BIOCHEMICAL PHARMACOLOGY COMMENTARIES

The role of epigenetics in the development of obesity

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Abbreviations: T2D: type 2 diabetes; GWAS: genome-wide association studies; SNP: single nucleotide polymorphism; 5mC: 5-methylcytosine; 5hmC: 5-hydroxymethylcytosine; 5fC: 5-formylcytosine; 5caC: 5-carboxylcytosine; FA: fatty acid; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; BMI: body mass index; SAT: subcutaneous abdominal adipose tissue; DNMT: DNA methyltransferase; PTMs: post-translational modifications; HAT: histone acetyltransferases; HDAC: histone deacetylase; SIRT: sirtuin; HMT: histone methyltransferase; PRMT: histone N-methyltransferase; HDM: histone demethylase; LSD1: lysine specific demethylase; JHDM: Jumonji C (JmjC) domain-containing histone demethylase; SAM: S-Adenosyl methionine; HFD: high fat diet; WAT: white adipose tissue; BAT: brown adipose tissue; ncRNA; non-coding RNA; lncRNA: long non-coding RNA; miRNA: microRNA; DOHAD: developmental origins of health and disease; SFA: saturated FA; TFA: trans- FA; OXPHOS: oxidative phosphorylation; mtDNA: mitochondrial DNA; URP^{mt}: mitochondrial unfolded protein response.

Abstract

The epidemic of obesity has become pandemic, putting a significant burden on the world's healthcare system. While the heritability of the disease is high, all the identified genetic variants associated to obesity account for a very small percentage of phenotypic variation. The origins of the obesity pandemic cannot be explained exclusively due to genetic factors. In recent years, epigenetic studies have offered valuable information for a deeper understanding of the steep increase in global obesity rates. Existing evidence indicate that environmental exposures induce alterations to the epigenome, leading to the transmission of obesity risk across generations. In this review, we provide insight into the epigenetic disturbances associated with obesity and discuss the impact of harmful diets, particularly calorie-dense foods, in the epigenetic regulation of obesity. The epigenetics of obesity is an expanding area of research, and current reports suggest potential in the use of epigenetic mechanisms as clinical biomarkers and therapeutic candidates.

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1. Introduction

Obesity rates are increasing rapidly worldwide, causing a major epidemiological change. The most recent data from the World Health Organization states that over 1.9 billion adults are overweight and over 650 million are obese. Overall, this represents about 13% of the world's adult population. Even more of a concern is the abrupt escalation in childhood obesity. If these trends continue, the global prevalence of overweight in children under 5 years might rise to 11% by 2025. This development is of great concern due to the significant social, economic and health impact of obesity.

Obesity is a complex disease involving excessive and/or abnormal body fat accumulation that is a health risk. It leads to serious conditions such as cardiovascular disease, type 2 diabetes (T2D), musculoskeletal disorders, and some types of cancer. From an evolutionary perspective, the rapid rise in obesity rates could be linked to the modern adoption of a sedentary lifestyle, coupled with an increase in dietary fat and sugar content [1].

However, it is extremely challenging to understand the precise determinants of obesity. Over the years, genetic studies in humans and experimental animal models have led to the discovery of some causal genes in rare monogenic forms of obesity. Moreover, genome-wide association studies (GWAS) have identified several susceptibility genetic variants associated with the disease. Yet genetics alone cannot explain the current obesity pandemic. Clearly, obesity arises from the complex interaction of susceptibility genes with multiple environmental factors (including stress, chemicals, pharmacological treatments, physical activity or diet). At molecular level, the epigenome is the flexible interface of gene-environment interactions.

Advances in the field of epigenetics highlight the influence of mitochondrial metabolism on the formation or modification of specific epigenetic marks that occur at nuclear level [2–4]. Furthermore, recent evidence suggests that epigenetic modifications may occur in mitochondria themselves [5–7]. While a wealth of studies have illustrated the strong connection between mitochondrial dysfunction and metabolic disease [8], the crosstalk between mitochondria and epigenetics in obesity has been mostly overlooked.

The aim of this commentary is to provide insight into the epigenetic disturbances associated with obesity, to describe how calorie-dense diets affect the epigenome and the consequences for health and disease over lifetime. In addition, we discuss concisely the involvement or mitochondrial epigenetics in obesity.

2. Epigenetic changes related to obesity

The molecular mechanism underlying obesity have received a lot of attention in biomedical and clinical research. While studies in twins and families have revealed the high heritability of the disease, the mouse genetics revolution we have experienced in recent decades has led to the discovery of some causal genes for monogenic obesity (including leptin [LEP], proopiomelanocortin [POMC] or melanocortin 4 receptor [MC4R], among others) [9]. The identification of these genes has underscored pathways to metabolic disease and has led to a deeper understanding of body weight regulation. However, for most individuals, a genetic predisposition to obesity has a polygenic basis.

In recent years, GWASs have uncovered numerous genes and single nucleotide polymorphisms (SNPs) associated with obesity [10], although only a handful have been robustly confirmed in subsequent studies. Nevertheless, genetics alone cannot explain the recent steady increase in worldwide obesity rates. First, only a very small percentage of obese individuals have mutations in the identified obesity genes. Second, it is rather unlikely that the human genome has changed so much in such a short period. At this point, it is clear that obesity stems from the complex interaction of susceptibility genes with multiple environmental factors. Hence there is growing interest in the role of epigenetics in obesity because it offers a tool to understand the link between genes and environment.

Epigenetic refers to heritable changes in gene activity that do not involve alterations to the DNA sequence. This term emerged from the work of Conrad Waddington in an attempt to explain how cells with the same genome exhibit different specializations [11]. For example, hepatocytes and neurons have the same DNA but are very different in terms of transcriptome and proteome and, hence, function. The definition of epigenetic today bears no resemblance to Waddington's original concept. Instead, the term is used to describe an

extra layer of information that exists beyond that encoded in the DNA sequence ("Epi-" means above or beyond in Greek). Epigenetic mechanisms encompass all post-translational modifications of histones and covalent modifications of DNA that define chromatin structure (generally referred to as epigenetic marks) (**Table 1**) [12,13]. In addition, non-coding RNAs have been described recently as part of the epigenome [14,15]. Through these epigenetic mechanisms, cells integrate environmental stimuli to coordinate a wide range of DNA processes, including gene transcription.

