

# Current Updates on the Understanding of the Role of DNA Methylation on Obesity

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**Abstract:** Obesity is a condition in which there is an accumulation of excess body fat leading to a weight far above the normal range that poses significant health risks. According to WHO, 8 billion people in the world were obese in 2022. Consequently, obesity has become a pandemic with negative impacts on both global health and economies. Obesity is influenced by various factors including environmental influences, lifestyle choices, gut microbiota, genetic factors, and epigenetic mechanisms such as DNA methylation. DNA methylation can affect an individual's phenotype and condition without altering their DNA sequence. It is the most extensively studied epigenetic alteration and it plays an important part in controlling gene activity associated with obesity. Numerous studies have indicated that DNA methylation is implicated in obesity, thus this review aims to elaborate the roles of DNA methylation to inform the development of preventive measures for obesity.

**Keywords:** DNA methylation, epigenetics, obesity, prevention

## Introduction

Obesity is characterized by the excessive accumulation of body fat leading to an individual's weight significantly exceeding normal levels which poses significant health risks. There are two World Health Organization (WHO) classifications of BMI: an individual is categorized as obese if the BMI is greater than or equal to 30 (kg/m<sup>2</sup>)<sup>1</sup> or if the BMI is greater than or equal to 25 (kg/m<sup>2</sup>) according to the WHO Asia-Pacific classification.<sup>2</sup> Obesity has become an epidemic with severe consequences for the global health and economy, with the increasing prevalence of obesity contributing to the decline in the quality and life expectancy of future generations.<sup>3</sup> According to WHO, in 2022, 8 billion of the world's population were obese.<sup>4</sup> In Indonesia, the 2018 National Basic Health Research and the 2023 Indonesian Health Survey reported that the prevalence of obesity in adults in Indonesia has consistently increased annually from 11.7% in 2007 to 21.8% in 2018, reaching a peak of 36.8% in 2023.<sup>5,6</sup>

Obesity is associated with an individual's genetic and epigenetic patterns. Despite the commonly attributed environmental factors to the trends of obesity in children, adolescents, and adults, there is variability in weight and fat mass among individuals. This indicates that adiposity or excess fat is influenced by the complex interplay between genetics, behavioral developments like dietary patterns and physical activity, and the environment, suggesting that epigenetic changes play a role in weight gain leading to obesity.<sup>7</sup> Moreover, obesity can increase the risk of several non-communicable diseases such as type 2 diabetes, hepatic steatosis, cardiovascular disease, stroke, dyslipidemia, hypertension, gallbladder problems, osteoarthritis, sleep disorders, respiratory problems, as well as various cancers (endometrial, breast, ovarian, prostate, liver, kidney, and colorectal).<sup>3</sup>

Understanding the epigenetic changes associated with obesity is crucial because it offers insights into how environmental and lifestyle factors can influence gene expression without altering the genetic code. This knowledge can lead to the development of targeted interventions and preventive strategies that could potentially mitigate the rising rates of obesity. Additionally, identifying specific epigenetic markers can aid in early diagnosis and personalized treatment plans

for individuals at risk of obesity, ultimately improving public health outcomes and reducing the economic burden associated with obesity-related diseases.

Epigenetics is a mechanism that connects environmental factors to alter gene activity without changing the gene sequence. For example, dietary habits and physical activity can modify an individual's phenotype by modulating DNA methylation, post-translational histone modifications, and gene regulation mediated by non-coding RNA (ncRNA) that regulates gene expression, cell differentiation, the stability and structure of the chromosome, etc. DNA methylation typically occurs at CpG sites. Methyltransferases such as DNMT1, DNMT3A, and DNMT3B catalyze methylation on DNA using S-adenosyl-L-methionine (SAM) as the methyl donor. Passive demethylation can occur when DNMT activity or methyl donor availability is reduced, while active demethylation involves TET enzymes which will catalyze oxidation reaction to form hydroxymethylation. Targeted passive demethylation occurs after that TET enzymes oxidize a methylated cytosine undergoes a conversion process to become hydroxymethyl-cytosine, which continues to persist until the S-phase, where DNMT1 does not recognize it, resulting in an unmethylated cytosine on the new strand. This allows TET enzymes to target cis-regulatory elements for site-specific demethylation.<sup>8</sup>

