Curr Atheroscler Rep (2017) 19:36
DOI 10.1007/s11883-017-0673-y

Connecting the Dots Between Fatty Acids, Mitochondrial Function, and DNA Methylation in Atherosclerosis

Silvio Zaina[1] · **Gertrud Lund**[2]

Abstract
Purpose of Review The quest for factors and mechanisms responsible for aberrant DNA methylation in human disease—including atherosclerosis—is a promising area of research. This review focuses on the role of fatty acids (FAs) as modulators of DNA methylation—in particular the role of mitochondrial beta-oxidation in FA-induced changes in DNA methylation during the progression of atherosclerosis.
Recent Findings Recent publications have advanced the knowledge in all areas touched by this review: the causal role of lipids in shaping the DNA methylome, the associations between chronic degenerative disease and mitochondrial function, the lipid composition of the atheroma, and the relevance of DNA hypermethylation in atherosclerosis.
Summary Evidence is beginning to emerge, linking the dynamics of FA type abundance, mitochondrial function, and DNA methylation in the atheroma and systemically. In particular, this review highlights mitochondrial beta-oxidation as an important regulator of DNA methylation in metabolic disease. Despite the many questions still unanswered, this area of research promises to identify mechanisms and molecular factors that establish a pathological gene expression pattern in atherosclerosis.

Keywords DNA methylation · Fatty acids · Beta-oxidation · Mitochondrial dysfunction · Atherosclerosis · Noncommunicable disease

This article is part of the Topical Collection on *Genetics and Genomics*

✉ Silvio Zaina
szaina@ugto.mx

✉ Gertrud Lund
gertrud.lund@cinvestav.mx

[1] Department of Medical Sciences, Division of Health Sciences, León Campus, University of Guanajuato, 20 de Enero no. 929, Col. Obregón, 37320 León, GTO, Mexico

[2] Department of Genetic Engineering, CINVESTAV Irapuato Unit, Km. 9.6 Libramiento Norte Carretera Irapuato-León, 36821 Irapuato, GTO, Mexico

Introduction

The review starts with an essential history and outline of the continuously evolving paradigms of DNA methylation and gene expression regulation. Subsequently, we present recent evidence linking DNA methylation dynamics with the abundance of FA types and the onset of mitochondrial dysfunction during the natural history of atherosclerosis.

DNA Methylation: Heritability and Reversibility

DNA methylation was first identified in higher eukaryotes in 1948 and has since been found to be widespread in virtually all organisms [1, 2]. In mammals, it occurs primarily in a CpG dinucleotide context, in particular within DNA regions of moderate or low-density CpG and GC content, while short regions of high CpG density and GC content, termed CpG islands (CGIs), are generally devoid of DNA methylation [3–5]. With respect to gene features, most methylation is found within transposon-derived sequences, while promoter regions and first exons are generally unmethylated [6]. Indeed, the lack of methylation within promoter regions reflects the fact that ~75% of promoter regions are within CGIs [5, 7, 8]. With respect to the effect of DNA methylation on transcription, there is a general consensus that methylation in CGI promoter regions is associated with gene silencing, and that

Published online: 22 July 2017

such methylation most probably is the consequence rather than the cause of transcriptional inactivation [9, 10]. Oppositely, methylation residing within the coding region—referred to as gene body methylation—tends to show a positive correlation with expression [9].

The fascination with DNA methylation lies in the fact that it is not only heritable but also reversible. This is exemplified by the dynamic changes in DNA methylation during germline and zygote development, where the bulk mammalian genome undergoes two rounds of demethylation followed by remethylation [11, 12]. Exceptions are selected repetitive elements of the intracisternal A-particle (IAP) family—in addition to single-copy loci that are often adjacent to those IAPs—that are refractory to germline DNA methylation reprogramming [13]. The enzymes that catalyze the addition of a methyl group to cytosine to form 5-methylcytosine (5mC) are known as DNA methyltransferases (DNMTs) and are largely defined by their capability of methylating previously unmethylated templates (DNMT3a and DNMT3b) or maintain existing methylation patterns (DNMT1) following DNA replication [14, 15]. Mammalian DNMTs preferentially target cytosines in a 5′-CG-3′ context (CpG dinucleotide). Conversely, the loss of DNA methylation occurs either passively due to lack of DNA methyltransferase activity or by an active removal mechanism mediated by ten-eleven translocation (TET) protein family members [16]. The latter catalyze the oxidation of 5mC to 5-hydroxymethyl-C (5hmC) that can be further oxidized to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). Since 5hmc is a poor substrate of DNMT1, this initial oxidation event can lead to replication-dependent passive loss of DNA methylation. Conversely, excision of demethylation intermediates 5fC and 5caC from DNA is an active process performed by thymine DNA glycosylases [16].

DNA Methylation in Disease

Following pioneering studies in cancer [17, 18], efforts from numerous laboratories show that a multitude of additional noncommunicable diseases show genome-wide changes in DNA methylation relative to a non-diseased state [19]. For example, obesity and its comorbidities non-alcoholic fatty liver disease (NAFLD), type 2 diabetes (T2D), and atherosclerosis are all associated with alterations in DNA methylation [20–24]. In most cases, these alterations represent absolute changes in DNA methylation. However, DNA methylation variation, that can be cell type-dependent [25•], is increasingly been recognized as an important parameter for identifying individuals with cancer, obesity, depression, and type I diabetes [25•, 26–28]. Furthermore, the vast majority of genome-wide methylation studies have restricted their methylation analysis of diseased and non-diseased tissues to a single developmental time point rendering it is impossible to assign a causal role of DNA methylation in disease. Exceptions are studies detailing the dynamic changes in DNA methylation before and during the development of atherosclerosis both in ApoE-null mice, a model of hyperlipidemia-induced atherosclerosis [29] and in humans [24, 30]. As for diabetes, DNA methylation profiles that are established in prediabetic and are maintained in diagnosed individuals have been identified in whole blood of both type 1 and type 2 diabetes mellitus patients [31, 32]. As for diabetes complications, a number of whole blood DNA methylated sites are associated with nephropathy risk in type 1 diabetes mellitus [33]. These studies have conclusively shown that alterations in DNA methylation profiles precede the disease.