2.1. DNA methylation

DNA methylation was the first epigenetic modification to be characterized. It refers to the addition of a methyl group to the fifth carbon of a cytosine (5-methylcytosine [5mC]). In most cases, 5mC is found in the context of symmetrical CpG dinucleotides [16]. DNA methylation has many biological functions, including X chromosome inactivation, monoallelic expression of imprinted genes, and transcriptional repression of transposon-derived sequences. The repressive role of DNA methylation in transcription has long been suggested, with a correlation between DNA methylation and gene silencing [17]. However, how DNA methylation leads to transcription inhibition is still a matter of debate, as the methyl mark *per se* does not seem to confer silencing.

DNA methylation is catalyzed by a family of enzymes called DNA methyltransferases (in mammals: DNTM1, DNTM3A and DNTM3B) (**Table 1**). While DNMT1 is responsible for the maintenance of DNA methylation during replication, DNTM3A and DNTM3B are in charge of creating *de novo* DNA methylation patterns [16]. Loss of DNA methylation can occur passively (during successive rounds of DNA replication in the absence of DNTM1 activity) or actively. The topic of active DNA demethylation is controversial, but several mechanisms have been proposed [18]. These include oxidative demethylation by the TET methylcytosine dioxygenases, which progressively oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [19].

In the context of obesity, DNA methylation is undoubtedly the most widely studied epigenetic mark, particularly in humans. *POMC* is a key component of the melanocortin

system; a complex neuroendocrine network that regulates food intake and energy balance. There is evidence in both children and adults that DNA hypermethylation at the *POMC* variably methylated region of intron2/exon3 is associated with obesity [20,21]. Studies that examined other regions of the *POMC* gene showed that altered DNA methylation status was associated with specific metabolic profiles (such as HDL cholesterol levels) but not with body weight [22]. Insulin-like growth factor 2 (*IGF2*) is a paternally expressed imprinted gene controlling the regulation of growth and body composition. Several reports have shown a significant correlation between *IGF2* hypomethylation and increased BMI [23,24]. Additional studies have revealed that BMI is associated with altered DNA methylation patterns of other melanocortin pathway genes and obesity-related genes; such as leptin receptor (LEPR), leptin (*LEP*), adiponectin (*ADIPOQ*), Insulin receptor substrate 1 (*IRS1*) [24,25]. DNA methylation patterns in circadian clock genes like *CLOCK*, *BMAL1* and *PER2* have been linked to obesity and metabolic syndrome as well. Interestingly, the percentage of methylation of *CLOCK* CpG sites was associated with the intake of monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) [26]. Epigenome-wide association studies performed in various human cohorts and ethnicities have identified several CpG sites where DNA methylation levels correlate with obesity [27,28]. The main findings were in the *HIF3A* and *CPT1A* loci. HIF3A is a component of hypoxia-inducible transcription factor (HIF) that plays a role in adipocyte differentiation and the cellular response to glucose and insulin. Increased DNA methylation at the *HIF3A* locus was shown in blood cells and in the adipose tissue of obese individuals, which suggests that perturbation of hypoxia-inducible pathways could play an important role in the response to obesity [27]. The biological significance of the *CPT1A* locus is hard to overstate. Carnitine palmitoyltransferase 1A (CPT1A) is the rate-limiting enzyme in the transport of long-chain FA into the mitochondria for oxidation. Several studies have linked genetic variation in *CPT1A* and obesity [29]. Significantly, methylation in intron 1 of CPT1A in blood T cells has been shown to correlate inversely with body mass index (BMI) [30].

Although they provide precious information, an important limitation of all these analyses is that they were performed almost exclusively in peripheral blood samples (whole blood or

leukocytes). Yet the epigenetic chromatin state in blood cells could presumably differ from that in other cell types. Therefore, several researchers have begun to explore epigenetic signatures in tissues relevant to energy metabolism, particularly adipose tissue [31–33]. Consistent with the aforementioned observations in blood, two studies showed methylated CpG sites in DNA from obese adipose tissue that were located in *CPT1A* [34] and *HIF3A* genes [32,34]. Another report discovered differential DNA methylation signatures in both omental and subcutaneous abdominal adipose tissue (SAT) in response to gastric bypass surgery and weight loss [33]. In SAT, lower CpG DNA methylation was observed after weight loss in several genes previously associated with obesity (*e.g. MC4R* and *LEPR*). Concomitantly, genes involved in epigenetic regulation (such as *DNMT3A* and *3L*) showed decrease methylation after weight loss as well. In a follow up study, another laboratory compared DNA methylation patterns in fat cells from women two years after bypass surgery [31]. Interestingly, 27% of genes linked to adipogenesis displayed differentially methylated DNA sites in post-obese patients when compared to never-obese women. Although, these differences were not accompanied by differential expression of the annotated genes. Epigenetic analysis of skeletal muscle has also provided interesting results. A 2017 study revealed that the number of DNA methylation changes induced by differentiation from muscle stem cells to myotubes was approximately 3-fold higher in obese patients. Among the genes that showed both differential DNA methylation and expression, were genes previously associated with obesity and metabolic diseases (*e.g. IL18 and PNPLA2*). These data suggest an epigenetic reorganization during myogenesis that is influenced by an obese environment [35].

