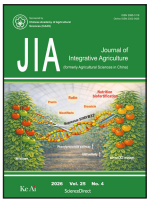
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REVIEW

Methyl donor micronutrients orchestrate lipid metabolism: The role of DNA methylation modification

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Highlights

- DNA methylation is a pivotal epigenetic mechanism linking methyl donor intake to lipid metabolism, dynamically regulating fat formation, deposition, and breakdown in livestock.
- Methyl donors such as folate and betaine reshape DNA methylation patterns of core lipid genes (e.g., PPARγ, SREBP1), directly influencing intramuscular fat and meat quality.
- Balanced methyl donor supply promotes lipid homeostasis and meat traits, whereas insufficient or excessive intake disrupts methylation, impairing metabolism and product quality.

Abstract

DNA methylation, a key epigenetic modification, plays a crucial role in regulating lipid metabolism. Consistent correlations have been observed between aberrant DNA methylation patterns and lipid metabolic disorders. Emerging evidence indicates that methyl donor micronutrients could influence DNA methylation patterns, consequently exerting an influence on lipid metabolism. Specifically, the deficiency or excesses of methyl donor micronutrients (folate, choline, betaine, B vitamins and methionine) have been associated with altered DNA methylation patterns linked to lipid metabolism. These alteration in DNA methylation levels, occurring globally and within promoter regions, could affect gene expression related to lipid metabolism. However, the mechanisms through which methyl donor micronutrients regulate lipid metabolism *via* the DNA methylation modification and the role of methyl donor micronutrients supplementation on DNA methylation profiles remain unclear. In this review, we summarized the regulatory role of DNA methylation in lipid metabolism, and highlighted recent findings investigating the impact of methyl donor micronutrients on lipid metabolism, as well as DNA methylation-mediated adipogenesis and adipose deposition. Taken together, this review deepened our understanding of how the complex interplay between methyl donor micronutrients, DNA methylation, and lipid metabolism, and provides valuable information for accurately regulating lipid metabolism of livestock and poultry, thereby improving meat quality, and promoting the development of animal husbandry.

Keywords: DNA methylation, epigenetic, lipid metabolism, methyl donor micronutrients

1. Introduction

In recent years, as people's living standards continuously improved, meat quality has become an increasingly critical factor in consumers' purchasing decisions. Consequently, the production of high-quality meat has become a top priority for the livestock industry. Fat deposition is a crucial determinant of pork quality, involving multiple processes such as fat intake, transport, synthesis, and catabolism (Han *et al.* 2025). These processes are intricately connected to genes that regulate fat synthesis and catabolism, as well as related signaling networks. The fat deposition was regulated by genetic, environmental, and nutritional factors (Li Z *et al.* 2022). Epigenetic modifications, including DNA methylation, histone modification, and chromatin remodeling, play pivotal roles in regulating gene expression and significantly

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affect fat deposition (Panera *et al.* 2022). Notably, among these modifications, DNA methylation, especially at CpG dinucleotides, play a key role in regulating gene transcription (Wang S *et al.* 2023).

DNA methylation was catalyzed by DNA methyltransferases (DNMTs), which added a methyl group to the carbon-5 position of cytosine bases in DNA chains, forming 5-methylcytosine (Mattei *et al.* 2022). This process regulated the expression levels of specific genes, either by interacting with transcription factors or altering chromatin conformation. DNA methylation, acting as a link between genes and environmental influences (Skinner *et al.* 2010), played a crucial role in controlling gene expression during development (Cedar *et al.* 2022). Recent studies showed that aberrant patterns in DNA methylation could disrupt gene expression, leading to diverse phenotypes that were directly associated with lipid metabolism disorders (Samblas *et al.* 2019). Methyl donor micronutrients, such as methionine (Met), betaine, choline, folate (FA), vitamin B12 (VB_{12}), and other VBs, were exhibited the ability to modify DNA methylation levels either globally or at specific regions by inducing the formation of methyl donor through one-carbon metabolism (Romano *et al.* 2017). Such alterations could affect the expression of genes related to the health of animals, participated in the regulation of lipid metabolism (Zhang 2018; Liu Q *et al.* 2022). The interaction between micronutrients and DNA methylation was known as “Nutri-epigenomics” (Remely *et al.* 2015). Nutritional interventions targeting epigenetics could provide important strategies for improving lipid metabolism in the body. DNA methylation served as a critical epigenetic link between lipid metabolism and diets, which was the main focus of this review.

The intricate interplay between micronutrients and DNA methylation offered a compelling avenue for understanding and potentially improving lipid metabolism. However, comprehensive investigations in this promising field were required to deepen our understanding the role of nutrition in regulating epigenetics, especially DNA methylation. For instance, how micronutrients mediated the expression of lipid metabolism-related genes by regulating the level of DNA methylation modification to improve lipid metabolism lipid metabolism required further exploration to uncover the underlying regulatory mechanisms. Therefore, this review overviewed the latest studies that provided potential mechanisms of micronutrients induced-DNA methylation reprogramming in lipid metabolism. It also highlighted the association among micronutrients, DNA methylation profiles, and gene expression levels.

2. DNA methylation and lipid metabolism

DNA methylation was a crucial epigenetic modification that regulated gene expression patterns, subsequently affecting lipid metabolism by adding methyl groups to DNA molecules. Generally, DNA hypermethylation on the gene promoters was often associated with gene repression, whereas DNA hypomethylation typically correlated with increased DNA binding of transcription factors and chromatin proteins (Ma and Kang 2019). As expected, a study on the mouse adipocyte methylome identified hypermethylation in the promoter regions of several genes (e.g., phosphoenolpyruvate carboxykinase1 (*PCK*1), transcription factor 7-like 2, and protein kinase B), which were significantly negatively correlated with gene expression in mouse adipocytes exposed to high-fat diet (HFD) (Multhaup *et al.* 2015). The promoters of peroxisome proliferator-activated receptor-γ (*PPAR*γ) gene were gradually demethylated during adipogenesis in 3T3-L1 cell line, correlating with an upregulation of the *PPAR*γ mRNA expression (Fujiki *et al.* 2013). This suggested that a potential negative feedback regulatory mechanism between DNA methylation levels and target gene expression, which might be one of the important mechanisms by which cells responded to environmental changes and maintained homeostasis. The involvement of DNA methylation changes in the regulation of key adipogenic genes further emphasized the role of epigenetic modifications in lipid metabolism.

The DNA methylation modifications on the gene promoters were orchestrated by a conserved group of enzymes known as DNMTs (including DNMT1, DNMT3a, and DNMT3b), which were involved in various forms of metabolic perturbation (Jeltsch and Jurkowska 2014). Understanding the role of these DNMTs in metabolic processes was crucial, as their dysregulation had been implicated in various metabolic disorders. Specifically, alterations in DNMTs activity in response to environmental factors, such as diet, could lead to changes in gene methylation patterns and subsequent effects on gene expression. Notably, a dynamic upregulation of DNMT1 and DNMT3a by HFD might have induced hypermethylation of the *Klb* promoter and subsequent downregulation of *Klb* expression, inhibiting Fibroblast growth factor 15 (*FGF*15)/*FGF*21/*Klb* signaling, and resulting in the development of hepatic lipid accumulation (Wang S *et al.* 2023). This discovery illustrated the intricate interplay between diet, DNMTs, DNA methylation, and lipid metabolism, the necessity for further exploration into the mechanisms by which dietary factors influenced DNMTs activity, DNA methylation patterns, and their subsequent effects on lipid metabolism. Furthermore, DNMTs were associated with insulin resistance induced by lipid metabolism disorder. DNMT3a had been identified as a novel epigenetic mediator of insulin resistance *in vitro* and *in vivo*. *FGF*21, a key metabolic target gene of DNMT3a in adipocytes, could rescue DNMT3a-mediated insulin resistance. Elevated DNA methylation at the *FGF*21 promoter regions in Dnmt3a overexpressed 3T3-L1 cells reduced *FGF*21 expression, leading to decreased insulin sensitivity (You *et al.* 2017). Similarly, *FGF1* could modulate lipid metabolism and effectively reduce the insulin resistance *via* downregulating DNMT3a activity through inhibition of *P*53, reducing the recruitment of DNMT3a to the insulin-like growth factor binding protein 2 (*IGFBP*2) genomic locus. This leading to decreased *IGFBP*2 gene methylation and increased mRNA and protein expression in HFD-fed mice (Wang J *et al.* 2023). This suggested that DNMT3a could be considered a potential regulatory target, and modulating its activity might have helped improve lipid metabolism disorders and insulin resistance. It was worth noting that modulating the expression of DNMT3b had also demonstrated significant impacts on lipid metabolism. Silencing DNMT3b had been shown to ameliorate hyperlipidemia by reducing

the expression level of DNMT3b. This reduction led to a decrease forkhead box protein P3(Foxp3)-TSDR methylation levels, and an increase in the mRNA and protein expression levels of Foxp3, reduced production of inflammation factors (Zhu *et al.* 2022), this might have contributed to improving lipid metabolism homeostasis. However, Dexamethasone treatment, by inhibiting the Wnt/β-catenin pathway, caused significant upregulation of mRNA and protein levels of CCAAT/enhancer-binding protein alpha (*C/EBP*α) by blocking DNMT3b binding to *C/EBP*α promoter, leading to demethylation in C3H10T1/2 cells, thereby promoting lipid deposition (Li *et al.* 2013). In summary, DNMTs (including DNMT1, DNMT3a, and DNMT3b) played a central role in the process of DNA methylation modifications in gene promoter regions, and these modifications were closely related to multiple aspects of lipid metabolism. Specifically, upregulating the expression of DNMT1 and DNMT3a could have increased the methylation levels of the *Klb* and *FGF*21 genes, thereby reducing their expression. Conversely, downregulating the expression of DNMT3b could have reduced its binding to *C/EBP*α, lowering the methylation level of this gene and subsequently upregulating the expression of *C/EBP*α, which promoted lipid deposition. Furthermore, downregulating the expression of DNMT3a and DNMT3b could have also decreased the methylation levels of the *IGFBP*2 and *Foxp*3 genes, upregulating their expression and contributing to the improvement of lipid metabolism homeostasis. The mechanism of action of DNMT3b was relatively complex, potentially regulating lipid metabolism through multiple methylation modification pathways, and its specific mechanisms still required further investigation and exploration. Overall, DNMTs, as potential regulatory targets, played a significant role in modulating the methylation levels and expression levels of genes related to lipid metabolism, thereby influencing the lipid metabolic process.

