Low
Krichner, 2016, Molecular Metabolism	Low	Unclear	Low	High	Low
Kvaloy, 2018, Scientific Reports	Low	Low	Low	Low	Low
S. Li, 2019, IJO	Low	Low	Low	Low	Low
W. Li, 2019, IJO	Low	Low	Low	Low	Low
Mansego, 2014, Anales de la Real Academica Nacional de Farmacia	Low	Low	Low	Unclear	Unclear
Mansego, 2015, International Journal of Molecular Science	Low	Low	Low	Low	Low
Meeks, 2017, Clinical Epigenetics	Low	Low	Low	Low	Low
Mendelson, 2017, PLOS Medicine	Low	Low	Low	Low	Low
Milagro, 2011, FASEB J	Low	Low	Low	High	Unclear
Nagashima, 2019, Scientific Reports	Low	Low	Low	High	Unclear
Ollikainen, 2015, Clinical Epigenetics	Low	Low	Low	Low	Low
Orozco, 2018, Human Molecular Genetics	Low	Low	Low	High	Low
Pietilaeinen, 2016, IJO	Low	Unclear	Low	Unclear	Unclear
Ramos-Lopez, 2018, Appetite	Low	Low	Low	Low	Low
Rönn, 2015, Human Molecular Genetics	Low	Low	Low	Unclear	Low
Samblas, 2019, European Journal of Nutrition	Low	Low	Low	High	High
Sayols-Baixeras, 2017, Epigenetics	Low	Low	Low	Low	Low
Shah, 2015, Am J Hum Genet	Low	Low	Low	Unclear	Low
Sun, 2019, Circulation	Low	Low	Low	Low	Low
Wahl, 2017, Nature	Low	Low	Low	Low	Low
Wilson, 2017, IJO	Low	Low	Low	Low	Low
Xu, 2018, Biol Psychol	Low	Low	Low	Low	Low

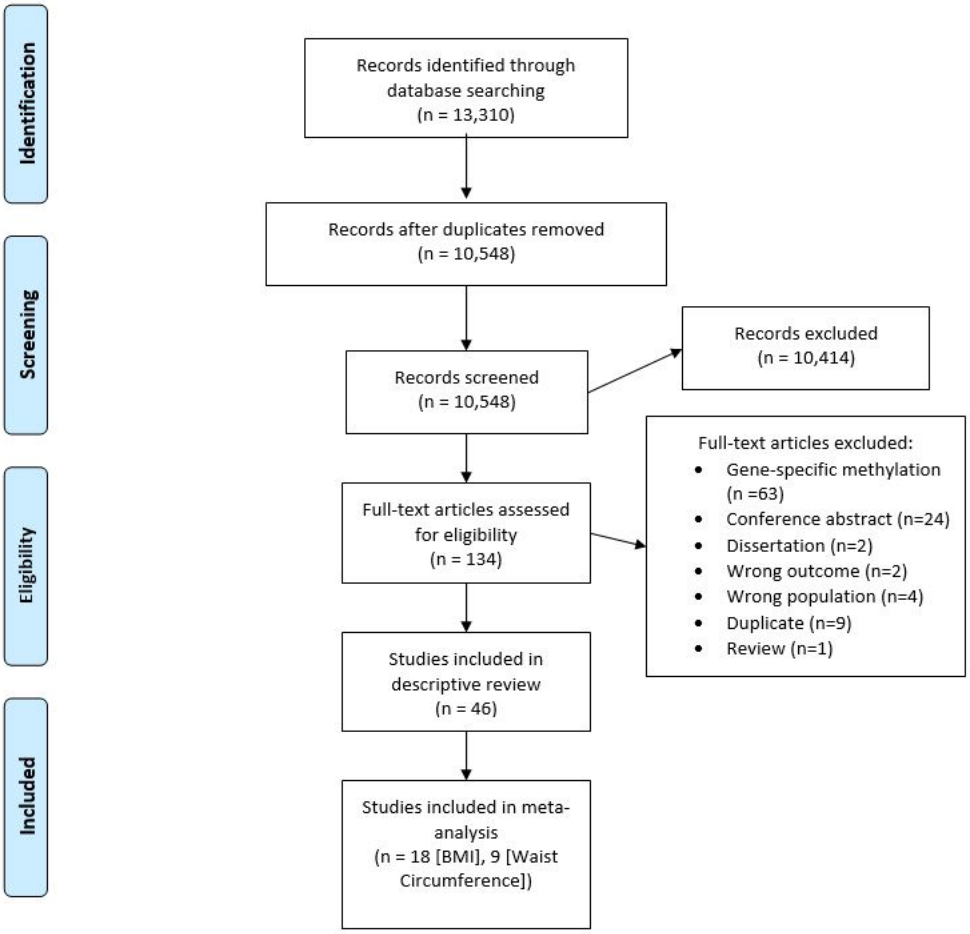


Figure 2.1. *Flow diagram of studies*

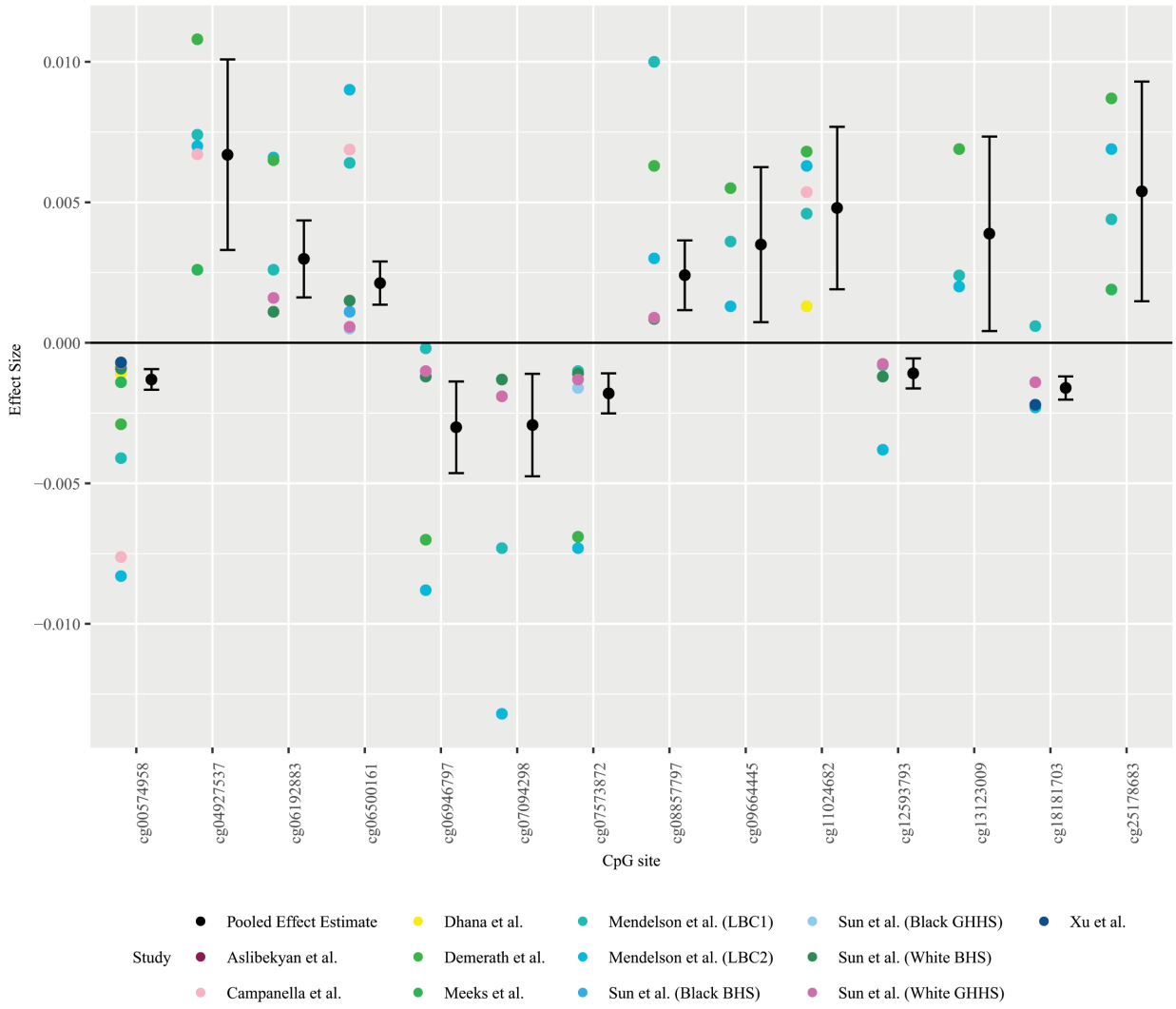
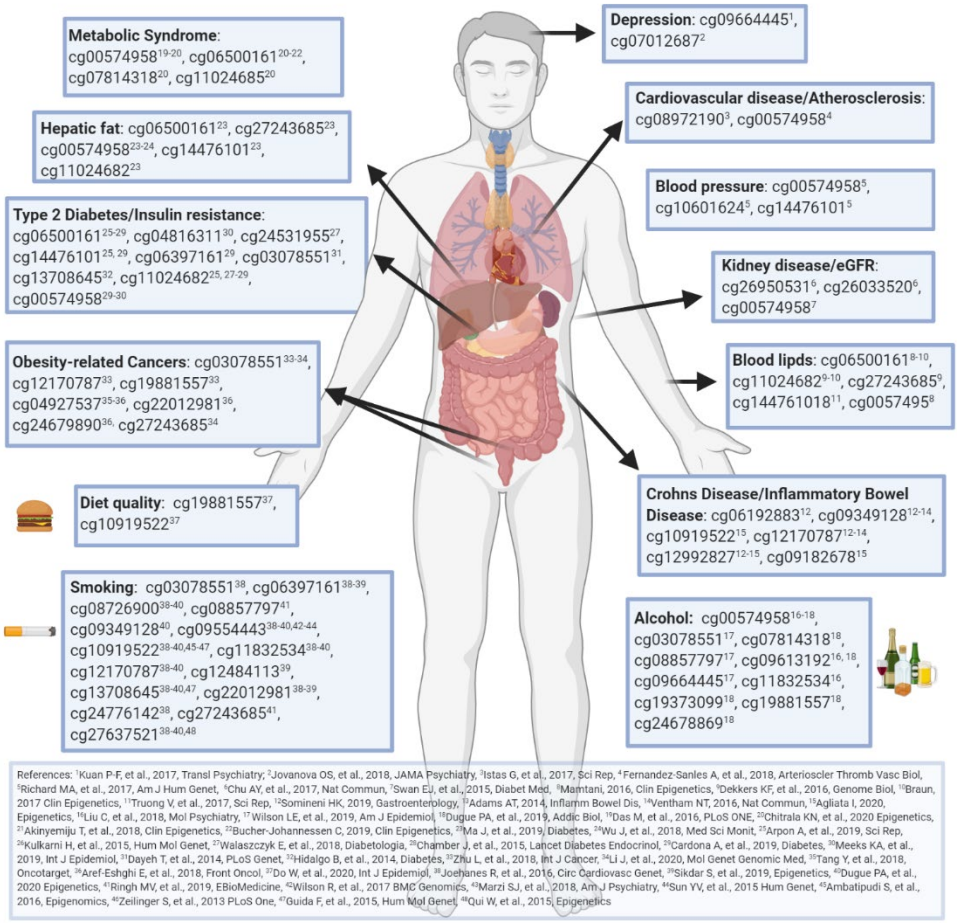


Figure 2.2. *Random-effect meta-analysis of CpG sites that were significant in 5 or more studies.*



References: [1]Kuan P-F, et al., 2017, Transl Psychiatry; [2]Jovanova OS, et al., 2018, JAMA Psychiatry, [3]Istas G, et al., 2017, Sci Rep, [4]Fernandez-Sanles A, et al., 2018, Arterioscler Thromb Vasc Biol, [5]Richard MA, et al., 2017, Am J Hum Genet, [6]Chu AY, et al., 2017, Nat Commun, [7]Swan EJ, et al., 2015, Diabet Med, [8]Mamtani, 2016, Clin Epigenetics, [9]Dekkers KF, et al., 2016, Genome Biol, [10]Braun, 2017 Clin Epigenetics, [11]Truong V, et al., 2017, Sci Rep, [12]Somineni HK, 2019, Gastroenterology, [13]Adams AT, 2014, Inflamm Bowel Dis, [14]Ventham NT, 2016, Nat Commun, [15]Agliata I, 2020, Epigenetics, [16]Liu C, et al., 2018, Mol Psychiatry, [17]Wilson LE, et al., 2019, Am J Epidemiol, [18]Dugue PA, et al., 2019, Addic Biol, [19]Das M, et al., 2016, PLoS ONE, [20]Chitrala KN, et al., 2020 Epigenetics, [21]Akinyemiju T, et al., 2018, Clin Epigenetics, [22]Bucher-Johannessen C, 2019, Clin Epigenetics, [23]Ma J, et al., 2019, Diabetes, [24]Wu J, et al., 2018, Med Sci Monit, [25]Arpon A, et al., 2019, Sci Rep, [26]Kulkarni H, et al., 2015, Hum Mol Genet, [27]Walaszczyk E, et al., 2018, Diabetologia, [28]Chamber J, et al., 2015, Lancet Diabetes Endocrinol, [29]Cardona A, et al., 2019, Diabetes, [30]Meeks KA, et al., 2019, Int J Epidemiol, [31]Dayeh T, et al., 2014, PLoS Genet, [32]Hidalgo B, et al., 2014, Diabetes, [33]Zhu L, et al., 2018, Int J Cancer, [34]Li J, et al., 2020, Mol Genet Genomic Med, [35]Tang Y, et al., 2018, Oncotarget, [36]Aref-Eshghi E, et al., 2018, Front Oncol, [37]Do W, et al., 2020, Int J Epidemiol, [38]Joehanes R, et al., 2016, Circ Cardiovasc Genet, [39]Sikdar S, et al., 2019, Epigenetics, [40]Dugue PA, et al., 2020 Epigenetics, [41]Ringh MV, et al., 2019, EBioMedicine, [42]Wilson R, et al., 2017 BMC Genomics, [43]Marzi SJ, et al., 2018, Am J Psychiatry, [44]Sun YV, et al., 2015 Hum Genet, [45]Ambatipudi S, et al., 2016, Epigenomics, [46]Zeilinger S, et al., 2013 PLoS One, [47]Guida F, et al., 2015, Hum Mol Genet, [48]Qui W, et al., 2015, Epigenetics

Figure 2.3. *Previously associations among BMI-associated sites identified within this study and obesity-associated biomarkers and diseases*

CHAPTER 3

Methods

3.1 Study Population

Several study populations have been included in this dissertation and are further discussed with the methods of each Aim. The Women's Health Initiative (WHI) was included in all of the following Chapters and will be elucidated upon here.

Women's Health Initiative

The WHI is a large, U.S.-based cohort study of postmenopausal women, aged 50-79 at time of enrollment, consisting of two study arms: the Clinical Trial (CT) and the Observational Study (OS). The WHI was a national study examining the most common causes of morbidity and mortality in women. Three randomized-controlled trials make up the CT: hormone therapy, dietary modification and calcium/vitamin (1). Within this dissertation, three ancillary studies from the WHI were included in the analyses. These include: Epigenetic Mechanisms of Particulate Matter-Mediated Cardiovascular Disease (EMPC), the Integrative Genomics for Risk of Coronary Heart Disease and Related Phenotypes in WHI cohort (BAA23), and Bladder Cancer and Leukocyte Methylation (AS311). EMPC (n=2200) assessed epigenetic mechanisms underlying associations between ambient particulate matter air pollution and cardiovascular disease within the WHI CT (2). A subset of women from EMPC (n=200) have DNA methylation measured at 3 or 6 years following their baseline sample. BAA23 was a case-control study assessing predictors of coronary heart disease (CHD) within the WHI CT (n=1664) and OS (n=443), where cases were identified using eight biomarkers of CHD. AS311 is a matched case-control study of bladder cancer among women within the WHI CT (n = 426) and OS (n = 456).

3.2 Data Analyses

Expression quantitative trait methylation loci

Examining how DNA methylation associates with gene expression can provide insight into the functional implications of significant sites. In the following aims, the association between significant methylation sites and mRNA transcription were examined using summary statistics from an epigenome-wide association study (EWAS) of mRNA transcription in the Multi-Ethnic Study of Atherosclerosis (MESA) and the Grady Trauma Project (GTP) (2). In these studies, results were only reported for suggestive significance ($p<1E-6$) and highly significant ($p<1E-11$) associations.

References

1. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. Controlled clinical trials 1998;19(1):61-109.
2. Kennedy EM, Goehring GN, Nichols MH, Robins C, Mehta D, Klengel T, Eskin E, Smith AK, Conneely KN. An integrated -omics analysis of the epigenetic landscape of gene expression in human blood cells. BMC genomics 2018;19(1):476. doi: 10.1186/s12864-018-4842-3.

CHAPTER 4

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 4,355 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 HumanMethylation 450 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, 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. 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 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 dysglycemia. These findings may provide insight into pathways through which diet influences chronic disease.

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 US (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 high fat overfeed or Mediterranean diets (6-8), finding some differences in either mean gene methylation or cytosine-phosphate-guanine (CpG) site-specific methylation. Two cross-sectional studies examined individual dietary factors, dietary fat and fiber, respectively with genome-wide DNAm, and reported differential methylation among genes potentially related to metabolism, though neither 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. Moreover, no previous studies have examined diet quality based on measures combining dietary factors shown to contribute to chronic disease progression. 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 Women's Health Initiative (WHI) is a large, U.S.-based cohort study of postmenopausal women, aged 50-79 at time of enrollment, consisting of two study arms: the Clinical Trial (CT) and the Observational Study (OS). Three randomized-controlled trials make up the CT: hormone therapy, dietary modification and calcium/vitamin D (11). The WHI ancillary studies in the discovery cohort included: *Epigenetic Mechanisms of Particulate Matter-Mediated Cardiovascular Disease* (EMPC), the *Integrative Genomics for Risk of Coronary Heart Disease and Related Phenotypes in WHI cohort* (BAA23), and *Bladder Cancer and Leukocyte Methylation* (AS311). EMPC (n=2200) assessed epigenetic mechanisms underlying associations between ambient particulate matter air pollution and cardiovascular disease within the WHI CT (12). BAA23 was a case-control study assessing predictors of coronary heart disease (CHD) within the WHI CT (n=1664) and OS (n=443), where cases were identified using eight biomarkers of CHD. AS311 is a matched case-control study of bladder cancer among women within the WHI CT (n = 426) and OS (n = 456). Diet was measured using a food frequency questionnaire (FFQ) designed by WHI investigators (13).

The replication cohort was derived from the TwinsUK cohort, a large registry of male and female twins between the ages of 19-82 in the United Kingdom (16). This study examined peripheral blood DNA methylation in 497 monozygotic (MZ) and 74 dizygotic (DZ) female twins (17-19). Diet was assessed using the EPIC-Norfolk FFQ (20). DNA methylation in adipose

tissue was also examined in 168 MZ and 232 DZ female twins (37% overlapping with blood methylation replication sample).

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) (13). 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 (14). 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 two years and one 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. Probes on the X and Y chromosomes (11091 probes), probes with detection p-values > 0.01 in >10% of samples, and samples with detection p-values > 0.01 in >1% of probes were excluded (probes excluded prior to analysis). Probe signals showing multiple clusters, which tend to occur when signals are driven by an underlying single nucleotide polymorphism (SNP) or genetic variant, were identified using the gaphunter function in the *minfi* package and removed (1567 probes in EMPC, 3069 in BAA23, and 1898 in AS311) (15). Additional probes were filtered based on general filtering recommendations from Zhou et al. (18334 probes) (16). All methylation data were normalized using beta-mixture quantile (BMIQ) normalization (17).

To examine outliers in the discovery cohort, we conducted a principal component analysis including a random sample of more than half the cytosine-phosphate-guanine (CpG) sites without any missing values (30K of 53K) and the full study sample. The first and second principal components explained 16% and 8.74% of the variation in methylation. Outliers were detected by individuals more than 4 standard deviations away from the mean. Thirteen individuals were identified as outliers. Sensitivity analyses excluding these individuals were conducted. 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 to 100 (lower score indicates poor diet) using the AHEI-2010, which evaluates foods and nutrients strongly predictive of chronic disease (18). 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 (19) and in other settings (18, 20).

Data Analysis

We used R software to conduct all analyses. The discovery analysis flow chart is included as **Figure 4.1**. Overall, 834 women were excluded due to missing dietary intake, implausible dietary intake or overlapping samples. The final discovery cohort included 4,355 women. Epigenome-wide association study (EWAS) meta-analysis was conducting 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, 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 (21). 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.

Additional Post-Hoc Analyses

To examine the degree to which each ancillary study contributed to the study, we examined how the results changed when excluding individual WHI studies. While associations between bladder cancer and diet quality are not well-established (22), if bladder cancer cases in AS311 have differential diet quality, it may confound the relationship between diet and methylation. A sensitivity analysis excluding cases from the AS311 cohort was conducted and compared to the full sample analysis. Additionally, to assess the degree to which adjustment for body mass index (BMI) may have influenced the analysis, we conducted a secondary epigenome-wide association study (EWAS) unadjusted for BMI. Given the potential for socioeconomic status (SES) to confound the relationship between diet quality and methylation, we conducted an EWAS adjusted for income status as a proxy for SES (3 level factor variable reduced from 7 level factor). In a sample of women from AS311 (n=664), no principal components of genetic relatedness were available and EWAS models between diet quality and

DNA methylation were run unadjusted for the top three principal components. We additionally examined results excluding these women.