Over the last years, the interest in fasting and its metabolic benefits has received a lot of attention. Hjort and colleagues studied recently the effects of 36-hours fasting on DNA methylation of *LEP* and *ADIPOQ* in human adipose tissue from men born with a normal weight (NBW) and men born with low birth weight (LBW) [36]. People born with a LBW have an increased risk of metabolic disease and respond differently to fasting than NBW people. The study found that fasting increased methylation of *LEP* and *ADIPOQ* CpG sites only in NBW men. In fact, LBW men showed a higher degree of *LEP* and *ADIPOQ* methylation at

baseline compared with NBW men, which did not increase further in response to fasting. This study suggests a lower epigenetic flexibility in LBW men, which may perhaps contribute to increased susceptibility to obesity and metabolic disorders.

What emerges from all these data is that obesity induces increased variability in DNA methylation landscapes. Furthermore, data from tissues relevant to obesity indicate a role for DNA methylation in the disease pathophysiology. Together, the studies published so far support an impact of BMI on the DNA methylation patterns of candidate genes for obesity.

2.2. Histone modifications

Histones are well-conserved proteins that organize and package DNA into chromatin. There are five families of histones: H1, H2A, H2B, H3 and H4. The basic unit of chromatin, named the nucleosome, is made from DNA wrapped around an octamer of histones (two copies each of H2A, H2B, H3, and H4). Chromatin architecture, and eventually access to DNA for gene transcription, is largely controlled by various post-translational modifications (PTMs) of histone tails. Some histone PTMs disrupt histone-DNA interactions, making DNA accessible to transcriptional machinery and subsequent transcription. Conversely, histone PTMs that strengthen histone-DNA interactions create tightly packed chromatin, resulting in gene silencing.

To date, several histone PTMs have been discovered. Among them, acetylation, and methylation are the most studied. Together, these sets of modifications make up what is known as the histone code: a complex network of histone PTMs that dictate the transcriptional state of a genomic region [37]. Each of these PTMs are added or removed from histone amino acid residues by specific enzymes, typically named “writers” and “erasers” (**Table 1**). The addition of acetyl groups on a lysine or an arginine residue is catalyzed by specific histone acetyltransferases (HAT); HATs are divided into 2 major types: Type A (acetylate nuclear histones) and Type B (acetylate newly synthetized histones in the cytoplasm). Histone deacetylases (HDACs) can remove the acetyl groups from histone tails. HDACs are classified in four classes. Class I HDACs (HDAC1, HDAC2, HDAC3 and HDAC8); class II HDACs (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10); class III HDACs or

Sirtuins (SIRTs) (SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6 and SIRT7); and class IV HDAC (HDAC11). Histone methylation occurs mainly on arginine, lysine and histidine residues, and is catalyzed by specific histone methyltransferases (HMT); SET-domain containing, and DOT1-like methyltransferases are specific for lysine, while N-methyltransferases (PRMT) are specific for arginine. Specific histone demethylases (HDM) can remove the methyl groups from histone tails. There are two groups of histone demethylases; the lysine specific demethylase (LSD1 or KDM1A) and the Jumonji C (JmjC) domain-containing proteins (JHDMs) (for detailed information about different histone modifications and their functions the reader is referred to [38]). Remarkably, the catalytic activities of these "writers" and "erasers" depends on specific metabolites [39]. For instance, glucose and FA catabolism generates acetyl-CoA that is the universal cofactor for histone acetylation. Similarly, HMTs utilize S-Adenosyl methionine (SAM) cycle-derived methyl groups to methylate histone tails. Therefore, changes in key metabolic pathways are likely to affect histone marks.

The role of histone PTMs in obesity has been systematically studied in cell culture and animal models. Dynamic remodeling of histone PTMs has been shown to control the expression of key regulatory genes (*Pref-1*, *C/EBPβ*, *C/EBPα*, *PPARγ2* and *aP2*) during adipocyte differentiation [40]. In murine models of obesity, expression of tumor necrosis factor (*Tnfα*) and monocyte chemotactic protein 1 (*Ccl2*) in the liver has been associated to increased histone lysine acetylation at those genes [41]. In opposition, caloric restriction has been associated with increased histone acetylation and transcription of *Glut4* gene in adipose tissue of obese mice [42]. The expression of several HDACs (such as *Hdac3, Hdac4, Hdac5, Hdac8* and *Hdac11*) is modulated by fasting and high-fat diet (HFD) in the hypothalamus, the region of the brain that controls whole-body energy metabolism [43]. HDAC5 has been proposed to mediate hypothalamic leptin action [44]. Another study showed that a broad inhibition of class I HDACs corrects an array of metabolic disturbances in HFD-fed obese diabetic mice [45]. Furthermore, lack of *Hdac9* or *Hdac11* in mice has been shown to increase whole-body energy expenditure and protect against HFD-induced obesity [46,47]. Histone methylation has also been implicated in regulating metabolism. A study showed increased accumulation of H3K36me2 (a repressive mark) in the liver of diet-

induced obese (DIO) mice [48]. Deficiency of JHDMA2 (a H3K9 specific HDM), has been shown to induce metabolic alterations in white adipose tissue (WAT), brown adipose tissue (BAT) and skeletal muscle, leading to obesity and hyperinsulinemia [49]. Likewise, adipose-specific inactivation of JMJD2B (another H3K9/H3K36 HDM) has been linked to obesity and metabolic abnormalities [50]. The HDM LSD1 has been shown to promote BAT thermogenesis via different mechanisms. On one hand, LSD1 promotes *Ucp1* transcription [51]. On the other hand, LSD1 represses the glucocorticoid pathway [52]. Finally, point mutations in the HMT *Mll2* gene result in hyperglycemia and hyperinsulinemia in mice [53] and are associated with some cases of congenital hyperinsulinism in humans [54].

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With all these data collected in animal models, histone modifications emerge as interesting therapeutic targets. HDAC inhibitors have already been used in humans to treat cancer and inflammatory disease. Future studies are needed to evaluate whether they could also be suitable candidates for the treatment of obesity and metabolic syndrome.