Research had shown that DNA methylation could undergo dynamic and reversible changes during the process of lipid deposition, primarily regulated by DNMTs. For instance, the mRNA level of DNMT1 was rapidly elevated and promoted differentiation at the early stage of differentiation of 3T3-L1 preadipocyte differentiation, while inhibited lipogenesis at the late stage (Yang *et al.* 2016). Interestingly, pharmacological inhibition of DNA methylation by 5-aza-2′-deoxycytidine (an inhibitor that inhibits DNMTs activity) led to demethylation on the Wnt10a promoter, an antiadipogenic factor. Upregulation of Wnt10a downregulates the mRNA expression of adipogenic markers genes (*C/EBP*α, *PPAR*γ2, fatty acid synthase (*FAS*) and adipocyte fatty acid binding protein (aP2)) and ultimately supresses adipogenesis at the early stage of differentiation (Chen *et al.* 2016; Yang *et al.* 2016). However, 5-aza-2′-deoxycytidine markedly enhanced the expression of lipogenic markers, such as reduced the DNA methylation on the sterol regulatory element-binding proteins 1c (*SREBP*-1c) promoter and increasing its expression, and its downstream target FAS, acetyl-CoA carboxylase (*ACC*), and stearoyl-CoA desaturase-1 (*SCD1*), and enhancing lipogenesis at late stage of differentiation (Yang *et al.* 2016). This might have been due to DNMT3a played distinct roles in different stages of adipocyte development and function. Therefore, regulating DNMTs at different stages of adipocyte development could have had contrasting effects on lipid metabolism. However, the data regarding the potential role of DNMTs in regulating lipid deposition through their impact on DNA methylation levels were conflicting. For instance, Guo *et al.* (2016) reported that the mRNA expression level of DNMT3a in preadipocytes gradually upregulated in the early stage of adipogenesis and then decreased significantly after 24 h of the induction. In contrast, Yang *et al.* (2016) observed a slight increase in DNMT3a expression only at the mature adipocyte stage after 8 days of differentiation. In addition, the knockout of DNMT3a in mature adipocytes protected mice from developing insulin resistance (You *et al.* 2017). Conversely, adipocyte progenitor cell lines lacking DNMT3a exhibited aberrant differentiation, leading to constitutive DNA hypomethylation, a persistent increase in leptin levels, adipose tissue expansion, weight gain, and resulted in insulin resistance (Tovy *et al.* 2022). This disparity in research findings could have potentially been attributed to the different types of adipose cells involved, illustrating the intricate nature of lipid metabolism and the diverse roles that DNMTs might play within various adipocyte subpopulations. In summary, DNMT1, DNMT3a, and DNMT3b played vital roles in establishing DNA methylation patterns and regulating adipogenesis. The regulation of lipid deposition by DNMTs likely involved multiple, interconnected mechanisms that might have varied depending on the stage of adipogenesis, the specific adipocyte subpopulations, and the overall metabolic context. The aberrant patterns in DNA methylation caused by DNMTs could lead to disturbed gene expression, resulting in diverse phenotypes, which were directly associated with lipid metabolism disorders. These discrepancies in phenotypes might have been due to the complex regulatory network between DNA methylation, gene expression, and adipogenic signaling pathways, as well as the diverse roles played by different DNMTs at various stages of the process. There was a need to further elucidate the regulatory mechanisms of these interactions and their impact on lipid metabolism. Therefore, based on the dynamic changes of DNA methylation in livestock and poultry at different developmental stages, we can effectively regulate the lipid deposition process by modulating the activity of DNMTs, which subsequently influences the methylation level and expression level of target genes, ultimately achieving the goal of improving meat quality.

Lipid deposition was discovered to be associated with the secretion of adipokines, and dysregulation of adipokine secretion could lead to the abnormal lipid metabolism. However, DNA methylation might regulate the secretion of these adipokines. For instance, DNA methylation could regulate the expression and secretion of leptin. Leptin, an important adipokines produced by adipose tissue, not only acted on the hypothalamus to suppress appetite and increased energy expenditure, but also appeared to have a direct influence on the epigenetic machinery, specifically through its interaction with the *JAK2*/*STAT3* signaling pathway (Zeng *et al.* 2024). Its expression and circulating levels were significantly positively associated with the amount of body

fat mass (Perakakis *et al.* 2021), and its secretion primarily dependent on the production rate of leptin gene transcription and translation (Zhao S *et al.* 2020). Research suggested that the DNA methylation level at the promoter region of the leptin gene was found to be inversely correlated with body weight (Sadashiv *et al.* 2021), and reduced leptin methylation could account for obesity-related leptin upregulation (Stöger 2006). This might be due to the fact that leptin inhibited the expression of Ten-Eleven-Translocation 2 (TET2) through the *JAK*2/*STAT*3 signaling pathway, while TET2, by interacting with the transcription factor *C/EBP*α, reduced the methylation level of the leptin promoter, thereby promoting the expression of the leptin gene (Zeng *et al.* 2024). This suggested that leptin's inhibitory effect on TET2 expression *via* the *JAK2*/*STAT3* signaling pathway indicated a negative feedback loop in leptin. Inconsistently, another study found that a HFD induced hypermethylation of the leptin promoter in gonadal adipose tissue, which was positively correlated with leptin gene expression (Zwamborn *et al.* 2017). Long-term maternal HFD feeding in rats resulted in leptin hypermethylation, increased plasma leptin level and higher birth weight of offspring (Milagro *et al.* 2009). Likewise, a study indicated that maternal obesity was only associated with elevated DNA methylation of the leptin promoter on fetal side (Nogues *et al.* 2019). The inconsistencies in these findings might have been because leptin promoter DNA methylation was not sufficient to decrease the leptin expression to normal level (Zwamborn *et al.* 2017), and could also be attributed to the fact that different tissues might have responded differently to epigenetic modifications. It was evident that the DNA methylation level at the promoter region of the leptin gene significantly impacted leptin gene expression levels and lipid deposition. However, the relationship between leptin gene promoter methylation and leptin expression in different tissues under various nutritional levels requires further investigation. Similarly, DNA methylation could regulate the expression and secretion of adiponectin. Adiponectin played a pivotal role in the maintenance of lipid homeostasis and regulated various metabolic processes, exerted its effects through interactions with its receptors, adiponectin receptor1 (*ADIPOR*1) and *ADIPOR*2 (Nogues *et al.* 2019). Studies found that obesity-induced proinflammatory cytokines promoted the expression and enzymatic activity of DNMT1, leading to DNA hypermethylation within the *ADIPOQ* promoter's R2 region. Promoter hypermethylation resulted in the suppression of adiponectin expression, thereby affecting lipid homeostasis (Kim *et al.* 2015). Conversely, suppressing DNMT1 activity with a DNMT inhibitor reduced the methylation level at the Adipoq promoter's R2 region, leading to increased adiponectin level (Kim *et al.* 2015). This evidence demonstrates that DNMT1 played a crucial regulatory role in the expression and secretion of adiponectin. By targeting DNMT1 or manipulating DNA methylation patterns of adiponectin, it might have been possible to restore adiponectin levels and improve lipid metabolism. The intricate relationship between adiponectin DNA methylation and its mRNA expression had a profound influence on lipid metabolism.