Results

Demographic characteristics are described by quartile of AHEI-2010 (**Table 4.1**). Older women had a higher AHEI-2010 score (indicating a healthier diet) compared to younger women. Those with higher BMI and obesity had a lower AHEI-2010 score. White women had a higher AHEI-2010 score compared to African American and Hispanic women. Smoking status did not differ by quartile of diet quality.

In the discovery analysis (n=4,355), AHEI-2010 was significantly associated with methylation levels of 428 CpG sites (**Figure 4.2**). 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 higher than 56.7 and women in quartile 1 (worst diet quality) had AHEI-2010 scores lower than 42.7. Women consuming the best diet had an average difference in methylation of 0.001 at the significant sites compared to those consuming the worst diet (**Figure 4.3**).

Replication in whole blood

419 of the 428 significant sites passed QC in the TwinsUK cohort and were tested for replication in whole blood samples from 571 women. AHEI-2010 score was significantly associated with methylation at 24 sites with a $p < 0.05$ and a consistent direction of effect (**Table 4.2**), more sites than would be expected by chance (binomial test $p = 2.7 \times 10^{-4}$). None of the sites were significant after FDR adjustment. In sensitivity analyses, TwinsUK cohort was restricted to individuals with diet quality and methylation measures taken within two years (n=447) or one year (n=361). In replication analyses restricting individuals to within two years,

16 sites replicated, with 12 of which identified in the full analysis. In analyses restricting to within one year, 14 sites replicated, with 8 of which identified in the full analysis.

Replication in adipose tissue

421 of 428 CpG sites were examined in the adipose tissue of 400 female twins in the TwinsUK cohort. 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 (**Table 4.3**). None of the sites were 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) (23). The 428 CpGs identified in the discovery cohorts were associated with expression of 412 genes in the eQTM database ($p < 1x10^{-5}$) (23), for a total of 1,842 CpG-transcript associations. Gene ontology analysis of these 412 genes revealed enrichment for 343 ontologies (FDR<0.05), 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 ($p=9.02 \times 10^{-6}$).

In sensitivity analyses excluding individual ancillary studies, diet quality remained associated with 319, 0 and 36 sites when excluding EMPC, BAA23 and AS311, respectively. The correlation between effect sizes for the original 428 sites from the full analysis and the analysis excluding EMPC, BAA23 and AS311 was 0.9841, 0.9807 and 0.9948, respectively. In

sensitivity analyses excluding bladder cancer cases in the AS311 study, diet quality was associated with differential DNA methylation at 1737 CpG sites, 315 of which were identified in the primary analysis, with 21 of the replicated sites. The correlation between effect sizes for the 428 original sites was 0.9963. In analyses unadjusted for BMI, diet quality was associated with 1851 CpG sites, which included all 428 CpG sites from the fully adjusted analyses, and the correlation between effect sizes from the two analyses was 0.9983. In EWAS adjusted for SES, 22 sites were associated with diet quality, including two which replicated and were further reviewed in the discussion (cg01676795 and cg12458003). The correlation between effect sizes for the original 428 sites was 0.9980. In EWAS models excluding results unadjusted for principal component of genetic relatedness, 21 sites remained associated with diet quality. The correlation between effect sizes for the original 428 sites was 0.9951. In analyses excluding the thirteen outliers identified in the principal component analysis, 387 sites were found significant, with 372 among the original 428 sites. The correlation between effect sizes for the 428 sites originally identified sites was 0.9998.

Discussion

Diet quality was associated with 428 CpG sites in the discovery cohort of postmenopausal 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 (24, 25), cg12458003 in the body of *NFASC* (26), cg20954977 in the transcription start site of *B3GNT7* (27), and cg01676795 in the body of *POR (28)*. 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, aligning with our findings since poor diet is associated with higher BMI. cg01101459 has also been associated with chronic low

grade inflammation (29) with a positive association between methylation and C-reactive protein (CRP). CRP is another cardiometabolic risk factor playing a direct role in disease progression (30), which has been found to associate with diet patterns (31-34), such that poorer diets can lead to elevated CRP. cg01676795 has been found to associate with dysglycemia in several studies (28, 35). In these studies, higher methylation was positively associated with fasting insulin (28) and hemoglobin A1c (28, 35), 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 (36), air pollution (37), smoking (38) and vitamin B12 supplementation (39). *SEL1L* has been shown to play a significant role in lipid metabolism as a regulator of lipoprotein lipase (LPL) secretion (40, 41). *SEL1L* knock-out mouse models have elevated fibroblast growth factor 21 (FGF21), a critical metabolic hormone regulating growth, nutrient metabolism and insulin (42), and elevated levels have been associated with obesity (43) and have predicted myocardial infarction (44, 45). 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 gene expression (46). 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 (23), which would align with our study. As this gene may play a protective role against metabolic disturbances, the higher methylation patterns found associated with poor diet would be deleterious. Methylation was also shown to associate with expression in *ABHD* gene in the MESA study. *ABHD* has been shown to play a catabolic role in medium-chain and oxidatively-truncated phospholipids (47, 48).

The 428 CpG sites identified in the discovery cohort were also found to associate with differential expression of 412 genes in 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 an upstream effect of diet on cardiometabolic diseases. While we adjusted for differences in cell composition (49), 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. Improving diet quality has been shown to improve inflammatory profiles and decrease inflammatory markers such as CRP and TNFα (33, 34). Moreover, one replicated site was previously associated with CRP levels (29).

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 socioeconomic status). While all of these analyses resulted in a change in the number of significant sites (ranging from 0-1851 CpG sites), any change in significance was likely due to change in power as there was very little change in the effect size (correlation of effect sizes between analyses was above 0.98 for all analyses).

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 preadolescents 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.

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.(50) In our study, the best diet had as large as a ~2% difference in β-value compared to the worst diet. 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 (51-53).

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. We included women from the TwinsUK cohort with methylation measured within three 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 two years or one year. The TwinsUK cohort also

differed from the WHI cohorts as they were younger and racially homogenous, 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 (24-29, 35, 36). Future research should utilize more precise and unbiased estimates of diet quality through use of dietary biomarkers and metabolomic indices to fully elucidate the role of diet quality on the epigenome.

References

1. 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 (London, England) 2016;388(10053):1659-724. doi: 10.1016/s0140-6736(16)31679-8.
2. Mozaffarian D. Dietary and Policy Priorities for Cardiovascular Disease, Diabetes, and Obesity: A Comprehensive Review. Circulation 2016;133(2):187-225. doi: 10.1161/circulationaha.115.018585.
3. Prince C, Hammerton G, Taylor AE, Relton CL, Anderson EL, Davey Smith G, Timpson NJ, Richmond RC, Munafò MR. Investigating the impact of cigarette smoking behaviours on DNA methylation patterns in adolescence. Human Molecular Genetics 2018;28(1):155-65. doi: 10.1093/hmg/ddy316.
4. Madrigano J, Baccarelli A, Mittleman Murray A, Wright Robert O, Sparrow D, Vokonas Pantel S, Tarantini L, Schwartz J. Prolonged Exposure to Particulate Pollution, Genes Associated with Glutathione Pathways, and DNA Methylation in a Cohort of Older Men. Environmental Health Perspectives 2011;119(7):977-82. doi: 10.1289/ehp.1002773.
5. Nitert MD, Dayeh T, Volkov P, Elgzyri T, Hall E, Nilsson E, Yang BT, Lang S, Parikh H, Wessman Y, 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(12):3322-32. doi: 10.2337/db11-1653.
6. Perfilyev A, Dahlman I, Gillberg L, Rosqvist F, Iggman D, Volkov P, Nilsson E, Riserus U, Ling C. Impact of polyunsaturated and saturated fat overfeeding on the DNA-methylation pattern in human adipose tissue: a randomized controlled trial. The American journal of clinical nutrition 2017. doi: 10.3945/ajcn.116.143164.
7. Arpón A, Milagro FI, Razquin C, Corella D, Estruch R, Fitó M, Marti A, Martínez-González MA, Ros E, Salas-Salvadó J, 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(1):15. doi: 10.3390/nu10010015.
8. Arpon A, Riezu-Boj JI, Milagro FI, Marti A, Razquin C, Martinez-Gonzalez MA, Corella D, Estruch R, Casas R, Fito M, et al. Adherence to Mediterranean diet is associated with methylation changes in inflammation-related genes in peripheral blood cells. Journal of physiology and biochemistry 2016;73(3):445-55. doi: 10.1007/s13105-017-0552-6.
9. Chen L, Dong Y, Wang X, Hao G, Huang Y, Gutin B, Zhu H. Epigenome-Wide Association Study of Dietary Fiber Intake in African American Adolescents. Molecular nutrition & food research 2018;62(12):e1800155. doi: 10.1002/mnfr.201800155.
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. European journal of human genetics : EJHG 2015;23(5):654-62. doi: 10.1038/ejhg.2014.139.
11. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. Controlled clinical trials 1998;19(1):61-109.
12. Gondalia R, Baldassari A, Holliday KM, Justice AE, Méndez-Giráldez R, Stewart JD, Liao D, Yanosky JD, Brennan KJM, Engel SM, et al. Methylome-wide association study provides evidence of particulate matter air pollution-associated DNA methylation. Environment International 2019:104723. doi: https://doi.org/10.1016/j.envint.2019.03.071.

13. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. Annals of epidemiology 1999;9(3):178-87.
14. Bowyer RCE, Jackson MA, Pallister T, Skinner J, Spector TD, Welch AA, Steves CJ. Use of dietary indices to control for diet in human gut microbiota studies. Microbiome 2018;6(1):77. doi: 10.1186/s40168-018-0455-y.
15. Andrews SV, Ladd-Acosta C, Feinberg AP, Hansen KD, Fallin MD. “Gap hunting” to characterize clustered probe signals in Illumina methylation array data. Epigenetics & Chromatin 2016;9(1):56. doi: 10.1186/s13072-016-0107-z.
16. Zhou W, Laird PW, Shen H. Comprehensive characterization, annotation and innovative use of Infinium DNA methylation BeadChip probes. Nucleic Acids Research 2016;45(4):e22-e. doi: 10.1093/nar/gkw967.
17. Teschendorff AE, Marabita F, Lechner M, Bartlett T, Tegner J, Gomez-Cabrero D, Beck S. A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. Bioinformatics (Oxford, England) 2013;29(2):189-96. doi: 10.1093/bioinformatics/bts680.
18. Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, Stampfer MJ, Willett WC. Alternative Dietary Indices Both Strongly Predict Risk of Chronic Disease. The Journal of Nutrition 2012;142(6):1009-18. doi: 10.3945/jn.111.157222.
19. George SM, Ballard-Barbash R, Manson JE, Reedy J, Shikany JM, Subar AF, Tinker LF, Vitolins M, Neuhouser ML. 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. American journal of epidemiology 2014;180(6):616-25. doi: 10.1093/aje/kwu173.
20. 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. Journal of the Academy of Nutrition and Dietetics 2018;118(1):74-100.e11. doi: 10.1016/j.jand.2017.08.024.
21. Grundberg E, Meduri E, Sandling JK, Hedman AK, Keildson S, Buil A, Busche S, Yuan W, Nisbet J, Sekowska M, et al. Global analysis of DNA methylation variation in adipose tissue from twins reveals links to disease-associated variants in distal regulatory elements. American journal of human genetics 2013;93(5):876-90. doi: 10.1016/j.ajhg.2013.10.004.
22. Potter J, Brown L, Williams RL, Byles J, Collins CE. Diet Quality and Cancer Outcomes in Adults: A Systematic Review of Epidemiological Studies. International journal of molecular sciences 2016;17(7). doi: 10.3390/ijms17071052.
23. Kennedy EM, Goehring GN, Nichols MH, Robins C, Mehta D, Klengel T, Eskin E, Smith AK, Conneely KN. An integrated -omics analysis of the epigenetic landscape of gene expression in human blood cells. BMC genomics 2018;19(1):476. doi: 10.1186/s12864-018-4842-3.
24. Hannon E, Knox O, Sugden K, Burrage J, Wong CCY, Belsky DW, Corcoran DL, Arseneault L, Moffitt TE, Caspi A, et al. Characterizing genetic and environmental influences on variable DNA methylation using monozygotic and dizygotic twins. PLoS genetics 2018;14(8):e1007544. doi: 10.1371/journal.pgen.1007544.
25. Wahl S, Drong A, Lehne B, Loh M, Scott WR, Kunze S, Tsai PC, Ried JS, Zhang W, Yang Y, et al. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. Nature 2017;541(7635):81-6. doi: 10.1038/nature20784.
26. Mendelson MM, Marioni RE, Joehanes R, Liu C, Hedman ÅK, Aslibekyan S, Demerath EW, Guan W, Zhi D, Yao C, 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(1):e1002215-e. doi: 10.1371/journal.pmed.1002215.
27. Demerath EW, Guan W, Grove ML, Aslibekyan S, Mendelson M, Zhou YH, Hedman AK, Sandling JK, Li LA, Irvin MR, 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(15):4464-79. doi: 10.1093/hmg/ddv161.
28. Cardona A, Day FR, Perry JRB, Loh M, Chu AY, Lehne B, Paul DS, Lotta LA, Stewart ID, Kerrison ND, et al. Epigenome-Wide Association Study of Incident Type 2 Diabetes in a British Population: EPIC-Norfolk Study. Diabetes 2019. doi: 10.2337/db18-0290.
29. Ligthart S, Marzi C, Aslibekyan S, Mendelson MM, Conneely KN, Tanaka T, Colicino E, Waite LL, Joehanes R, Guan W, et al. DNA methylation signatures of chronic low-grade inflammation are associated with complex diseases. Genome Biol 2016;17(1):255. doi: 10.1186/s13059-016-1119-5.
30. Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM, Teeling JL, Blaak EE, Fenech M, Vauzour D, et al. Low-grade inflammation, diet composition and health: current research evidence and its translation. The British journal of nutrition 2015;114(7):999-1012. doi: 10.1017/S0007114515002093.
31. Dias JA, Wirfält E, Drake I, Gullberg B, Hedblad B, Persson M, Engström G, Nilsson J, Schiopu A, Fredrikson GN, et al. A high quality diet is associated with reduced systemic inflammation in middle-aged individuals. Atherosclerosis 2015;238(1):38-44. doi: https://doi.org/10.1016/j.atherosclerosis.2014.11.006.
32. Barbaresko J, Koch M, Schulze MB, Nöthlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. Nutrition reviews 2013;71(8):511-27. doi: 10.1111/nure.12035.
33. 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. Journal of the Academy of Nutrition and Dietetics 2018;118(11):2135-43. doi: 10.1016/j.jand.2018.05.014.
34. Piccand E, Vollenweider P, Guessous I, Marques-Vidal P. Association between dietary intake and inflammatory markers: results from the CoLaus study. Public health nutrition 2019;22(3):498-505. doi: 10.1017/S1368980018002355.
35. Soriano-Tárraga C, Consortium obotG, Jiménez-Conde J, Consortium obotG, Giralt-Steinhauer E, Consortium obotG, Mola-Caminal M, Consortium obotG, Vivanco-Hidalgo RM, Consortium obotG, et al. Epigenome-wide association study identifies TXNIP gene associated with type 2 diabetes mellitus and sustained hyperglycemia. Human Molecular Genetics 2015;25(3):609-19. doi: 10.1093/hmg/ddv493.
36. Cheng Y, Monteiro C, Matos A, You J, Fraga A, Pereira C, Catalan V, Rodriguez A, Gomez-Ambrosi J, Fruhbeck G, et al. Epigenome-wide DNA methylation profiling of periprostatic adipose tissue in prostate cancer patients with excess adiposity-a pilot study. Clinical epigenetics 2018;10:54. doi: 10.1186/s13148-018-0490-3.
37. de F C Lichtenfels AJ, van der Plaat DA, de Jong K, van Diemen CC, Postma DS, Nedeljkovic I, van Duijn CM, Amin N, la Bastide-van Gemert S, de Vries M, et al. Long-term Air Pollution Exposure, Genome-wide DNA Methylation and Lung Function in the LifeLines Cohort Study. Environmental health perspectives 2018;126(2):027004-. doi: 10.1289/EHP2045.
38. Joehanes R, Just AC, Marioni RE, Pilling LC, Reynolds LM, Mandaviya PR, Guan W, Xu T, Elks CE, Aslibekyan S, et al. Epigenetic Signatures of Cigarette Smoking. Circulation Cardiovascular genetics 2016;9(5):436-47. doi: 10.1161/CIRCGENETICS.116.001506.

39. Yadav DK, Shrestha S, Lillycrop KA, Joglekar CV, Pan H, Holbrook JD, Fall CH, Yajnik CS, Chandak GR. Vitamin B12 supplementation influences methylation of genes associated with Type 2 diabetes and its intermediate traits. Epigenomics 2018;10(1):71-90. doi: 10.2217/epi-2017-0102.
40. . Internet: https://www.genecards.org/cgi-bin/carddisp.pl?gene=SEL1L.
41. Sha H, Sun S, Francisco AB, Ehrhardt N, Xue Z, Liu L, Lawrence P, Mattijssen F, Guber RD, Panhwar MS, et al. The ER-associated degradation adaptor protein Sel1L regulates LPL secretion and lipid metabolism. Cell Metab 2014;20(3):458-70. doi: 10.1016/j.cmet.2014.06.015.
42. Bhattacharya A, Sun S, Wang H, Liu M, Long Q, Yin L, Kersten S, Zhang K, Qi L. Hepatic Sel1L-Hrd1 ER-associated degradation (ERAD) manages FGF21 levels and systemic metabolism via CREBH. The EMBO Journal 2018;37(22):e99277. doi: 10.15252/embj.201899277.
43. Zhang X, Yeung DCY, Karpisek M, Stejskal D, Zhou Z-G, Liu F, Wong RLC, Chow W-S, Tso AWK, Lam KSL, et al. Serum FGF21 Levels Are Increased in Obesity and Are Independently Associated With the Metabolic Syndrome in Humans. Diabetes 2008;57(5):1246-53. doi: 10.2337/db07-1476.
44. 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(6):e0129791. doi: 10.1371/journal.pone.0129791.
45. Shen Y, Zhang X, Xu Y, Xiong Q, Lu Z, Ma X, Bao Y, Jia W. Serum FGF21 Is Associated with Future Cardiovascular Events in Patients with Coronary Artery Disease. Cardiology 2018;139(4):212-8. doi: 10.1159/000486127.
46. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nature reviews Genetics 2012;13(7):484-92. doi: 10.1038/nrg3230.
47. Long JZ, Cisar JS, Milliken D, Niessen S, Wang C, Trauger SA, Siuzdak G, Cravatt BF. Metabolomics annotates ABHD3 as a physiologic regulator of medium-chain phospholipids. Nature chemical biology 2011;7(11):763-5. doi: 10.1038/nchembio.659.
48. 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(4):792-802. doi: 10.1016/j.bbalip.2013.01.002.
49. Houseman EA, Molitor J, Marsit CJ. Reference-free cell mixture adjustments in analysis of DNA methylation data. Bioinformatics (Oxford, England) 2014;30(10):1431-9. doi: 10.1093/bioinformatics/btu029.
50. Breton CV, Marsit CJ, Faustman E, Nadeau K, Goodrich JM, Dolinoy DC, Herbstman J, Holland N, LaSalle JM, Schmidt R, 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(4):511-26. doi: 10.1289/ehp595.
51. Argos M, Chen L, Jasmine F, Tong L, Pierce BL, Roy S, Paul-Brutus R, Gamble MV, Harper KN, Parvez F, et al. Gene-Specific Differential DNA Methylation and Chronic Arsenic Exposure in an Epigenome-Wide Association Study of Adults in Bangladesh. Environmental health perspectives 2015;123(1):64-71. doi: doi:10.1289/ehp.1307884.
52. Everson TM, Punshon T, Jackson BP, Hao K, Lambertini L, Chen J, Karagas MR, Marsit CJ. Cadmium-Associated Differential Methylation throughout the Placental Genome: Epigenome-Wide Association Study of Two U.S. Birth Cohorts. Environmental health perspectives 2018;126(1):017010. doi: doi:10.1289/EHP2192.
53. Lee MK, Hong Y, Kim S-Y, London SJ, Kim WJ. DNA methylation and smoking in Korean adults: epigenome-wide association study. Clinical epigenetics 2016;8(1):103. doi: 10.1186/s13148-016-0266-6.