2.3. Non-coding RNAs

Non-coding RNAs (ncRNAs) represent a very large share of the transcriptome. However, these RNA molecules are not translated into protein. In general, ncRNA are divided into three categories: (i) small nuclear/nucleolar RNAs (snRNAs and snoRNAs), (ii) interference RNA, including micro RNAs (miRNAs), and (iii) long ncRNAs (lncRNAs) [14,15]. miRNA are short molecules (20 to 40 nucleotides) that regulate gene expression by blocking protein translation or inducing mRNA degradation. In contrast, lncRNAs (>200 nucleotides) have been associated with reprogramming chromatin states [14]. Over the last decade, high-throughput methods and sophisticated bioinformatics analyses have contributed to the identification of new ncRNA transcripts, yet only few of them have been functionally characterized and confirmed in experimental models.

Several miRNAs are differentially expressed in WAT of obese subjects when compared to non-obese individuals. For instance, expression of miR-17-5p and miR-132 differ significantly between obese and non-obese omental fat, and correlate with BMI, fasting blood glucose, and glycosylated hemoglobin [55]. In SAT, expression of miR-21 is higher in

obese patients when compared to lean controls and positively correlates with BMI [56]. A recent study measured miRNAs expression in SAT from 19 individuals with severe obesity, before and after a weight loss intervention (hypocaloric diet and exercise). This intervention led to up-regulation of miR-29a-3p and miR-29a-5p and down-regulation of miR-20b-5b [57]. In addition to the human studies, research in mice have suggested that diet-induced obesity changes the expression of many miRNAs in adipose tissue. Indeed, HFD-feeding for 5 months causes up-regulation of miR-342-3p, miR-142-3p, miR-142-5p, miR-21, miR-146a, miR-146b, miR-379 and down-regulation of miR-122, miR-133b, miR-1, miR-30a, miR-192, miR-203 [58].

Recently, numerous studies have suggested that miRNAs play an important role in adipocyte differentiation and therefore contribute to the pathogenesis of obesity. The first study suggesting a role for miRNA in the regulation of fat cells was in *Drosophila,* showing that miR-14 inhibits fat metabolism by targeting p38 and MAPK [59]. Since then, the role of miRNA in adipocyte biology has been explored substantially in cell lines, rodents [58] and humans [55]. An array of miRNAs have been shown to potentially promote adipogenesis through different molecular mechanisms; miR-143 promotes human adipocyte differentiation acting via MAPK signaling pathway [60], miR-17 promotes adipocyte differentiation by inhibiting Rb2/p130 [61], miR-26b targets the phosphatase PTEN to enhance adipocyte differentiation [62], and miR-21 regulates adipogenesis through the modulation of TGF-β pathway in mesenchymal stem cells (MSC) derived from human adipose tissue [63]. Conversely, several other miRNAs have been reported to interfere with adipocyte differentiation; miR-130 inhibits PPARγ expression to suppress adipogenesis [64], miR-22 and miR-138 inhibit adipogenic differentiation of human adipose tissue-derived MSCs by repressing HDAC6 and adenovirus EID-1 (a nuclear receptor coregulator) respectively [65,66].

A collection of interesting papers has recently established the role of adipose tissue as a major source of circulating miRNAs that affect the function of other metabolic organs [67,68]. Consequently, many of the miRNAs described above have been also found in the blood of obese individuals, which highlights the possibility that variations in cell-free

circulating miRNAs could be used as non-invasive biomarker for the disease and, potentially, as early diagnosis tool [55]. Additional studies have reported the presence of miRNAs in other body fluids such as serum, urine and saliva [69], but these sources have been less investigated.

Locked nucleic acid (LNA) modified anti-miRNAs have been safely used in humans to treat some conditions. Based on this notion, Seeger *et al.* showed that inhibition of the adipogenesis-promoting miR-21 using LNA was sufficient to decrease body weight and adipocyte size in obese mice [70]. Similarly, a very recent study has shown that inhibition of miR-324-5p reduces body weight in mice [71]. Together, these finding have highlighted the role of miRNAs in obesity and their clinical relevance as potential biomarkers and therapeutic targets.

In humans, some lncRNAs have been described as altered between lean and obese individuals, without further functional characterization. For instance, circulating lncRNA-p5549, lncRNA-p21015, and lncRNA-p19461 are inversely correlated with BMI, waist circumference and fasting insulin levels [72]. A recent GWAS analysis in children with severe obesity highlighted a SNP located in the lncRNA RMST [73]. Numerous lncRNA have been identified to be important regulators of adipocyte biology *in vitro*. The lncRNA ADNCR targets miR-204, which inhibits adipocyte differentiation via PPARγ repression [74]. In bone marrow-derived MSCs, lncRNA H19 reduces the expression of *Hdac4*, *Hdac5* and *Hdac6* and inhibits adipocyte differentiation [75]. Moreover, WAT lncRNA H19 expression is reduced in obese humans and its expression in BAT protects mice from diet-induced obesity [76]. In 3T3-L1 cells, the lncRNA NEAT1 has been shown to interact with SRp40 to regulate PPARγ2 splicing during adipogenesis [77] and the lncRNA U90926 has been shown to promote adipocyte differentiation via transactivation of *Pparγ2* [78].

Nonetheless, our knowledge about lncRNA in obesity is still in its infancy. Unexpectedly, many lncRNAs do not show the same pattern of interspecies conservation as protein-coding genes. Consequently, the functional interpretation of newly discovered lncRNA remains challenging. We are still a long way from establishing the relevance of lncRNAs as therapeutic targets for the amelioration of obesity and related metabolic disease.

3. Influence of environmental factors on the epigenome: focus on diet

In recent years, remarkable breakthroughs in epigenetic research have coincided with increased interest in how environmental factors affect health. Many external factors cause profound changes in epigenetic landscapes across numerous tissues associated with metabolic disease; these include chemical stressors (metals, air pollution or endocrine-disruptive chemicals such as Bisphenol A, etc.), unhealthy habits (tobacco, high alcohol intake, sedentarism, etc.), pharmacological factors and diet [79].