DNA Methylation Dynamics in Atherosclerosis

Gene-specific and more recently epigenome-wide association studies (EWAS) conducted in blood cells describe DNA methylation profiles that potentially predict, or are associated with, human cardiovascular disease. The reader is referred to two extensive reviews of the topic, one recently published [34, 35]. On the other hand, studies addressing vascular DNA methylation dynamics across specific stages of the natural history of the human atheroma are relatively scarce. A microarray, whole genome sequencing, and RNAseq-based systematic survey of vascular DNA methylation during lesion progression from the early stable phase (AHA grade III) to advanced stable and symptomatic phase revealed a dynamic DNA methylome (Fig. 1). A set of CpGs underwent significant differential methylation in comparison with donor-matched lesion-free portions of the same vessel type. Those profiles were constant in grade III to VII plaques, indicating that they arose at a yet unidentified early stage of lesion progression [24]. The vast majority of that CpG set was hypermethylated in the atherosclerotic portion of the aorta and mapped to genes involved in smooth muscle cell biology. This latter feature was later independently confirmed by comparative epigenomics [36]. Furthermore, significant hypermethylation of *Alu*, a repeat family representing ~11% of the human genome, was observed in atherosclerotic portions of the aorta. A further CpG set displayed a methylation drift that increased with lesion severity [30]. Similar to the previously described CpG set, the drift was mainly towards hypermethylation but involved genes regulating macrophage function. Taken together, the data indicate that hypermethylation is the predominant DNA methylome signature in stable human plaques. Independent mouse model studies, in which the DNA methylome drift was controlled by biochemical or molecular manipulation of DNMT and TET2 activity, convincingly assign a causal role to DNA hypermethylation in atherosclerosis [37–40]. On the other hand, the DNA methylome of symptomatic, bona fide post-rupture carotid plaques displayed a global reversal, i.e., a loss of methylation

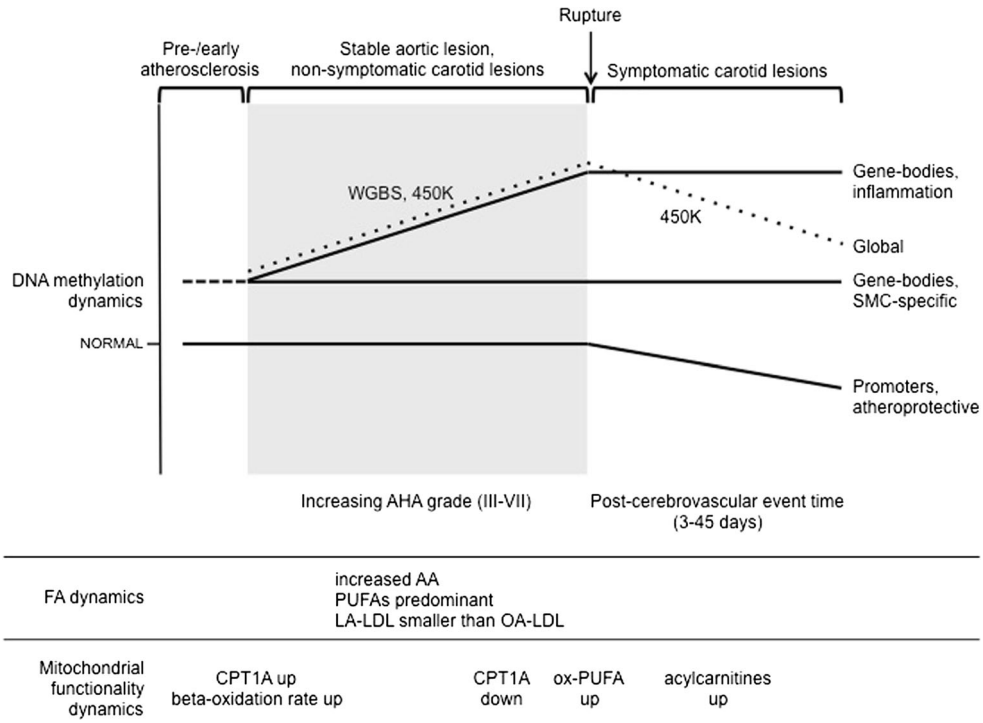

Fig. 1 Schematic view of available information linking DNA methylation, FA abundance, and mitochondrial activity dynamics in atherosclerosis. See the text for references and symbols

that correlates with the time elapsed from the cerebrovascular event [41]. Rather than normalizing the profiles of loci that undergo hypermethylation during the stable phase, loss of methylation in symptomatic plaques preferentially targets promoters of atheroprotective genes. RNAseq data revealed transcriptional activation of demethylated genes, including *TET2*. Therefore, the post-cerebrovascular event wave of DNA hypomethylation is consistent with the previous observations that the ruptured plaque spontaneously reverts to a relatively stable structure [42].

Fatty Acids, DNA Methylation, and Mitochondrial Function

Lifestyle choices—such as diet and level of physical activity—in addition to environmental contaminants or socioeconomic status play important roles in the development and modulation of DNA methylation profiles in noncommunicable diseases [43, 44]. Given the heritability of DNA methylation, a lifestyle factor-induced alteration in DNA methylation at any particular developmental time point has the potential to be retained following DNA replication, both within, or across, generations. Indeed, inheritance of DNA methylation patterns acquired during fetal development may form part of the mechanism behind the fetal origins of adult disease [45], a hypothesis proposed by Barker and colleagues that was originally based on the inverse relationship between death from coronary artery disease and birth weight [46]. In addition to DNA methylation [47–49], histone modifications [50, 51], inheritance of small noncoding RNAs [52–54], and mitochondrial dysfunction [55, 56•] have each been associated with specific transgenerationally transmitted phenotypes. Interestingly, a recent study shows that ovaries of mice exposed to high-fat diet show impaired mitochondrial function and loss of methylation [57••]. This suggests an important, unexplored cross talk between mitochondrial function and DNA methylation in transgenerational phenomena.