Researchers already identified hundreds of epigenetic mechanisms, regulated by numerous enzymes, contributing to intricate epigenetic regulation. Histones are composed of protein subunits H2A, H3, H3B, and H4 and serve as central structures around which DNA coils to form nucleosomes, the fundamental units of chromatin. These histones undergo modifications such as acetylation, methylation, ubiquitination, or phosphorylation. These specific changes, alone or combined, alter the structure of nucleosomes, thereby controlling access to the DNA by transcriptional machinery. Methylation and acetylation are the most frequently occurring histone modifications, leading to their extensive study. Histone acetylation, modulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), is one of the most common histone modifications. Acetylated histones facilitate a more open chromatin structure, enabling transcriptional machinery access to DNA. Histones can also be methylated on lysines and arginines by the enzyme histone methyltransferases (HMTs), with demethylation catalyzed by enzyme histone demethylases (HDMs). Histone methylation typically induces gene silencing by recruiting DNMTs, followed by methyl-binding proteins and HDACs. However, histone methylation can also enhance the activity of positive transcriptional elements, such as enhancers or promoters.<sup>9</sup>

ncRNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), play crucial roles in gene expression regulation linked to obesity. miRNAs like miR-30, miR-26b, miR-199a, and miR-148a are elevated in obese individuals and contribute to adipogenesis. Elevated levels of miR-17-5p, miR-132, miR-21, and miR-221 in obese individuals correlate with higher body mass index and metabolic dysfunction. Silencing these miRNAs reduces adipogenesis and improves metabolic function. lncRNAs, such as GYG2P1, lncRNA-p21015, and lncRNA-p5549, are downregulated in obesity and correlate negatively with body mass index and metabolic markers.<sup>3</sup> Other lncRNAs like RP11-20G13.3, lnc-dPrm16, and MIST also impact adipogenesis, inflammation, and lipid metabolism. Therefore, the role of epigenetics and epigenetic changes is crucial in metabolism and the development of obesity.

DNA methylation is a marker of epigenetic changes associated with gene expression related to metabolic conditions such as obesity. Numerous studies have highlighted the involvement of DNA methylation in obesity, indicating specific genes and pathways that are epigenetically modified in obese individuals. However, the mechanisms by which these modifications influence obesity-related traits and how they can be manipulated for therapeutic purposes are not yet fully understood. This study aims to provide a comprehensive review of the current knowledge on DNA methylation in obesity, highlighting recent advances, gaps in the existing research, and potential areas for future investigation. By synthesizing the latest findings, this review will contribute to a deeper understanding of the role of DNA methylation in obesity and inform the development of more effective prevention and treatment strategies.

## Materials and Methods

The databases *PubMed* and *Cochrane* were searched from August to December 2023 using the following MeSH keywords: (Obesity OR Obese) AND (DNA Methylation OR DNA methylation OR DNA Methylations OR DNA methylations OR Methylated DNA OR methylated DNA). The inclusion criteria were original research articles that described the impact and mechanism of DNA methylation on obesity and were published in the last 10 years

(January 2013–January 2023). Articles whose full text or abstract could not be accessed, articles in languages other than English, and articles that did not mention DNA methylation or obesity were excluded.

The research methodology is outlined in Figure 1 and involved: (1) a literature search based on scientific articles using the strategy detailed above, (2) removing duplicate entries by examining the article titles, (3) screening the literature by reviewing both the title and abstract, discarding those that meet the exclusion criteria, (4) assess the eligibility of literature by examining the abstract and full text of each article, discarding those that do not meet the inclusion criteria, and (5) analyze and synthesize the relevant articles.