During human development, the first three months after fertilization are a critical period in which developmental plasticity is possible. In contrast to the genome, the epigenome is very dynamic during this stage. Epigenetic changes can be passed on mitotically (through cell division) or meiotically (germline meiosis). Therefore, environmental factors can shape the epigenetic programming of parental gametes, fetus and early postnatal development to facilitate regulation of tissue gene expression throughout the life course into adulthood. A comprehensive overview of the external factors affecting the epigenome is beyond the scope of this commentary. Consequently, here we focus specifically on the impact of nutritional factors (both pre- and postnatal) on the epigenome and their relationship to obesity.

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3.1. The epigenetics of fetal development

Compelling evidence has revealed that the intrauterine environment influences the development of the fetus [80]. From an evolutionary perspective, plasticity during early gestational development might be crucial for anticipated adaptation of the fetus to its environment. However, the Developmental Origins of Health and Disease (DOHAD) hypothesis proposes that adverse effects during the critical peri-conceptual, fetal and early infant phases of life predispose to poor health during adulthood [81]. The first evidence supporting the DOHAD hypothesis came from epidemiological studies that uncovered a strong association between low birth weight and metabolic disease later in life [82,83]. Subsequent epidemiological studies described similar findings in those exposed to overnutrition during intrauterine life [84]. At this time, studies in rodents and humans have

384 confirmed that adverse maternal conditions (including over- and undernutrition) can trigger
385 epigenetic modifications in the offspring that mediate a predisposition to obesity and
386 metabolic disease [24,81,85]. The focus of current DOHAD research is on delineating the
387 epigenetic mechanisms involved in transmitting these fetal-programmed traits to the
388 following generations.

389 Several studies in rats have suggested that nutritional insults during pregnancy might affect
390 the appetite and energy balance regulation of the offspring. For example, the *Pomc*
391 promoter appears to be less methylated in the offspring of rats fed a low-protein diet.
392 Conversely, neonatal overfeeding induces hypermethylation of the *Pomc* promoter and
393 obesity in post-weaning rats [86,87]. A more recent study has revealed a two-step
394 epigenetic mechanism (DNA methylation linked with histone PTMs) that inhibits *Pomc*
395 expression only in the obesity-prone offspring of HFD-fed dams [88]. Interestingly, this
396 report proposes an explanation of the fact that some individual offspring escape the
397 malprogramming caused by unfavorable maternal nutrition.

398 Numerous reports in rodents have evaluated the consequences of maternal HFD on
399 offspring health (reviewed elsewhere [89]). Additionally, a few reports have explored
400 associated epigenetic alterations. For instance, maternal HFD changes hypothalamic insulin
401 receptor (*InsR*) expression and promoter DNA methylation in adult offspring [90]. It also
402 changes DNA methylation and gene expression of dopamine and opioid-related genes,
403 which alters appetite regulation and induces a preference for energy-dense foods in
404 postnatal life [91]. Likewise, maternal diet-induced obesity impairs skeletal development in
405 adult offspring [92]. Finally, HFD during pregnancy causes a higher predisposition to develop
406 hepatic steatosis and inflammation, which has been linked to persistent changes in the DNA
407 methylation of key liver metabolic genes, such as *Fgf21* and *Ppargc1β* [93].

408 In addition to the effects of adverse nutrition during fetal development, overfeeding during
409 the suckling period also results in adult-onset overweight and obesity. A 2013 study
410 revealed that overnutrition during lactation leads to hypermethylation of *Irs1* and *Glut4*
411 promoters, which correlates with lower expression of these genes in the skeletal muscle of

412 adult rats [94]. These observations suggest that epigenetic modifications of key insulin-
413 signaling pathway genes might contribute to the pathophysiology of insulin resistance.

414 In humans, limited studies have linked maternal exposure during peri-conception and
415 pregnancy to metabolic disease in offspring. The investigation of the Dutch famine effects
416 provide evidence for inheritance of epigenetic marks (specifically DNA methylation
417 patterns) [24]. Other examples support the hypothesis that developmental changes in DNA
418 methylation correlate with childhood obesity. For example, DNA analyses from umbilical
419 cord tissue samples revealed that the methylation status of the promoter region of specific
420 "obesity genes" is associated with a child's subsequent adiposity [95]. In another study,
421 gestational diabetes was concomitant with genome-wide DNA methylation changes in the
422 placenta and the offspring's umbilical cord blood [96].

423 Compared with the maternal effects, the impact of paternal diet on offspring health has
424 received limited attention. Nevertheless, during the last decade some studies have
425 discovered that poor paternal diet is associated with altered phenotypic outcomes in
426 progeny [97,98]. HFD feeding of male mice has been shown to modify sperm DNA
427 methylation and small ncRNA signatures [97,99,100]. Strikingly, transfer of sperm miRNA
428 from HFD-fed males into naïve embryos was sufficient to induce the development of obesity
429 in the resulting offspring [101]. In humans, obesity may contribute to increased rates of
430 abnormal sperm parameters and male infertility, which indicates the deleterious effects of
431 HFD on reproductive parameters [102,103]. Yet the specific effects of paternal diet on
432 offspring health have been difficult to study in men due to the concurrence of other
433 confounding lifestyle factors (for example, smoking and physical activity levels) [104].

434 *3.2. Transgenerational epigenetic inheritance*

435 Intergenerational transmission of a phenotype implies direct exposure of parental and
436 subsequent generations to the stressor. For example, when a pregnant mother is fed a HFD,
437 three generations are effectively exposed to this insult simultaneously: the mother (F0), the
438 fetal offspring (F1) and the developing germ cells within the F1 fetus (sperm and eggs that
439 will become the F2). In contrast, transgenerational inheritance refers to the germline

transmission of information between generations in the absence of any environmental exposure (**Figure 1**). If exposure occurs during pregnancy, transgenerational effects will be present even in the F3 generation. If exposure occurs solely before conception, transgenerational effects will appear in the F2 generation.