With respect to diet, much research has focused on the differential role of monounsaturated, polyunsaturated, and saturated fatty acids in metabolic diseases (MUFAs, PUFAs, and SFAs, respectively). Current dietary recommendations for lowering disease risk are based on total reduction in fat content, in particular reducing SFA and trans fat and increasing PUFA consumption [58]. However, several recent studies have highlighted the lack of any association between total fat and/or SFA consumption and mortality from chronic degenerative disease [59–64] that may in part be explained by FA-specific effects and FA source [58, 64, 65]. At the molecular level, in vitro studies have uncovered differential effects of specific FA types on DNA methylation [66–68, 69•]. For example, the SFA palmitic acid (PA) and the PUFA arachidonic acid (AA) provoke increases in DNA methylation in distinct cell types, while the effects of the MUFA oleic acid (OA) are smaller and in the opposite direction. A comparative study of methylation arrays of cells stimulated with AA, OA, and PA shows that there is considerable overlap between genomic targets of AA- and PA-induced methylation, but to a lesser degree compared to OA [69•]. Importantly, we know that the AA-induced hypermethylated response in cell culture is dependent on known regulators of mitochondrial fatty acid oxidation, i.e., on the activity of the histone deacetylase sirtuin 1 (SIRT1), the transcription factor peroxisome proliferator activated

receptor-alpha (PPARα), and carnitine palmitoyl transferase (CPT1) [69•]. Likewise, PA induces hypermethylation of the promoter of PPAR gamma coactivator 1-alpha (PGC-1α)—a coactivator of PPARs—to a greater extent than OA in primary human myotubes [67]. Interestingly, PGC-1α promoter methylation is also increased in skeletal muscle from type 2 diabetes mellitus (T2DM) individuals and correlates inversely with mitochondrial DNA (mtDNA) content, a reduction in mitochondrial transcription factor A (TFAM), a key regulator of mtDNA number, in addition to reduced expression of proteins from the mitochondrial respiratory chain [67].

In vivo exposure to specific FAs also differentially impacts on global DNA methylation in peripheral blood or adipose tissue [70, 71, 72•, 73–75]. In a human cohort discordant for BMI, overweight (OW) and obese (OB) individuals showed reduced levels of global DNA methylation and PUFA—but increased levels of SFA and MUFA—relative to individuals with normal BMI [72•]. Importantly, neither OW nor OB individuals showed abnormalities in blood parameters typically associated with metabolic disease such as blood pressure, lipoprotein, triglyceride, and glucose content. This suggested that hypomethylation in blood may be an early indicator of metabolic disease, possibly reflecting undiagnosed fatty liver disease [76•]. As described previously, in cell culture, both SFAs and PUFAs (i.e., PA, the most abundant SFA in human blood and AA, respectively) are associated with increased levels of beta-oxidation-dependent DNA methylation, and the genomic targets of DNA methylation changes between these FAs are similar [68, 69•]. Therefore, the observed hypomethylation in OW and OB individuals is more likely the result of reduction in mitochondrial beta-oxidation rather than a low PUFA intake. The reduction in beta-oxidation could either result from defects in mitochondrial beta-oxidation pathways or from the reciprocal nature between glucose and lipid metabolism [77], i.e., that high concentrations of glucose can inhibit fatty acid oxidation via malonyl-CoA inhibition of CPT1. In support of the latter, hypomethylation observed in obese individuals with and without T2D showed upregulation of acetyl-CoA carboxylase (ACC) [78••], an enzyme complex that catalyzes the carboxylation of acetyl-CoA to malonyl-CoA. Notably, in that study, the liver methylome of OB individuals with or without T2DM also showed lower levels of DNA methylation compared to non-obese. This again suggests that obesity-associated DNA hypomethylation precedes or is present at an early stage in the development of T2DM.

Fatty Acids, DNA Methylation, and Mitochondrial Function in Atherosclerosis

Lipids have been characterized in plaques by imaging techniques that yield good noninvasive data but do not reach the detail of lipidomics in profiling the >300 lipids contained in the atherosclerotic plaque [79]. Lipidomics is a relatively new discipline that has been applied to animal models, but corresponding studies in humans are still scarce. For an extensive review of lipidomics in vascular biology, see the work by Kolovou and colleagues [80•]. The data presented in the following paragraph are based on FA composition in the atheroma, therefore may, or may not, match accepted outcomes of dietary interventions with specific FA types.

AA is more abundant in diabetic compared to nondiabetic patient plaques, thus appears to directly follow lesion severity [81•]. Also, the AA precursor LA is high in stable plaques. PUFAs and MUFAs are directly and inversely associated with global DNA methylation in peripheral blood, respectively [72•], and are therefore expected to show inverse abundances in the hypermethylated plaque milieu and associations with lesion size/severity. Accordingly, 18-carbon di- and tri-unsaturated, and ω-6 PUFAs are the two most abundant FA types in rabbit plaque [82]. Furthermore, a recent report shows that genetically lowering PUFAs by fatty acid desaturase 1 (FADS1) gene disruption decreases atherosclerotic lesion size in mice [83••]. On the other hand, atheroprotective liver X receptor agonists increase FADS1 and PUFAs [84], thus indicating that the responses to PUFAs are certainly influenced by a complex set of factors including diet and physiological conditions. As for MUFAs, two recent studies indicate that supplementation with oils enriched in that FA class decreases atherosclerosis in rodent models [85, 86]. Data on low-density lipoprotein (LDL) FA composition is consistent with opposite pathological effects of PUFAs and MUFAs, as LA-rich LDL particles are smaller and therefore in principle more atherogenic than OA-rich counterparts [87].