Tables & Figures

Table 4.1. *Demographic and study characteristics by quartile of the Alternative Healthy Eating Index 2010 (AHEI-2010). Counts and means (standard deviation) 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 0 to 42.7, Q2 is 42.8 to 49.2, Q3 is 49.3 to 56.7, and Q4 is greater than 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	161	176	205	297	
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 mean (SD)	64.0 (7.11)	62.4 (7.1)	64.1 (7.1)	64.3 (7.0)	65.1 (7.0)	<0.0001
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 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	

Table 4.2*. Replicated CpG sites associated with diet quality in the Women's Health Initiative (WHI) and TwinsUK. Models were adjusted for age, ethnicity (WHI), smoking status, body mass index (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	

***Table 4.3**. Replicated sites in the adipose tissue of TwinsUK cohort.*

CpG Label	**Effect Estimate**	**Standard Error**	**T-statistic**	**P-value**	**n**	**FDR**	**Reference Gene**
cg06563086	-0.00967	0.004329	-2.23	0.025	400	0.91	
cg15778054	-0.01021	0.004788	-2.13	0.033	400	0.91	*KCTD14*
cg16379999	-0.00997	0.005006	-1.99	0.046	400	0.91	*SEL1L*
cg19719391	-0.01046	0.005073	-2.06	0.041	400	0.91	

Figure 4.1. *Flow chart of discovery sample exclusion by ancillary study.*

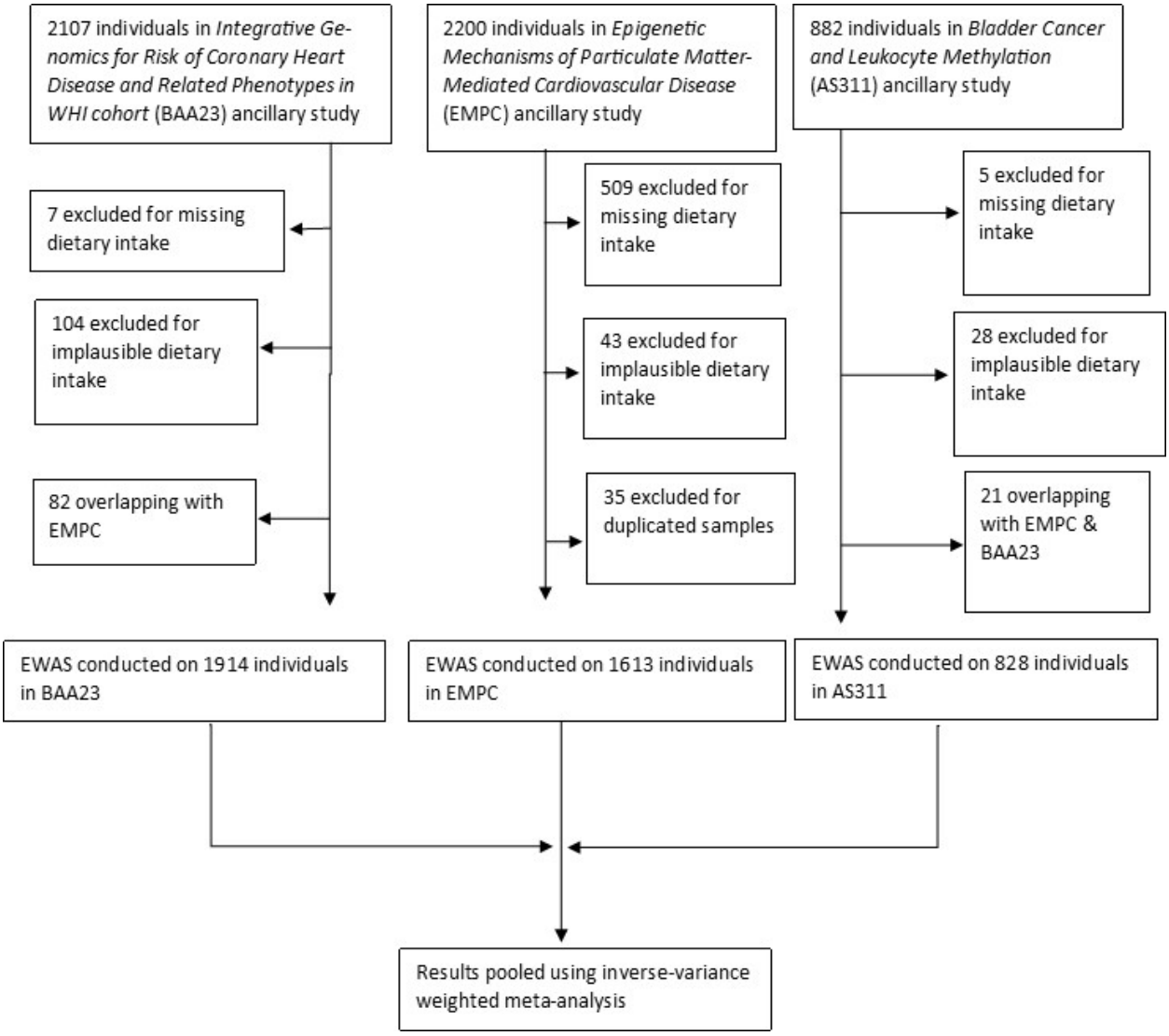


***Figure 4.2**. Manhattan Plot of the EWAS of diet quality. The x-axis represents chromosomal position and the y-axis represents P-values on the –log$_{10}$ scale for each CpG site. The line denotes the threshold for significance P = 4.8 x 10^{-5}.*

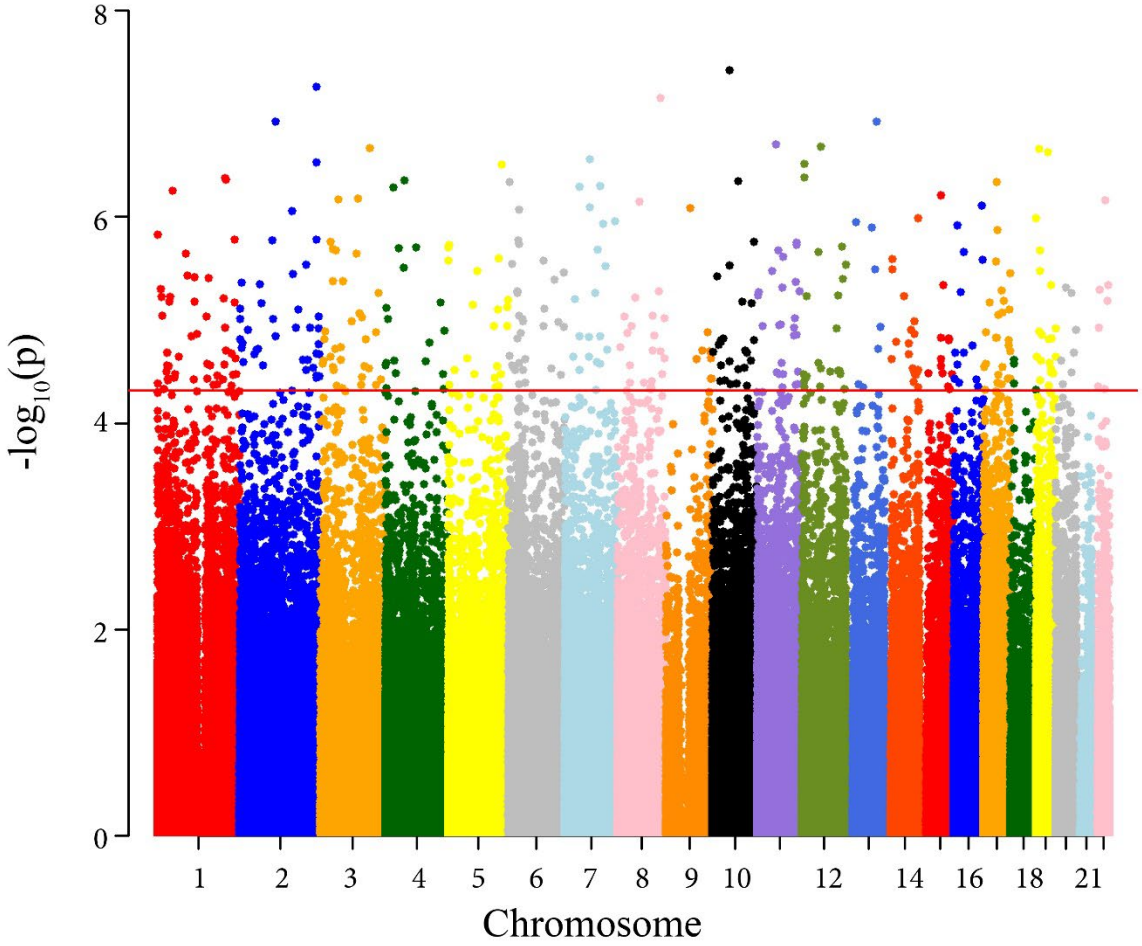
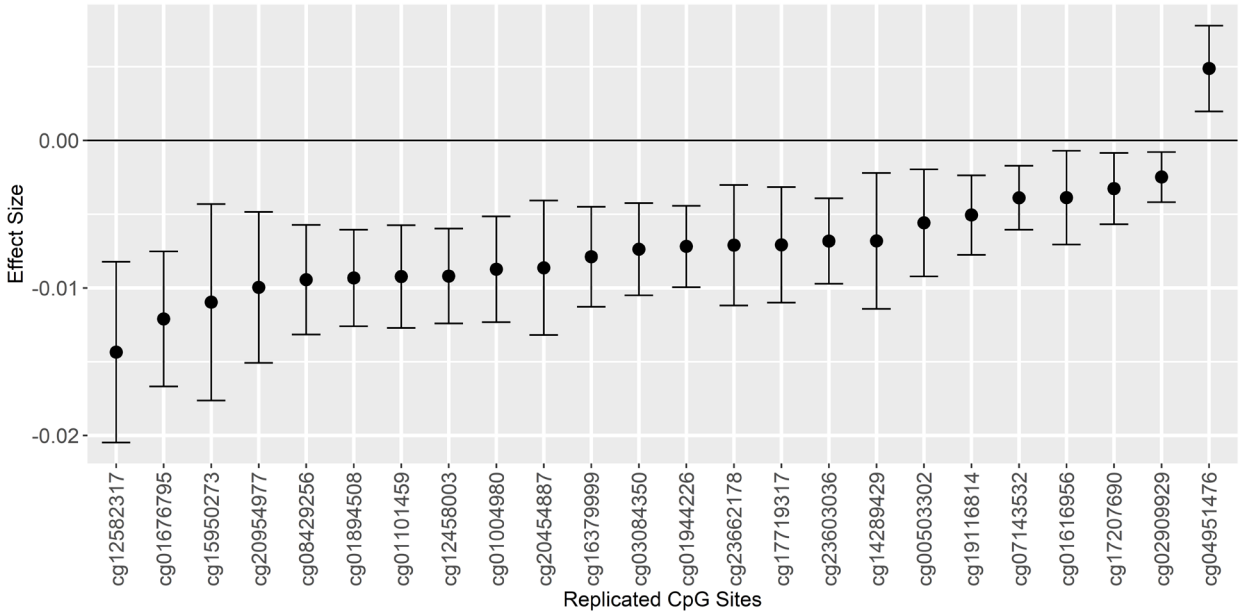


Figure 4.3. *Difference in β-value of replicated CpG sites comparing the best diet score (AHEI-2010 > 56.7) to the worst diet score (AHEI-2010 < 42.7).*



CHAPTER 5

Epigenome perturbations associated with obesity

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Introduction

Globally, the prevalence of obesity is rising with an estimated 600 million adults obese, representing 12% of the population. Obesity has been found to accompany a multitude of molecular and metabolic perturbations including impaired cell signaling, insulin resistance, hyperlipidemia, and hypertension (1-3). Ultimately these perturbations can lead to the early onset of chronic diseases with obesity accounting for 37% of the risk of type 2 diabetes (4) and 67-85% of the risk of cardiovascular disease (5). With a growing obese population, it is increasingly important to understand the molecular mechanisms dysregulated by obesity to further elucidate both early markers of disease progression and novel therapeutic targets.

Epigenetic mechanisms are molecularly mediated changes in gene function which do not change the DNA sequence. DNA methylation, the most widely characterized epigenetic mechanism, occurs when a methyl group attaches to the cytosine in a cytosine-guanine nucleotide (CpG) pair (6). DNA methylation has been shown to influence gene expression by blocking transcription factor binding and recruiting chromatin remodelers (7). As a functional mechanism influencing gene expression, DNA methylation may be on a disease pathway and could provide insight into important therapeutic targets. DNA methylation has also become an important biomarker of health, for example with the development of the epigenetic clock. The epigenetic clock provides an estimate for an individual's age based on the methylation status of several CpG sites. More importantly, individuals whose DNA methylation deviate from their actual chronological age, such that their epigenetically predicted age is higher than their actual age,

have been shown to have higher rates of cancer, cardiovascular disease, diabetes, and mortality (8). All of these properties may be relevant in the relationship between DNA methylation and obesity.

Several studies have examined the relationship between DNA methylation and body mass index (BMI) (9-18). Obesity has been significantly associated with differential DNA methylation as both a cause and a consequence of the disease (10). While several large scale studies have identified a number of sites associated with obesity, examining methylation at a genome-wide scale requires large sample sizes as DNA methylation tends to have small effect sizes (19). Thus a goal of this study is to conduct the largest epigenome-wide association study (EWAS) meta-analysis of BMI to identify novel sites associated with obesity using summary statistics from nine population based cohort studies. With the identification of novel sites, we may be better able to predict BMI as well as to reveal unique molecular signatures of various BMI phenotypes. Previous studies have found methylation explaining between 4.7-18% of the variance in BMI (10, 18, 20, 21). In conducting the largest EWAS, we may have better predictive capacity by incorporating the novel CpG sites identified in the EWAS meta-analysis. As such, a secondary aim of this study is to examine whether BMI-associated CpG sites can predict BMI. As with epigenetic age, deviations from epigenetically predicted BMI may be associated with several relevant health outcomes, and could be used as an informative metric of overall health and/or a predictor of future cardiovascular disease. Thus we examined whether individuals whose BMI was poorly predicted by DNA methylation (DNA methylation over predicts their actual BMI or DNA methylation under predicts their actual BMI) have differential metabolic health status.

Methods

Participants

We used data from 17,034 participants from six published EWAS studies of individuals of European descent (n=11,220), with a small minority in individuals of African descent (n=2,587)

and South Asians (n=2,680). This study includes data from the Atherosclerosis Risk in Communities (ARIC), Melbourne Collaborative Cohort Study (MCCS), Lifelines DEEP, Lothian Birth Cohort (LBC) 1921 and 1936, Bogalusa Heart Study (BHS), the Research on Obesity and Diabetes among African Migrants (RODAM) study, the Kooperative Gesundheitsforschung in der Region Augsburg (KORA), the London Life Sciences Prospective Population Study (LOLIPOP), and Italian cardiovascular component of the European Prospective Investigation into Cancer and Nutrition (EPICORE). ARIC includes data from 2097 African American men and women aged 45-64 years recruited from four US communities: Forsyth County, NC; Jackson, MS; Minneapolis, MN; Washington County; MA. Participants were followed up for up to 5 visits. DNA methylation derived from visit 2. The MCCS consists of 5361 men and women aged 40-69 years from the Melbourne region. The study was composed of participant's included in six prior nested case-control studies of prostate, colorectal, lung or kidney cancer, urothelial cell carcinoma or mature B-cell neoplasms. Controls were matched on sex, year of birth, country of birth, baseline sample type and smoking status (lung cancer study only). The LBC consists of individuals born in 1921 and 1936 living in the Lothian region. This study includes the baseline examination of 550 individuals from LBC 1921 (average age 79) and 1091 LBC from 1936 (average age 70). Lifelines DEEP is a sub cohort of the LifeLines study consisting of 752 individuals from the Netherlands. The BHS study is a long-term cohort study focused on cardiovascular disease. This study includes 1,485 adult participants from the study (995 non-Hispanic white and 490 African American) recruited during 2006-2010. The RODAM study is a study of Ghanaians recruited from Ghana, London, Amsterdam and Berlin. From the total study population, 736 were included in the EWAS of BMI. The KORA cohort is a population-based health study examining individuals living in the region of Augsburg in Southern Germany. The F3 and F4 surveys were follow up examinations of the original cohort taken in 2004-2005 and 2006-2008, respectively. DNA methylation was measured in 1709 participants from F4 and 285 participants from F3. The LOLIPOP study is a prospective cohort study of Indian Asian and European men and women recruited in West

London, United Kingdom between 2003 and 2008. DNA methylation was measured in 2,680 participants free from type 2 diabetes using peripheral blood collected at enrollment. EPICOR was a nested case-control study from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Italy cohort, recruited during 1994-1998. DNA methylation was measured in peripheral blood collected at enrollment in 292 controls.

Secondary analyses were conducted in three ancillary studies from the Women's Health Initiative (WHI): *Epigenetic Mechanisms of Particulate Matter-Mediated Cardiovascular Disease* (EMPC, aka AS315), the *Integrative Genomics for Risk of Coronary Heart Disease and Related Phenotypes in WHI cohort* (BAA23), and *Bladder Cancer and Leukocyte Methylation* (AS311). EMPC (n=2200) assessed epigenetic mechanisms underlying associations between ambient particulate matter air pollution and cardiovascular disease within the WHI CT. BAA23 was a case-control study assessing predictors of coronary heart disease (CHD) within the WHI CT (n=1664) and OS (n=443), where cases were identified using eight biomarkers of CHD. AS311 is a matched case-control study of bladder cancer among women within the WHI CT (n = 426) and OS (n = 456). In the WHI, individuals were excluded if BMI and DNA methylation were not measured within the same year. Extreme levels of BMI < 17 and >75 were excluded.

BMI, DNA methylation and covariates

BMI was defined as weight in kg/height in m^2. Methodologies obtaining weight and height differed among the studies, however all used standard methods. One study transformed BMI values to obtain a normal distribution (17). In the WHI, weight was measured on a balance beam scale to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Relevant variables in our replication analysis will include race/ethnicity, age, physical activity and smoking status. Race/ethnicity, smoking and physical activity were self-reported. Smoking status was defined as current, former or never.

DNA methylation was measured in several cell types including CD4+ T-cells, leucocytes, mononuclear cells and the whole blood. DNA methylation in all studies was measured using the Illumina 450K Infinium Methylation BeadChip. DNA methylation was estimated as the proportion of methylated probes relative to combined unmethylated and methylated probes for a specific CpG sites defined as the β-value. Quality control procedures of the previous studies have been reported on in detail and they did not differ substantially. In the WHI data, all methylation data was quality controlled and normalized using beta-mixture quantile normalization. In replication analyses, chip and row were included as technical covariates in all models to adjust for batch effects. Cell composition was estimated using methods derived by Houseman et al.(22).

Statistical Analysis

Our primary meta-analytic method was weighted sum of Z-score meta-analysis. This method utilizes Z-scores and the direction of effect to determine significant sites. Significance was defined by p-value < $1x10^{-7}$. This was chosen as the primary method for meta-analysis since the studies did not all have equivalent exposure-outcome definition (DNA methylation defined as exposure in two studies and outcome in four studies) and BMI was transformed in one study.

The significant sites were examined for replication within WHI. Models were stratified by ancillary study. Covariates in this analysis included age, race/ethnicity, cell composition, the top three principal components of genetic relatedness, smoking status, clinical trial arm and case-control status (BAA23 and AS311). To account for potential chip-to-chip differences in measurement and to adjust for batch effects, chip was included as a random effect for each BeadChip in our model. Stratified analyses were combined using inverse-variance weighted meta-analysis. Significance will be defined by false discovery rate q-value < 0.05.

BMI Prediction Score

To examine the degree to which methylation can predict BMI and the secondary cardiometabolic outcomes associated with BMI, we used elastic net regression models with the significant sites to predict log-normalized BMI. The WHI cohorts was randomly divided into test and training set (75% and 25%, respectively) with an equal BMI distribution. We used elastic net regression on the training set with 10-fold cross validation. Predicted values were compared to actual BMI values. Using the significant sites and coefficients remaining in the elastic net regression, a DNA methylation prediction score was developed by multiplying the coefficient by the individual β-value and summing all the sites for an individual. The DNA methylation score was examined for how well it predicted obesity status (BMI ≥ 30) using the sensitivity and specificity.

Using the predicted BMI values, we examined the patterns among outliers in the prediction model. Individuals were split into categories based on the difference between predicted BMI and actual BMI. Accurately predicted individuals were defined as those whose difference between predicted and actual BMI between -0.08 to 0.08. To determine outliers, individuals outside of this were be split into two groups: difference below -0.08 (individuals whose predicted BMI is less than their actual BMI or low epigenetic BMI) and difference above 0.08 (individuals whose predicted BMI is more than their actual BMI or high epigenetic BMI). These thresholds were defined based on the 10% and the 90% distribution of the difference score. Using these categories, we examined cardiometabolic differences including waist circumference, triglycerides, HDL-cholesterol, LDL-cholesterol, and blood glucose among these categories using linear regression models regressing log-normalized cardiometabolic markers on DNA methylation prediction category adjusted for age, race/ethnicity, smoking status and physical activity.

Sensitivity Analyses

We examined several sites for interaction by self-reported race/ethnicity and BMI using linear mixed-effect models adjusting for age, cell composition, smoking status, WHI study randomization arm, case-control status, row with a random effect for chip.

Results

Our discovery analysis included 17,058 participants from 9 cohorts (**Table 5.1**). The definition of BMI and DNA methylation differed with several transforming these values in the models. The covariates in the model also differed with all studies adjusting for age and sex, and the majority adjusting for cell composition and smoking status. When pooling results from all studies, 1265 CpG sites were associated with BMI (**Figure 5.1**, $p < 1E-7$). Of the 1265 sites, 1254 were analyzed in WHI with 1238 replicating in WHI (FDR q-value < 0.05).

We examined how these sites associated with differential gene expression in the GTP and MESA cohort. The 1238 CpG sites associated with 1103 CpG-mRNA associations in MESA and 79 CpG-mRNA associations in GTP. Relevant top pathways included cellular response to low-density lipoprotein particle stimulus, tumor necrosis factor-mediated signaling and positive regulation of stress activated MAPK cascade. One site associated with the same mRNA transcript in both cohorts (**Table 5.2**).

In race stratified models, 936 and 130 CpG sites were associated with individuals from European and African descent, respectively. Of the 130, 43 unique sites were only significant in African populations. We examined these sites for interaction in the WHI EA and AA individuals. We found that five CpG sites had a significant interaction with BMI by race/ethnicity (**Table 5.3**). Two sites were quantitative trait methylation loci in the GTP cohort: cg25212453 negatively associated with *TNFRSf13B* and *COCH* and cg08122652 negatively associated with *LGALS3BP* and *OTOF*.