DNA methylation also played a pivotal role in regulating meat quality, particularly by direct or indirect modulating genes involved in lipid metabolism, which directly impacted intramuscular fat (IMF) content and fatty acid composition — a critical factor influencing meat tenderness, juiciness, and flavor. Recent research showed that significant differences in meat quality among pig breeds, which were related to intramuscular fat content, with obese-type breeds exhibiting greater intramuscular fat content (Ryu *et al.* 2008; Wu *et al.* 2013; Ma J *et al.* 2022). Research highlighted significant disparities in DNA methylation levels between adipose tissues of obese and lean pig breeds. These variations were mirrored in differentially methylated genes (DMGs) associated with lipid metabolism across different pig breeds. For instance, Yang *et al.* (2017a) conducted a systematic comparative analysis of skeletal muscle in obese-type Tongcheng and lean-type Landrace (LD) pigs, and identified 112 DMGs that were hypermethylated. These genes, including enoyl-CoA hydratase domain containing 2, fatty acyl-CoA reductase 1, uncoupling protein-3, UDP-glucose ceramide glucosyltransferase, and ST6GALNAC6, were related to fatty acid metabolism and lipid biosynthesis and exhibited downregulated expression in LD pigs with low intramuscular fat content. Li *et al.* (2021) observed a significantly negative correlation between DNA methylation levels and expression levels of 41 DMGs related to lipid metabolism in longissimus dorsi muscles of Wannanhua and Yorkshire pigs. This explained the significant correlation between intramuscular fat content and DMGs related to lipid metabolism in different pig breeds. This suggested that the differences in meat quality traits between the two pig breeds were closely related to DNA methylation, further emphasizing the important regulatory role of epigenetics in the formation of high-quality pork. DNA methylation exhibited dynamic regulation of lipid metabolism-related genes across different developmental stages. The Laiwu pig, characterized by its abundant IMF content, exhibited the highest rate of IMF deposition during the period from 120- to 240-d-age. Notably, differences in DNA methylation patterns were observed in fat biosynthesis-related DMGs such as fatty acid synthase (*FASN*), parvalbumin, inhibitor Of DNA binding 2, SH3 And PX domains 2B, and early growth response 1 when comparing the 120- and 240-d-age time points (Wang *et al.* 2017). Similarly, in hens, later laying-period individuals exhibited elevated global DNA methylation, particularly in the promoters of ATP binding cassette subfamily A member 1, collagen type VI alpha 1 chain, and glutathione S-transferase theta 1, resulting in transcriptional downregulation and enhanced intramuscular fat deposition, compared to juvenile hens (Zhang *et al.* 2017). The aforementioned research unveiled the complex associations between DNA methylation patterns, IMF deposition, and meat quality traits, which exhibited spatiotemporal specificity. It is evident that by regulating the methylation status of candidate genes with differential methylation during the critical window of IMF deposition through epigenetic means, their expression levels could be influenced, thereby modulating IMF deposition and enhancing meat quality. This method offered new insights for precisely improving pork quality. In addition, the DNA methylation pattern was intimately linked to the composition of fatty

acids in pigs. For instance, hypermethylation of key gene promoters (e.g., *SCD1*, *C/EBP*α, *C/EBP*β) suppressed gene expression, leading to reduced saturated fatty acid content and fat deposition in Landrace pigs compared to Rongchang pigs (Zhang *et al.* 2016). This suggested that DNA methylation patterns contributed to the differences in meat quality traits between these breeds. Optimizing the fatty acid composition in pigs could have a beneficial impact on human health. It was evident that DNA methylation played a crucial role in regulating intramuscular fat content and fatty acid composition in pigs. These discoveries not only deepened our understanding of the epigenetic regulatory mechanisms governing fat deposition in pigs but also provided novel perspectives and strategies for enhancing pork quality by mediating target genes through DNA methylation, with potential beneficial implications for human health.

3. Methyl nutrients, DNA methylation and lipid metabolism

In organisms, the process of DNA methylation depended on methyl nutrients such as Met, betaine, FA, and other B vitamins. These methyl donors played a central role in one-carbon metabolism, directly or indirectly participated in the synthesis of SAM and influenced DNA methylation patterns. This further regulated the expression of genes related to lipid metabolism, thereby affecting physiological processes such as lipid synthesis, transport, and oxidation.

3.1. Folate, DNA methylation and lipid metabolism

FA, serving as a crucial single-carbon donor, was a central nutrient for one-carbon metabolism, actively participating in S-adenosylmethionine (SAM)-dependent DNA methylation processes. The interconversion of FA and its derivatives formed a core pathway in one-carbon metabolism (Mc Auley *et al.* 2018). FA, obtained from dietary sources and synthesized by intestinal bacteria, was absorbed in the small intestine, where it was hydrolyzed into its monoglutamine form in the small intestinal mucosa (Clare *et al.* 2019). Upon reaching the target cells, FA monoglutamates were transformed into biochemically active 5-methyltetrahydrofolate (5-MTHF). In the Met cycle, 5-MTHF reacted with homocysteine (Hcy), facilitated by VB_{12} and Met synthase (MTR), to produce tetrahydrofolate (THF) and Met. The FA cycle was then completed through further conversions, including the transformation of THF to 5,10-methenyltetrahydrofolate *via* the B_6-dependent enzyme serine hydroxymethyltransferase; the further irreversible reduction of 5,10-methenyltetrahydrofolate to 5-MTHF was facilitated by methylenetetrahydrofolate reductase (MTHFR) and the B_2-dependent enzyme methylenetetrahydrofolate (Mentch and Locasale 2016).

Numerous studies consistently highlighted the significant association between FA, DNA methylation and lipid metabolism involving intricate interactions within the one-carbon metabolism pathway (Champier *et al.* 2012; Crider *et al.* 2012; Ly *et al.* 2012; Da Silva *et al.* 2014). FA influenced DNA methylation patterns (Ly *et al.* 2012) and gene expression related to lipid metabolism, obesity, and one-carbon metabolism (Da Silva *et al.* 2014). Studies demonstrated that dietary FA promoted the enzymatic conversion of Hcy to Met, reduced the plasma Hcy concentrations, and maintained cellular SAM concentrations, decreased hyperhomocysteinemia (HHcy) and hepatic Hcy-lated protein levels, restored Stx17 expression and autophagy, stimulated β-oxidation of fatty acids, and improved hepatic metabolism in mice (Da Silva *et al.* 2014; McBreairty *et al.* 2016; Robinson *et al.* 2018; Tripathi *et al.* 2022). *C/EBP*α acting synergisticaly with *PPAR*γ, promoted differentiation of preadipocytes by activating the expression of genes involved in lipid metabolism. FA supplementation significantly upregulated the expression of genes involved in one-carbon metabolism (DNMTs and MTHFR), resulting in greater methylation of the *PPAR*γ, *C/EBP*α, *p*53, and *p*16, and dose-dependently decreased transcript levels of *PPAR*γ, *C/EBP*α and *FAS*, thereby enhancing adipocyte proliferation while reducing per-cell lipid accumulation (Sie *et al.* 2013; Yu *et al.* 2014). Likewise, FA treatment significantly increased the expression of the folate transporter PCFT and DNA methylation-related genes MTHFD2 and DNMT1, which were crucial for DNA methylation and epigenetic regulation. It also disturbed the mTOR and downstream *SREBP*-1-*PI3K* pathways, and elevated the expression of *PGC*-1α, *FABP*1, *CREB*, PPAR-alpha (*PPAR*α), and *ACOX*1 in hepatocytes, inhibited lipid deposition (Du *et al.* 2023). Moreover, FA supplementation reduced serum Hcy level, reduced the abundance of DNMT3a and DNMT1, decreased methylation of *ADCY*3, *RAPGEF*4, *FTO*, *PPAR*D, *PPAR*g, and *PRDM*16 genes associated with obesity and insulin secretion, and increased mRNA expression of *ADCY*3 and *RAPGEF*4. These effects altered the methylation pattern of genes, resulting in reduced fat mass in obese mice fed a HFD (Li *et al.* 2018). Activation of AMP-activated protein kinase (*AMPK*) could inhibit adipocyte differentiation and promote lipolysis. FA was able to promote *AMPK* and *ACC* phosphorylation, enhanced the expression of carnitine palmitoyltransferase1 (*CPT-1*), thereby leading to enhance fatty acid oxidation in HFD-induced mice (He *et al.* 2022). This inverse relationship between promoter methylation and gene expression suggested that altered lipid storage due to FA supplementation may led to reduced DNA methylation of the promoters of *CPT1* promoters, though the detailed molecular mechanism remains to be studied. Overall, these findings emphasized the importance of FA in modulating lipid metabolism through the regulation of genes associated with DNA methylation and implied potential therapeutic implications for lipid metabolism disorder.

In addition, FA supplementation during pregnancy also appeared to affect DNA methylation patterns within specific genomic regions in offspring, significantly influenced lipid utilization (Skjærven *et al.* 2018). In Sprague-Dawley rat studies, maternal FA intake reduced serum Hcy levels, elevated serum and liver triglyceride (TG) levels, which were linked to increased DNA methylation patterns and decreased mRNA and protein expression levels of hepatic adipose triglyceride lipase (*ATGL*) and adipose lipoprotein lipase (*LPL*) genes in male offspring of FA supplementation dams fed HFD, indicating a potential impact on lipid homeostasis

in the offspring (Yang *et al.* 2017b). Maternal methyl donor supplementation exhibited a lower plasma Hcy concentration, and increased average daily gain (ADG), body weight (BW), carcass traits, and meat quality of the offspring (Zhao *et al.* 2019a). This may be related to the alteration of methylation patterns in the offspring by methyl donors. Maternal methyl donor supplementation throughout gestation could increase serum SAM levels, the liver mRNA level of betaine–homocysteine methyltransferase (BHMT) and glycine N-methyltransferase (GNMT), as well as the birthweight and postnatal growth rate of offspring. These increases may be increased associated with elevated expression of the *IGF-1* gene, along with the enhanced DNA methylation of *IGF-1* gene promotor (Jin *et al.* 2018). The findings highlighted the intricate interplay between maternal FA status, epigenetic modifications, and lipid metabolism in the next generation. These findings underscored the importance of FA balanced supplementation during pregnancy to avoid potential adverse effects on the lipid metabolism of offspring, and further research was needed to better understand the mechanisms involved in regulating lipid metabolism.