Recent data highlight the involvement of FA-induced DNA methylation profiles in atherosclerosis. AA induces DNA hypermethylation in a cultured monocyte model, and a subset of the targeted CpGs is significantly enriched in loci that are hypermethylated in stable atherosclerosis [69•]. In the same study, OA elicited the opposite profiles compared to AA. Notably, etomoxir, a CPT1 inhibitor, nearly completely abolished AA-induced DNA hypermethylation. Mitochondrial dysfunction has been long recognized as an important phenomenon in atherosclerosis [88]. A recent review is a useful updated panorama of the field [89•]. Also, work published in the last year showed that mitochondrial dysfunction induces posttranslational modifications of actin that favor colocalization of mitochondria and the inflammasome [90•]. Yet, evidence linking the dynamics of mitochondrial functionality and atheroma progression is relatively scarce. Expression and epigenetic data for CPT1A, a critical enzyme for FA entry into mitochondria, support the idea that DNA hypermethylation observed in hyperlipidemia and early atherosclerosis coincides with, or is a short-term memory of, abnormally high mitochondrial activity [24]. Differential methylation and expression of the *CPT1A* gene is a recurrent finding in EWAS. In particular, two studies described a direct association of circulating triglycerides (TGs) with *CPT1A* expression [91••,

92]. Although with the *caveat* that neither study details the prevalence of atherosclerosis in the corresponding cohorts, the data suggest that atherosclerosis risk or its early stages coincide with lipid-induced increased mitochondrial functionality. Importantly, one of the two EWAS concludes that changes in DNA methylation are the consequence of lipid levels, not the opposite, in the studied cohort [91••]. The data echo the global DNA hypermethylation induced by TG-rich lipoproteins in cultured macrophages [93]. Conversely, a deterioration of mitochondrial functionality should coincide with the shift towards DNA hypomethylation observed in advanced, rupture-prone plaques [41]. Indeed, acylcarnitines, a marker of incomplete beta-oxidation, are increased in symptomatic plaques [94•]. Furthermore, oxidized PUFAs, a by-product of stalled beta-oxidation in the oxidizing milieu of dysfunctional mitochondria, are a hallmark of the necrotic core of unstable plaques [95]. *CPT1A* expression follows a consistent trend, as it decreases in advanced, compared to early atherosclerotic lesions in ApoE-null mice [96]. Direct experimental data on the effects of etomoxir on atherosclerosis progression are to our knowledge not available and would be extremely valuable.

A further important issue is macrophage phenotype. Macrophages have been classified into pro-inflammatory or repairing/resolving phenotypes (M1 or M2, respectively). The relevance and controversies of this classification are explained in detail in a recent review [97•]. M2 macrophages rely on beta-oxidation more than M1 counterparts and following the line of reasoning presented here should peak when the lesion DNA is hypermethylated. Yet, current macrophage phenotype censing in the atheroma is not conclusive enough to support any such correlation.

The data thus suggest that de novo methylation of at least a subset of loci differentially methylated in atherosclerosis is dependent of beta-oxidation. It follows a model of DNA methylome shaping by FAs in atherosclerosis, in which AA and possibly other PUFAs [72•] initially induce aortic DNA hypermethylation supported by active beta-oxidation. As disease progresses, mitochondrial overload results in mitochondrial dysfunction, decreased beta-oxidation, and DNA hypomethylation. Experimental evidence convincingly supporting this model includes plaque lipid content data—particularly individual FAs and markers of mitochondrial functionality—obtained across atherosclerosis progression stages.

The above outlined associations between DNA methylation, FAs, and mitochondrial function are based on donor-matched pairs of atherosclerotic and normal vascular samples. In that sample type, atherosclerosis is marked by a relative DNA hypermethylation. Yet, comparisons between atherosclerotic arteries and control samples from distinct donors suggest a more complicated scenario, as these studies have detected DNA hypomethylation in atherosclerosis in humans [98, 99]. A *caveat* in the latter studies is that atherosclerotic and control arteries were of different anatomic locations, thus introducing a potential confounder. Indeed, the comparison of whole blood DNA of healthy and coronary artery disease patients revealed hypermethylation in the latter [100]. Nonetheless, the data suggest that cardiovascular risk factors might impose DNA hypomethylation in the pre-atherosclerotic arterial wall, and that hypermethylation is a subsequent event that marks the appearance and progression of the stable atheroma. This scenario is supported by evidence that two cardiovascular risk factors, obesity and diabetes, are marked by DNA hypomethylation and reduced mitochondrial FA metabolism [31, 72•, 101]. Thus, the relative increase in mitochondrial functionality in atherosclerosis compared to pre-atherosclerosis may reflect the energy-demanding, atheroma milieu-specific active inflammatory process. Indeed, epigenomics data available for donor-matched and non-donor-matched samples suggest that the early "priming" effect and the atheroma-specific events target distinct gene sets and are therefore non-overlapping pathobiological processes [24, 30, 99].

Taken together, the above data prompt the speculative hypothesis that the pre-atherosclerosis, obesity/diabetes-associated DNA hypomethylation, and the hypomethylation wave observed in symptomatic plaques possibly result from two distinct processes. As discussed above, the former may reflect a shift from FAs to carbohydrate metabolism, accompanied by ACC-driven inhibition of CPT1 activity [78••]. It is tempting to speculate that related phenomena may underlie the widespread DNA hypomethylation observed in cancer, where glucose metabolism predominates (Warburg effect [102]). The latter DNA hypomethylation event might result from beta-oxidation overload and mitochondrial dysfunction. These are testable hypotheses that might provide important insights into disease epigenetics. Lastly, based on the evidence discussed in the "Fatty Acids, DNA Methylation, and Mitochondrial Function" section, the dietary fatty acid composition may represent an important determinant of DNA methylation profiles via the specific activation or repression of genes involved in mitochondrial beta-oxidation.