We examined how well DNA methylation predicted BMI. After model tuning, using elastic-net regression, 398 sites remained in the model. These sites accounted for 32% of the

variance in WHI in the test set. The addition of age, ethnicity, physical activity, and cell composition only marginally improved the adjusted R^2 (**Table 5.4**). In the full cohort, these sites accounted for 36% of the variance in BMI. We examined how well the predictors from Mendelson et al. (10) predicted BMI in the WHI cohort. In the full WHI cohort, the 83 CpG sites accounted for 29% of the variance.

Using these 397 CpG sites to predict obesity, the sensitivity was 0.82 and the specificity was 0.57 with and area under the curve of 0.79 (**Figure 5.2**). Individuals were categorized based on how well methylation predicted BMI (**Figure 5.3**). Individuals with high epigenetic BMI had significantly higher blood glucose (**Figure 5.4,** p<2E-16) and triglycerides (**Figure 5.5**, p=9.24E-08), lower HDL-cholesterol (**Figure 5.6**, p=1.06E-07) and LDL-cholesterol (p=0.04) compared by accurate epigenetic BMI. Individuals with low epigenetic BMI had no differential association with metabolic health parameters as accurate epigenetic BMI.

Discussion

This study identified a unique methylomic signature of BMI and obesity. In the WHI, the majority of the sites identified in the discovery cohort were replicated and found to influence several metabolic and inflammatory pathways. Moreover, we found five CpG sites which are differentially associated with BMI between non-Hispanic whites and African Americans, two of which may play a significant role in gene expression of inflammatory pathways. Several sites were also able to predict BMI as well as several other cardiometabolic risk factors. Finally, individuals whose BMI was overpredicted by their methylome were found to have poorer metabolic health including higher blood glucose and triglycerides and lower HDL-cholesterol compared to accurately predicted individuals.

The 1238 CpG sites annotated to 742 unique genes. Additionally, 147 of these genes were annotated to more than one CpG site with 382 CpG sites associated with 147 genes. With the large sample size, we were able to discover 685 novel CpG sites which had not previously

been identified in EWAS of BMI as well as 553 CpG sites which have previously been identified in the literature. The 1238 CpG sites were associated with differential gene expression in MESA and GTP. In the gene ontology analysis of the differentially expressed transcripts, the most significant pathways which enriched were immune response pathways.

We were additionally able to examine how these associations changed when stratified by race/ethnicity. We examined this as epidemiological ethnic differences in adiposity have been well established. While African Americans have been shown to have the higher risk for cardiovascular diseases compared to non-Hispanic whites, they have consistently shown to have lower visceral adipose tissue, a potentially advantageous metabolic factor (23). Moreover, at the same level of BMI, African Americans tend to have lower body fat percentage compared to non-Hispanic whites (24). Five CpG sites were found to have a differential association with BMI between non-Hispanic white individuals and African Americans in the WHI cohort. Of these five sites, two CpG sites were associated with differential expression in four mRNA transcripts, which may regulate inflammatory pathways and hearing. *TNFRSF13B* and *LGALS3BP* were differentially expressed in association with two CpG sites. These two genes have been shown to be regulators of NF-kappa-B signaling. *TNFRSF13B* encodes a member of the TNF receptor subfamily, which is required to activate NFAT, AP and NF-kappa-B (25) and has been associated with metabolic syndrome (26). *LGALS3BP* is a scaffolding molecule of TRAF molecules which then influence NF-kappa-B signaling (27) and has been shown to be upregulated with obesity (28).

Our study found a positive association between BMI and methylation in cg25212453 and cg08122652 (in WHI) and a negative association between methylation in these two sites and expression in *LGALS3BP* and *TNFRSF13B* (in GTP). Thus, as BMI increases in African Americans, gene expression may be decreasing in these sites, suggestion a potentially advantageous effect on inflammatory profiles in African Americans. Low-grade inflammation in obesity is a hallmark of the disease, which leads to significant metabolic dysregulation (29). There

is some epidemiological data that suggests this individuals of African descent may not be as prone to an increased inflammatory profile when living with obesity. TNF-α has showed no association with obesity in both African Americans and West Africans (30). Similarly, another study found a weaker association between inflammatory markers and adiposity in West Africans compared to European Whites (31). However, not all studies have a reduced association between obesity and inflammatory markers in African Americans (32). Nevertheless, our study may provide some mechanistic explanation to these differences in the relationship between inflammation and adiposity in individuals of African descent. Since expression of both *LGALS3BP* and *TNFRSF13B* would lead to increased inflammation, individuals of African descent may be protected from these effects due to methylation in cg25212453 and cg08122652, which are associated with downregulation as BMI increases.

These results need to be interpreted with caution given several important limitations. In the discovery analysis, we stratified this analysis based on race/ethnicity as it was defined within each of the individual studies. This differed between studies with most based on self-report of race/ethnicity. Thus it is unclear whether we are identifying molecular differences due to ancestry or social construct. Moreover, these populations are not homogenous, with African Americans, Ghanians, and European-residing Ghanians. Nevertheless, our interaction and expression results were conducted in African American populations from the WHI and GTP. These results may only be generalizable for this population. The racial disparities in the US may be an underlying cause of these results, as opposed to differences in ancestry. For example in the US, African Americans are much more likely to live in poverty compared to non-Hispanic whites (33). In our results, we may be identifying compensatory mechanisms of social stressors which may be driven by environmental exposures associated with racial disparities (ambient particulate matter exposure, stress) as well as obesity.

We also found that DNA methylation was highly predictive of BMI explaining 32% of the variance in BMI. Previous studies have found methylation explaining between 4.7-18% of the variance in BMI (10, 18, 20, 21). DNA methylation has been found to be an accurate predictor of current BMI and poor predictor of future BMI (21). Outliers in the epigenetic BMI model predicted a unique phenotype. Individuals with high epigenetic BMI or whose BMI was over predicted by the epigenetic markers had worse cardiometabolic markers compared to accurately predicted. This may suggest that epigenetic BMI prediction may be identifying individuals with poor health regardless of their BMI and this suggests these sites may be useful biomarkers to examine.

There are some limitations worth noting. In the EWAS, weighted-sum of Z-score meta-analysis may not be stringent enough method to detect differential methylation as the underlying hypothesis is that one population parameter differs from the null. However, for this reason, we used stringent p-value for detecting significant sites ($p<1E-7$). Moreover, replicating in another population (WHI) and applying an FDR adjustment to the replication p-value give us more confidence in our results.

Overall, this study had several important discoveries. We identified novel sites associated with BMI and found a unique molecular profile associated with obesity in individuals of African descent. We additionally found that epigenetic markers can predict BMI well and it may be able to distinguish individuals with whose metabolic health do not align with their BMI. Future studies should examine whether BMI-associated methylation is differential by metabolic health status.

References

1. Asghar A, Sheikh N. Role of immune cells in obesity induced low grade inflammation and insulin resistance. Cell Immunol 2017;315:18-26. doi: 10.1016/j.cellimm.2017.03.001.
2. O'Flanagan CH, Bowers LW, Allot EH, Hursting SD. Molecular and metabolic mechanisms underlying the obesity–cancer link. Edtion ed. Energy Balance and Obesity: IARC Working Group, 2017.
3. Hall JE, Carmo JMd, Silva AAd, Wang Z, Hall ME. Obesity-Induced Hypertension. Circulation research 2015;116(6):991-1006. doi: doi:10.1161/CIRCRESAHA.116.305697.
4. Narayan KMV, Boyle JP, Thompson TJ, Gregg EW, Williamson DF. Effect of BMI on Lifetime Risk for Diabetes in the U.S. Diabetes care 2007;30(6):1562-6. doi: 10.2337/dc06-2544.
5. Khan SS, Ning H, Wilkins JT, Allen N, Carnethon M, Berry JD, Sweis RN, Lloyd-Jones DM. Association of Body Mass Index With Lifetime Risk of Cardiovascular Disease and Compression of Morbidity. JAMA cardiology 2018;3(4):280-7. doi: 10.1001/jamacardio.2018.0022.
6. Smith ZD, Meissner A. DNA methylation: roles in mammalian development. Nature reviews Genetics 2013;14(3):204-20. doi: 10.1038/nrg3354.
7. Greenberg MVC, Bourc'his D. The diverse roles of DNA methylation in mammalian development and disease. Nature Reviews Molecular Cell Biology 2019;20(10):590-607. doi: 10.1038/s41580-019-0159-6.
8. Fransquet PD, Wrigglesworth J, Woods RL, Ernst ME, Ryan J. The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. Clinical epigenetics 2019;11(1):62. doi: 10.1186/s13148-019-0656-7.
9. Demerath EW, Guan W, Grove ML, Aslibekyan S, Mendelson M, Zhou Y-H, Hedman ÅK, Sandling JK, Li L-A, Irvin MR, et al. Epigenome-wide association study (EWAS) of BMI, BMI change and waist circumference in African American adults identifies multiple replicated loci. Human Molecular Genetics 2015;24(15):4464-79. doi: 10.1093/hmg/ddv161.
10. Mendelson MM, Marioni RE, Joehanes R, Liu C, Hedman ÅK, Aslibekyan S, Demerath EW, Guan W, Zhi D, Yao C, 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 medicine 2017;14(1):e1002215. doi: 10.1371/journal.pmed.1002215.
11. Al Muftah WA, Al-Shafai M, Zaghlool SB, Visconti A, Tsai PC, Kumar P, Spector T, Bell J, Falchi M, Suhre K. Epigenetic associations of type 2 diabetes and BMI in an Arab population. Clinical epigenetics 2016;8:13. doi: 10.1186/s13148-016-0177-6.
12. Aslibekyan S, Demerath EW, Mendelson M, Zhi D, Guan W, Liang L, Sha J, Pankow JS, Liu C, Irvin MR, et al. Epigenome-wide study identifies novel methylation loci associated with body mass index and waist circumference. Obesity (Silver Spring, Md) 2015;23(7):1493-501. doi: 10.1002/oby.21111.
13. Campanella G, Gunter MJ, Polidoro S, Krogh V, Palli D, Panico S, Sacerdote C, Tumino R, Fiorito G, Guarrera S, et al. Epigenome-wide association study of adiposity and future risk of obesity-related diseases. International journal of obesity (2005) 2018;42(12):2022-35. doi: 10.1038/s41366-018-0064-7.
14. Meeks KAC, Henneman P, Venema A, Burr T, Galbete C, Danquah I, Schulze MB, Mockenhaupt FP, Owusu-Dabo E, Rotimi CN, et al. An epigenome-wide association study in whole blood of measures of adiposity among Ghanaians: the RODAM study. Clinical epigenetics 2017;9:103-. doi: 10.1186/s13148-017-0403-x.
15. Wahl S, Drong A, Lehne B, Loh M, Scott WR, Kunze S, Tsai P-C, Ried JS, Zhang W, Yang Y, et al. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. Nature 2017;541(7635):81-6. doi: 10.1038/nature20784.

16. Sun D, Zhang T, Su S, Hao G, Chen T, Li Q-Z, Bazzano L, He J, Wang X, Li S, et al. Body Mass Index Drives Changes in DNA Methylation. Circulation research 2019;125(9):824-33. doi: doi:10.1161/CIRCRESAHA.119.315397.
17. Geurts YM, Dugue PA, Joo JE, Makalic E, Jung CH, Guan W, Nguyen S, Grove ML, Wong EM, Hodge AM, et al. Novel associations between blood DNA methylation and body mass index in middle-aged and older adults. International journal of obesity (2005) 2018;42(4):887-96. doi: 10.1038/ijo.2017.269.
18. Shah S, Bonder Marc J, Marioni Riccardo E, Zhu Z, McRae Allan F, Zhernakova A, Harris Sarah E, Liewald D, Henders Anjali K, Mendelson Michael M, et al. Improving Phenotypic Prediction by Combining Genetic and Epigenetic Associations. The American Journal of Human Genetics 2015;97(1):75-85. doi: https://doi.org/10.1016/j.ajhg.2015.05.014.
19. Breton CV, Marsit CJ, Faustman E, Nadeau K, Goodrich JM, Dolinoy DC, Herbstman J, Holland N, LaSalle JM, Schmidt R, 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(4):511-26. doi: 10.1289/ehp595.
20. McCartney DL, Hillary RF, Stevenson AJ, Ritchie SJ, Walker RM, Zhang Q, Morris SW, Bermingham ML, Campbell A, Murray AD, et al. Epigenetic prediction of complex traits and death. Genome Biol 2018;19(1):136. doi: 10.1186/s13059-018-1514-1.
21. Reed ZE, Suderman MJ, Relton CL, Davis OSP, Hemani G. The association of DNA methylation with body mass index: distinguishing between predictors and biomarkers. Clinical epigenetics 2020;12(1):50. doi: 10.1186/s13148-020-00841-5.
22. Houseman EA, Molitor J, Marsit CJ. Reference-free cell mixture adjustments in analysis of DNA methylation data. Bioinformatics (Oxford, England) 2014;30(10):1431-9. doi: 10.1093/bioinformatics/btu029.
23. Després J-P. Body Fat Distribution and Risk of Cardiovascular Disease. Circulation 2012;126(10):1301-13. doi: doi:10.1161/CIRCULATIONAHA.111.067264.
24. Dugas LR, Cao G, Luke AH, Durazo-Arvizu RA. Adiposity is not equal in a multi-race/ethnic adolescent population: NHANES 1999-2004. Obesity (Silver Spring, Md) 2011;19(10):2099-101. doi: 10.1038/oby.2011.52.
25. . Internet: https://www.genecards.org/cgi-bin/carddisp.pl?gene=TNFRSF13B (accessed 12-15-2020.
26. Zhang Y, Kent Jr. JW, Olivier M, Ali O, Broeckel U, Abdou RM, Dyer TD, Comuzzie A, Curran JE, Carless MA, et al. QTL-based association analyses reveal novel genes influencing pleiotropy of metabolic syndrome (MetS). Obesity 2013;21(10):2099-111. doi: https://doi.org/10.1002/oby.20324.
27. Xu G, Xia Z, Deng F, Liu L, Wang Q, Yu Y, Wang F, Zhu C, Liu W, Cheng Z, et al. Inducible LGALS3BP/90K activates antiviral innate immune responses by targeting TRAF6 and TRAF3 complex. PLOS Pathogens 2019;15(8):e1008002. doi: 10.1371/journal.ppat.1008002.
28. Challa TD, Straub LG, Balaz M, Kiehlmann E, Donze O, Rudofsky G, Ukropec J, Ukropcova B, Wolfrum C. Regulation of De Novo Adipocyte Differentiation Through Cross Talk Between Adipocytes and Preadipocytes. Diabetes 2015;64(12):4075-87. doi: 10.2337/db14-1932.
29. Reilly SM, Saltiel AR. Adapting to obesity with adipose tissue inflammation. Nature Reviews Endocrinology 2017;13(11):633-43. doi: 10.1038/nrendo.2017.90.
30. Doumatey AP, Lashley KS, Huang H, Zhou J, Chen G, Amoah A, Agyenim-Boateng K, Oli J, Fasanmade O, Adebamowo CA, et al. Relationships Among Obesity, Inflammation, and Insulin Resistance in African Americans and West Africans. Obesity 2010;18(3):598-603. doi: https://doi.org/10.1038/oby.2009.322.

31. Hakim O, Bello O, Ladwa M, Peacock JL, Umpleby AM, Charles-Edwards G, Amiel SA, Goff LM. The Link between Obesity and Inflammatory Markers in the Development of Type 2 Diabetes in Men of Black African and White European Ethnicity. Nutrients 2020;12(12):3796.
32. Carroll JF, Fulda KG, Chiapa AL, Rodriquez M, Phelps DR, Cardarelli KM, Vishwanatha JK, Cardarelli R. Impact of Race/Ethnicity on the Relationship Between Visceral Fat and Inflammatory Biomarkers. Obesity 2009;17(7):1420-7. doi: https://doi.org/10.1038/oby.2008.657.
33. Carnethon MR, Pu J, Howard G, Albert MA, Anderson CAM, Bertoni AG, Mujahid MS, Palaniappan L, Taylor HA, Willis M, et al. Cardiovascular Health in African Americans: A Scientific Statement From the American Heart Association. Circulation 2017;136(21):e393-e423. doi: doi:10.1161/CIR.0000000000000534.

Tables and Figures

Table 5.1. *Study characteristics of discovery analyses.*

Reference	Study Population	N	Exposure	Outcome	Sample	Covariates
Demerath, et al., 2015, Hum Mol Genet	Atherosclerosis Risk in Communities (ARIC)	2097	BMI	DNA methylation β-value	Leucocytes	Age, sex, study center, total white blood cell differentials, education, household income, cigarette smoking, current alcohol use, leisure physical activity, cell composition via Housman, top 10 PCs of genetic relatedness and batch effects (row, plate number and chip number)
Geurts, et al., 2018, Int J Obes	Melbourne Collaborative Cohort Study (MCCS)	5361	BMI Z-score	DNA methylation M-values	Mononuclear cells	Age, sex, smoking status, country of birth, sample type, white blood cell composition (Houseman), and study, plate and chip included as random effects.
Meeks, et al., 2017, Clin Epigenetics	Research on Obesity and Diabetes among African Migrants (RODAM) study,	541	BMI	DNA methylation M-values	Whole Blood	Age, sex, recruitment site, cell composition (Houseman), hybridization batch, array position and first principal component of genetic relatedness
Shah, et al., 2015, Am J Hum Genet	Lothian Birth Cohort (LBC) and Lifelines DEEP	2116	Log-transformed DNA methylation	BMI Z-score	Whole Blood	Age, sex, batch effects, complete blood cell count adjusted for in sensitivity analyses

Sun, et al., 2019, Circulation	Bogalusa Heart Study (BHS)	1485	BMI	DNA methylation β-value	Whole Blood	Age, sex, current smoking status and estimated white blood cell counts included as fixed effects with batch array as a random effect
Wahl, et al., 2017, Nature	KORA, LOLIPOP, EPICORE	5458	DNA methylation β-value	BMI	Whole Blood	Top 20 principal components of control probes, cell composition, age, gender, smoking status, physical activity index and alcohol consumption

Table 5.2. *CpG-mRNA association in both GTP and MESA*

Cohort	CpG site	Status	Annotated Gene	Beta	SE	P
GTP	cg25653947	Promoter	*TOP1MT*	0.12576092	0.01941799	3.495e-10
MESA	cg25653947	Promoter	*TOP1MT*	0.223428	0.01623042	4.232e-40

Table 5.3. *Interaction between BMI and race/ethnicity in WHI between non-Hispanic whites and African Americans.*

CpG Site	Effect Estimate	Standard Error	Z-score	P-value
cg25652701	-0.00058	0.000136	-4.29275	1.76E-05
cg25212453	0.000501	0.000164	3.050552	0.002284
cg08122652	0.000889	0.00036	2.466811	0.013632
cg27113059	-0.00023	9.59E-05	-2.36333	0.018112
cg15391590	-0.00024	0.00011	-2.17865	0.029358

Table 5.4. *Predicting BMI from DNA methylation using elastic net regression*

Predictors	RMSE	Adjusted R^2
398 CpG sites	0.0702687	0.316954
398 CpG sites + Age	0.0699664	0.3229309
398 CpG sites + Age + Ethnicity	0.06991631	0.3474048
398 CpG sites + Age + Ethnicity + Cell Composition	0.069918	0.3473717

Table 5.5*. Outliers in the prediction model compared to log-normalized cardiometabolic risk factors. Model adjusted for race/ethnicity, smoking status, age and physical activity.*

	Estimate	SE	P-value
Waist Circumference (n=4356)			
High Epigenetic BMI	7.27E-03	8.52E-03	0.39

Low Epigenetic BMI	7.49E-03	1.06E-02	0.47
Blood Glucose (n=3823)			
High Epigenetic BMI	1.39E-01	1.43E-02	<2E-16
Low Epigenetic BMI	-1.978E-02	2.10E-02	0.34
Blood Triglycerides (n=3829)			
High Epigenetic BMI	1.43E-01	2.67E-02	9.24E-08
Low Epigenetic BMI	-7.36E-02	3.88E-02	0.057
HDL-cholesterol (n=3832)			
High Epigenetic BMI	-7.85E-02	1.47E-02	1.06E-07
Low Epigenetic BMI	1.89E-02	2.11E-02	0.37
LDL-cholesterol (n=3740)			
High Epigenetic BMI	-3.38E-02	1.69E-02	0.04
Low Epigenetic BMI	7.37E-03	2.33E-02	0.75