One well-established form of transgenerational epigenetic transmission is genomic imprinting. These epigenetic marks are established in the oocytes or sperm of the parents and maintained in the somatic cells of the progeny. Conversely, the hypothetical importance of non-imprinted transgenerational inheritance has been proposed recently and is still a controversial matter in mammals, and humans in particular [105]. Paternal HFD has been implicated in the transgenerational amplification of obesity and T2D [106]. In addition, maternal diet insults during gestation lead to inheritance of metabolic disease across generations [85,107].

Another important aspect to consider is the role of oocyte mitochondria epigenetics, since mitochondrial DNA (mtDNA) is inherited exclusively from the mother. For instance, Saben *et al.* showed that a high fat/high sugar diet during gestation and lactation leads to mitochondrial dysfunction and impaired insulin signaling in F1-F3 offspring fed a normal diet [108]. Although speculative, these results suggest that aberrant mitochondria might be passed through the maternal germline.

3.3. Epigenetic disturbances during adulthood

High intake of energy-dense food is a long-established risk factor contributing to obesity. While nutrients such as carbohydrates, fat and proteins, provide us the building blocks and energy necessary to maintain normal physiological functions, over-nutrition has adverse consequences to our health. Nutrients can directly regulate gene expression by modulating the activity of transcription factors. For example, long-chain fatty acids (FA) bind PPARs to regulate their transactivating activities [109]. In addition, current evidence suggests that epigenetic mechanisms are also very sensitive to nutrient availability. Consequently, understanding how food consumption influence the epigenome, and how this in turn can

affect gene expression, is an important step towards better nutritional interventions for the treatment of metabolic disease.

In mice, diet-induced obesity has been associated with increased methylation of the *Pparg* promoter, reduced *Pparg* expression and increased adipocyte dysfunction [110]. Likewise, exposure to HFD has been shown to induce fat-specific alterations in the DNA methylome [111]. Rats fed a high carbohydrate diet have shown altered histone acetylation of the *Pomc* and *Npy* locus in the hypothalamus [112]. The acetyl-CoA required for histone acetylation can be generated from various nutrients. A recent *in vitro* study showed that only lipids induce histone hyperacetylation, even in the presence of high glucose [113]. These findings suggest that the source of acetyl-CoA might specifically regulate histone acetylation and hence gene expression. Further investigations would determine if a similar phenomenon occurs *in vivo.* Nonetheless, another study reported that HFD feeding in mice decreases tissue acetyl-CoA levels and acetylation of specific histones in WAT [114]. In 2017, Nie and colleagues performed a very comprehensive profile of liver histone PTMs in prediabetic HFD-fed mice. The results reported 15 histone marks that are differentially abundant in the liver of HFD-fed mice compared to control ones. Interestingly, the HFD-induced rise in H3K36me2 (a repressive mark) was reversed by metformin treatment, which supports the idea that this epigenetic mark might play a role in the development of T2D [115]. Few studies have reported that diet can influence the expression or function of many miRNAs and lncRNAs. An interesting report showed that lifespan-extending dietary interventions (such as a low-fat diet or caloric restriction) largely repressed the expression of specific miRNAs, lncRNAs, and transposable elements in mice liver [116].

In recent years, the profile of FAs intake has change dramatically from diets with high content in MUFA and PUFA to a "Western-type" diet characterized by a high content in saturated FA (SFA) and trans FA (TFA). This nutritional transition is associated with the rising obesity pandemic, which have been linked to aberrant epigenetic changes. A study in humans associated industrial TFA consumption with plasmatic HDL cholesterol levels and concentrations of HDL-carried miRNAs. Importantly, these miRNAs were associated with lipid metabolism regulation [117]. Other studies have investigated the role of unsaturated

FA in the prevention and treatment of metabolic disease. In humans, n-3 PUFA supplementation for 6 months changed the methylation pattern of 308 CpG sites (93% hypermethylated and 7% hypomethylated). Using pathway analysis systems, it was reported that these epigenetic changes were associated with inflammatory pathways, lipid metabolism and T2D pathways [118]. In line with this evidence, another study found that n-3 PUFA-rich fish oil supplementation in obese subjects improved body weight loss and increased the methylation levels of *PDK4*, *CD36* and *FADS1* CpG sites [119]. Arpón *et al.* investigated, within the PREDIMED (*PREvención con DIeta MEDiterránea*) study, the effect of Mediterranean diet (MD) supplemented with extra virgin olive oil (EVOO) or nuts. The study found that MD+nuts favors the hypermethylation of carnitine palmitoyltransferase 1B (*CPT1B*) gene and MD+EVOO induce hypomethylation of Guanine Nucleotide Binding Protein, G Protein (*GNAS/GNASAS*) gene [120]. In rodents, n-3 PUFA supplementation in HFD-induced obesity models decreases leptin (*Lep*) mRNA expression and ameliorate leptin resistance [121].

Finally, we should reflect on the impact of gut microbial metabolites on the epigenome (recently reviewed elsewhere [122]). Long-term dietary choices affect the diversity and function of the gut microbiota, which ultimately influences the bioavailability of dietary elements and cofactors of epigenetic reactions. For example, butyrate is a short-chain FA generated from the gut fermentation of nonabsorbable dietary fiber that has multiple beneficial effects for health. It has been shown that butyrate promotes histone acetylation by inhibiting HDACs activity [123]. As HDAC1 inhibits the BAT thermogenic program, butyrate might increase energy expenditure by promoting the expression of thermogenesis-related genes in BAT through HDAC1 inhibition [124,125].

4. Mitochondria and epigenetics in obesity

Mitochondria are the powerhouse of the cell. In addition to the production of most cellular ATP through oxidative phosphorylation (OXPHOS), mitochondria modulate numerous signaling pathways that are critically important for the maintenance of cellular homeostasis. Mitochondrial function coordinates glucose and FA oxidation and controls intermediate metabolism. Mitochondrial abnormalities have been found to be implicated in pathological

phenotypes, including obesity and metabolic syndrome; these include defects in mitochondrial biogenesis, number, morphology, and dynamics (fusion and fission).