Conclusion

Although patchy and challenged by unanswered questions, evidence is emerging that links DNA methylation, FA abundance, and mitochondrial function in atherosclerosis and related risk-enhancing disorders. If supported by further experimental testing, those findings might lead to effective prevention or therapeutic strategies for cardiovascular disease.

Acknowledgements GL and SZ have received funding from the Kellogg's Institute for Nutrition and Health, "Research Projects in Nutrition 2016" grant no. 100. Due to the extent of available literature and the multidisciplinary nature of this review, readers are in some instances referred to review articles. We apologize to all authors whose work was not directly cited.

Compliance with Ethical Standards

Conflict of Interest Gertrud Lund and Silvio Zaina each declare no conflicts of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki Declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Hotchkiss RD. The quantitative separation of purines, pyrimidines, and nucleosides by paper chromatography. J Biol Chem. 1948;175:315–32.
2. Suzuki MM, Bird A. DNA methylation landscapes: provocative insights from epigenomics. Nat Rev Genet. 2008;9:465–76.
3. Cooper DN, Taggart MH, Bird AP. Unmethylated domains in vertebrate DNA. Nucleic Acids Res. 1983;11:647–58.
4. Bird A, Taggart M, Frommer M, Miller OJ, Macleod D. A fraction of the mouse genome that is derived from islands of nonmethylated, CpG-rich DNA. Cell. 1985; 40:91–9.
5. Edwards JR, O'Donnell AH, Rollins RA, et al. Chromatin and sequence features that define the fine and gross structure of genomic methylation patterns. Genome Res. 2010;20:972–80.
6. Edwards JR, Yarychkivska O, Boulard M, Bestor TH. DNA methylation and DNA methyltransferases. Epigenetics Chromatin. 2017;10:23.
7. Zhang MQ, Ioshikhes IP. Large-scale human promoter mapping using CpG islands. Nat Genet. 2000;26:61–3.
8. Lister R, Pelizzola M, Dowen RH, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. Nature. 2009;462:315–22.
9. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet. 2012;13:484–92.
10. Bestor TH, Edwards JR, Boulard M. Notes on the role of dynamic DNA methylation in mammalian development. Proc Natl Acad Sci. 2015;112:6796–9.
11. Seisenberger S, Peat JR, Hore TA, Santos F, Dean W, Reik W. Reprogramming DNA methylation in the mammalian life cycle: building and breaking epigenetic barriers. Philos Trans R Soc B Biol Sci. 2012;368:20110330.
12. Tang WWC, Kobayashi T, Irie N, Dietmann S, Surani MA. Specification and epigenetic programming of the human germ line. Nat Rev Genet. 2016;17:585–600.
13. Hackett JA, Sengupta R, Zylicz JJ, Murakami K, Lee C, Down TA, et al. Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. Science. 2013;339:448–52.
14. Bestor TH. Activation of mammalian DNA methyltransferase by cleavage of a Zn binding regulatory domain. EMBO J. 1992;11:2611–7.
15. Pradhan S, Bacolla A, Wells RD, Roberts RJ. Recombinant human DNA (cytosine-5) methyltransferase. I. Expression, purification, and comparison of de novo and maintenance methylation. J Biol Chem. 1999;274:33002–10.
16. Wu H, Zhang Y. Mechanisms and functions of Tet protein-mediated 5-methylcytosine oxidation. Genes Dev. 2011;25:2436–52.
17. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature. 1983;301:89–92.
18. Gama-Sosa MA, Slagel VA, Trewyn RW, Oxenhandler R, Kuo KC, Gehrke CW, et al. The 5-methylcytosine content of DNA from human tumors. Nucleic Acids Res. 1983;11:6883–94.
19. Zoghbi HY, Beaudet AL. Epigenetics and human disease. Cold Spring Harb Perspect Biol. 2016;8:a019497.
20. Feinberg AP, Irizarry RA, Fradin D, et al. Personalized epigenomic signatures that are stable over time and covary with body mass index. Sci Transl Med. 2010;2:49ra67.
21. Wang X, Zhu H, Snieder H, et al. Obesity related methylation changes in DNA of peripheral blood leukocytes. BMC Med. 2010;8:87.
22. Murphy SK, Yang H, Moylan CA, et al. Relationship between methylome and transcriptome in patients with nonalcoholic fatty liver disease. Gastroenterology. 2013;145:1076–87.
23. Dayeh T, Volkov P, Salö S, et al. Genome-wide DNA methylation analysis of human pancreatic islets from type 2 diabetic and non-diabetic donors identifies candidate genes that influence insulin secretion. PLoS Genet. 2014;10:e1004160.
24. Zaina S, Heyn H, Carmona FJ, et al. DNA methylation map of human atherosclerosis. Circ Cardiovasc Genet. 2014;7:692–700.
25.• Paul DS, Teschendorff AE, Dang MANN, et al. Increased DNA methylation variability in type 1 diabetes across three immune effector cell types. Nat Commun. 2016;7:13555. **This article highlights the importance of cell type on DNA methylation variability in the analysis of genome and gene-specific methylation profiles.**
26. Hansen KD, Timp W, Bravo HC, et al. Increased methylation variation in epigenetic domains across cancer types. Nat Genet. 2011;43:768–75.
27. Xu X, Su S, Barnes VA, De Miguel C, Pollock J, Ownby D, et al. A genome-wide methylation study on obesity: differential variability and differential methylation. Epigenetics. 2013;8:522–33.
28. Córdova-Palomera A, Fatjó-Vilas M, Gastó C, Navarro V, Krebs M-O, Fañanás L. Genome-wide methylation study on depression: differential methylation and variable methylation in monozygotic twins. Transl Psychiatry. 2015;5:e557.
29. Lund G, Andersson L, Lauria M, Lindholm M, Fraga MF, Villar-Garea A, et al. DNA methylation polymorphisms precede any histological sign of atherosclerosis in mice lacking apolipoprotein E. J Biol Chem. 2004;279:29147–54.
30. Valencia-Morales Mdel P, Zaina S, Heyn H, et al. The DNA methylation drift of the atherosclerotic aorta increases with lesion progression. BMC Med Genomics 2015;8:7.
31. Rakyan VK, Beyan H, Down TA, et al. Identification of type 1 diabetes-associated DNA methylation variable positions that precede disease diagnosis. PLoS Genet. 2011;7:e1002300.
32. Toperoff G, Aran D, Kark JD, et al. Genome-wide survey reveals predisposing diabetes type 2-related DNA methylation variations in human peripheral blood. Hum Mol Genet. 2012;21:371–83.