***Figure 5.1**. Manhattan plot of the association between DNA methylation and BMI.*

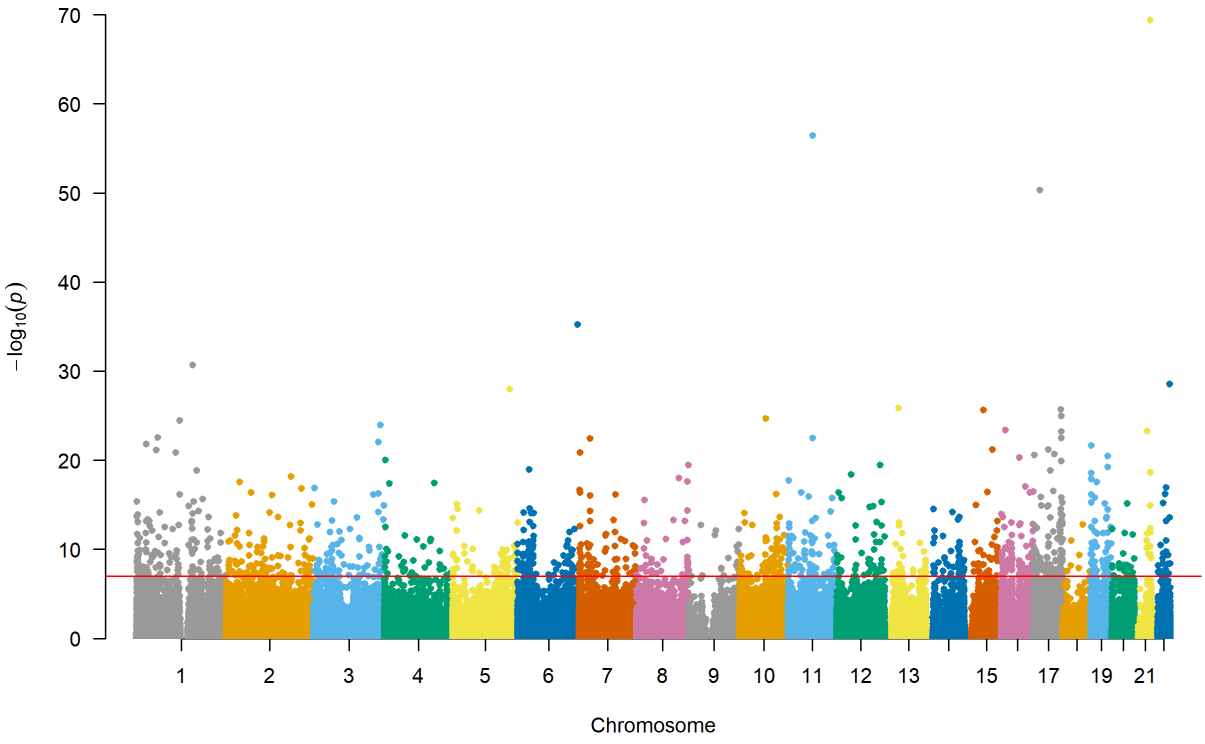


Figure 5.2. *Receiver operating characteristic curve showing the performance of the DNA methylation prediction score identifying obesity. AUC denotes area under the curve. Y-axis is the sensitivity (true positive rate) and the x-axis is 1-specificity (false positive rate).*

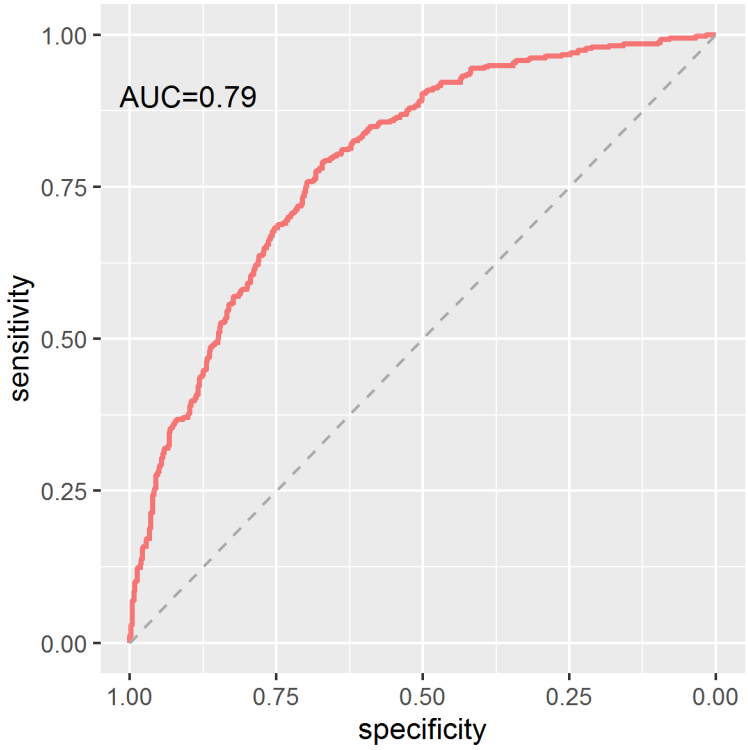


Figure 5.3. *Scatter plot of predicted BMI from elastic net regression of 398 CpG sites by actual BMI. Individuals categorized based on the residual of predicted BMI regressed on actual BMI.*

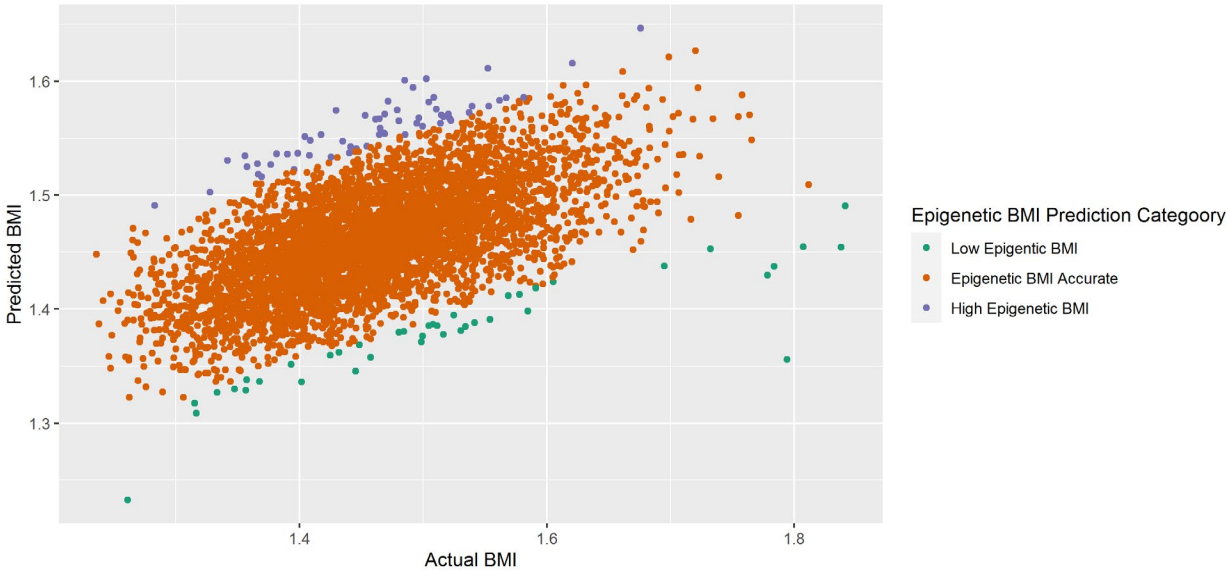


Figure 5.4. *Boxplot of the association between epigenetic prediction category and blood glucose (mg/dL).*

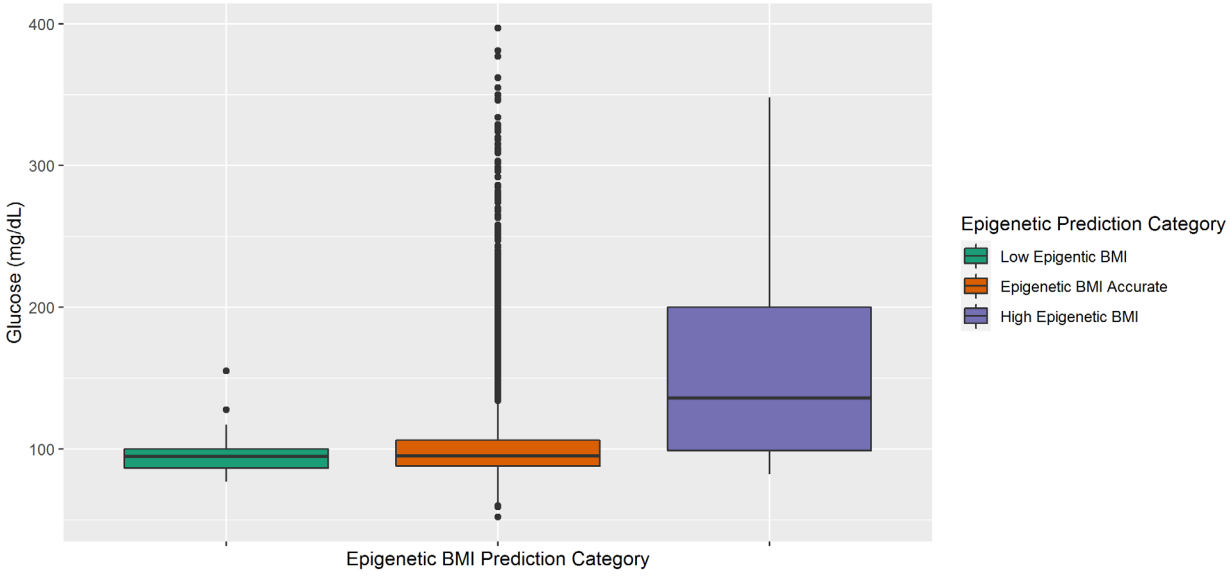


Figure 5.5. *Boxplot of the association between epigenetic prediction category and blood triglycerides (mg/dL).*

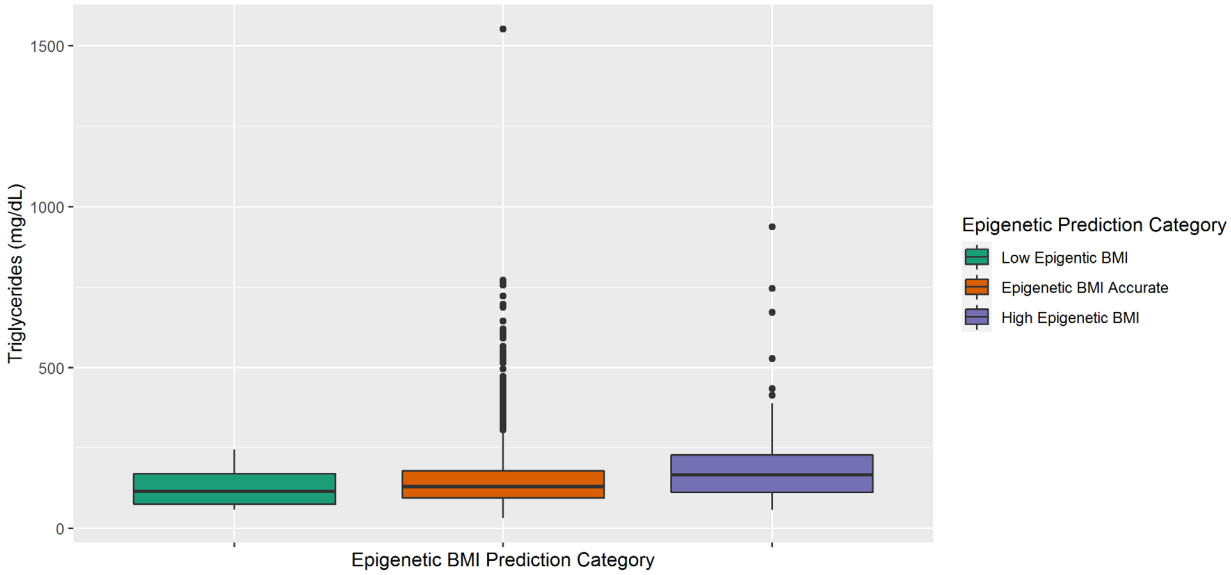
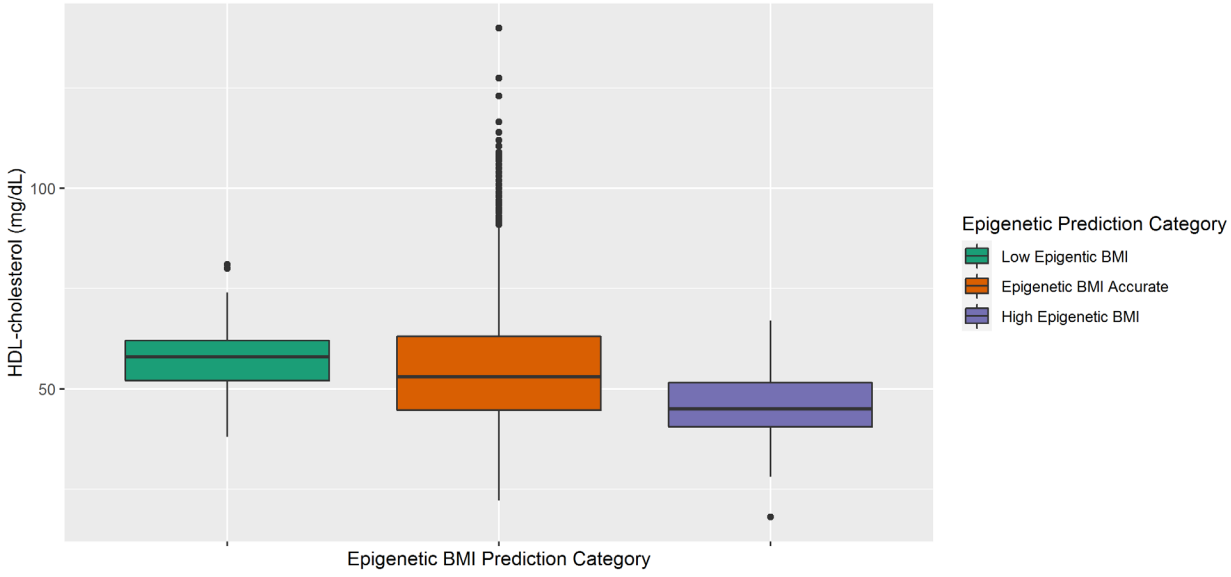


Figure 5.6. *Boxplot of the association between epigenetic prediction category and HDL-cholesterol (mg/dL).*



CHAPTER 6

Associations between DNA methylation and BMI vary by metabolic health status: a potential link to disparate cardiovascular outcomes

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Abstract

Context

As a measure of adiposity, increasing body mass index correlates with coronary heart disease (CHD). However, metabolic health has been shown to modify the relationship between BMI and CHD. The mechanisms underlying this relationship are poorly understood. DNA methylation may be able to distinguish biomarkers or pathways which underlie this interaction.

Objective

The purpose of this study was to examine whether metabolic health influences the relationship between body mass index (BMI) and blood DNA methylation.

Methods

The discovery study population was derived from two population based cohort studies and replicated in another cohort study. Metabolic health was examined continuously as a pooled Z-score (MHZ) of the clinical measures used in the ATP-III criteria. Generalized linear models regressed methylation β-values on the interaction between BMI and MHZ (BMIxMHZ) adjusted for BMI, MHZ, cell composition, chip number and location, study characteristics, top three ancestry principal components, smoking, age, ethnicity, and sex. Significance was set at FDR q< 0.05. Significant sites were replicated and examined for association with CHD.

Results

Among the 429,566 sites examined, BMIxMHZ was associated with differential DNAm at 22 CpG sites (FDR<0.05), with one site (cg18989722) replicating. Three of the 22 sites were associated with incident CHD. Risk of incident CHD increased by 9% and decreased by 6-11% per 0.01 unit increase in DNA methylation β-value at these sites.

Conclusions

The interaction between BMI and MHZ was associated with differential DNA methylation at 22 sites, one of which replicated and three of which were predictive of incident CHD over 25 years. These sites are located in genes related to NF-kappa-B signaling, suggesting a potential role for inflammation between DNA methylation and BMI-associated metabolic health.

Background

Obesity rates continue to rise with 41.1% of women in the United States living with obesity in 2016 (1). While obesity is most typically defined as body mass index (BMI) > 30 kg/m^2, limitations in the use of BMI have been noted, including variation in associations with health outcomes by race/ancestry, physical activity, and age (2, 3), as well as some reports finding no association between higher risk categories of BMI (overweight and middle obesity) and mortality (4, 5). These conflicting reports have motivated several studies to examine whether differential phenotypes of obesity exist and whether examining BMI in isolation of additional metabolic health parameters is a sufficient metric of overall health.

A growing body of evidence has found heterogeneity in obesity, with some phenotypes exhibiting differential risk for cardiovascular outcomes. Metabolically healthy obesity (MHO) has been defined as obesity with less than two or three metabolic health risk factors. Some but not all studies have found MHO to be associated with reduced risk of cardiovascular outcomes compared to metabolically unhealthy obesity (MUO) (6-11). In a recent systematic review, MHO had higher risk of cardiovascular events than metabolically healthy, normal weight participants (RR 1.45, 95% CI: 1.20-1.70), but had lower risk to metabolically unhealthy normal weight (RR: 2.07, 95% CI: 1.62-2.65) and MUO individuals (RR: 2.31, 95% CI: 1.99-2.69) (12). These findings suggest that metabolic health status may differentially influence the relationship between BMI and health outcomes. Examining the molecular underpinnings of this phenotype may guide our understanding of this epidemiological phenomenon by identifying the biological mechanisms which may be leading to a reduction in risk of health outcomes associated with obesity. Additionally, identifying biomarkers of MHO, particularly if they can identify individuals more likely to remain in MHO, would be advantageous for more targeted interventions.

Epigenetic mechanisms, such as DNA methylation, are important biological features to examine in the context of chronic diseases such as obesity and metabolic health. Changes to DNA methylation can induce changes in gene expression in causal disease pathways potentially

mediating or modifying differential health outcomes. Obesity has been widely examined and shown to associate with prolific methylation changes in the blood and adipose tissue (13-15). Similarly, metabolic syndrome and metabolic health risk factors have been found to associate with differential methylation (16-20). Indeed the mouse model which is used to represent MHO is developed from deletion of the *BRD2* gene, which is a primary epigenetic regulator of histone acetylation (21). However, no studies have integrated these phenotypes to examine how BMI-associated methylation varies by metabolic health status. Particularly since DNA methylation has been reported to mediate the relationship of obesity to increased cardiovascular outcomes (22), evaluating the epigenome may provide insight into pathways contributing to the differences in outcomes. The purpose of this study is to examine whether BMI associates with methylation differentially according to metabolic health status (**Figure 6.1**).

Methods

A summary of the methods is included in **Figure 6.2**.

Study population

Two cohorts were used in the discovery phase: the Women's Health Initiative (WHI) and the Atherosclerosis Risk in Communities study (ARIC). Data from three WHI ancillary studies were included: *Epigenetic Mechanisms of Particulate Matter-Mediated Cardiovascular Disease* (EMPC, aka AS315), the *Integrative Genomics for Risk of Coronary Heart Disease and Related Phenotypes in WHI cohort* (BAA23), and *Bladder Cancer and Leukocyte Methylation* (AS311). EMPC assessed epigenetic mechanisms underlying associations between ambient particulate matter air pollution and cardiovascular disease within the WHI Clinical Trials (CT, n=2200). BAA23 was a case-control study assessing predictors of coronary heart disease (CHD) within the WHI CT (n=1664) and OS (n=442), where cases were identified using eight biomarkers of CHD. AS311 is a matched case-control study of bladder cancer among women within the WHI CT (n = 405) and OS (n = 455) (23).

ARIC included data from two ancillary studies of African Americans (AA) and European Americans (EA). DNA methylation was measured in 2879 and 1100 ARIC AA and EA in visit 2 (1990-1992) or visit 3 (1993-1995). ARIC is an ongoing prospective cohort study investigating the etiology of CHD in four US communities: Forsyth County, NC; Jackson, MS; Minneapolis, MN; Washington County; MA. Participants were aged 45 to 64 and followed up for trends in coronary heart disease in each community over 15 years with 7 study visits (24, 25).

The replication cohort derived from the Multi-Ethnic Study of Atherosclerosis (MESA) study. MESA is a longitudinal, population cohort study designed to examine risk factors for and the progression of CHD. Participants aged 45-84 years without clinically apparent CHD were recruited between July 2000 and August 2002 from 6 regions in the US: Winston-Salem, NC; Northern New York, NY; Baltimore, MD; St. Paul, MN; Chicago, IL; and Los Angeles, CA. DNA methylation was derived from peripheral blood mononuclear cell samples at Exam 1 or Exam 5 in a random sample of 1,200 non-Hispanic white, African American, Hispanic and Chinese American participants (26, 27).

Measurements

In WHI, weight, height, waist circumference and blood pressure (BP) were measured at the physical exam. In ARIC, these measurements were taken at Visit 2 or 3. BMI was calculated as weight (kg)/height $(m)^2$. Waist circumference was measured to the nearest 0.5 cm. Two BP measurements were collected (systolic/diastolic). Biochemical measurements were analyzed in blood samples collected after a 12-hour fast. These include triglycerides (TG), high-density lipoprotein cholesterol (HDL), and fasting glucose.

Metabolic Health Exposures

Metabolic health was examined in two ways, dichotomously and continuously. Metabolic risk was dichotomously defined by presence of three or more components of metabolic syndrome using the Adult Treatment Panel III (ATP III) criteria (**Table 6.1**). Thus, MUO and MHO referred to the presence of three or more and less than three components, respectively. Metabolic health

was also examined continuously as a Z-score of the clinical measures used in the ATP III criteria. For each metabolic parameter, for example TG, the Z-score for TG was created by (TG – mean TG)/standard deviation (TG) of the population. Then all the clinical parameter Z-scores were combined to define a metabolic health Z-score (MHZ). For HDL, the inverse of HDL was used as a higher MHZ is indicative of poorer health. Since the association of BMI with CpG site DNA methylation has been shown to linear, we examined BMI continuously. Individuals were excluded if metabolic health parameters and DNA methylation were not measured within the same year.