Notably, Different levels of FA, whether deficient or excessive, had distinct effects on DNA methylation levels and lipid metabolism. FA deficiency elevated the hepatic or plasma concentrations of Hcy and S-adenosylhomocysteine (SAH); decreased erythrocyte FA level, reduced hepatic SAM concentrations and the ratio of SAM/SAH, and resulted in global DNA hypomethylation (Halsted *et al.* 2002; Purohit *et al.* 2007). This deficiency decreased in choline level due to increased utilization of choline as a methyl group source, and reduced the synthesis of *de novo* phosphatidylcholine (PC) through phosphatidylethanolamine N-methyltransferase pathways (Pogribny *et al.* 2013). FA deficiency also significantly reduced the erythrocyte folate concentration, and increased the plasma Hcy level, impaired methylation capacity and PC synthesis, and induced the expression of genes (*GOS2*, *SAA*3, *MAOA*, *C-MYC*, *PFKFB*2, *SNX*10, *THRSP*) involved in hepatic lipid synthesis (Champier *et al.* 2012). Conditions that limited the production of SAM or PC activated *SREBP*-1a, which stimulating *de novo* fatty acid synthesis (Walker *et al.* 2011). FA deficiency also inhibited adipocyte development, hindered the secretion of adipokines like leptin, and led to disruption in lipid accumulation. Additionally, a diet choline: FA-deficient reduced the expression of MTHFR, methionine adenosyltransferase 1α (MAT1A), adenosyl-homocysteinase (AHCY), and methylenetetrahydrofolate dehydrogenase 1 (MTHFD1). This deficiency was associated with a decreased level and promoter binding of the transcription factor *C/EBP*β, which directly regulated their transcription. Consequently, this led to increased hepatic TG and lipid accumulation (Pogribny *et al.* 2013). The suppression of phosphatidylethanolamine methylation caused by FA deficiency could be compensated for by an abundant provision of choline and methionine (McNeil *et al.* 2008). Similarly, in offspring exposed to a maternal protein-restricted diet during pregnancy and fed with 1 mg kg^{-1} FA in a HFD, hepatic *PPARα* methylation was significantly was significantly reduced, while the expression of *PPAR*α and its respective targets, acyl-CoA oxidase (*AOX*) and *CPT*-1 were elevated compared to those on a maternal normal diet (Burdge *et al.* 2009). Therefore, it could be concluded that FA played a significant role in the regulation of lipid metabolism, and its deficiency had detrimental effects on various aspects of lipid homeostasis.

On the other hand, excessive FA intake increased plasma FA level in mice without causing a decrease in plasma Hcy, indicated incomplete FA metabolism (Christensen *et al.* 2015). Elevated levels of unmetabolized FA in the plasma led to the cellular accumulation of dihydrofolate, inhibited MTHFR expression and activity. MTHFR deficiency resulted in the decreasing of SAM and 5-MTHF, thereby impeding the remethylation of Hcy, reduced methylation capacity, and altering the expression of lipid regulators (Cholesterol 7α-hydroxylase, *CYP7A*1), inflammation (Serum amyloid A2), one-carbon metabolism (MTR, Choline dehydrogenase), and lipid metabolism (nuclear receptor subfamily 1, group H, member 4 (*NR1H*4), *SREBF*1/2, *PPAR*α, and Carnitine O-octanoyltransferase), potentially increasing the risk of liver disease (Christensen *et al.* 2015; Leclerc *et al.* 2019). Supplementing with 5-MTHF effectively raised plasma FA level without caused an increase in un-metabolized FA (Prinz-Langenohl *et al.* 2009). Furthermore, excessive FA supplementation (5 mg kg^{-1}) in a HFD led to weight gain and hepatic lipid accumulation. This was attributed to impaired fatty acid oxidation in the liver, evidenced by significantly increased PPARα methylation, decreased expression of *PPAR*α, *CPT*-1, and *AOX* in the liver. Additionally, adipose tissue in these conditions exhibited higher mRNA levels of *PPAR*γ2 and *LPL*, along with lower expression of hormone-sensitive lipase (HSL), compared to the offspring of rats that received FA supplementation (1 mg kg^{-1}) during pregnancy (Burdge *et al.* 2009). In addition, excessive FA intake, resulted in prominent hepatic lipid accumulation. Mechanistically, increased level of arginase-1 caused lipid metabolism disorder in rat offspring by regulating the nitric oxide synthase-3(NOS3)-*AMPK*α pathway, leading to lipid accumulation in hepatocytes (Liu Z *et al.* 2022), this may be related to the alteration of methylation patterns caused by excessive intake of folic acid. Evidently, FA deficiency or excess could impair methylation capacity, disrupt lipid metabolism. In summary, the impact of FA on the expression of genes through methylated modification pathways further underscored its role in regulating lipid metabolism (Fig. 1). However, the mechanism by which FA regulates methylation levels under different dosage conditions, thereby affecting lipid metabolism, is not fully understood, and further research is necessary. Therefore, understanding the intricate interactions between FA, SAM, methyltransferase, DNA methylation level of target gene, and lipid metabolism could lead to a potential avenue for therapeutic intervention in lipid metabolic disorders.

3.2. Methionine, DNA methylation and lipid metabolism

Met played a crucial role in the intricate interplay between DNA methylation and lipid metabolism. As a methyl donor in one-carbon metabolism, Met indirectly enhanced the DNA methylation reaction catalyzed by DNMTs by promoting

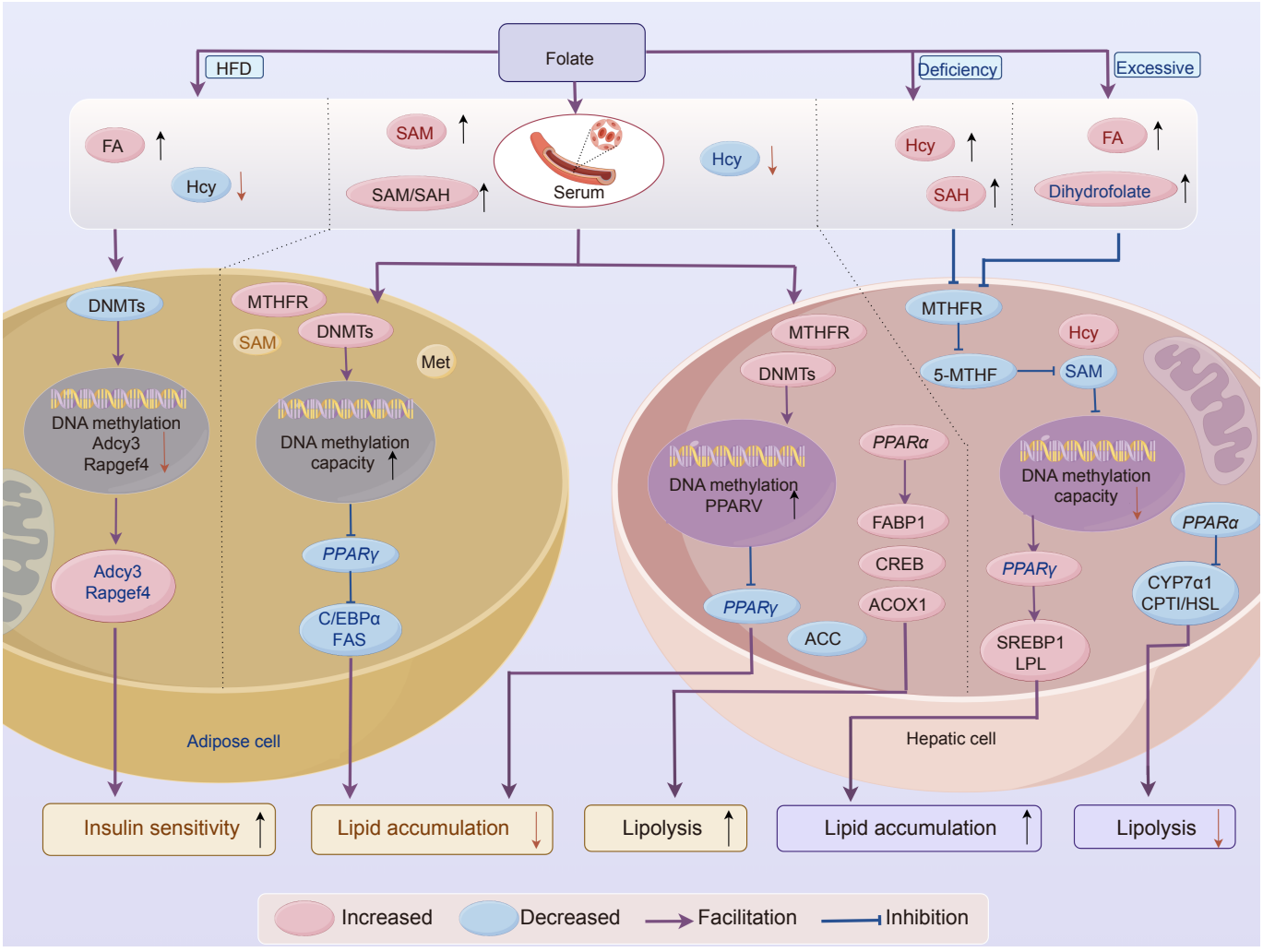


Fig. 1 Schematic illustration of the mechanism by which folate (FA) regulates lipid metabolism through DNA methylation modification (by Figdraw). The activity of *PPAR*γ is modulated by DNA methylation, thus affecting lipid metabolism. FA regulates the expression of genes related to lipid metabolism by affecting the activity of methyltransferase and the level of DNA methylation modification of genes related to lipid metabolism. FA supplementation can increase methyltransferase activity, inhibit *PPAR*γ, and reduce fat accumulation. However, when FA is deficient or excessive, it could reduce S-adenosylmethionine (SAM), activate *PPAR*γ, inhibit *PPAR*α, and promote lipid deposition.