## Results

The literature search retrieved 234 articles ( $n = 123$  from *PubMed* and  $n = 111$  from *Cochrane*). Duplicates were identified and removed using Microsoft Excel by matching titles, authors, and publication dates and no duplicates were identified during this process. Two independent reviewers then screened the remaining articles based on titles and abstracts for relevance. Disagreements were resolved through discussion and consensus. From this process, 11 articles that discussed the impact of DNA methylation on the development of obesity were selected for inclusion in the review (Table 1). All selected articles indicated a correlation between DNA methylation and obesity across different body tissues. The selection process involved a detailed examination of the full texts to ensure they met all inclusion criteria.

In 2013, study explored whether variations in DNA methylation are a significant feature of obesity, similar to cancer risk. Researchers utilized peripheral blood leukocytes and the Infinium Human Methylation 450K BeadChip to examine whole-genome methylation patterns over 470,000 CpG sites in peripheral blood samples from 48 obese and 48 non-obese African-American adolescents, aged 14–20 years. The study identified numerous CpG sites with differentially variable CpG sites (DVCs) and differentially methylated CpG sites (DMCs). DVCs exhibited more variability in obese individuals, and both DVCs and DMCs independently predicted obesity status in validation sets. Genes containing DMCs and DVCs were significantly enriched with genes linked to obesity and its associated conditions, including hypertension, dyslipidemia, type 2 diabetes, and various cancers. This supports their contributions to the development and progression

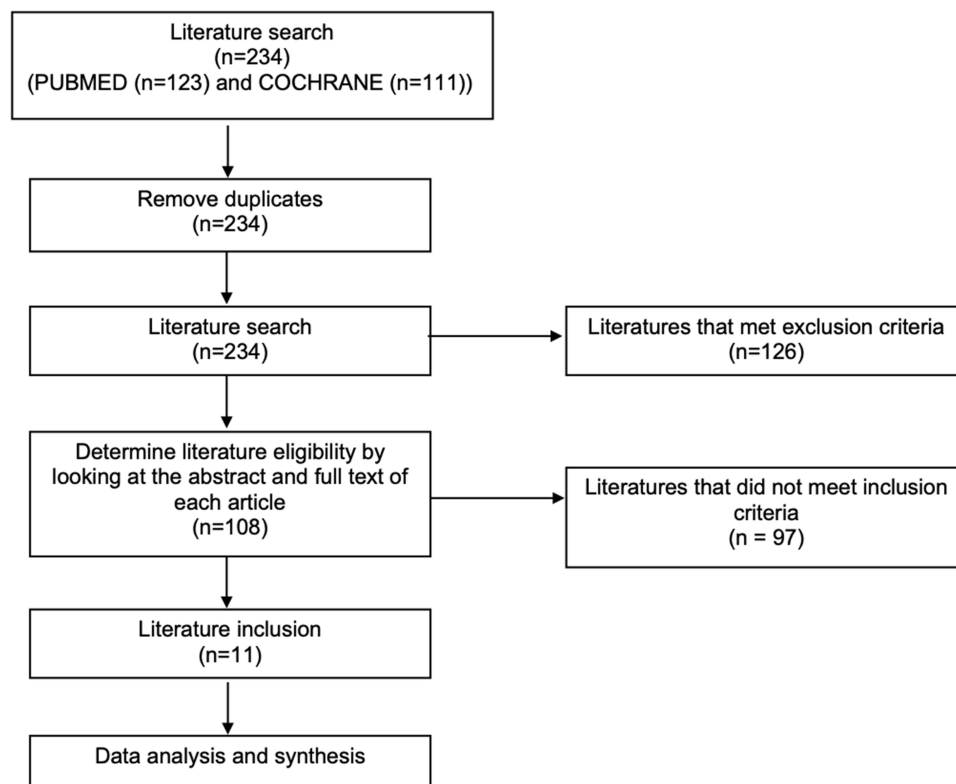


Figure 1 Literature review process.