Covariates

Age, race/ethnicity (White, African American, Hispanic/Latino, Asian, American Indian, other), and smoking status (current/former or never) were self-reported. Physical activity was measured by the Baecke questionnaire in ARIC (28) and a self-administered questionnaire in WHI (29) and expressed as total energy expended from light, moderate, or vigorous intensity recreational physical activity which includes walking, mild, moderate and strenuous physical activity in kcal/week/kg (MET-hours/week).

Outcome in Secondary Analyses

In significant sites identified through EWAS, DNA methylation at cytosine and guanine nucleotide pair (CpG) sites was examined as a predictor of incident CHD in the WHI. CHD was defined by incident myocardial infarction or CHD death. Medical records were reviewed and acute, hospitalized myocardial infarction was identified on the basis of cardiac pain, electrocardiogram, and biomarker data; then physician-adjudicated. Further details regarding the review, classification, and adjudication of CHD in WHI (31) have been described.

DNA methylation

In the all cohorts, DNA was extracted from peripheral blood leucocytes collected at visit-specific fasting blood draws (32). In the WHI and ARIC cohorts, DNA methylation was measured using the Illumina HM450K Infinium Methylation BeadChip. In the MESA cohort, DNA methylation was measured via the Illumina MethylationEPIC BeadChip array. DNA methylation was estimated

as the proportion of methylated probes relative to combined unmethylated and methylated probes for a specific CpG sites defined as the β-value (which is defined as the percentage of probes methylated divided by the combined methylated and unmethylated probes ranging from 0 [unmethylated] to 1 [methylated]). All methylation data were normalized using beta-mixture quantile normalization (33). Technical covariates included plate, chip, and row to adjust for batch effects and cell composition, which was estimated using the reference-based Houseman method (34). Quality control procedures included exclusion of probes with multi-modal signals as detected by the gaphunter function in the minfi package in R (https://www.r-project.org/) excluding 54636 probes. After quality control, 428278 probes remained in the analysis and were examined.

Statistical Analysis

We used R (https://www.r-project.org/) for all analyses. We calculated means and standard deviations or counts and proportions for study population characteristics. In the EWAS, all models were stratified by cohort (EMPC, BAA23, AS311 in WHI) and race (AA and EA in ARIC) and pooled using inverse-variance weighted meta-analysis. BMI was examined continuously. To examine the differential impact of metabolic health status on BMI, linear regression models were used regressing the methylation β-value on the interaction term for BMI and metabolic health status, adjusting for each higher-level variable (BMI and metabolic health) and covariates. We conducted two EWAS with metabolic health status defined dichotomously (BMIxMH) and continuously (BMIxMHZ). Covariates in all models included cell composition, the top 3 principal components of genetic relatedness, race/ethnicity (WHI), sex (ARIC), smoking status (current/former or never) and age. Study-specific covariates included trial study and randomization arm (EMPC, BAA23, AS311) and case-control status (BAA23, AS311). To adjust for batch effects, the DNA methylation array was included as a random effect for each BeadChip in our model. Significant CpG sites were identified by the interaction p-value at a false discovery rate (FDR) q-value <0.05.

Results identified in the discovery cohorts were replicated in the MESA cohort using linear regression models as previously described. Significant CpG sites were examined using the same linear regression model as above examining BMIxMHZ. Models were adjusted for DNA methylation array number and row location, cell composition, principal components of genetic relatedness, race/ethnicity, age, sex, alcohol consumption and smoking. Significant replication was defined at $p < 0.05$ and a consistent direction of effect.

Outcomes Analyses

Multivariate Cox proportional hazard ratios were used to examine whether significant sites identified through EWAS (exposure) were associated with incident CHD in WHI. Individuals with a history of (or incident) myocardial infarction or coronary revascularization (angioplasty; stent; bypass) before measurement of DNA methylation were excluded. Covariates included age, race/ethnicity, smoking status, case-control status (BAA23 and AS311), DNA methylation array, row, and cell composition in the reduced model. In the full model, we adjusted for the covariates in the reduced model as well as physical activity and diet quality. Significant sites were defined by $p < 0.05$.

Gene Expression

To elucidate the potential functional implications of the identified CpGs, gene expression information was obtained for each of the significant sites identified in the replication analysis and those associated with incident CHD. Specifically, for each of these CpGs, previously published gene expression quantitative trait methylation loci (eQTMs) summary statistics in blood from MESA and the Grady Trauma Project (GTP) were examined (35). This population from MESA had minimal overlap with the MESA population examined in replication analyses.

Sensitivity analyses

As metabolic health status is constructed from a number of metabolic parameters, differences in methylation may be driven by individual metabolic parameters. To assess the degree that individual metabolic parameters influence methylation at significant sites, we

reanalyzed associations between BMIxMHZ status and methylation excluding individual metabolic parameter in the MHZ score and compared the effects to the original estimates obtained through EWAS. For the significant sites, we also examined changes in effect size when adjusting for lipid, hypertension and glycemic medication use. We additionally repeated the primary EWAS analysis adjusting for physical activity.

Results

Demographic characteristics of the cohorts have been described in **Table 6.2**. Overall, 7497 participants were included in the discovery analysis. We identified no statistically significant differential associations between CpG methylation and BMI by dichotomized metabolic health status (BMIxMH). When metabolic health status was examined continuously (MHZ), 22 CpG sites were associated with BMIxMHZ (FDR q-value < 0.05, **Table 6.3**, **Figure 6.3**). For ease of interpretation, we described the direction of effect in the 22 significant sites in the models examining BMIxMH. In 13 of the 22 sites, an increase in BMI was associated with an opposite direction of effect in the coefficient in metabolically healthy vs unhealthy individuals. In the replication analysis, one site associated with BMIxMHZ ($p < 0.05$ in a consistent direction, **Table 6.3**). cg18989722 inversely associated with BMIxMHZ or the coefficient increased and decreased with every one unit increase BMI in metabolically healthy and metabolically unhealthy individuals, respectively.

Given the differential relationship between MHO and cardiovascular disease, we examined whether significant CpG sites predicted incident myocardial infarction over 25 years in the WHI. After excluding individuals from WHI with a history of cardiovascular disease, 3746 individuals remained (BAA23 n=1823, EMPC n=1775, AS311 n=148). When predicting incident myocardial infarction, we initially examined whether the interaction between BMIxMHZ associated with incident CHD, adjusting for age, smoking status and ethnicity. BMIxMHZ was significantly associated with incident CHD (HR: 1.014, 95% CI: 1.004, 1.03, p-value = 0.009, **Figure 6.4**). cg18989722 was not associated with incident CHD. However, when examining the 22 sites from

the discovery analysis, three sites were associated with incident CHD ($p < 0.05$, **Figure 6.5, Table 6.4**).

Among the replicated site and the three sites associated with incident CHD, we examined whether they were associated with differential gene expression in two cohorts, GTP and MESA. cg18989722 was associated with differential expression of *PTGS1* and cg16461485 was associated with differential expression of *TNFRSF13B*.

In sensitivity analyses, we examined the change in the association when adjusted for physical activity. No sites were significantly associated with BMIxMHZ. However, this may be due in part to a change in power as the correlation between effect sizes in the 22 significant sites was 0.97 and effect sizes were generally larger in the physical activity adjusted analyses (Supplemental Figure 1, ARIC EA n=929, ARIC AA n=2173, EMPC n=1676, BAA23 n=1969, AS311 n=163). When examining the change in the effect size when leaving out one of the clinical parameters from the MHZ score, HDL-cholesterol was the most significantly different (effect estimate correlation=0.89). In the 22 sites, effect sizes and Z-scores changed minimally when adjusted for lipid, hypertension and glycemic medications (all correlations in effect size=0.97 and all correlations in Z-scores=0.95). We examined the influence of individual ancillary studies on the results by examining the change in significance and effect size when ancillary studies (BAA23, EMPC, AS311, ARIC AA and ARIC EA) were individually excluded from the analysis. Significance changed moderately with exclusion of each study with 30, 20, 25, 20 and 27 significant sites when BAA23, EMPC, AS311, ARIC AA and ARIC EA were excluded, respectively. Differences in effect size were minor (correlation with main analysis= 0.99) in all studies except with exclusion of ARIC AA (correlation with main analysis = 0.86).

Discussion

In this study, we found 22 CpG sites were associated with BMIxMHZ in the WHI and ARIC cohorts, with one site replicating in a consistent direction in MESA. Among the 22 sites, two CpG

sites associated inversely and one CpG site associated positively with incident CHD in the WHI cohort.

The one site replicated in MESA, cg18989722, is located in the body of the *TRAPPC9* gene. *TRAPPC9* has a role in NF-kappa-B signaling by activating NF-kappa-B through increased phosphorylation of the IKK complex (28). *TRAPPC9* encodes NIBP, which binds to IKK/NIK to enhance NF-kappa-B activation. *TRAPPC9* has recently been identified as an imprinted gene primarily expressing the maternal allele (29). *TRAPPC9* knock-out mice exhibit a rare intellectual disability accompanied by an increase in fat mass and body weight (29), suggesting that expression of this gene may protect against obesity. Several CpG sites in *TRAPPC9* have been identified in an EWAS of childhood adiposity (30, 31). As gene body methylation has often been cited as an indicator of an active gene (32), our findings are in alignment with previous reports of protection against obesity since individuals with lower BMIxMHZ had higher methylation in this site.

This site was also associated with increased gene expression of the *PTGS1* gene. *PTGS1* (also known as *COX1*) catalyzes the conversion of arachinodate to prostaglandin protein and is inhibited by anti-inflammatory drugs. In our sensitivity analysis, when adjusted for lipid medication use including peripheral vasodilators such as aspirin, the effect size moderately changed (-8.36×10^{-5} in unadjusted models and -5.25×10^{-5} in adjusted models). However, the Z-score was smaller (-6.54 in unadjusted models and -2.55 in adjusted models). This suggests some attenuation in the relationship between BMIxMHZ and DNA methylation is potentially modified by medication use.

Methylation in three sites was associated with incident CHD over 25 years in the WHI cohort: cg16461485 located in the body of *SELT*, cg02851049 located in the body of *POLR3K*, and cg20210586 in the body of *TRIM39.* None of these sites have been identified in previous EWAS. We also found that cg16461485 associated with reduced gene expression of *TNFRSF13B*, which encodes the tumor necrosis factor (TNF) receptor superfamily member 13B,

also known as the transmembrane activator and CAML interactor (TACI). This protein activates NFAT, AP1 and NF-kappa-B (33). TACI knock-out mice were protected against high fat diet induced inflammation and dysglycemia, which may be mediated by a shift in adipose tissue macrophages from M1 to M2, which tend to promote a phenotype of insulin sensitivity (34). These findings further support the role that methylation in cg16461485 exhibiting a protective effect.

Given the molecular functions of these genes, differential inflammatory mechanisms (potentially mediated by the NF-kappa-B pathway) may account for the observed differences in health outcomes by BMIxMHZ. This is consistent with several studies which have found that MHO may be due to an uncoupling of obesity and insulin resistance. Indeed, MHO has been associated with lower inflammatory markers including CRP, TNF-α, IL-6, and plasminogen activator inhibitor-1 (35, 36). Several studies have also observed a unique relationship between inflammatory markers and adiposity in individuals of African descent, where these markers do not appear to be as sensitive to adiposity compared to individuals of European descent (37, 38). This may explain the significant differences observed when we exclude the ARIC AA cohort in sensitivity analyses.

There are several important limitations to this study. Given the cross sectional design, we cannot determine any causal association and may be at risk for reverse causality, if methylation is contributing to changes in BMI or levels of the metabolic risk factors included in our score. Moreover, metabolic risk factors may also be a product of duration of obesity, since several studies have found MHO to be a transitory state (6, 7, 39). However, understanding the CpG site specific differences in populations with MHO would still be advantageous to identify biological mechanisms that may be driving the differences in outcomes. Another limitation includes the potential for confounding by cell composition. Obesity and several of the metabolic health parameters associate with inflammation (40). While we controlled for cell composition using the methods of Houseman et al (41), we may not be able to account for rarer cell types and the identified CpG sites may be a reflection of these differences in cell composition associated with differential inflammatory profiles associated with these disease exposures. While we found novel

relationships between three CpG sites and CHD, none of these sites replicated in an external population, suggesting that other confounding factors may be causing the association. Nevertheless, the limited replication may be due in part to limited power as the replication analysis had the power to detect effect sizes as low as 0.01 and the effect sizes from our discovery EWAS were much lower (**Supplemental Table 7**). A strength of this study is examination of unique interactions between BMI and metabolic health in three population-based cohort studies and their impact on gene expression and CHD outcomes.

Overall, we found four CpG sites which may have a differential relationship with BMI in metabolically healthy vs unhealthy individuals. Our study findings may align with several studies suggesting that differential inflammatory mechanisms may account for differences in metabolic risk factors associated with increasing BMI. Future research studies could benefit from examining longitudinal changes in methylation associated with change in metabolic health status to determine the direction of effect.

References

1. Hales CM CM, Fryar CD, Ogden CL. Prevalence of obesity among adults and youth: United States, 2015–2016. 2017(NCHS data brief, no 288).
2. Winter JE, MacInnis RJ, Wattanapenpaiboon N, Nowson CA. BMI and all-cause mortality in older adults: a meta-analysis. The American journal of clinical nutrition 2014;99(4):875-90. doi: 10.3945/ajcn.113.068122.
3. Evans EM, Rowe DA, Racette SB, Ross KM, McAuley E. Is the current BMI obesity classification appropriate for black and white postmenopausal women? International Journal of Obesity 2006;30(5):837-43. doi: 10.1038/sj.ijo.0803208.
4. Flegal KM, Kit BK, Orpana H, Graubard BI. Association of All-Cause Mortality With Overweight and Obesity Using Standard Body Mass Index Categories: A Systematic Review and Meta-analysis. Jama 2013;309(1):71-82. doi: 10.1001/jama.2012.113905.
5. Xu H, Cupples LA, Stokes A, Liu C-T. Association of Obesity With Mortality Over 24 Years of Weight History: Findings From the Framingham Heart Study. JAMA Network Open 2018;1(7):e184587-e. doi: 10.1001/jamanetworkopen.2018.4587.
6. Mongraw-Chaffin M, Foster MC, Kalyani RR, Vaidya D, Burke GL, Woodward M, Anderson CAM. Obesity Severity and Duration Are Associated With Incident Metabolic Syndrome: Evidence Against Metabolically Healthy Obesity From the Multi-Ethnic Study of Atherosclerosis. The Journal of clinical endocrinology and metabolism 2016;101(11):4117-24. doi: 10.1210/jc.2016-2460.
7. Mongraw-Chaffin M, Foster MC, Anderson CAM, Burke GL, Haq N, Kalyani RR, Ouyang P, Sibley CT, Tracy R, Woodward M, et al. Metabolically Healthy Obesity, Transition to Metabolic Syndrome, and Cardiovascular Risk. J Am Coll Cardiol 2018;71(17):1857-65. doi: 10.1016/j.jacc.2018.02.055.
8. Stefan N, Häring HU, Schulze MB. Metabolically healthy obesity: the low-hanging fruit in obesity treatment? The lancet Diabetes & endocrinology 2018;6(3):249-58. doi: 10.1016/s2213-8587(17)30292-9.
9. Hamer M, Stamatakis E. Metabolically Healthy Obesity and Risk of All-Cause and Cardiovascular Disease Mortality. The Journal of Clinical Endocrinology & Metabolism 2012;97(7):2482-8. doi: 10.1210/jc.2011-3475.
10. Guo F, Garvey WT. Cardiometabolic disease risk in metabolically healthy and unhealthy obesity: Stability of metabolic health status in adults. Obesity (Silver Spring) 2016;24(2):516-25. doi: 10.1002/oby.21344.
11. Roberson LL, Aneni EC, Maziak W, Agatston A, Feldman T, Rouseff M, Tran T, Blaha MJ, Santos RD, Sposito A, et al. Beyond BMI: The “Metabolically healthy obese” phenotype & its association with clinical/subclinical cardiovascular disease and all-cause mortality -- a systematic review. BMC public health 2014;14(1):14. doi: 10.1186/1471-2458-14-14.
12. Eckel N, Meidtner K, Kalle-Uhlmann T, Stefan N, Schulze MB. Metabolically healthy obesity and cardiovascular events: A systematic review and meta-analysis. European journal of preventive cardiology 2015;23(9):956-66. doi: 10.1177/2047487315623884.
13. Wang X, Zhu H, Snieder H, Su S, Munn D, Harshfield G, Maria BL, Dong Y, Treiber F, Gutin B, et al. Obesity related methylation changes in DNA of peripheral blood leukocytes. BMC Medicine 2010;8(1):87. doi: 10.1186/1741-7015-8-87.
14. Dick KJ, Nelson CP, Tsaprouni L, Sandling JK, Aïssi D, Wahl S, Meduri E, Morange P-E, Gagnon F, Grallert H, et al. DNA methylation and body-mass index: a genome-wide analysis. The Lancet 2014;383(9933):1990-8. doi: https://doi.org/10.1016/S0140-6736(13)62674-4.

15. Sayols-Baixeras S, Subirana I, Fernández-Sanlés A, Sentí M, Lluís-Ganella C, Marrugat J, Elosua R. DNA methylation and obesity traits: An epigenome-wide association study. The REGICOR study. Epigenetics 2017;12(10):909-16. doi: 10.1080/15592294.2017.1363951.
16. Barres R, Zierath JR. DNA methylation in metabolic disorders. The American journal of clinical nutrition 2011;93(4):897S-900S. doi: 10.3945/ajcn.110.001933.
17. Samblas M, Milagro FI, Martínez A. DNA methylation markers in obesity, metabolic syndrome, and weight loss. Epigenetics 2019;14(5):421-44. doi: 10.1080/15592294.2019.1595297.
18. Braun KVE, Dhana K, de Vries PS, Voortman T, van Meurs JBJ, Uitterlinden AG, Hofman A, Hu FB, Franco OH, Dehghan A, et al. Epigenome-wide association study (EWAS) on lipids: the Rotterdam Study. Clinical epigenetics 2017;9(1):15. doi: 10.1186/s13148-016-0304-4.
19. Dhana K, Braun KVE, Nano J, Voortman T, Demerath EW, Guan W, Fornage M, van Meurs JBJ, Uitterlinden AG, Hofman A, et al. An Epigenome-Wide Association Study of Obesity-Related Traits. American journal of epidemiology 2018;187(8):1662-9. doi: 10.1093/aje/kwy025.
20. Walaszczyk E, Luijten M, Spijkerman AMW, Bonder MJ, Lutgers HL, Snieder H, Wolffenbuttel BHR, van Vliet-Ostaptchouk JV. DNA methylation markers associated with type 2 diabetes, fasting glucose and HbA1c levels: a systematic review and replication in a case-control sample of the Lifelines study. Diabetologia 2018;61(2):354-68. doi: 10.1007/s00125-017-4497-7.
21. Wang F, Deeney JT, Denis GV. Brd2 gene disruption causes "metabolically healthy" obesity: epigenetic and chromatin-based mechanisms that uncouple obesity from type 2 diabetes. Vitam Horm 2013;91:49-75. doi: 10.1016/B978-0-12-407766-9.00003-1.
22. Mendelson MM, Marioni RE, Joehanes R, Liu C, Hedman ÅK, Aslibekyan S, Demerath EW, Guan W, Zhi D, Yao C, 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(1):e1002215-e. doi: 10.1371/journal.pmed.1002215.
23. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. Controlled clinical trials 1998;19(1):61-109.
24. Sharrett AR. The Atherosclerosis Risk in Communities (ARIC) Study. Introduction and objectives of the hemostasis component. Annals of epidemiology 1992;2(4):467-9.
25. INVESTIGATORS TA. THE ATHEROSCLEROSIS RISK IN COMMUNIT (ARIC) STUI)Y: DESIGN AND OBJECTWES. American journal of epidemiology 1989;129(4):687-702. doi: 10.1093/oxfordjournals.aje.a115184.
26. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR, Jr., Kronmal R, Liu K, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. American journal of epidemiology 2002;156(9):871-81. doi: 10.1093/aje/kwf113.
27. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, JacobsJr. DR, Kronmal R, Liu K, et al. Multi-Ethnic Study of Atherosclerosis: Objectives and Design. American journal of epidemiology 2002;156(9):871-81. doi: 10.1093/aje/kwf113.
28. TRAPPC9 Gene. GeneCards.
29. Liang ZS, Cimino I, Yalcin B, Raghupathy N, Vancollie VE, Ibarra-Soria X, Firth HV, Rimmington D, Farooqi IS, Lelliott CJ, et al. Trappc9 deficiency causes parent-of-origin dependent microcephaly and obesity. PLoS genetics 2020;16(9):e1008916. doi: 10.1371/journal.pgen.1008916.
30. Huang RC, Garratt ES, Pan H, Wu Y, Davis EA, Barton SJ, Burdge GC, Godfrey KM, Holbrook JD, Lillycrop KA. Genome-wide methylation analysis identifies differentially methylated CpG loci associated with severe obesity in childhood. Epigenetics 2015;10(11):995-1005. doi: 10.1080/15592294.2015.1080411.
31. Rzehak P, Covic M, Saffery R, Reischl E, Wahl S, Grote V, Weber M, Xhonneux A, Langhendries J-P, Ferre N, et al. DNA-Methylation and Body Composition in Preschool Children: Epigenome-

Wide-Analysis in the European Childhood Obesity Project (CHOP)-Study. Scientific Reports 2017;7(1):14349. doi: 10.1038/s41598-017-13099-4.
32. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nature reviews Genetics 2012;13(7):484-92. doi: 10.1038/nrg3230.
33. . Internet: https://www.genecards.org/cgi-bin/carddisp.pl?gene=TNFRSF13B (accessed 12-15-2020.
34. Liu L, Inouye KE, Allman WR, Coleman AS, Siddiqui S, Hotamisligil GS, Akkoyunlu M. TACI-Deficient Macrophages Protect Mice Against Metaflammation and Obesity-Induced Dysregulation of Glucose Homeostasis. Diabetes 2018;67(8):1589-603. doi: 10.2337/db17-1089.
35. Karelis AD, Faraj M, Bastard J-P, St-Pierre DH, Brochu M, Prud'homme D, Rabasa-Lhoret R. The Metabolically Healthy but Obese Individual Presents a Favorable Inflammation Profile. The Journal of Clinical Endocrinology & Metabolism 2005;90(7):4145-50. doi: 10.1210/jc.2005-0482.
36. Phillips CM, Perry IJ. Does inflammation determine metabolic health status in obese and nonobese adults? The Journal of clinical endocrinology and metabolism 2013;98(10):E1610-9. doi: 10.1210/jc.2013-2038.
37. Doumatey AP, Lashley KS, Huang H, Zhou J, Chen G, Amoah A, Agyenim-Boateng K, Oli J, Fasanmade O, Adebamowo CA, et al. Relationships Among Obesity, Inflammation, and Insulin Resistance in African Americans and West Africans. Obesity 2010;18(3):598-603. doi: https://doi.org/10.1038/oby.2009.322.
38. Hakim O, Bello O, Ladwa M, Peacock JL, Umpleby AM, Charles-Edwards G, Amiel SA, Goff LM. The Link between Obesity and Inflammatory Markers in the Development of Type 2 Diabetes in Men of Black African and White European Ethnicity. Nutrients 2020;12(12):3796.
39. Power C, Thomas C. Changes in BMI, Duration of Overweight and Obesity, and Glucose Metabolism: 45 Years of Follow-up of a Birth Cohort. Diabetes Care 2011;34(9):1986-91. doi: 10.2337/dc10-1482.
40. Ferrante Jr AW. Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. Journal of Internal Medicine 2007;262(4):408-14. doi: 10.1111/j.1365-2796.2007.01852.x.
41. Houseman EA, Molitor J, Marsit CJ. Reference-free cell mixture adjustments in analysis of DNA methylation data. Bioinformatics (Oxford, England) 2014;30(10):1431-9. doi: 10.1093/bioinformatics/btu029.