the synthesis of SAM, which was crucial for maintaining genomic stability and regulating gene expression. This process resulted in the release of SAH and facilitated methylation reactions. It was worth noting that every step in the methionine cycle was closely connected, jointly maintaining the dynamic balance of the methylation reaction. Both SAH and Hcy, acting as competitive inhibitors in methyltransferase reactions, could have hindered the transition of SAM to SAH metabolism, exerted inhibitory effects on methylation pathways to potentially impaired the capacity of DNA methylation. Recycling SAH and Hcy back to Met was essential to address these inhibitory effects. For example, SAH was further broken down by SAH hydrolase, not only prevented its inhibitory effect on methyltransferases but also generated adenosine and Hcy (Zhou *et al.* 2016). Furthermore, the metabolic conversion of Hcy was also a crucial aspect of maintaining the methionine cycle and methylation capacity. Hcy reacted with VB_{12} and 5-MTHF to facilitate SAM synthesis, remove SAH, and generate THF and Met. This process completed the Met cycle, ensured overall methylation capacity and regulated gene expression (Mosca *et al.* 2021). Hcy was primarily derived from Met catabolism. A high-methionine diet, which resulted in a increase in total plasma Hcy, was reported to lower both total plasma cholesterol and HDL-C levels, ultimately led to a significant reduction in body weight in wild-type C57BL/6 mice (Velez-Carrasco *et al.* 2008). It was evident that the methionine cycle was particularly crucial for managing Hcy accumulation caused by a high-methionine diet. It helped prevent potential inhibitory effects of excessive Hcy levels on the methylation pathway, ensuring the stability of DNA methylation patterns.

The availability of Met influenced the DNA methylation patterns of genes involved in lipid synthesis, fatty acid oxidation, and related pathways, which were associated with the expression activity of DNMTs. High-Met supplementation might downregulate DNMT1 gene, and

caused hypomethylation in the fatty-acid-binding protein 4 (*FABP*4) promoter region by elevating levels of SAH or Hcy, upregulated *FABP*4 mRNA expression, thus facilitating lipid deposition in ApoE (–/–) mice (Yang *et al.* 2015). Interestingly, the recovery of DNA methylation state was dependent on Dnmt3b, which might upregulate DNMT3b gene expression following DNMT1 ablation. This results in increased global DNA methylation and altered DNA methylation levels of specific genes, down-regulated free fatty acid receptor 1 (*FFAR*1) mRNA expression (Elliott *et al.* 2016; Rosa Velazquez *et al.* 2020). Additionally, it led to altered expression of one-carbon and lipid metabolism genes, such as downregulation of *PPAR*g, apolipoprotein A5 (*ApoA*5), and *FFAR*1, and upregulation of *FABP*4. These changes ultimately caused lipid accumulation in the liver (Aissa *et al.* 2014, 2022; Yang *et al.* 2015). The mechanism may involve regulating the level of DNA methylation modification by affecting DNMTs, which further influences the expression of genes related to lipid metabolism, thereby impacting lipid metabolism. The addition of FA, VB_{12} and VB_6 to the high-Met diet was showed to counteract the adverse HHcy-induced hypomethylation in the *FABP*4 promoter by increased the level of DNMT1, decreased *FABP*4 expression, result in a significant decrease in the LDL levels (Yang *et al.* 2015), reduced plasma SAH or Hcy concentration and prevented hepatic DNA hypomethylation (Pogribny *et al.* 2005; Da Silva *et al.* 2014). This suggested that methionine could influence DNA methylation patterns by regulating the expression of DNMTs, thereby affecting lipid metabolism.

Met played a pivotal role in lipid metabolism of the body tissues, its availability in the diet was showed to impact various aspects of lipid homeostasis through DNA methylation modifications (Blachier *et al.* 2020). Osorio *et al.* (2016) reported that feeding Met to peripartal cows resulted in lower global hepatic DNA methylation, potentially improved lipid metabolism through SAM synthesis, activation of *PPAR*α-regulated signaling pathways and up-regulated of *PPAR*α target genes (angiopoietin-like protein 4 (*ANGPTL*4), *FGF*21, and *PCK*1). L-Met supplementation in rats on a the high-fat-diet-induced led to significant alterations in DNA methylation patterns by inhibited the expression of DNMT1. These changes affected the regulation of genes involved in lipid metabolism, such as *FASN*, resulting in notable alterations in lipid metabolism. Specifically, there was a reduction in hepatic triglyceride (TG) levels and circulating free fatty acid (FFA) levels. This effect was attributed to the hepatic silent information regulator 1 (*SIRT*1)/*AMPK* signaling pathway, which was associated with the beneficial effects of L-Met (Navik *et al.* 2019, 2022). Overall, supplementation with Met could influence the global DNA methylation as well as the methylation levels of specific genes, regulated gene expression related to lipid oxidation and transport. The changes in these gene methylation patterns played a crucial role in regulating lipid synthesis and lipid oxidation pathways.

However, the research results on the effects of different dietary Met levels on mRNA expression of lipid metabolism genes through DNA methylation modifications were inconsistent. The high-Met diet specifically increased DNA methylation level in the *PPARg* promoter region and downregulated *PPARg*, which might inhibit lipid deposition in the liver of mice compared to those fed a Met-deficiency diet, potentially related to the upregulation of DNMT3b mRNA levels (Aissa *et al.* 2022). A Met-deficiency diet specifically down-regulated DNMT1 mRNA level, increased the overall genomic DNA methylation, decreased DNA methylation levels in the *ApoA*5 and *PPARg* promoter regions and downregulated Apoa5 in the liver of female Swiss albino mice compared to the control diet, this could be associated with increase in liver triglyceride content (Aissa *et al.* 2022). Met deficiency increased hepatic levels of SAH and Hcy, and also altered the expression of one-carbon and lipid metabolism genes in mice. It increased TG level by upregulating lipogenesis (malic enzyme 1 and *FASN*) and fatty acid transport (*CD36*) in the dorsal subcutaneous adipose tissue of the pig (Aissa *et al.* 2014; Castellano *et al.* 2015). Met deficiency also enhanced lipid accumulation by *AMPK-PGC-1α* signaling pathway in skeletal muscle of the pigs (Hasek *et al.* 2010; Wu *et al.* 2019). This could also be due to Met deficiency affected DNA methylation patterns of lipid metabolism genes, thereby regulated lipid metabolism. However, FA, betaine, or a combination of them could equally resynthesize Met by donating a methyl group to Hcy for protein synthesis when dietary Met was limit, sequentially changed in DNA methylation patterns, ultimately the maintenance of lipid homeostasis (McBreairty *et al.* 2016; Robinson *et al.* 2018). These findings demonstrated a significant impact of Met restriction on promoting lipid deposition in adipose tissue or the liver. Interestingly, the impact of Met diets could lead to conflicting outcomes. Conversely, studies indicated that Met deficiency reduced SAM level, elevated SAH and Hcy levels, and decreased genomic DNA methylation level, upregulated FGF21 gene and protein levels, increased fatty acid oxidation, and decreased lipid synthesis in liver of rats (Sanchez-Roman *et al.* 2011; Perrone *et al.* 2012). It appeared that Met deficiency was considered to increase *de novo* lipogenesis, lipolysis and fatty acid oxidation through DNA methylation modifications, with a result of enhance lipid metabolism (Zhou *et al.* 2016). In summary, these findings underscored the complexity of the relationship between Met, DNA methylation, and lipid metabolism, highlighted the potential of nutritional interventions to modulate these processes and improved metabolic outcomes (Fig. 2). Met might exert beneficial effects on lipid metabolism by DNA methylation modulating multiple signaling pathways, particularly the AMPK signaling pathway, making it a promising as a potential nutritional supplement for regulating lipid metabolism.