**Table I** The Role of DNA Methylation in Obesity

No.	Subject and Sample Size	Sample	Number of CpG Sites	Method Used for DNA Methylation Measurement	Results	References
1	Obese (n=48)	Peripheral blood leukocytes	23,305	Illumina Infinium Human Methylation 450K Beadchip	The number of CpG sites associated with obesity is 23,305.	[10]
	Lean (n=48)					
2	European (n=2707)	Whole blood	278	Illumina Infinium Human Methylation 450K Beadchip	Epigenome-wide analysis identifying a total of 278 CpG sites showed that BMI is associated with widespread changes in DNA methylation.	[11]
	South Asean (n=2680)					
3	Participants BMI > 18.50 kg/m <sup>2</sup> (n=3,156)	Whole Blood	237	Illumina Infinium Human Methylation 450K Beadchip	DNA methylation was associated with both BMI and waist circumference. A total of 237 CpGs associated with either BMI or WC were detected.	[12]
4	Women with obesity (n=11)	Peripheral blood leukocytes	16,064	Illumina Infinium Human Methylation 450K Beadchip	The dietary intervention resulted in alterations in the DNA methylation levels at 16,064 CpG sites. Nonetheless, even with the hypocaloric intervention, a subset of 878 CpGs (associated with 649 genes) remained notably different in obese women compared to those with normal weight.	[13]
	Normal-weight women (n=24)					
5	Female with obesity (n=18)	Whole blood	7	Pyrosequencing (Pyromark Q24)	Weight-loss program, including resistance training, may be associated with decreased visceral fat area and increased CpG methylation level.	[14]
6	Low carbohydrate diet (n=30)	Whole blood	47	Illumina Human Methylation 850 Bead Chips	Body weight loss after an 18-month lifestyle intervention was connected to distinct methylation patterns. Additionally, disparities in methylation among the identified genes might serve as predictive indicators for effective weight loss therapy, thus enhancing tailored treatments for obesity in patients	[15]
	Low carbohydrate diet + physical activity (n=30)					
	Low fat diet (n=30)					
	Low fat diet + physical activity (n=30)					
7	Children (n=92)	Saliva	17	Illumina Infinium Human Methylation 450K Beadchip	DNA methylation in saliva could potentially forecast future childhood obesity among Hispanic children. NFR1 may warrant further investigation as a potential target for understanding obesity in this population.	[16]
8	Obese men (n=20)	Human sperm	3264	Illumina Infinium Human Methylation 450K Beadchip	Obesity in men was linked to changes in the methylation patterns of sperm DNA, which can affect the accuracy of reprogramming in some sperm cells, indicating a potential impact on spermatogonia.	[17]
	Normal BMI (n=47)					

9	Obese (n=4)	Whole blood	9371	Infinium Methylation EPIC Bead Chips	Pyrosequencing results showed significant changes in methylation levels at specific CpG sites within the CRTCI (CpG1 and CpG2-cg11660071) and INHBB (CpG2) genes among obese patients compared to healthy controls.	[18]
	Normal control (n=4)					
10	Subcutaneous adipocytes (n=95)	Subcutaneous adipocytes	4485	Illumina Infinium Human Methylation 450K Beadchip	DNA methylation is associated with BMI and waist circumference, and DNA methylation is a crucial determinant of human obesity and related metabolic complications	[19]
	Visceral adipocytes (n=95)	Visceral adipocytes	445			
11	Adult female Göttingen Minipigs Ellegaard (n=17)	Arterial blood	27	MSP-PCR	Differentially methylated region in an intronic region of the PPARGC1B gene was found with total of 1236 cfDNA DMRs were associated with obesity.	[20]

**Abbreviations:** BMI, body mass index; cfDNA, cell free DNA; CpG, cytosine and guanine nucleotides; DMRs, differentially methylated regions; MSP-PCR, methylation specific PCR; WC, waist circumference.