Tables and Figures

Table 6.1. *ATP III Clinical Identification of Metabolic Syndrome*

Clinical Measure	Defining Level
Waist Circumference	≥102 cm in men or ≥88 cm in women
Triglycerides	≥150 mg/dL or drug treatment for elevated triglycerides
High Density Lipoprotein (HDL)	<40 mg/dL in men or <50 mg/dL in women or drug treatment for reduced HDL
Blood Pressure	≥130/85 mmHG or drug treatment for hypertension
Glucose	≥110 mg/dL or drug treatment for elevated glucose

Table 6.2. *Demographic characteristics of each ancillary study in WHI and ARIC. Means (sd) or proportions have been included.*

	EMPC (n=1833)	BAA23 (n=1977)	AS311 (n=167)	ARIC EA	ARIC AA
Clinical Trial Participant					
Yes	1833	1543	119	-	-
No	0	434	0	-	-
Case/Control status (BAA23)					
Case	0	987	0	-	-
Control	0	990	0	-	-
Case/Control status (AS311)					
Case	0	0	91	-	-
Control	0	0	76	-	-
Age mean (SD)	63.2 (7.1)	64.6 (7.1)	66.2 (7.2)	59.9 (5.4)	56.6 (5.9)
Ethnicity					
White	922	944	99		
African American	474	631	49		
Hispanic/Latino	260	402	16		
Asian or Pacific Islander	107	0	2		
American Indian or Alaskan Native	41	0	1		
Other	29	0	0		
Smoking Status					
Former and current	853	913	99	610	1351
Never	963	1048	67	449	1110
Metabolic Health Status					
Metabolically Healthy	1254	1163	109	662	1177

Metabolically Unhealthy	579	814	58	397	1284
BMI*Metabolic Health Z-score mean (SD)	2.96 (30.38)	2.92 (31.59)	-2.5 (2.3)	0 (1)	0 (1)
BMI mean (SD)	29.5 (5.9)	29.8 (6.1)	29.3 (6.9)	26.2 (4.3)	30.1 (6.2)
BMI Categories					
Underweight	7	13	0	20	18
Normal	417	420	41	435	444
Overweight	641	680	67	429	912
Obese	768	864	59	175	1087
Waist Circumference mean (SD)	89.5 (13.8)	90.7 (13.7)	89.1 (15.1)	94.5 (12.8)	101.4 (15.1)
Triglycerides mean (SD)	153 (88.2)	146.9 (83.4)	143.8 (82.5)	140.4 (83.6)	117.3 (77.7)
HDL-Cholesterol mean (SD)	58 (15.1)	52.1 (13.2)	53.2 (13.0)	52.3 (18.2)	53.3 (17.3)
Systolic Blood Pressure mean (SD)	128 (18)	132.1 (17.8)	132.5 (16.6)	118.7 (18.0)	127.3 (20.6)
Diastolic Blood Pressure mean (SD)	75.3 (9.4)	76.4 (9.3)	76.3 (8.5)	68.5 (9.7)	75.2 (10.7)
Blood Glucose mean (SD)	103 (31.1)	108.6 (41.3)	105.2 (37.2)	105.9 (28.6)	129.3 (64.3)

Table 6.3. *CpG sites identified in the discovery cohort.*

	WHI and ARIC						MESA			
CpG site	**Effect Size**	**Standard Error**	**Zscore**	**p-value**	**Metabolically Healthy**	**Metabolically Unhealthy**	**T.statistic**	**P.value**	**Holm.sig**	**FDR**
cg00868074	0.000315	6.90E-05	4.753387	2.00E-06	-	+	0.813911	0.416087	FALSE	0.693479
cg02851049	-8.85E-05	1.04E-05	-7.91227	2.53E-15	-	-	-1.71765	0.086487	FALSE	0.693479
cg05441596	-5.74E-05	1.11E-05	-4.95087	7.39E-07	+	-	-0.04166	0.966786	FALSE	0.97337
cg06344952	-9.11E-05	1.46E-05	-5.78557	7.23E-09	+	-	0.033399	0.97337	FALSE	0.97337
cg07226317	-9.17E-05	1.62E-05	-5.15533	2.53E-07	+	-				
cg08082299	-0.0001	1.73E-05	-5.59187	2.25E-08	-	-	1.122892	0.262028	FALSE	0.693479
cg10057841	-9.86E-05	1.56E-05	-5.99443	2.04E-09	+	-	0.364646	0.715531	FALSE	0.954042
cg11553983	-6.96E-05	1.36E-05	-4.75996	1.94E-06	+	-	-0.21812	0.827428	FALSE	0.97337
cg15062225	-0.00014	2.05E-05	-6.52431	6.83E-11	+	-	-1.10901	0.267963	FALSE	0.693479
cg16461485	-0.00012	2.06E-05	-5.22882	1.71E-07	+	+	0.920949	0.357526	FALSE	0.693479
cg16543390	6.71E-05	1.38E-05	5.257168	1.46E-07	+	+	0.88978	0.374016	FALSE	0.693479
cg18298785	-8.52E-05	1.40E-05	-5.57895	2.42E-08	+	-	-0.70114	0.483547	FALSE	0.743918
cg18989722	-8.36E-05	1.20E-05	-6.54025	6.14E-11	+	-	-2.04684	0.041201	FALSE	0.693479
cg19572849	-4.99E-05	8.96E-06	-5.63193	1.78E-08	-	-	0.551567	0.581494	FALSE	0.830705
cg20210586	-0.0001	1.39E-05	-6.96531	3.28E-12	+	-	-0.10473	0.916631	FALSE	0.97337

cg21880445	-7.90E-05	1.44E-05	-5.22636	1.73E-07	-	-	-0.95512	0.339982	FALSE	0.693479
cg22076143	-0.0001	1.27E-05	-7.61932	2.55E-14	-	+	0.874629	0.3822	FALSE	0.693479
cg24460625	-7.26E-05	1.11E-05	-6.37718	1.80E-10	-	-	-1.36572	0.172646	FALSE	0.693479
cg24720717	6.22E-05	1.22E-05	4.945594	7.59E-07	-	+	-1.07273	0.283915	FALSE	0.693479
cg24827562	-8.71E-05	9.83E-06	-8.41337	3.98E-17	+	-	0.092298	0.926499	FALSE	0.97337
cg26206680	-6.13E-05	9.90E-06	-5.73111	9.98E-09	-	-	0.9877	0.323782	FALSE	0.693479
cg27004639	5.20E-05	9.14E-06	5.644086	1.66E-08	-	-	0.9877	0.323782	FALSE	0.693479

Table 6.4. *Significant CpG sites associated with incident CHD in WHI over 25 years*

CpG Site	HR	95% CI	p-value	FDR
cg02851049	0.897052	(0.812154, 0.990825)	0.032217	0.249169
cg20210586	1.094975	(1.003004, 1.19538)	0.042661	0.249169
cg16461485	0.941783	(0.888621, 0.998125)	0.043042	0.249169

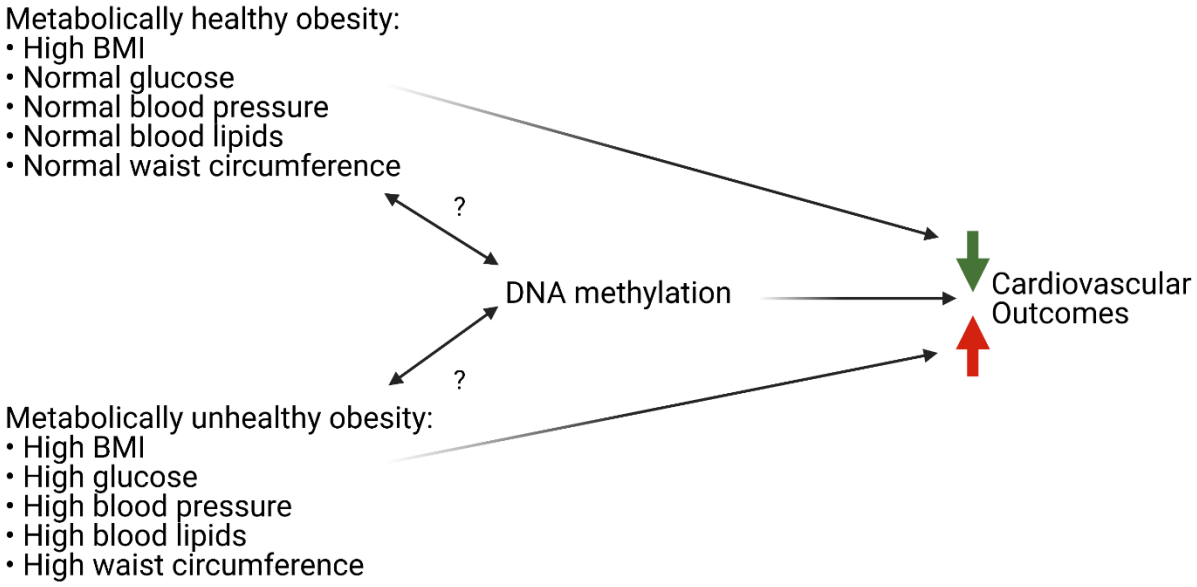


Figure 6.2*. Conceptual framework research question*

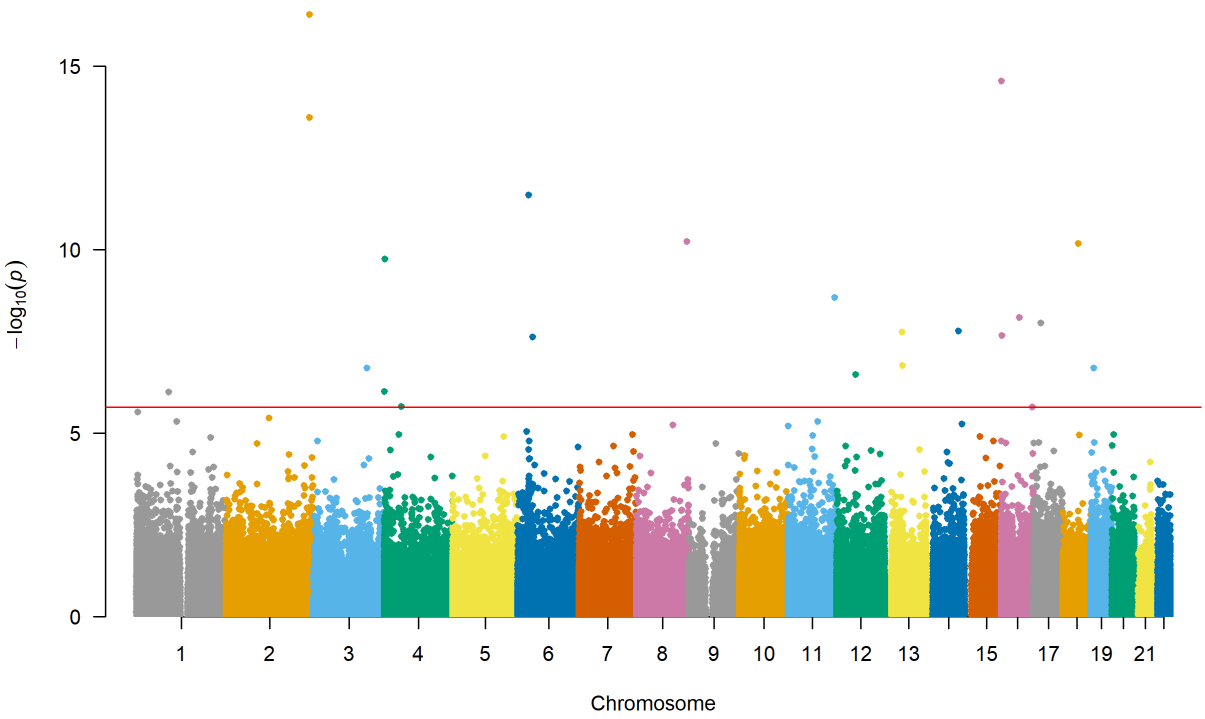


***Figure 6.2**. Manhattan plot of the association between the interaction of BMI and metabolic health Z-score and DNA methylation. Significant sites identified as those above the red line (p < 2x10-6).*

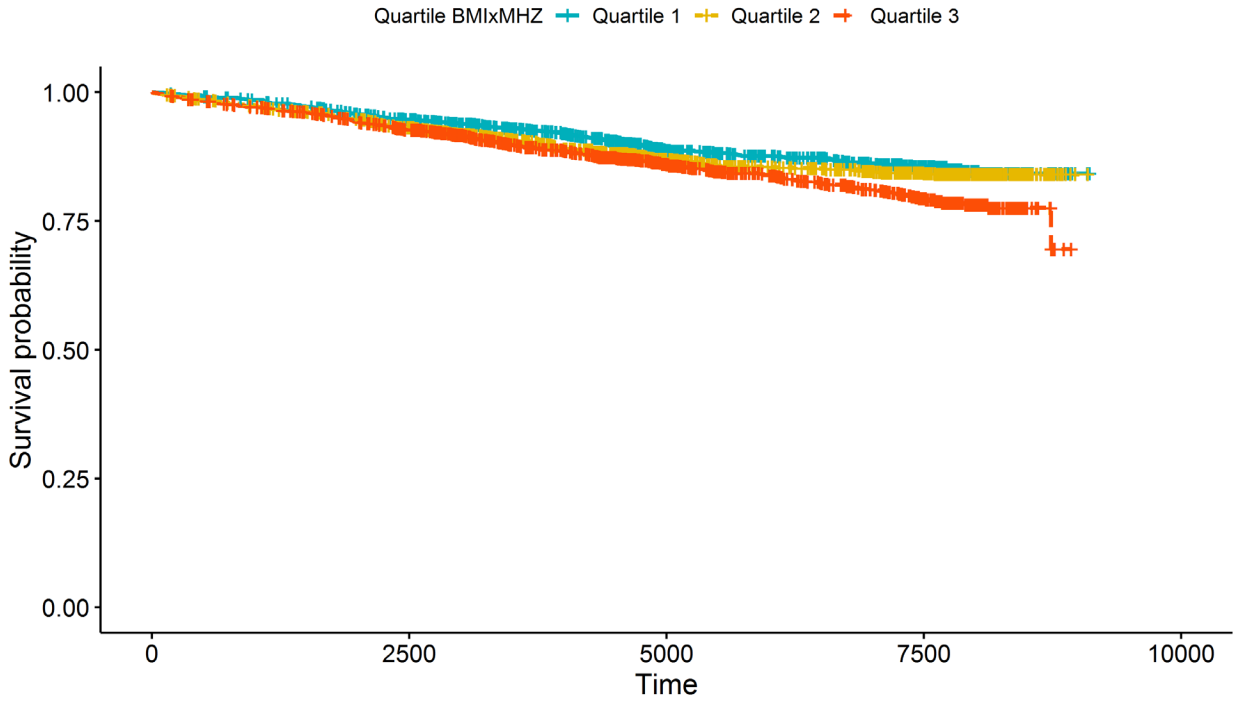
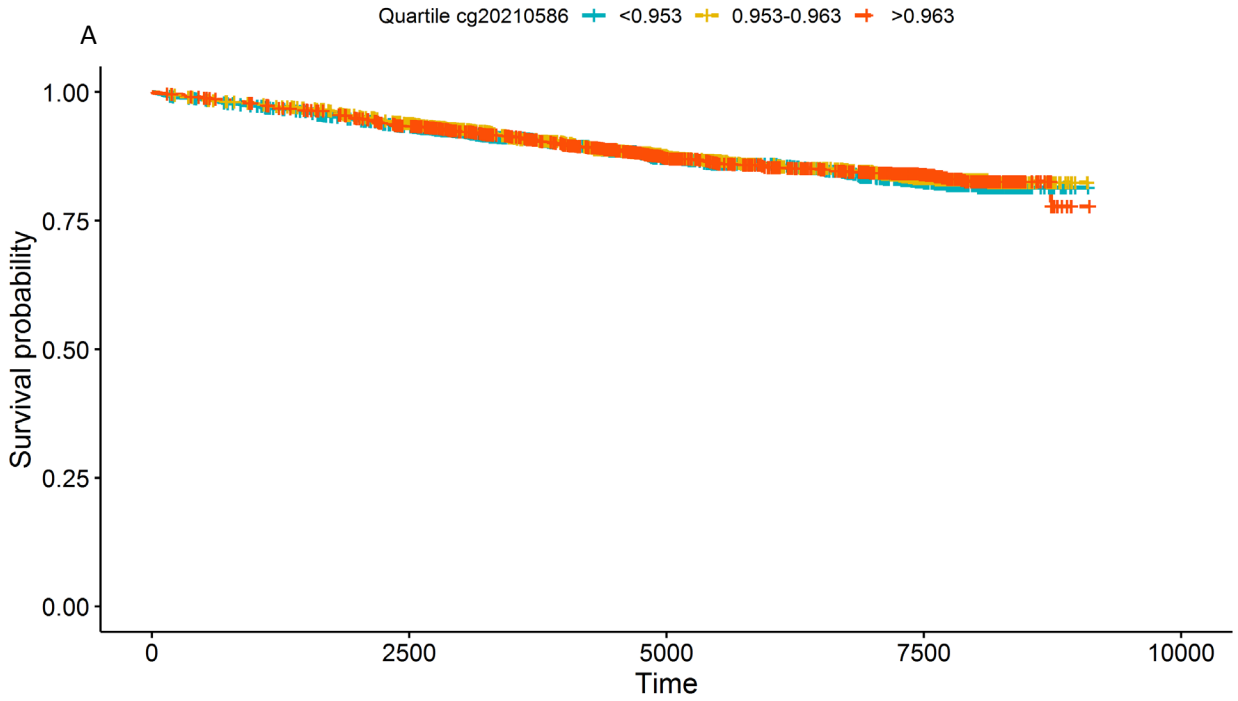


Figure 6.3. *Probability of incident CHD by quartile of BMIxMHZ in WHI cohort over 25 years.*