3.3. Betaine, DNA methylation and lipid metabolism

Betaine, a universal methyl donor for numerous methylation reactions synthesized endogenously from choline, played a significant role in the intricate connection between DNA methylation and lipid metabolism. It served as an efficient methyl donor in one-carbon metabolism, participated in methyl metabolism by providing methyl groups for the remethylation of Hcy to Met, and then participated in the Met cycle, which further contributed to the synthesis of SAM, would improve DNA methylation, as evidenced by various

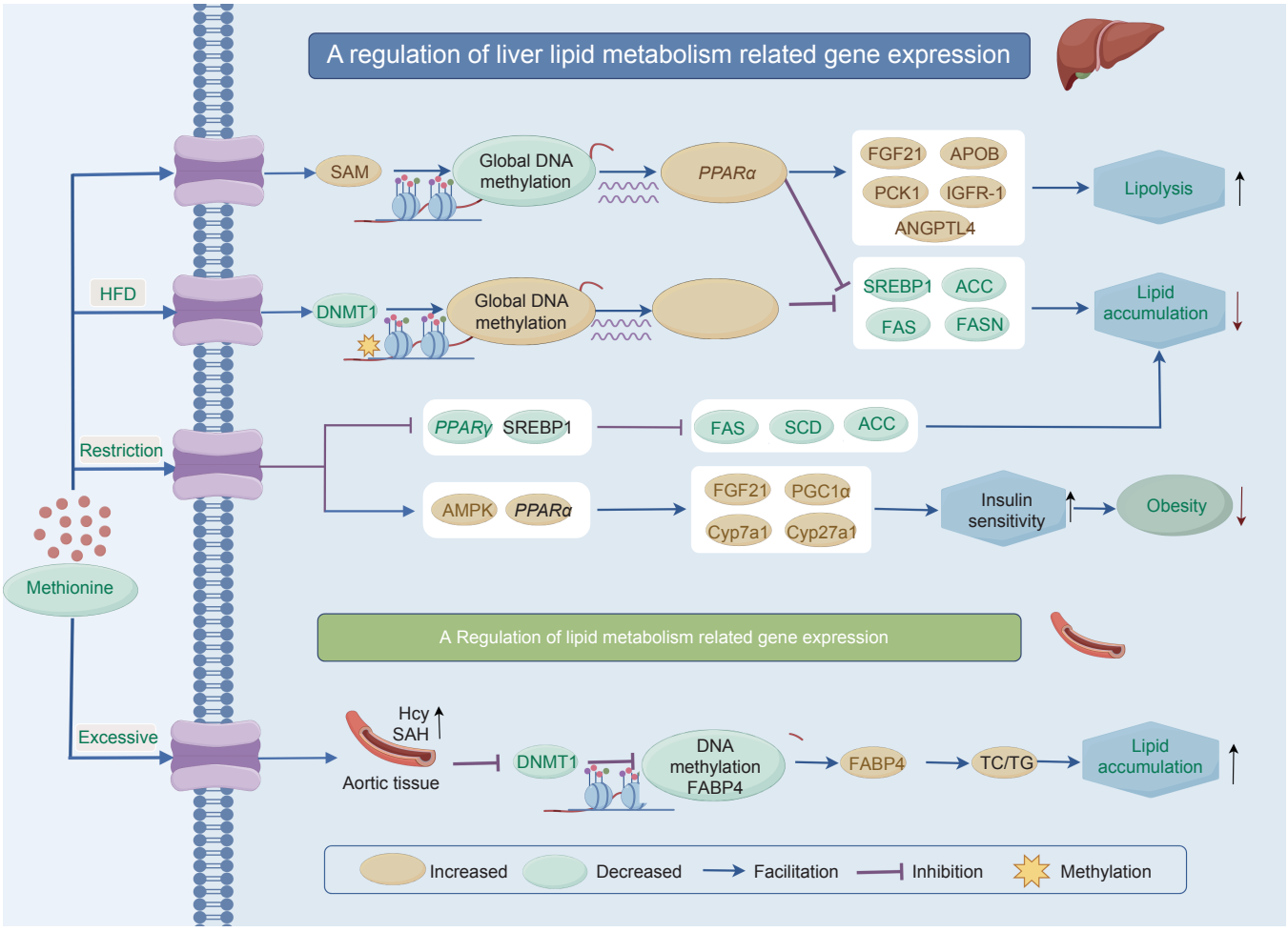


Fig. 2 Schematic illustration of the mechanism by which Met regulates lipid metabolism through DNA methylation modification (by Figdraw). Met regulates genes related to lipid metabolism through DNA methylation modification. Met supplementation could affect the level of DNA methylation modification, activate *PPARα*, and promote liver lipidolysis. However, when Met is deficient, *PPARα* could be inhibited, lipid synthesis gene expression could be up-regulated, and liver lipid deposition could be promoted.

studies (Cai *et al.* 2014b). Notably, betaine, more efficient than choline and Met as a methyl donor, exhibited the ability to promote fat mobilization, fatty acid oxidation, and altered body fat content and distribution. While betaine acutely reduced the increase in Hcy after Met loading (Olthof *et al.* 2003; Olthof and Verhoef 2005). Within the typical dietary intake range, betaine helped to prevent lipid metabolism disorders by decreasing hepatic or plasma concentrations of Hcy and SAH, improved liver SAM, and increased the ration of SAM:SAH, ultimately altering DNA methylation patterns, (Ahn *et al.* 2016; Lu *et al.* 2023).

Research indicated that betaine supplementation could exert an influence on epigenetic regulation, altering the DNA methylation levels of genes associated with lipid metabolism. Betaine supplementation had the potential to induce alterations in the expression of genes related to liver lipid synthesis and transport by reducing methylation levels within specific regions of gene promoters, including *SREBP*-1, *FAS*, *ACC*, *SCD*-1, *ApoB*, and microsomal triglyceride transfer protein (*MTTP*) markedly up-regulated at the mRNA level (Omer *et al.* 2020). These changes were associated with an increase in glucocorticoid receptor binding on these promoters, resulting in elevated concentrations of TG in both the liver and plasma of hens (Omer *et al.* 2020). Interestingly, these findings in the liver were paralleled by observations in the small intestine. The small intestine, a primary site of lipid absorption and metabolism, exhibited a significant up-regulation in the expression of proteins related to lipid metabolism and transport (*MTTP*, *ApoA*4, *FABP*1, and *FABP*2) in response to betaine supplementation in HFD-induced mice (Hu *et al.* 2022). The observation that *MTTP*, a protein crucial for the assembly and secretion of triglycerides, was upregulated in both studies suggested a possible common regulatory mechanism involving DNA methylation. Despite its overall positive effects, betaine was observed to increase plasma SAH concentration and decrease SAM, led to a reduced ratio of SAM:SAH, depressed liver methylation, increased the expression of genes involved in β-oxidation (*FGF*21 and *PPARα*), and exerted significant effects on lipid metabolism in Met or choline-deficient diet-fed rats (Abu Ahmad *et al.* 2019). A Met restriction diet with added betaine increased hepatic ApoB and betaine-homocysteine methyltransferase (*BHMT*) mRNA levels, V-LDL secretion, and decreased hepatic TG (Sparks *et al.* 2006). These findings suggested

that Betaine influenced the expression of genes related to lipid synthesis and transport by regulating the methylation levels of specific gene promoter regions, thereby modulating lipid metabolism. Under dietary conditions deficient in methionine or choline, the supplementation of betaine was particularly crucial, as it could exert protective effects against lipid metabolism disorders. Furthermore, betaine participated in regulating the activity of key transcription factors such as *PPAR*γ and *PPAR*α and the expression of their target genes by DNA methylation modification, further impacting fatty acid oxidation and lipid deposition processes. In instances of HFD-induced disruption of hepatic excessive lipid deposition, betaine supplementation was found to reverse dysregulation of lipogenic genes (*PPAR*γ, *CD36*) and rectify disrupted expression of methyltransferase genes (BHMT, GNMT, DNMT1), it partly alleviated the HFD-induced increase in plasma and hepatic levels of TG and TCH, as well as the level of LDL-C *via* the *PPAR*γ/*CD*36 pathway (Li Y *et al.* 2022). Betaine treatment inhibited forkhead box O1 (*FOXO*1) binding to the *PPAR*γ promoter and *PPAR*γ transcriptional activity, decreased mRNA expression levels of their downstream lipogenic target genes including *FAS* and *ACC* in db/db mice (Kim *et al.* 2016). These effects may be associated with modifications in CpG methylation on the promoters of lipogenic genes. Conversely, betaine supplementation increased the expression of *PPAR*γ in both the Longissimus lumborum and Biceps femoris muscles of obese pigs. Accompanying, there were increases in the mRNA levels of *SCD*, *LPL*, and heat-fatty acid binding protein (*H-FABP*), along with a decrease in HSL expression, specifically altered lipid synthesis, thereby improved meat quality (Albuquerque *et al.* 2017). Whether betaine regulated lipid metabolism in muscle tissues of obese pigs through DNA methylation modifications, remained to be further validated the specific mechanisms. Betaine's protective effect against hepatic lipid deposition was observed to correlate with a reduction in *PPAR*α promoter methylation and an upregulation of *PPAR*α target gene expression (*CPT*-1, Cytochrome P450 2E1 (*CYP2E*1)). Furthermore, *PPAR*α methylation was positively correlated with hepatic betaine and Hcy concentrations in mice (Wang *et al.* 2013). This indicated that betaine might participate in regulating the lipid metabolism process in the liver by affecting the methylation status of *PPAR*α. Betaine supplementation was linked to the attenuation of hepatic steatosis in HFD-fed mice, wherein it reduced *MTTP* promoter methylation, upregulated both *PPAR*α and *MTTP*, downregulated *FAS* and Acyl-CoA oxidase (*ACOX*) mRNA expression levels, and increased genomic DNA methylation in the liver, ultimately inhibiting fatty acid synthesis in liver (Wang *et al.* 2022). Consistent with the aforementioned research results, betaine supplementation prevented hepatic lipid accumulation induced by a HFD in Sprague-Dawley rats. This was achieved through increased hepatic BHMT, which significantly upregulated the expression of genes (*PPAR*α and *CPT-1*) associated with β-oxidation, enhanced hepatic lipid export and fatty acid oxidation in rats (Xu *et al.* 2015). This may be related to the fact that betaine altered the methylation levels of lipolysis and oxidative genes. Betaine and its precursors enhanced lipid homeostasis in obesity through their involvement in lipolysis and oxidative gene demethylation (Sivanesan *et al.* 2018). The studies highlighted that betaine regulated the expression of methyltransferases, altered the methylation levels of genes related to lipid synthesis and lipid oxidation, affected the expression of genes related to lipid metabolism, and thereby regulated the lipid metabolism process. In summary, betaine built a bridge between DNA methylation and lipid metabolism, played a crucial role in regulating methylation capacity, gene expression related to lipid metabolism, and hepatic lipid metabolic. The impact of betaine on epigenetic modifications and lipid metabolic pathways was evident. As an effective methyl donor, the mechanism by which betaine regulated lipid metabolism through DNA methylation was complex, involving not only changes in multiple methyltransferases and gene expression but also being closely related to dietary nutritional levels.