of obesity. The study concluded that differential variability is a crucial feature in obesity-related methylation changes, and future epigenetic research on obesity should use both mean-based and variance-based statistics.<sup>10</sup>

A large-scale study in 2017 was conducted using DNA methylation data from 450,000 CpG sites with more than 10,000 complete blood samples, identifying 187 CpG sites associated with body mass index (BMI). This research revealed that changes in DNA methylation in the blood are primarily a consequence of obesity, rather than a contributing factor. These conclusions were also confirmed in adipose tissue. The study emphasized that methylation sites were enriched with functional genomic elements in various tissues. Key methylation markers identified gene expression patterns at 38 loci related to lipid metabolism, nutrient transport, and inflammatory pathways. Furthermore, disruptions in DNA methylation were found to predict the onset of type 2 diabetes, with a relative risk of 2.3 for each standard deviation increase in the methylation risk score.<sup>11</sup>

In the same year, a study utilized Illumina 450K DNA methylation profiling of whole blood samples to investigate its correlation with obesity, identifying 94 CpG sites linked to body mass index (BMI) and 49 CpG sites related to waist circumference. Obesity, known for its association with increased risks of various diseases and its global epidemic status, exhibits high heritability, yet genome-wide association studies have only partially explained its genetic variability. It is hypothesized that epigenetic plays a significant role in this unexplained variability. The aim of the study was to identify differential methylation patterns associated with obesity using an epigenome-wide association approach. The research began with a discovery phase involving 641 participants from the REGICOR study and was subsequently validated with 2515 participants from the Framingham Offspring Study. Illumina HumanMethylation450 BeadChip was employed to assess blood DNA methylation levels, followed by meta-analysis using fixed-effects methods and functional pathway analysis with Ingenuity Pathway Analysis software. The study confirmed 94 CpG sites correlated with BMI and 49 CpG sites correlated with waist circumference, distributed among 95 genetic loci. Furthermore, it uncovered 70 previously unidentified CpG sites associated with BMI and 33 CpG sites associated with waist circumference. Together, these CpG sites accounted for variation 25.94% of the variation in BMI and 29.22% of the variation in waist circumference, respectively, within the sample of REGICOR. Cross-validation with GIANT genome-wide association data revealed that 65 of the 95 validated loci included Tag SNPs linked to BMI. Pathway analyses highlighted implications for neurological, psychological, endocrine, and metabolic pathways, underscoring the potential of epigenetic mechanisms in elucidating the multifaceted nature of obesity.<sup>12</sup>

In 2019, a study examining obese women undergoing a hypocaloric dietary intervention found that this intervention partially restored DNA methylation patterns associated with obesity at 16,064 CpG sites. Despite the intervention, 878 CpG sites associated with 649 genes remained significantly different in women with severe obesity compared to women of normal weight. The research included 11 women with severe obesity who underwent evaluation before and after a six-week dietary intervention, with their outcomes compared to those of normal-weight women of similar age. Genome-wide analysis of DNA methylation was conducted using the Infinium Human Methylation 450 BeadChip assay, applying specific thresholds for statistical significance. The dietary intervention altered CpG sites associated with pathways including cancer, cell cycle, MAPK, Rap1, and Ras signaling. However, persistent changes in the 878 CpG sites highlighted pathways related to cadherin and Wnt, angiogenesis signaling, and p53 by glucose deprivation. The findings suggest that while short-term hypocaloric interventions can partially restore obesity-related DNA methylation patterns, complete reversal may depend on the extent of weight loss achieved through dietary intervention, emphasizing the impact of lifestyle patterns on an individual's condition.<sup>13</sup>