33. Bell JT, Tsai PC, Yang TP, et al. Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. PLoS Genet. 2012;8: e1002629.
34. Turunen MP, Aavik E, Ylä-Herttuala S. Epigenetics and atherosclerosis. Biochim Biophys Acta. 2009;1790:886–91.
35. Sayols-Baixeras S, Irvin MR, Elosua R, Arnett DK, Aslibekyan SW. Epigenetics of lipid phenotypes. Curr Cardiovasc Risk Rep. 2016;10:31.
36. Houseman EA, Kelsey KT, Wiencke JK, Marsit CJ. Cell-composition effects in the analysis of DNA methylation array data: a mathematical perspective. BMC Bioinformatics. 2015;16:95.
37. Dunn J, Qiu H, Kim S, et al. Flow-dependent epigenetic DNA methylation regulates endothelial gene expression and atherosclerosis. J Clin Invest. 2014;124:3187–99.
38. Cao Q, Wang X, Jia L, et al. Inhibiting DNA methylation by 5-Aza-2′-deoxycytidine ameliorates atherosclerosis through suppressing macrophage inflammation. Endocrinology. 2014;155: 4925–38.
39. Yu J, Qiu Y, Yang J, Bian S, Chen G, Deng M, et al. DNMT1-PPARγ pathway in macrophages regulates chronic inflammation and atherosclerosis development in mice. Sci Rep. 2016;6:30053.
40. Liu R, Jin Y, Tang WH, Qin L, Zhang X, Tellides G, et al. Ten-eleven translocation-2 (TET2) is a master regulator of smooth muscle cell plasticity. Circulation. 2013;128:2047–57.
41. Zaina S, Gonçalves I, Carmona FJ, Gomez A, Heyn H, Mollet IG, et al. DNA methylation dynamics in human carotid plaques after cerebrovascular events. Arterioscler Thromb Vasc Biol. 2015;35: 1835–42.
42. Peeters W, Hellings WE, De Kleijn DP, De Vries JP, Moll FL, Vink A, et al. Carotid atherosclerotic plaques stabilize after stroke: insights into the natural process of atherosclerotic plaque stabilization. Arterioscler Thromb Vasc Biol. 2009;29:128–33.
43. Vaiserman A. Epidemiologic evidence for association between adverse environmental exposures in early life and epigenetic variation: a potential link to disease susceptibility? Clin Epigenetics. 2015;7:96.
44. Barrès R, Zierath JR. The role of diet and exercise in the transgenerational epigenetic landscape of T2DM. Nat Rev Endocrinol. 2016;12:441–51.
45. Zheng J, Xiao X, Zhang Q, Yu M. DNA methylation: the pivotal interaction between early-life nutrition and glucose metabolism in later life. Br J Nutr. 2014;112:1850–7.
46. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet (London, England). 1989;2:577–80.
47. Burdge GC, Hoile SP, Uller T, Thomas NA, Gluckman PD, Hanson MA, et al. Progressive, transgenerational changes in offspring phenotype and epigenotype following nutritional transition. PLoS One. 2011;6:e28282.
48. Öst A, Lempradl A, Casas E, et al. Paternal diet defines offspring chromatin state and intergenerational obesity. Cell. 2014;159: 1352–64.
49. Radford EJ, Ito M, Shi H, et al. In utero effects. In utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism. Science. 2014;345:1255903.
50. Gaydos LJ, Wang W, Strome S. H3K27me and PRC2 transmit a memory of repression across generations and during development. Science. 2014;345:1515–8.
51. Strakovsky RS, Zhang X, Zhou D, Pan Y-X. The regulation of hepatic Pon1 by a maternal high-fat diet is gender specific and may occur through promoter histone modifications in neonatal rats. J Nutr Biochem. 2014;25:170–6.
52. Fernandez-Twinn DS, Alfaradhi MZ, Martin-Gronert MS, Duque-Guimaraes DE, Piekarz A, Ferland-McCollough D, et al. Downregulation of IRS-1 in adipose tissue of offspring of obese mice is programmed cell-autonomously through post-transcriptional mechanisms. Mol Metab. 2014;3:325–33.
53. Fullston T, Ohlsson Teague EMC, Palmer NO, DeBlasio MJ, Mitchell M, Corbett M, et al. Paternal obesity initiates metabolic disturbances in two generations of mice with incomplete penetrance to the F2 generation and alters the transcriptional profile of testis and sperm microRNA content. FASEB J. 2013;27:4226–43.
54. de Castro Barbosa T, Ingerslev LR, Alm PS, et al. High-fat diet reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the offspring. Mol Metab. 2016;5:184–97.
55. Zander-Fox DL, Fullston T, McPherson NO, Sandeman L, Kang WX, Good SB, et al. Reduction of mitochondrial function by FCCP during mouse cleavage stage embryo culture reduces birth weight and impairs the metabolic health of offspring1. Biol Reprod. 2015;92:124.
56.• Saben JL, Boudoures AL, Asghar Z, Thompson A, Drury A, Zhang W, et al. Maternal metabolic syndrome programs mitochondrial dysfunction via germline changes across three generations. Cell Rep. 2016;16:1–8. **Highlights the important role of mitochondria in transgenerational inheritance of metabolic sydrome induced by high fat/high sucrose diet in mice.**
57.•• Hou Y-J, Zhu C-C, Duan X, Liu H-L, Wang Q, Sun S-C. Both diet and gene mutation induced obesity affect oocyte quality in mice. Sci Rep. 2016;6:18858. **Important evidence that the ovary exposed to high fat diet and displays DNA hypomethylation.**
58. Zock PL, Blom WAM, Nettleton JA, Hornstra G. Progressing insights into the role of dietary fats in the prevention of cardiovascular disease. Curr Cardiol Rep. 2016;18:111.
59. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. Am J Clin Nutr. 2010;91:535–46.
60. Chowdhury R, Warnakula S, Kunutsor S, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk. Ann Intern Med. 2014;160:398–406.
61. de Souza RJ, Mente A, Maroleanu A, et al. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. BMJ. 2015;351:h3978.
62. Harcombe Z, Baker JS, Cooper SM, Davies B, Sculthorpe N, DiNicolantonio JJ, et al. Evidence from randomised controlled trials did not support the introduction of dietary fat guidelines in 1977 and 1983: a systematic review and meta-analysis. Open Hear. 2015;2: e000196.
63. Ramsden CE, Zamora D, Majchrzak-Hong S, Faurot KR, Broste SK, Frantz RP, et al. Re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from Minnesota Coronary Experiment (1968–73). BMJ. 2016;353:i1246.
64. Praagman J, Beulens JW, Alssema M, Zock PL, Wanders AJ, Sluijs I, et al. The association between dietary saturated fatty acids and ischemic heart disease depends on the type and source of fatty acid in the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort. Am J Clin Nutr. 2016;103:356–65.
65. Hu FB, Stampfer MJ, Manson JE, Ascherio A, Colditz GA, Speizer FE, et al. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. Am J Clin Nutr. 1999;70:1001–8.
66. Flores-Sierra J, Arredondo-Guerrero M, Cervantes-Paz B, Rodríguez-Ríos D, Alvarado-Caudillo Y, Nielsen FC, et al. The trans fatty acid elaidate affects the global DNA methylation profile of cultured cells and in vivo. Lipids Health Dis. 2016;15:75.
67. Barrès R, Osler ME, Yan J, Rune A, Fritz T, Caidahl K, et al. Non-CpG methylation of the PGC-1alpha promoter through DNMT3B controls mitochondrial density. Cell Metab. 2009;10:189–98.