In maternal supplementation, betaine exhibited profound effects on the epigenetic regulation of genes involved in hepatic lipogenesis, lipid deposition, and cholesterol metabolism. Maternal dietary betaine supplementation during gestation attenuated hepatic lipogenesis in neonatal piglets, resulted in lower liver TG content. This mechanism involved an elevation the ratio of SAM:SAH in the liver, accompanied by DNA hypermethylation on hepatic lipogenic gene promoters (*ACC*, *FAS*, *SCD*, or *SREBP*-1c) and significant downregulation of the expression of these genes (Cai *et al.* 2016). Additionally, it also reduced hepatic TG concentration associated with the upregulation of the DNA methylation on the promoter of hepatic lipogenic genes (*ACC*1, and *SCD*1) and the downregulation of these genes expression the addition of maternal betaine in dexamethasone-induced rats (Zhao *et al.* 2018). Meanwhile, studies showed that maternal betaine supplementation might also influence cholesterol metabolism in the offspring, affect hepatic cholesterol content and the expression of related genes. Maternal betaine supplementation significantly enhanced hepatic cholesterol contents accompanying with the down-regulation of hepatic *CYP7α*1 mRNA expression level by hepatic hypermethylation of *CYP7α*1 gene promoter, and the up-regulation of hepatic the protein content of low-density lipoprotein receptor (LDLR) and BHMT expression at the mRNA and protein level in offspring rats (Zhao *et al.* 2019b). Betaine alleviated HFD-induced hepatic cholesterol accumulation by maternal dietary supplementation, upregulated genes such as LXR-α and Cholesterol-27alpha-hydroxylase (*CYP27α*1). This up-regulation coincided with decreased CpG methylation on the promoters of these genes (Hu *et al.* 2017). Maternal betaine supplementation significantly downregulated the *ATGL* and *HSL* expression at both mRNA and protein levels by hypermethylation of *ATGL* and *HSL* gene promoters in dexamethasone-induced pregnant rats (Zhao N *et al.* 2020). Betaine increased hepatic cholesterol content through maternal dietary supplementation, and downregulated the mRNA levels of cholesterol-related genes (e.g., *SREBP*-2, *LDLR*, 3-hydroxy-3-methylglutaryl CoA reductase (*HMGCR*)) by inducing CpG island hypermethylation of *HMGCR* and *LDLR*, upregulated the mRNA expression of *CYP27α*1 and *CYP7*α1, thus contributing to improve cholesterol homeostasis in neonatal piglets (Cai *et al.* 2014a). Overall, these findings suggested that maternal betaine intake

demonstrated potential as an epigenetic modulator influencing hepatic lipid homeostasis, cholesterol metabolism and related gene expression patterns in the offspring. By altering DNA methylation patterns, betaine could effectively modulate the expression of key genes, ultimately influencing hepatic lipogenesis, lipid deposition, and cholesterol homeostasis in the offspring. This underscored the potential of maternal betaine supplementation as a strategy to improve lipid metabolic in the offspring through epigenetic modifications. In conclusion, betaine's impact on DNA methylation and its subsequent influence on lipid metabolism underscored its crucial role as a key regulator in the complex network linking DNA methylation, and lipid metabolism (Fig. 3).

4. Analysis of methyl nutrients-mediated methylation regulation mechanism

Met, betaine, FA, and other B vitamins each played distinct roles in DNA methylation modification, collectively maintaining the stability of DNA methylation in the body. Among them, Met served as a key methyl donor in one-carbon metabolism, primarily catalyzed by methionine adenosyltransferase to generate SAM, which then participated in the process of DNA methylation. After providing a methyl group, SAM was converted to SAH, which was subsequently hydrolyzed to Hcy. As an intermediate metabolite of Met metabolism, Hcy acted as an effective inhibitor of methyltransferases, exerting feedback inhibition on DNMTs activity, thereby regulating the rate and extent of DNA methylation. To recycle Hcy back to Met, FA and VB_{12} were required. This remethylation process allowed the synthesis of SAM, thereby alleviating Hcy's inhibitory effect on DNMT activity, completing the Met cycle, and maintaining the dynamic balance of methylation reactions. It was evident that the stability of DNA methylation, as maintained by Met, relied on a sufficient supply of other nutrients. FA itself did not directly provide methyl groups but was first converted to the active form, 5-methyltetrahydrofolate (5-MTHF). In the presence of coenzymes such as vitamins B_{12}, B_6, and B_2, 5-MTHF participated in the remethylation

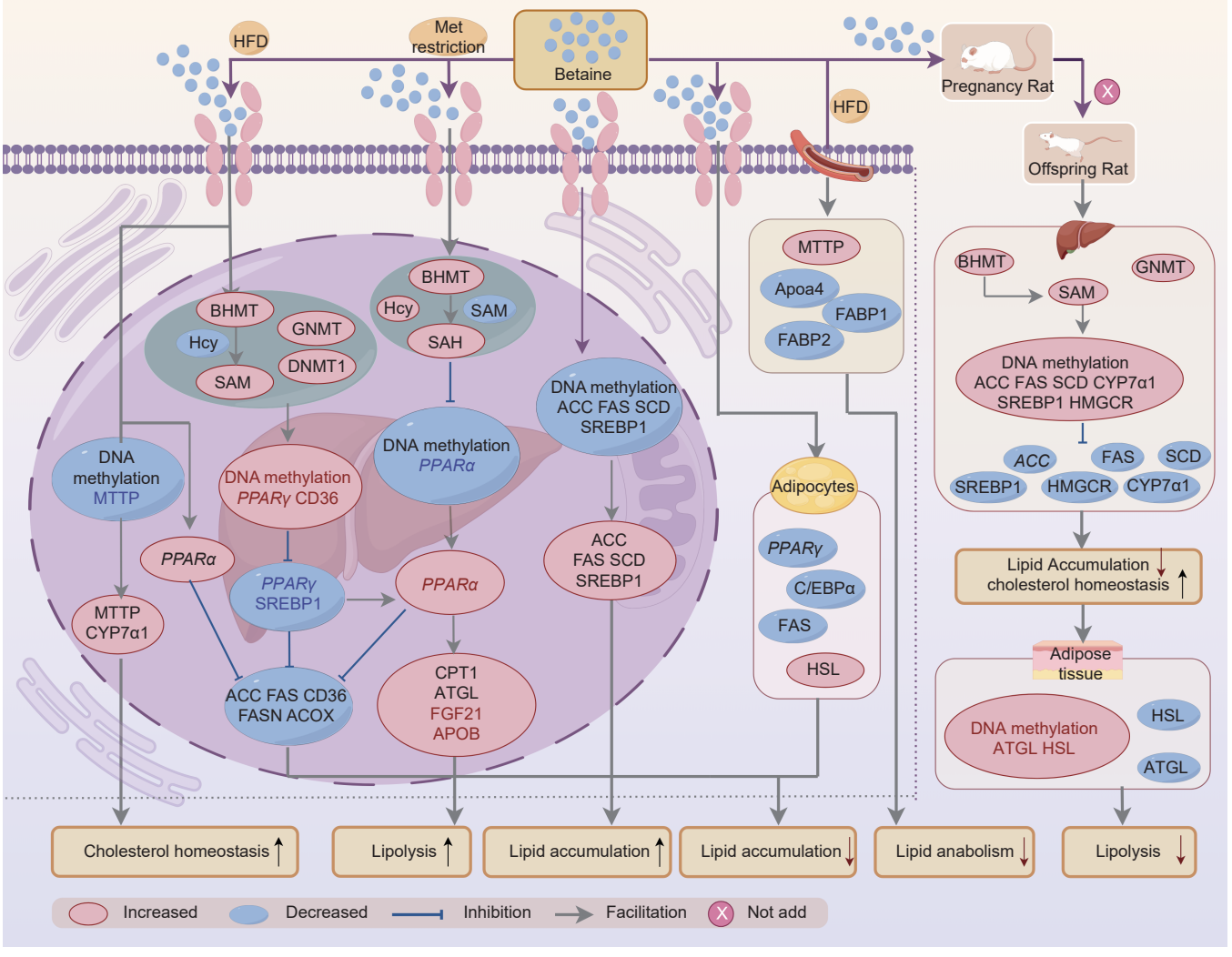


Fig. 3 Schematic illustration of the mechanism by which betaine regulates lipid metabolism through DNA methylation modification (by Figdraw). Betaine regulates genes related to lipid metabolism through DNA methylation modification. Betaine supplementation could increase S-adenosylmethionine (SAM)-level, inhibit *PPARγ*, activate *AMPK* and *PPARα*, reduce liver fat accumulation and enhance lipolysis. When AMPK is activated, it could phosphorylate and inhibit *ACC*, causing a increase in *CPT-1* levels.