In a study, an increase in methylation was observed in a group of Japanese women who followed an exercise program, specifically in the CpG3 region. Their objective was to measure methylation levels on FTO promoter and correlate it with 6-month weight-loss intervention program. The participants were divided randomly into two groups, one receiving standard treatment and the other receiving additional resistance training. Each participant underwent assessments of exercise tolerance, metabolic parameters, and body composition. The results indicated a significant reduction in methylation level at CpG1 and overall CpG in the normal treatment group, whereas in the resistance training group, methylation level at CpG3 was increased. Moreover, factors independently influencing %CpG3 in the resistance training group included changes in visceral fat area (%VFA) ( $\beta = -0.568$ ,  $P = 0.007$ ,  $R^2 = 0.527$ ) and participation in resistance

training ( $\beta = 0.517$ ,  $P = 0.012$ ,  $R^2 = 0.527$ ). They concluded that 6-month weight-loss program correlated with reductions in visceral fat area and alterations in FTO methylation levels.<sup>14</sup>

These results were corroborated by another study conducted in the same year, which showed that a combined exercise and diet program influences DNA methylation changes in patients with obesity and aids in weight loss, with 32 out of the 47 CpG sites examined being associated with weight changes. The study aimed to address the challenge of significant variability in individual responses to dietary and physical activity interventions. To explore this, DNA methylation analysis was performed on blood samples of 120 participants who were predominantly male, with an average age of 49 years and a mean BMI of 30.2 kg/m<sup>2</sup> who participated in 18-month intervention program. Participants followed either a low-carbohydrate diet or a low-fat diet, with or without physical activity. The analysis compared participants with the most significant weight loss to those who did not respond, revealing notable DNA methylation differences in several genes, such as *CRISP2*, *LRR27*, and *SLFN12*. A comprehensive analysis of the epigenome for proportional weight loss has identified 15 CpG sites that were inversely related to weight change following the intervention, including *NCOR2* and *NUDT3*. A preliminary DNA methylation score proved to be a better predictor of successful weight loss (AUC ROC = 0.95–1.0) compared to traditional predictors like age and BMI (AUC ROC = 0.56). In that study, they concluded that lifestyle intervention is linked to particular methylation patterns and could act as indicators for predicting the success of weight loss management, thereby enhancing patient-tailored obesity treatments.<sup>15</sup>

In 2020, a unique study conducted by Rushing et al explored the association between DNA methylation in saliva and obesity. This study investigated whether baseline DNA methylation in saliva could predict childhood obesity in a cohort of Hispanic children aged 3 to 5 years over a three-year period. Initial saliva samples were obtained from 92 participants in the Growing Right Onto Wellness (GROW) trial using the DNA saliva collection kit, with selection criteria based on the maternal obesity status of the participants. The results showed that initial methylation levels of CpG1307483 (*NRF1*) demonstrated a significant association with the incidence of childhood obesity at the 36-month follow-up (OR = 2.98,  $p = 0.04$ ). These findings suggest that baseline salivary methylation of *NRF1* is a significant predictor of childhood obesity, independent of baseline BMI-Z. This study supports the potential of using saliva as a non-invasive approach for collecting DNA and performing epigenetic analyses, highlighting *NRF1* as a target for further investigation in obesity research.<sup>16</sup>

In 2021, a study investigated the impact of obesity on sperm DNA methylation in 20 obese men compared to 47 men with normal BMI to assess potential implications for offspring development. Using Infinium Human Methylation 450 Bead Chips, the study identified 3264 sperm CpG sites that showed a significant association with BMI ( $p < 0.05$ ). These sites were notably enriched in genes associated with transcriptional modulation, cancer dysregulation, etc. Bisulfite sequencing of cloned alleles revealed that methylation differences were not randomly distributed but rather present in a subset of spermatozoa. The findings suggest that male obesity alters sperm DNA methylation profiles, potentially affecting reprogramming fidelity in spermatogonia.<sup>17</sup>