Mitochondria have their own genome (the mtDNA), a circular double-stranded molecule that encodes 37 genes: 13 proteins that are part of the mitochondrial OXPHOS complexes, 2 rRNAs and 22 tRNAs. The rest of the proteins that encompass the mitochondrial machinery are encoded in nuclear genes [126]. The mtDNA is redundant, consisting of multiples copies in each mitochondrion.

The correct function of mitochondria depends on the coordination of mitochondrial and nuclear genomes. Signaling from the nucleus to the mitochondria promotes biogenesis and regulates OXPHOS to meet cellular energy needs. Signaling from mitochondria to the nucleus can control the expression of nuclear genes to reprogram cellular metabolism. Environmental factors can modify this crosstalk through epigenetic mechanisms. For example, DNA methylation reprogramming in *PPARGC1A* (the gene encoding PGC1α, a master regulator of mitochondrial biogenesis and function) has been observed in tissues from obese patients [127], suggesting that epigenetic regulation of mitochondrial function might play a role in the pathophysiology of the disease. Conversely, extreme dietary conditions like HFD affect the mitochondrial pathways that generate acetyl-CoA [128,129], which in turn might alter the cellular levels of histone acetylation hence affecting gene expression.

In this section, we discuss briefly how mitochondrial metabolism affects the epigenome and how epigenetic mechanisms can regulate mitochondrial function. Moreover, we debate recent data indicating that dysfunctional mtDNA methylation could underlie disease.

4.1. The interplay between mitochondrial function and epigenetics

Epigenetic changes influence the function of mitochondria [4]. For example, many nuclear genes encoding key mitochondrial proteins are regulated by DNA methylation in the context of obesity (*e.g. PPARGC1A, CPT1A* or *ACACA*) [30,127]. Additionally, nuclear miRNAs have been shown to control the activity of mtDNA encoded genes [130] and regulate mitochondrial calcium ($_{m}Ca^{2+}$) handling [131]. Finally, the lysine-specific histone

demethylase LSD1 has received a lot of attention in obesity research as it regulates mitochondrial function and oxidative metabolism, especially in adipose tissue [132].

On the other hand, mitochondrial function is essential to provide the intermediate metabolites required to generate and modify nuclear epigenetic marks (**Figure 2**). For example, levels of acetyl-CoA, which are strictly dependent on mitochondrial function and energy status, are crucial for the function of HATs. Short-chain FA such as those produced as byproducts of FA oxidation are known to inhibit HDACs [2]. The levels of reactive oxygen species (ROS) produced in the mitochondria have been shown to inhibit HDMs [133]. Mitochondrial dysfunction caused by inducible depletion of mtDNA decreases acetylation of specific histone H3 marks [134]. Likewise, depletion of mtDNA also resulted in significant changes in the nuclear DNA methylation pattern [135], and mtDNA haplotypes have been associated with altered DNA methylation maps [136].

HMTs and DNMTs use SAM as a precursor in methyl group transfer. Therefore, mitochondrial function can regulate histone and DNA methylation by indirectly controlling the synthesis of SAM (**Figure 2**). Conversely, the JMJD family of lysine HDMs and TET DNA demethylases require α-ketoglutarate (α-KG), Fe(II) and oxygen to remove the methyl group. Besides, both families of enzymes are inhibited by succinate and fumarate, and therefore citric acid cycle dysregulation affects their activity [137,138]. Recent evidence has shown in myofibroblasts that $[_{m}Ca^{2+}]$ causes changes in the bioavailability of α-KG, which drives JMJD-dependent histone demethylation for activation of specific genes that control cell differentiation [139].

Mitochondrial dysfunction triggers the mitochondrial unfolded protein response (UPR^{mt}), a cellular stress response design to maintain mitochondrial homeostasis. In invertebrates, UPR^{mt} is a positive regulator of lifespan. Merkwirth *et al*. have shown in *C. elegans* that during mitochondrial stress the HDMs JMJD-1.2 and JMJD-3.1 remove repressive histone marks (H3K27me2 and H3K27me3) from specific gene loci thus allowing the activation of the UPR^{mt} [140]. Interestingly, the JMJD-1.2 and JMJD-3.1 murine homologs (PHF8 and JMJD3, respectively) correlate as well with lifespan and UPR^{mt} activation [140].

Nonetheless, the epigenetic control of mitochondrial function during stress in mammals requires further investigation.

4.2. Epigenetic modifications of mtDNA

Methylation of mtDNA has been a matter of debate since the 1970s, but accumulating evidence firmly suggests that it is a real phenomenon. Currently, mtDNA methylation adds a hypothetical layer of epigenetic regulation to mitochondrial function.

In this regard, it has been reported that a transcript variant of DNMT1 translocates to the mitochondria, where it presumably regulates the expression of mtDNA genes [5]. In addition, TET1 and TET2 have also been located in the mitochondria [6]. In the context of obesity, epigenetic modification of liver mtDNA has been associated with the severity of non-alcoholic fatty liver disease [7]. Moreover, mtDNA methylation and copy number have been linked to body composition in humans [141]. Nonetheless, the function and physiological role or mtDNA methylation is still unknown and requires further investigation.

5. Conclusions and future directions

It is highly unlikely that our genome has changed so much in recent decades that it predisposes the entire world population to obesity. However, environmental factors may have interacted with our genome to influence human disease. This review highlights the important role of epigenetic mechanisms in the obesity pandemic.

