

Mitochondrial alteration in type 2 diabetes and obesity

An epigenetic link

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Abbreviations: AMPK, 5' adenosine monophosphate-activated protein kinase; BMI, body mass index; COX7A1, cytochrome c oxidase subunit VIIa polypeptide 1; CpG, cytosine-guanine dinucleotide; CREB, cAMP response element-binding protein; DNMT1, DNA methyltransferase 1; HDAC, histone deacetylase; MEF2, myocyte enhancer factor 2; Mfn, mitofusin; mtDNA, mitochondrial DNA; MT-ND6, mitochondrial NADH dehydrogenase 6; NAFLD, nonalcoholic fatty liver disease; NCoR, nuclear receptor corepressor; NDUFB6, NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6; NRF, nuclear respiratory factor; OXPHOS, oxidative phosphorylation; PGC1 α , peroxisome proliferator-activated receptor γ coactivator 1 α ; PKA, protein kinase A; PPARGC1A, the gene encoding PGC1 α ; ROS, reactive oxygen species; SIRT1, sirtuin (silent mating type information regulation 2 homolog) 1; T2DM, type 2 diabetes mellitus; Tfam, mitochondrial transcription factor A; UCP, uncoupling protein; 3'UTR, 3'-untranslated region.

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The growing epidemic of type 2 diabetes mellitus (T2DM) and obesity is largely attributed to the current lifestyle of over-consumption and physical inactivity. As the primary platform controlling metabolic and energy homeostasis, mitochondria show aberrant changes in T2DM and obese subjects. While the underlying mechanism is under extensive investigation, epigenetic regulation is now emerging to play an important role in mitochondrial biogenesis, function, and dynamics. In line with lifestyle modifications preventing mitochondrial alterations and metabolic disorders, exercise has been shown to change DNA methylation of the promoter of PGC1 α to favor gene expression responsible for mitochondrial biogenesis and function. In this article we discuss the epigenetic mechanism of mitochondrial alteration in T2DM and obesity, and the effects of lifestyle on epigenetic regulation. Future studies designed to further explore and integrate the epigenetic mechanisms with lifestyle modification may lead to interdisciplinary interventions and novel preventive options for mitochondrial alteration and metabolic disorders.

Introduction

Type 2 diabetes mellitus (T2DM) and obesity are growing epidemics due to the current lifestyle of consumption of energy-rich foods and physical inactivity.¹⁻³ Surplus nutrient supply overloads and hyperpolarizes mitochondria, the primary metabolic platform and energy generator in mammalian cells, leading

to the accumulation of incompletely oxidized substrates or intermediates (e.g., fatty acids and diacylglycerol) and overproduction of reactive oxygen species (ROS) (Fig. 1).^{2,4-9} As such, stress-sensitive kinases such as c-Jun N-terminal kinase and protein kinase C are activated and impair insulin action (insulin resistance) by phosphorylating insulin receptor substrates (Fig. 1).^{2,5,7,8} Accordingly, a decrease in mitochondrial function or mitochondrial DNA (mtDNA) copy number has been correlated with insulin resistance and dysregulated lipid metabolism.^{7,10-13} A vicious cycle may develop due to negative feedback regulation loops. For instance, insulin resistance can dysregulate electron transport chain and impair mitochondrial function and biogenesis.^{14,15} In addition, ROS induces further mitochondrial damage and leads to pancreatic β -cell dysfunction and insulin secretory deficiency, thereby deteriorating metabolic disturbance and energy imbalance in various tissues (Fig. 1).¹⁶⁻¹⁹ Nevertheless, properly restricted calorie intake or maintaining a physically active lifestyle can significantly improve mitochondrial integrity and function and protect against metabolic syndromes.²⁰⁻²² The interactions between mitochondria and lifestyle stimuli shown in these studies suggest that epigenetic regulation may underlie mitochondria-mediated metabolism and energy homeostasis. Indeed, DNA methylation of the promoters of genes encoding PGC1 α and Tfam (the regulators of mitochondrial biogenesis), and microRNAs that regulate mitochondrial function and dynamics have been identified in obese

and diabetic subjects.^{13,23-28} Recently DNA methyltransferase 1 (DNMT1) was found to translocate to the mitochondria and control the expression of transcripts from the heavy and light strands of mtDNA, which is accompanied with the presence of cytosine 5-hydroxymethylcytosine and 5-methylcytosine in mtDNA.²⁹ In addition, mitochondrial microRNAs have been discovered in purified mitochondria, which may regulate various cellular processes including RNA turnover, apoptosis, cell cycle, and nucleotide metabolism.^{30,31} Thus, understanding the epigenetic mechanism of mitochondrial alteration in obesity and diabetes may open a new window of prevention or therapeutics for the metabolic diseases. In this article we discuss the latest epigenetic studies and mechanisms of mitochondrial alteration in diabetes and obesity, and review the emerging evidence of lifestyle modifications influencing the epigenetic traits.