68. Hall E, Volkov P, Dayeh T, Bacos K, Rönn T, Nitert MD, et al. Effects of palmitate on genome-wide mRNA expression and DNA methylation patterns in human pancreatic islets. BMC Med. 2014;12:103.
69.• Silva-Martínez GA, Rodríguez-Ríos D, Alvarado-Caudillo Y, et al. Arachidonic and oleic acid exert distinct effects on the DNA methylome. Epigenetics. 2016;11:321–34. **An analysis of FA-specific effects on beta-oxidation-dependent DNA methylation.**
70. Aslibekyan S, Wiener HW, Havel PJ, Stanhope KL, O'Brien DM, Hopkins SE, et al. DNA methylation patterns are associated with n-3 fatty acid intake in Yup'ik people. J Nutr. 2014; doi:10.3945/jn.113.187203.
71. 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 pre-adolescents. Eur J Hum Genet. 2015;23:654–62.
72.• de la Rocha C, Pérez-Mojica JE, Zenteno-De León S, et al. Associations between whole peripheral blood fatty acids and DNA methylation in humans. Sci Rep. 2016;6:25867. **Details BMI-dependant associations between FA content and DNA methylation in metabolically healthy individuals.**
73. Marchlewicz EH, Dolinoy DC, Tang L, et al. Lipid metabolism is associated with developmental epigenetic programming. Sci Rep. 2016;6:34857.
74. Tremblay BL, Guénard F, Rudkowska I, Lemieux S, Couture P, Vohl M-C. Epigenetic changes in blood leukocytes following an omega-3 fatty acid supplementation. Clin Epigenetics. 2017;9:43.
75. Perfilyev A, Dahlman I, Gillberg L, Rosqvist F, Iggman D, Volkov P, et al. Impact of polyunsaturated and saturated fat overfeeding on the DNA-methylation pattern in human adipose tissue: a randomized controlled trial. Am J Clin Nutr. 2017;105:991–1000.
76.• Ollikainen M, Ismail K, Gervin K, et al. Genome-wide blood DNA methylation alterations at regulatory elements and heterochromatic regions in monozygotic twins discordant for obesity and liver fat. Clin Epigenetics. 2015;7:39. **Points to an association between fatty liver disease and blood methylation profiles in twins discordant for BMI and fatty liver.**
77. McGarry JD, Mannaerts GP, Foster DW. A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. J Clin Invest. 1977;60:265–70.
78.•• Kirchner H, Sinha I, Gao H, et al. Altered DNA methylation of glycolytic and lipogenic genes in liver from obese and type 2 diabetic patients. Mol Metab. 2016;5:171–83. **This study shows that hypomethylation is a characteristic of obese individuals before or at an early stage in the development of type 2 diabetes and shows that hypomethylation is associated with upregulation of glycolysis and de novo lipogenesis.**
79. Erlinge D. Near-infrared spectroscopy for intracoronary detection of lipid-rich plaques to understand atherosclerotic plaque biology in man and guide clinical therapy. J Intern Med. 2015;278:110–25.
80.• Kolovou G, Kolovou V, Mavrogeni S. Lipidomics in vascular health: current perspectives. Vasc Health Risk Manag. 2015;11:333–42. **A comprehensive view of the relevance of lipidomics for cardiovascular disease research.**
81.• Ménégaut L, Masson D, Abello N, et al. Specific enrichment of 2-arachidonoyl-lysophosphatidylcholine in carotid atheroma plaque from type 2 diabetic patients. Atherosclerosis. 2016;251:339–47. **A description of the atheroma lipidome.**
82. Bojic LA, McLaren DG, Shah V, Previs SF, Johns DG, Castro-Perez JM. Lipidome of atherosclerotic plaques from hypercholesterolemic rabbits. Int J Mol Sci. 2014;15:23283–93.
83.•• Powell D, Gay J, Smith M, et al. Fatty acid desaturase 1 knockout mice are lean with improved glycemic control and decreased development of atheromatous plaque. Diabetes Metab Syndr Obes Targets Ther. 2016;9:185. **By genetic manipulation, the work demonstrates the metabolic effects of the alteration of FA pool composition.**
84. Varin A, Thomas C, Ishibashi M, et al. Liver X receptor activation promotes polyunsaturated fatty acid synthesis in macrophages: relevance in the context of atherosclerosis. Arterioscler Thromb Vasc Biol. 2015;35:1357–65.