The accumulating evidence that lifestyle and nutrition affect the epigenetic inheritance of disease risk could explain the rapidly increasing obesity rates. The epigenetics of obesity is an expanding area of research, but important questions remain; 1) Much of what we know about the role of epigenetics in the development of obesity has come from studies of inbred mice. This approach has become a valuable strategy to minimize genetic and environmental confounds. However, previous literature suggests that inbreeding depression might influence environmentally induced phenotypes [142]. This is a critical experimental limitation to be considered when studying epigenetic inheritance using inbred animal models. 2) Most of the research on the epigenetics side of obesity has focused on DNA methylation, particularly in humans. Nonetheless, the role of histone PTMs and chromatin

609 structure remains imprecise. Likewise, a growing number of studies have identified ncRNAs
610 with a potential function in metabolic regulation, but their mechanisms of action will need
611 elucidation. 3) A large body of work supports the developmental and transmittable origins
612 of obesity. However, much less is known about the exact epigenetic mechanisms involved.
613 Paternal contribution seems likely to be substantial and will need to be explored in more
614 detail. 4) The effects of calorie-dense diets have been investigated mostly in the context of
615 obesity, yet the impact of the diet *per se* is still unclear. In the future, studies investigating
616 the consequences of short-term poor-quality diets on the epigenome will help clarify this
617 question. 5) Understanding the complex interaction between mitochondria and the
618 epigenome will open novel interventions in which mitochondrial dysfunction could be
619 managed to treat obesity and other metabolic diseases. 6) Mitochondrial dynamics
620 regulates mitochondrial metabolism and preserves mitochondrial quality, thereby
621 indirectly modulating nuclear epigenetics. Besides, dysregulation of mitochondrial
622 dynamics is found in a variety of metabolic diseases. Nonetheless, it is still unknown
623 whether genes encoding mitochondrial fusion/fission proteins are susceptible to epigenetic
624 control in the context of obesity.

Disclosure statement

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Table 1. List of main epigenetic modifications and modifying enzymes.

Epigenetic modifications/Enzymes	Description
DNA methylation	Addition of a methyl group (CH_3) to the 5th carbon of a cytosine nucleobase (5mC), that is located next to a guanine nucleobase (CpG). Whilst methylation typically occurs at CpGs, non-CpG methylation has also been observed.
DNA methyltransferases (DNMTs)	Family of enzymes that catalyze the transfer of a methyl group to DNA. DNMT1 is responsible for the maintenance of DNA methylation during replication. DNTM3A and DNTM3B are in charge of creating *de novo* DNA methylation patterns.
TET methylcytosine dioxygenases	Three enzymes (TET1, TET2 and TET3) that actively oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC).
Histone acetylation	Addition of an acetyl group (CH_3CO) to the lysine (K) residues of histone proteins.
Histone acetyltransferases (HATs)	A class of enzymes responsible for acetylation of histones. HATs are often not specific to individual K residues, yet fulfill specific functions. The broad catalytic action of HATs requires localization to the correct genomic region, which is controlled by the non-catalytic domain of the enzyme.
Histone deacetylases (HDACs)	A family of enzymes responsible for deacetylating histone proteins. Many HDAC isoforms exist in eukaryotic cells (*e.g*. HDAC3, HDAC4, HDAC5, HDAC8, HDAC11), which raises questions about their specificity or redundancy of functions.
Sirtuins (SIRTs)	Class III HDACs that can catalyze NAD-dependent histone lysine deacetylation. Among the seven mammalian SIRTs, only the class I members (SIRT1–3) have shown robust deacetylase activity.
Histone methylation	Addition of a methyl group to amino acids of histone proteins. Histone methylation has been well characterized on arginines (R) and lysines (K).
Histone methyltransferases (HMTs)	Enzymes that catalyze the transfer of one, two, or three methyl groups to K (lysine-specific HMTs) and R (arginine-specific HMTs) residues of histone proteins.
Histone demethylases (HDMs)	Enzymes that actively remove the methyl groups from K and R residues of histone proteins. HDMs are categorized into two families: amino oxidase homolog lysine demethylase 1 (LSD1 or KDM1) and JmjC domain-containing histone demethylases (JHDMs).
miRNAs	Small (~22 nt) non-coding RNAs that regulate post-transcriptional gene expression through negative regulation or mRNA degradation.
lncRNAs	Long (≥200 nt) non-coding RNAs that regulate gene expression through different mechanisms, including chromatin remodeling.

Figure legends

Figure 1. Epigenetic inheritance across generations: Intergenerational vs transgenerational. Intergenerational transmission of a phenotype implies direct exposure to the stressor of parental and subsequent generations (fetus and primordial germ cells). In contrast, transgenerational inheritance refers to the germline transmission of information between generations in the absence of any environmental exposure. For example, when a pregnant mother is subjected to an environmental insult (e.g. high-fat diet (HFD)), three generations are effectively exposed simultaneously: the mother (F0), the fetal offspring (F1) and the developing germ cells within the F1 fetus (sperm and eggs that will become the F2). In such cases, only effects that would be visible in and beyond F3 would be perceived as truly transgenerational. Conversely, if exposure occurs solely before conception transgenerational effects will appear in the F2 generation.

Figure 2. Crosstalk between mitochondrial function and epigenetic modifications. Mitochondrial function is essential to provide cofactors for many epigenetic reactions. Acetyl-CoA derived from glucose and fatty acids (FA) is the source of acetyl groups used by histone acetyltransferases (HATs). Mitochondrial function also regulates the levels and redox status of Flavin adenine dinucleotide (FAD), and nicotinamide adenine dinucleotide (NAD^+), which are essential cofactors of histone demethylases (HDMs) and Siruins (SIRTs) respectively. FAD and NAD^+ are reduced to $FADH_2$ and NADH during the citric acid cycle and β-oxidation, and then they are oxidized again during OXPHOS. α-Ketoglutarate (a-KG) generated in the citric acid cycle is also a cofactor of the HDMs and TETs. Finally, S-adenosyl methionine (SAM) is the source of methyl groups used by DNA and histone methyltrasnferases (DNMTs and HMTs). SAM is generated in the cytosol through the coupling of the folate and methionine (Met) cycles, and in the mitochondria it sustains the one carbon (One-C) metabolism. Recent studies suggest that SAM can be used in the mitochondria to methylate mtDNA.