of Hcy, indirectly contributing to SAM synthesis and DNA methylation. In comparison, betaine acted as an efficient methyl donor, directly providing methyl groups for the remethylation of Hcy to Met. Unlike FA, whose role as a methyl donor was more indirect and dependent on the synergistic effects of other nutrients, betaine was more direct and efficient in this function. Betaine was capable of rapidly reducing plasma Hcy levels, altering DNA methylation patterns, and influencing gene expression. These methyl donors played a central role in one-carbon metabolism, directly or indirectly participating in the synthesis of SAM and influencing DNA methylation patterns. This further regulated the expression of genes related to lipid metabolism, thereby affecting physiological processes such as lipid synthesis, transport, and oxidation.

Met, FA, betaine, and other methyl donors exhibited significant differences in regulating lipid metabolism through DNA methylation, primarily reflected in their regulation of DNA methylation patterns. Met directly affected the expression of genes related to lipid metabolism by altering the DNA methylation levels of specific genes. Betaine changed the DNA methylation levels of specific genes by directly providing methyl groups and promoting the remethylation of Hcy, more effectively regulating the expression of genes related to lipid metabolism. FA maintained normal methylation reactions and gene expression by participating in one-carbon metabolism and methylation processes, indirectly regulating lipid metabolism. Other B vitamins, though having limited direct effects on lipid metabolism, were crucial for maintaining normal one-carbon metabolism and methylation reactions.

Under different nutritional conditions, the regulatory effects of methyl donor on lipid metabolism varied, primarily achieved by influencing the DNA methylation levels of related genes. Under regular dietary conditions, betaine mainly upregulated the expression level of BHMT, reduced SAM levels, decreased the DNA methylation levels of liver lipid metabolism-related genes (*ACC*, *SCD*, *SREBP*1, *FAS*), upregulating the expression levels of these genes and promoting lipid deposition. Conversely, the addition of betaine to a HFD increased the DNA methylation level of *PPAR*γ, downregulated the expression levels of genes such as *PPAR*γ, *FAS*, *ACC*, *CD*36, *FASN*, thereby inhibited lipid deposition. However, in situations of Met or choline deficiency, betaine supplementation decreased the DNA methylation level of *PPAR*α, increased the expression levels of *CPT*-1, *ATGL*, *FGF*21, *APOB*, which promoted liver lipid oxidation. Furthermore, different levels of Met, FA, and betaine intake had significantly different effects on lipid metabolism. An appropriate amount of M*et al*tered DNA methylation patterns by promoting SAM synthesis, which changed the DNA methylation levels or expression levels of lipid metabolism-related genes (*PPAR*α, *SIRT*1/*AMPK*, *SREBP*1, *FAS*), increased lipid oxidation, and reduced lipid deposition. Conversely, excessive Met supplementation might have downregulated DNMT1 gene expression, leading to hypomethylation of specific gene (e.g., *FABP*4) promoter regions, thereby upregulated the expression of these genes and promoting lipid deposition. Similarly, an appropriate amount of FA supplementation promoted the conversion of Hcy to Met, decreased plasma Hcy levels, increased SAM levels, elevated the SAM:SAH ratio, maintained a stable supply of SAM, and upregulated the expression of methylation-related genes such as DNMTs and MTHFR. This increase in methylation levels of lipid metabolism-related genes (e.g., *PPAR*γ), thereby led to the downregulation of these genes expression (*PPAR*γ, *C/EBP*α, and *FAS*), improved lipid metabolism, and reduced lipid deposition. Conversely, FA deficiency increased Hcy and SAH levels, downregulated MTHFR expression, decreased 5-MTHF levels, and affected the FA cycle, reduced Met and SAM production. This led to DNA hypomethylation, upregulated the expression of *PPAR*γ, *SREBP*1, and *LPL*, and promoted lipid deposition. Notably, excessive FA intake also led to DNA hypomethylation through the aforementioned pathways, causing abnormalities in lipid deposition. Additionally, excessive FA decreased lipid oxidation by increasing *PPAR*α methylation level and reducing the expression of *PPAR*α, *CPT*-1, and *HSL*. Nevertheless, these substances also regulated lipid metabolism through other pathways. In summary, moderate supplementation of Met or FA regulated the expression of *PPAR*γ and *PPAR*α pathways or their target genes through DNA methylation modification pathways, thereby enhancing lipid oxidation and reducing lipid deposition. Conversely, adding betaine to a normal diet promoted lipid deposition by regulating *PPAR*γ target genes methylation levels to upregulate their expression. However, adding betaine to a HFD or a Met- or choline-deficient diet reduced lipid deposition and promoted lipid oxidation by increasing the DNA methylation level of *PPAR*γ and decreasing that of *PPAR*α. Additionally, Excessive Met, FA deficiency, or an excessive diet could also had the potential to upregulate the expression of *PPAR*γ or downregulated the expression of *PPAR*α and its target genes through DNA methylation modification pathways, promoted lipid deposition and reducing lipid oxidation. Therefore, appropriate intake of methyl donor helped maintain normal lipid metabolism and DNA methylation levels; whereas excessive or insufficient intake may have led to lipid metabolism disorders and DNA methylation abnormalities. In practical applications, to achieve the best regulation effect on lipid metabolism, it is necessary to select appropriate methyl donors based on specific goals and reasonably adjust the intake of these methyl donor. Future research should further explore the specific mechanisms and synergistic relationships through which these methyl donors regulated lipid metabolism *via* DNA methylation modification. This could provide new ideas and methods for improving meat quality by regulating DNA methylation-mediated lipid metabolism, with the aim of achieving more breakthroughs and applications in the field of agricultural production.

5. Future trends

Accumulating evidence suggests that the process of lipid metabolism is closely related to changes in DNA methylation patterns. Changes in DNA methylation patterns may affect the expression levels of genes involved in lipid

metabolism pathways, thereby influencing processes such as adipogenesis, lipid deposition, and lipid oxidation. Methyl donor micronutrients (e.g., FA, Met, and choline) have been showed to modulate DNA methylation patterns associated with lipid metabolism, further highlighting the potential of methyl donor micronutrients interventions in improving lipid metabolism and overall metabolic health through DNA methylation modifications. In this review, we have summarized the pathway for methyl donor micronutrients regulated DNA methylation-mediated adipogenesis, lipidolysis, and adipose deposition. We also discussed the involvement of methyl donor micronutrients in lipid metabolism and conducted an analysis of the methylation regulation mechanisms mediated by these methyl nutrients. Overall, our review emphasizes the significance of DNA methylation as a crucial regulatory mechanism in lipid metabolism and highlights the potential impact of methyl donor micronutrients, and the interplay between methyl donor micronutrients intake, lipid metabolism, and DNA methylation. Therefore, given the significant role of methyl donor micronutrients and DNA methylation in lipid metabolism, further research is needed to identify the specific genes and pathways regulated by DNA methylation modifications mediated by methyl donor micronutrients in lipid metabolism, to elucidate the underlying mechanisms. Furthermore, explore the potential applications of using methyl donor micronutrients; the determination of optimal dosages in methyl donor micronutrients supplementation is crucial for enabling the development of targeted personalized nutrition regulation scheme for improving lipid metabolism and preventing metabolic disorder. In addition, integrating multi-omics approaches, such as metabolomics, transcriptomics, and proteomics, with DNA methylation studies could provide a comprehensive understanding of the complex interplay between methyl donor micronutrients, DNA methylation, and lipid metabolism. Such integrative analyses may uncover novel biomarkers and involved pathways. In conclusion, the future of research in understanding the impact of methyl donor micronutrients on lipid metabolism lies in uncovering specific gene targets and pathways, determining optimal dosages of supplementation, developing effective nutrition regulation scheme, and integrating multi-omics approaches. It is aim to provide an effective nutrition regulation scheme for accurately regulating lipid metabolism of livestock and poultry, improving meat quality, and promoting the development of animal husbandry.

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Declaration of competing of interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Ethical statements

This research did not involve animal ethics experiments.

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