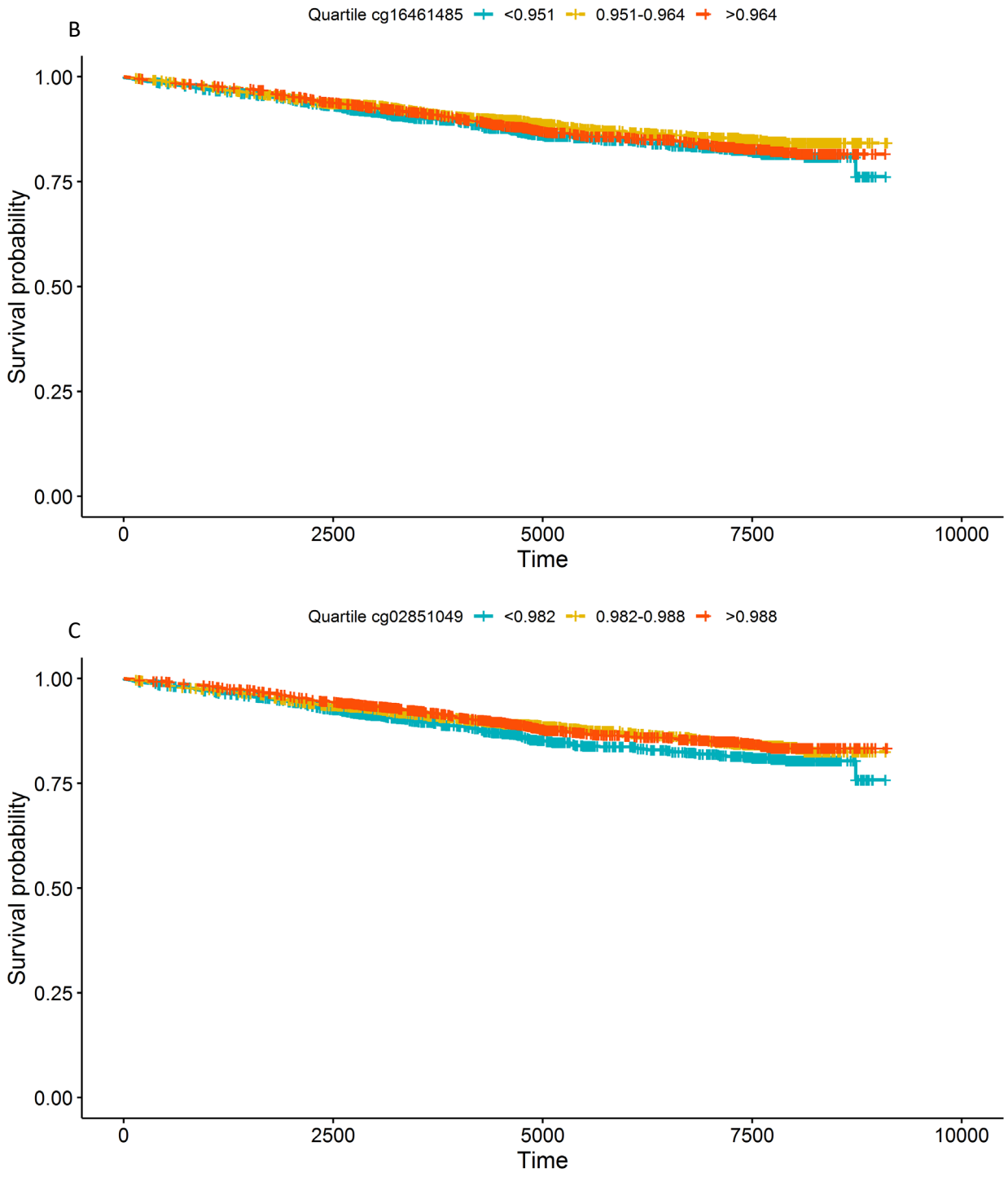


Figure 6.4. *Probability of incident CHD by quartile of CpG site methylation of cg20210586 (A), cg16461485 (B) and cg02851049 (C) over 25 years in WHI*

CHAPTER 7

Discussion

DNA methylation serves as a unique biological indicator of the physiological state of a person. Evaluating how DNA methylation associates with both extrinsic and intrinsic exposures can identify relevant biomarkers of health and elucidate the molecular mechanisms contributing to disease progression. Examining DNA methylation has shown utility as biomarkers in several clinical settings, including in early cancer detection (1) and predicting age-related disorders (2, 3). Using Mendelian Randomization methods, several studies have also identified DNA methylation as a mediator between various exposures and outcomes, including as mediating the effect of prenatal famine on adult cardiometabolic disease (4) and smoking on inflammation (5). These findings support the examination of DNA methylation in association with health outcomes. Diet quality and obesity represent critical contributors to a host of non-communicable diseases including type 2 diabetes, cardiovascular disease, cancer, and osteoarthritis. DNA methylation may be contributing to the relationship between diet and obesity and non-communicable disease progression. Additionally, examining DNA methylation could identify important biomarkers of these exposures. This dissertation evaluated whether DNA methylation was differentially associated with diet quality, metabolic health and obesity.

Nutrition and diet throughout the lifespan has been well known to play a role on the epigenome. However, the vast majority of studies have either focused on nutritional exposures in utero, specific nutrients or they have been underpowered to detect significant effects in individual CpG sites at a genome-wide threshold for significance. In chapter 4, we investigated how diet quality associated with differential methylation patterns in adult women in two large population-based cohort studies. We found significant differences in DNA methylation associated with diet quality as measured by the Alternative Healthy Eating Index – 2010 (AHEI-2010). Several of these CpG sites had previously been found to associate with cardiometabolic

disease risk factors including inflammation, obesity and dysglycemia. Ultimately, this study has established the important role of diet on the epigenome in adulthood.

One element which may have influenced our findings is the differential effect of nutrients on the epigenome. It has been speculated that nutrients of one-carbon metabolism would influence methylation patterns due to the fact that S-adenosylmethionine (SAM) acts as a methyl donor to de-novo methyltransferase. Folate, vitamin B12, choline, and vitamin B2 are essential nutrients in one-carbon metabolism. In practice, cross-sectional and prospective studies have had mixed findings (6-10). However, the relationship between these nutrients and DNA methylation may have implications for our study. In Aim 2, components of the AHEI-2010 comprising of these nutrients include vegetables, whole grains, nuts and legumes, and red/processed meats. As foods that increase one-carbon metabolism nutrients, it is plausible that these foods would influence methylation in the same direction. However, due to the way AHEI-2010 is scored where vegetables, whole grains and nuts and legumes increase AHEI-2010 score, and red/processed meats decrease AHEI-2010 score, we may be diluting the true association. Future research efforts should examine the relationship between the epigenome and individual dietary factors alongside combined diet quality estimates to assess which dietary factors may be driving the relationship between DNA methylation and diet quality.

One result from sensitivity analyses in chapter 4 was the important role that adiposity plays on the epigenome and how interrelated poor diet quality and obesity are. When examining the relationship between diet quality and the epigenome unadjusted for body mass index (BMI), the number of significant CpG sites rose from 400 to over 1800. This suggest adiposity has widespread effects on the epigenome and ultimately inspired the research in chapter 5 and 6.

In chapter 5, we conducted the largest epigenome-wide meta-analysis of BMI with 17,034 individuals in the discovery population. We found 1265 CpG sites were associated with BMI ($p<1x10^{-7}$). When replicated in the Women's Health Initiative (WHI), we found 1238 sites

remained associated with BMI (FDR q-value < 0.05). These included all the sites identified within the systematic review and meta-analysis of BMI in Chapter 2.2. Given the important relationship between BMI and diet quality, we examined the overlap in the CpG sites between the Aim 2 and 3. Thirty eight CpG sites were associated with both diet quality (in chapter 4) and BMI (in chapter 5). The relationship with BMI is likely representative of confounding by diet quality as none of the studies from the discovery analysis were adjusted for diet quality.

We also identified a unique signature of obesity in individuals of African descent. In this EWAS, 43 sites were uniquely associated in these models and not identified in EWAS of individuals of European descent. From these, five sites had a significantly different relationship with BMI between non-Hispanic whites and African Americans in the WHI. These sites may indicate relevant molecular pathways which differentiate how obesity influences health in this population. It has been well known that African Americans have a differential risk of metabolic risk factors and outcomes based on their BMI compared to non-Hispanic whites. For example, African Americans tend to have higher BMI compared to non-Hispanic whites (11). However, at these higher levels of BMI, they have lower body fat % compared to non-Hispanic whites or Asian individuals (12). This may be due to their distribution of adipose tissue in individuals of African descent, who tend to have higher subcutaneous and lower visceral adipose tissue (13). All of these findings suggest a differential relationship between BMI and metabolic risk factors by race. Our findings of a differential relationship between BMI and DNA methylation by race could be the cause or consequence of why BMI associates differentially with health outcomes in individuals of African descent.

We additionally examined how these five CpG sites associate with mRNA transcription in the Grady Trauma Project (GTP). Two sites associated with differential gene expression of genes relevant to adiposity-induced inflammation. In these sites, methylation was associated with the downregulation of potentially deleterious genes in the inflammatory cascade. This may

be identifying a protective mechanism in African Americans with obesity. Methylation may be downregulating two genes critical in the pathway activating NF-kappa-B, NFAT and AP1. In alignment with this, some recent studies have found no association between TNF- α and obesity in African Americans (14) and West Africans (15). In a systematic review examining CRP and obesity by race, women and North American/European whites had the strongest relationship between the two, with weaker relationship in individuals of African descent (16).

In Aim 2, we also found that DNA methylation was highly predictive of BMI with 398 sites accounting for 32% of the variance in BMI. The strongest risk factors and largest sources of variability in BMI are often cited as parental BMI and obesity in childhood (17). In models examining genetic predictor of BMI, genetic information accounts for around ~30% of the variation in BMI (17, 18). A large twin study found that genetic factors account for a large proportion of the variance, though this decreases with age (R^2 0.75 at age 20-29 and 0.59 at age 80 in women). Whereas, unique environmental effects increase with age (R^2 at age 20-29 0.25 and 0.40 at age 80 in women) (19). Similar to epigenetics, a substantial proportion of variation remains unaccounted for when only genetic effects are included. In prior epigenetic prediction models, the predictive ability ranged from 4-22% (20-22). In our study we may be accounting for some genetic variation that has previously been identified, through methylation quantitative trait loci, CpG sites that have been found to act dependently on SNPs. Shah et al. found that combined genetic and epigenetic effects accounted for 5-7% of the variance in BMI and they functioned in an additive manner, suggesting that these effects function independently (22). In Aim 2, we had a larger sample size and included CpG sites identified from a larger EWAS, which may be why we could account for a significantly larger proportion of the variance in BMI.

To examine whether “epigenetic BMI” could identify unique phenotypes of obesity, we examined outliers by their predicted BMI. Individuals whose BMI was over predicted by their

methylome had higher blood glucose and triglycerides and lower HDL-cholesterol compared to accurately predicted individuals. Similar to epigenetic age, “epigenetic BMI” could be a useful biomarker for more targeted interventions. Epigenetic age has been associated with a number of relevant exposures. Accelerated epigenetic age, when epigenetic age is higher than actual age, has been associated with cancer, neurological disorders, physical and cognitive health, and mortality (3, 23). This is a highly useful clinical biomarker that could identify individuals at risk of these diseases and allow for earlier intervention. “Epigenetic BMI” could similarly be a useful biomarker to identify metabolically healthy vs. unhealthy obese individuals.

Metabolically healthy obesity has been characterized from a subgroup of individuals who do not exhibit the typical cardiometabolic abnormalities associated with obesity. Several biological differences have been identified between metabolically healthy obese individuals including lower liver and visceral fat, higher leg fat and better insulin sensitivity, inflammatory markers and adipose tissue function (24). Metabolically healthy obesity has also been found to differentially influence cardiovascular disease and mortality. In a recent systematic review, metabolically healthy obesity had higher risk of cardiovascular events than metabolically healthy, normal weight participants (RR 1.45, 95% CI: 1.20-1.70), but had lower risk to metabolically unhealthy normal weight (RR: 2.07, 95% CI: 1.62-2.65) and obese individuals (RR: 2.31, 95% CI: 1.99-2.69) (25). Understanding the molecular mechanisms associated with metabolically healthy obesity could provide insight into the differential outcomes associated with this phenotype.

The study in Aim 3 sought to examine whether the interaction between BMI and metabolic health was associated with differential methylation patterns. We found that 22 sites were associated with the interaction between BMI and metabolic health Z-score in the WHI and the Atherosclerosis Risk in Communities (ARIC) cohort. When replicating the Multi-Ethnic Study of Atherosclerosis (MESA), one site replicated in a consistent direction, cg18989722. This study

also examined whether the 22 sites from the discovery analysis predicted incident coronary heart disease in the WHI over 25 years. Three sites were found to be predict CHD.

As a primary aim of this study was to examine the molecular underpinnings associated with metabolically healthy obesity, we examined both the functions of the genes annotated to each CpG site as well as how the four significant CpG sites associated with differential gene expression in two external cohorts (MESA and the Grady Trauma Project [GTP]). As in Aim 2, several genes in the NF-kappa-B pathway were identified within this study. One site was located in *TRAPPC9* which downregulates NF-kappa-B signaling and one site was associated with reduced gene expression of *TNFRSF13B*, which regulates TNF signaling in this pathway. These results are further reinforcing the potential relationship between DNA methylation and obesity-associated inflammation.

All of these findings represent important molecular signals that may be useful as potential biomarkers of disease or therapeutic targets in regulation of gene expression. However, DNA methylation represents only one epigenetic modification. Histone modifications and microRNA are two distinct mechanisms which have similar properties to methylation in their responsiveness to external signals and effects on chromatin modification. The N-terminal tails of histones can be prone to several modifications including acetylation, methylation, phosphorylation or ubiquination. Additionally, DNA methylation and histone modifications are biologically linked in carrying out their effects on gene expression, such that histones can direct DNA methylation patterns and DNA methylation may be a template for histone modifications (26). Similarly, DNA methylation has been shown to associate with differential microRNA expression, with 58 sites having been identified as causally regulating microRNA expression (27). Our studies were only able to examine DNA methylation as significantly fewer studies have assayed microRNA or histone modifications. However, future research studies should consider

examining these other epigenetic modifications to allow for better understanding of these modifications.

The overarching goal of this research was to identify molecular markers which may serve as useful biomarkers of various exposures or disease states and may underlie the exposure-disease relationship. We made several important discoveries including the novel identification of CpG sites associated with diet quality and BMI, with several of these sites in highly relevant metabolic genes. We also found that DNA methylation may be modifying inflammatory pathways associated with obesity in individuals of African descent and metabolically healthy obesity. Furthermore, we found that epigenetic BMI can serve as a potential biomarker distinguishing metabolically healthy and unhealthy individuals, which may have some utility for identifying “at risk” individuals. Overall, this work will help to further our understanding of the molecular dysregulation caused by poor diet, metabolic abnormalities and obesity.

References

1. Hao X, Luo H, Krawczyk M, Wei W, Wang W, Wang J, Flagg K, Hou J, Zhang H, Yi S, et al. DNA methylation markers for diagnosis and prognosis of common cancers. Proceedings of the National Academy of Sciences 2017;114(28):7414-9. doi: 10.1073/pnas.1703577114.
2. Horvath S. Epigenetic clock and biological age. Medizinische Genetik 2016;28(1):86-7. doi: 10.1007/s11825-016-0083-5.
3. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nature Reviews Genetics 2018;19(6):371-84. doi: 10.1038/s41576-018-0004-3.
4. Tobi EW, Slieker RC, Luijk R, Dekkers KF, Stein AD, Xu KM, Slagboom PE, van Zwet EW, Lumey LH, Heijmans BT. DNA methylation as a mediator of the association between prenatal adversity and risk factors for metabolic disease in adulthood. Science Advances 2018;4(1):eaao4364. doi: 10.1126/sciadv.aao4364.
5. Jhun MA, Smith JA, Ware EB, Kardia SLR, Mosley TH, Jr., Turner ST, Peyser PA, Park SK. Modeling the Causal Role of DNA Methylation in the Association Between Cigarette Smoking and Inflammation in African Americans: A 2-Step Epigenetic Mendelian Randomization Study. American journal of epidemiology 2017;186(10):1149-58. doi: 10.1093/aje/kwx181.
6. Chamberlain JA, Dugué P-A, Bassett JK, Hodge AM, Brinkman MT, Joo JE, Jung C-H, Makalic E, Schmidt DF, Hopper JL, et al. Dietary intake of one-carbon metabolism nutrients and DNA methylation in peripheral blood. The American journal of clinical nutrition 2018;108(3):611-21. doi: 10.1093/ajcn/nqy119.
7. Taylor RM, Smith R, Collins CE, Mossman D, Wong-Brown MW, Chan EC, Evans TJ, Attia JR, Smith T, Butler T, et al. Methyl-Donor and Cofactor Nutrient Intakes in the First 2-3 Years and Global DNA Methylation at Age 4: A Prospective Cohort Study. Nutrients 2018;10(3). doi: 10.3390/nu10030273.
8. Yadav DK, Shrestha S, Lillycrop KA, Joglekar CV, Pan H, Holbrook JD, Fall CH, Yajnik CS, Chandak GR. Vitamin B12 supplementation influences methylation of genes associated with Type 2 diabetes and its intermediate traits. Epigenomics 2018;10(1):71-90. doi: 10.2217/epi-2017-0102.
9. Ferrari A, Torrezan GT, Carraro DM, Aguiar Junior S. Association of Folate and Vitamins Involved in the 1-Carbon Cycle with Polymorphisms in the Methylenetetrahydrofolate Reductase Gene (MTHFR) and Global DNA Methylation in Patients with Colorectal Cancer. Nutrients 2019;11(6). doi: 10.3390/nu11061368.
10. Mandaviya PR, Joehanes R, Brody J, Castillo-Fernandez JE, Dekkers KF, Do AN, Graff M, Hanninen IK, Tanaka T, de Jonge EAL, et al. Association of dietary folate and vitamin B-12 intake with genome-wide DNA methylation in blood: a large-scale epigenome-wide association analysis in 5841 individuals. The American journal of clinical nutrition 2019;110(2):437-50. doi: 10.1093/ajcn/nqz031.
11. Hales CM CM, Fryar CD, Ogden CL. Prevalence of obesity among adults and youth: United States, 2015–2016. 2017(NCHS data brief, no 288).
12. Rao G, Powell-Wiley TM, Ancheta I, Hairston K, Kirley K, Lear SA, North KE, Palaniappan L, Rosal MC. Identification of Obesity and Cardiovascular Risk in Ethnically and Racially Diverse Populations. Circulation 2015;132(5):457-72. doi: doi:10.1161/CIR.0000000000000223.
13. Després J-P. Body Fat Distribution and Risk of Cardiovascular Disease. Circulation 2012;126(10):1301-13. doi: doi:10.1161/CIRCULATIONAHA.111.067264.
14. Doumatey AP, Lashley KS, Huang H, Zhou J, Chen G, Amoah A, Agyenim-Boateng K, Oli J, Fasanmade O, Adebamowo CA, et al. Relationships Among Obesity, Inflammation, and Insulin Resistance in African Americans and West Africans. Obesity 2010;18(3):598-603. doi: https://doi.org/10.1038/oby.2009.322.

15. Hakim O, Bello O, Ladwa M, Peacock JL, Umpleby AM, Charles-Edwards G, Amiel SA, Goff LM. The Link between Obesity and Inflammatory Markers in the Development of Type 2 Diabetes in Men of Black African and White European Ethnicity. Nutrients 2020;12(12):3796.
16. Choi J, Joseph L, Pilote L. Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. Obesity reviews : an official journal of the International Association for the Study of Obesity 2013;14(3):232-44. doi: 10.1111/obr.12003.
17. Loos RJF, Janssens ACJW. Predicting Polygenic Obesity Using Genetic Information. Cell metabolism 2017;25(3):535-43. doi: https://doi.org/10.1016/j.cmet.2017.02.013.
18. Goodarzi MO. Genetics of obesity: what genetic association studies have taught us about the biology of obesity and its complications. The Lancet Diabetes & Endocrinology 2018;6(3):223-36. doi: https://doi.org/10.1016/S2213-8587(17)30200-0.
19. Silventoinen K, Jelenkovic A, Sund R, Yokoyama Y, Hur Y-M, Cozen W, Hwang AE, Mack TM, Honda C, Inui F, et al. Differences in genetic and environmental variation in adult BMI by sex, age, time period, and region: an individual-based pooled analysis of 40 twin cohorts. The American journal of clinical nutrition 2017;106(2):457-66. doi: 10.3945/ajcn.117.153643.
20. Langdon RJ, Beynon RA, Ingarfield K, Marioni RE, McCartney DL, Martin RM, Ness AR, Pawlita M, Waterboer T, Relton C, et al. Epigenetic prediction of complex traits and mortality in a cohort of individuals with oropharyngeal cancer. Clinical epigenetics 2020;12(1):58. doi: 10.1186/s13148-020-00850-4.
21. Mendelson MM, Marioni RE, Joehanes R, Liu C, Hedman ÅK, Aslibekyan S, Demerath EW, Guan W, Zhi D, Yao C, 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 medicine 2017;14(1):e1002215. doi: 10.1371/journal.pmed.1002215.
22. Shah S, Bonder Marc J, Marioni Riccardo E, Zhu Z, McRae Allan F, Zhernakova A, Harris Sarah E, Liewald D, Henders Anjali K, Mendelson Michael M, et al. Improving Phenotypic Prediction by Combining Genetic and Epigenetic Associations. The American Journal of Human Genetics 2015;97(1):75-85. doi: https://doi.org/10.1016/j.ajhg.2015.05.014.
23. Fransquet PD, Wrigglesworth J, Woods RL, Ernst ME, Ryan J. The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. Clinical epigenetics 2019;11(1):62. doi: 10.1186/s13148-019-0656-7.
24. Blüher M. Metabolically Healthy Obesity. Endocrine reviews 2020;41(3):405-20. doi: 10.1210/endrev/bnaa004.
25. Eckel N, Meidtner K, Kalle-Uhlmann T, Stefan N, Schulze MB. Metabolically healthy obesity and cardiovascular events: A systematic review and meta-analysis. European journal of preventive cardiology 2015;23(9):956-66. doi: 10.1177/2047487315623884.
26. Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nature Reviews Genetics 2009;10(5):295-304. doi: 10.1038/nrg2540.
27. Huan T, Mendelson M, Joehanes R, Yao C, Liu C, Song C, Bhattacharya A, Rong J, Tanriverdi K, Keefe J, et al. Epigenome-wide association study of DNA methylation and microRNA expression highlights novel pathways for human complex traits. Epigenetics 2020;15(1-2):183-98. doi: 10.1080/15592294.2019.1640547.