85. Yang Z-H, Gordon SM, Sviridov D, Wang S, Danner RL, Pryor M, et al. Dietary supplementation with long-chain monounsaturated fatty acid isomers decreases atherosclerosis and alters lipoprotein proteomes in LDLr −/− mice. Atherosclerosis. 2017;262:31–8.
86. Kamalakkannan S, Tirupathi Pichiah P, Kalaiselvi S, Arunachalam S, Achiraman S. Emu oil decreases atherogenic plaque formation in cafeteria diet-induced obese rats. J Sci Food Agric. 2016;96:3063–8.
87. Degirolamo C, Shelness GS, Rudel LL. LDL cholesteryl oleate as a predictor for atherosclerosis: evidence from human and animal studies on dietary fat. J Lipid Res. 2008;50:S434–9.
88. Kim J, Wei Y, Sowers JR. Role of mitochondrial dysfunction in insulin resistance. Circ Res. 2008;102:401–14.
89.• Yu EP, Bennett MR. The role of mitochondrial DNA damage in the development of atherosclerosis. Free Radic Biol Med. 2016;100:223–30. **Describes relevant advances in the field of mitochondrial biology and atherosclerosis.**
90.• Yu J-W, Lee M-S. Mitochondria and the NLRP3 inflammasome: physiological and pathological relevance. Arch Pharm Res. 2016;39:1503–18. **Gathers up-to-date information on mitochondria-inflammasome functional interactions.**
91.•• Dekkers KF, van Iterson M, Slieker RC, et al. Blood lipids influence DNA methylation in circulating cells. Genome Biol. 2016;17:138. **A milestone that helps understanding the fundamental relationships between lipids and the DNA methylome.**
92. Irvin MR, Zhi D, Joehanes R, et al. Epigenome-wide association study of fasting blood lipids in the genetics of lipid-lowering drugs and diet network study. Circulation. 2014;130:565–72.
93. Rangel-Salazar R, Wickström-Lindholm M, Aguilar-Salinas CA, et al. Human native lipoprotein-induced de novo DNA methylation is associated with repression of inflammatory genes in THP-1 macrophages. BMC Genomics. 2011;12:582.
94.• Vorkas PA, Shalhoub J, Lewis MR, Spagou K, Want EJ, Nicholson JK, et al. Metabolic phenotypes of carotid atherosclerotic plaques relate to stroke risk: an exploratory study. Eur J Vasc Endovasc Surg. 2016;52:5–10. **An important description of metabolic markers of the carotid atheroma.**
95. Garbin U, Baggio E, Stranieri C, et al. Expansion of necrotic core and shedding of Mertk receptor in human carotid plaques: a role for oxidized polyunsaturated fatty acids? Cardiovasc Res. 2013;97:125–33.
96. Bisgaard LS, Mogensen CK, Rosendahl A, Cucak H, Nielsen LB, Rasmussen SE, et al. Bone marrow-derived and peritoneal macrophages have different inflammatory response to oxLDL and M1/M2 marker expression—implications for atherosclerosis research. Sci Rep. 2016;6:35234.
97.• Tabas I, Bornfeldt KE. Macrophage phenotype and function in different stages of atherosclerosis. Circ Res. 2016;118:653–67. **A must-read review by leading inflammation researchers.**
98. Castillo-Díaz SA, Garay-Sevilla ME, Hernández-González MA, Solís-Martínez MO, Zaina S. Extensive demethylation of normally hypermethylated CpG islands occurs in human atherosclerotic arteries. Int J Mol Med. 2010;26:691–700.

99. Aavik E, Lumivuori H, Leppänen O, et al. Global DNA methylation analysis of human atherosclerotic plaques reveals extensive genomic hypomethylation and reactivation at imprinted locus 14q32 involving induction of a miRNA cluster. Eur Heart J. 2014;36:993–1000.
100. Sharma P, Kumar J, Garg G, Kumar A, Patowary A, Karthikeyan G, et al. Detection of altered global DNA methylation in coronary artery disease patients. DNA Cell Biol. 2008;27:357–65.
101. Volkmar M, Dedeurwaerder S, Cunha DA, et al. DNA methylation profiling identifies epigenetic dysregulation in pancreatic islets from type 2 diabetic patients. EMBO J. 2012;31:1405–26.
102. Warburg O. On the origin of cancer cells. Science. 1956;123:309–14.