In 2021, another study highlighted an association between epigenetic changes in DNA methylation and obesity. Pyrosequencing revealed significant methylation changes in CpG regions of *CRTC1* in obese individuals compared to controls, aligning with the overall DNA methylation patterns. This research sheds new light on the pathological mechanisms driving obesity. Using the Illumina 850K methylation microarray, they analyzed the DNA methylation patterns in blood samples from both obese and normal individuals. The study uncovered a sum of 9371 sites with significant variations in methylation, with 7974 sites with increased methylation and 1397 sites with decreased methylation, across 4571 genes. Differential distribution of hypermethylated and hypomethylated sites was observed in gene structures and CpG islands, with 114 key sites identified in CpG islands. The RT-qPCR findings for *ZBTB7B* and *CRTC1* aligned with the methylation profiles, and pyrosequencing confirmed significant methylation alterations at *CRTC1* CpG sites and *INHBB* CpG sites in obese patients compared to normal controls.<sup>18</sup>

In 2023, a genomic analysis on adipocytes related to DNA methylation in obesity identified 4485 CpG sites in subcutaneous adipocytes and 445 CpG sites in visceral adipocytes associated with obesity, linked to over 500 target genes. This study highlights the pivotal impact of DNA methylation in obesity and its metabolic complications. Significant DNA methylation alterations were strongly linked to obesity were discovered in both subcutaneous and visceral adipocytes. These changes were connected to transcriptomic alterations at more than 500 target genes, and

potential between methylation and transcription factor interactions was identified. Using Mendelian Randomisation, the study inferred the influence of methylation patterns on obesity and related metabolic abnormality at 59 distinct loci. These results emphasize the significance of DNA methylation in influencing human obesity and related metabolic disorders, revealing mechanisms by which altered methylation can affect adipocyte functions.<sup>19</sup>

In the same year, a study using Göttingen Minipigs as animal models found that methylation in the intronic region of the PPARGC1B gene is associated with obesity. The study utilized nanopore sequencing to identify differentially methylated regions (DMRs) in circulating cell-free DNA (cfDNA) linked to obesity. A total of 1236 cfDNA DMRs were identified, with significant enrichment observed in pathways related to adipocytokine signaling, glucagon signaling, and cellular glucose homeostasis. Among these, a strong DMR was found in the PPARGC1B gene, which was validated using methylation-specific PCR (MSP-PCR) and showed a significant correlation with body weight ( $P < 0.05$ ). Interestingly, no DMRs were common between cfDNA and whole blood genomic DNA (gDNA), suggesting cfDNA originates from widespread tissue shedding. This study demonstrates the utility of nanopore sequencing in detecting differential methylation in low quantities of cfDNA, providing insights into the epigenetic mechanisms of obesity. Further research should focus on translating these findings to human studies and analyzing 5mC in somatic tissues to identify the precise location of pathological changes.<sup>20</sup>

## Discussion

Understanding the role of epigenetics is crucial in metabolic conditions such as obesity, in particular the epigenetic mechanism of DNA methylation, thus this review elaborated the roles of DNA methylation to inform the development of preventive measures for obesity.

## DNA Methylation as a Potential Marker for Overweight and Obesity

DNA methylation can increase or decrease depending on the function and protein encoded by genes that influence obesity, thus DNA methylation can repress or suppress gene expression. This is closely related to obesity considering the numerous genes that can be influenced by DNA methylation, thus potentially serving as markers for obesity.<sup>21</sup> The results from reviewed studies consistently identify specific CpG sites strongly correlated with obesity, BMI, and waist circumference.<sup>10,11</sup> For instance, studies have highlighted CpG sites in genes like NRF1, ADR1B, and PTPRN2, showing differential methylation patterns between obese and non-obese individuals, underscoring their potential as biomarkers for obesity risk.<sup>10,16</sup> Also, DNA methylation also has the potential to detect obesity in children, one example being the NRF1 gene.<sup>14</sup> Furthermore, the application of DNA methylation markers in diverse tissues such as saliva and sperm provides non-invasive avenues for obesity diagnostics and risk assessment,<sup>16,17</sup> thereby opening further insights and research to prevent and detect obesity.

DNA methylation is increasingly recognized as a promising biomarker for obesity-related traits. Quantitatively, these methylation markers explain a significant proportion of the variance in obesity-related traits. For example, a study found 94 CpG sites that are linked to BMI and 49 CpG sites that correlate with waist circumference. Furthermore, the AUC value, which represents the area under the ROC curve for distinguishing obese from non-obese individuals using these methylation markers, ranges from 0.75 to 0.85, indicating good predictive power.<sup>12</sup>

## Role of DNA Methylation in Obesity Prevention Through Exercise

Studies have shown that individuals engaging in an exercise program experience changes in DNA methylation profiles. A good exercise program can increase DNA methylation and induce specific changes in DNA methylation profiles, particularly influencing genes involved in metabolic pathways related to obesity.<sup>22–24</sup> The high level of DNA methylation will subsequently modify histones, resulting in histone methylation specifically at H3K9me3 and H3K27me3, as these regions are where both methylation and acetylation mechanisms can occur. Consequently, histone acetylation decreases, leading to the condensation of chromatin from its initial open state (euchromatin) to a more compact form (heterochromatin), thereby suppressing the expression of certain genes, especially those related to obesity.<sup>21</sup> Exercise can burn calories, particularly in adipose tissue, especially in those that store energy reserves in the form of fat, namely



triglycerides in white adipose tissue.<sup>25</sup> Therefore, lifestyle interventions, including structured exercise programs, leverage epigenetic mechanisms to effectively prevent obesity.

## Role of DNA Methylation in Obesity Prevention Through Dietary Intervention

Unhealthy eating habits can induce negative epigenetic modifications leading to conditions like obesity, including excessive intake and the types of food consumed. All studies indicated that selecting the type and amount of energy intake can help address obesity as assessed by BMI, waist circumference, and other factors, and improve and alter DNA methylation profiles in an individual.<sup>13,15</sup>

Excessive intake and poor food choices can lead to obesity by the increased production of acetyl-CoA,<sup>24</sup> leading to the formation of fatty acids and increased gene expression through histone acetylation, thus genes related to metabolism will be expressed leading to obesity. These eating habits can be reversed to suppress the expression of metabolism-related genes associated with obesity. Therefore, selecting and limiting excessive energy intake is one of the initial and most appropriate steps to address conditions related to obesity.<sup>26</sup> By selecting appropriate energy intake levels and types of foods, it is possible to modulate gene expressions involved in metabolic processes, thereby reducing obesity risks.

## DNA Methylation Serves as the Potential Approach in the Discovery of New Drugs to Prevent Obesity

DNA methylation significantly influences gene expression and numerous genes have been associated with obesity genes such as NRF1, ADR1B, PTPRN2, and others.<sup>10–20</sup> These genes are just a few examples indicating that there are still many genes to be explored. Manipulating DNA methylation levels through pharmacological interventions, including herbal medicines, presents a promising strategy for developing novel treatments for obesity.

Almost all studies included in this review indicated that epigenetic changes, specifically DNA methylation, serve as markers and one of the methods used for approaching, preventing, and treating obesity. Several studies also indicate that lifestyle patterns determine an individual's condition through epigenetics. Nonetheless, more studies are required considering the potential of DNA methylation in addressing obesity in various tissues. For instance, several studies examined epigenetic methylation DNA markers in various tissues such as blood, adipose tissue, saliva, and sperm, therefore, it is hoped that future studies will be conducted to address the issue of obesity that occurs both globally and in Indonesia.

## Conclusion

This review consolidates evidence supporting DNA methylation plays a critical role in modulating gene activity associated with obesity. As a significant epigenetic mechanism, DNA methylation can serve as a biomarker for identifying individuals at risk of obesity. Understanding the patterns of DNA methylation associated with obesity can facilitate the development of targeted preventive strategies, including personalized exercise and dietary interventions. Moreover, insights into DNA methylation can pave the way for novel therapeutic approaches, potentially leading to the discovery of new medications that modify epigenetic marks to combat obesity effectively. Therefore, advancing research in DNA methylation holds promise for more precise and effective prevention and treatment of obesity.

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## Disclosure

The authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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