Mitochondria in Energy Metabolism

Anabolism and catabolism converge on mitochondria, which convert nutrient influx into energy through ATP production and uncoupling protein (UCP)-mediated thermogenesis (Fig. 2A).^{8,32,33} Mitochondria also convert nutrients into chemical building blocks for macromolecule biosynthesis and energy storage (Fig. 2A).^{32,33} In response to increased nutrient supply, mitochondrial biogenesis and number is upregulated.³⁴ However, persistent nutrient surplus can override the adaptation and lead to mitochondrial overloading and dysfunction, which also holds true when mitochondrial capacity declines with aging.^{2,4,6,7} By contrast, calorie restriction has been shown to improve mitochondrial biogenesis and respiration efficiency, which reduces ROS production and promotes metabolic homeostasis.^{20,35} Mechanistically, mitochondrial biogenesis is regulated by energy sensors such as PKA/CREB, AMPK, and SIRT1 (Fig. 2B).^{33,36,37} During nutrient or energy depletion (e.g., fasting, exercise, or calorie restriction), the level of cAMP and ratio of AMP/ATP increase, which trigger the signaling cascades of PKA/CREB, AMPK, and SIRT1 that can

activate PGC1 α , the master regulator of mitochondrial biogenesis.³⁸ As a result, the downstream targets (i.e., NRF1, NRF2 and Tfam) are induced by PGC1 α , resulting in upregulation of mitochondrial biogenesis and activity (Fig. 2B).^{14,33,36}

Additional mechanisms that regulate mitochondrial number and integrity include the dynamic processes of mitochondrial fusion and fission (or division) (Fig. 2A).^{14,39} Although opposing, the fusion and fission processes work in concert to maintain mitochondrial morphology, size, and number.^{14,39} It has been shown that the fusion and fission can facilitate mixing and exchanging mitochondrial contents, including outer membranes and inner membranes, as well as matrix contents (e.g., mtDNA and metabolites),³⁹ thereby maintaining the integrity of the organelles. Dysregulation of the mitochondrial dynamics leads to inhibition of content mixing/exchange in addition to abnormal morphology, and it is associated with mtDNA instability and reduced respiratory capacity.^{14,39} It was found that high glucose (i.e., the mimic of hyperglycemia, a hallmark of T2DM) can upregulate mitochondrial fission protein Fis1, resulting in augmented mitochondrial

fragmentation and ROS overproduction.⁶ In addition, dysregulation of mitochondrial fusion protein (mitofusin, Mfn) was observed in insulin-resistant or diabetic subjects, suggesting that alterations in this regulatory pathway may contribute to the pathophysiology of insulin resistance and T2DM.^{14,40,41}

T2DM and obesity are characterized by impaired nutrient and energy control. In line with to such changes, the mitochondria have been found to adopt epigenetic mechanisms, including DNA methylation, histone modification, and microRNA, to carry the signals of biogenesis,^{13,23-26,42} functional regulation,⁴³⁻⁴⁵ and dynamics.²⁷ Below we discuss the epigenetic mechanisms in detail.

Epigenetic Regulation of Mitochondrial Biogenesis

As the key regulator of mitochondrial biogenesis (Fig. 2B),³⁸ PGC1 α is sensitive to environmental factors such as aerobic capacity, age, birth weight, and sex.⁴⁶ In addition, alcohol consumption has been correlated with specific single-nucleotide polymorphism in PPARGC1A (the PGC1 α -encoding gene) in African

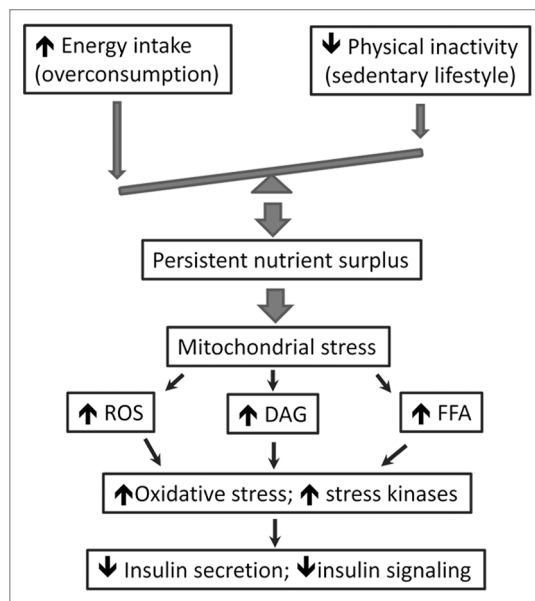


Figure 1. Mitochondrial stress in type 2 diabetes and obesity. The current lifestyle of overconsumption and physical inactivity poses a persistent nutrient surplus, leading to mitochondrial stress and the accumulation of reactive oxygen species (ROS) and metabolite intermediates (FFA and DAG) that can trigger oxidative stress and activation of stress-sensitive kinases. Impaired insulin secretion and sensitivity (i.e., the hallmarks of T2DM) occur as a result of stress-induced β -cell dysfunction and insulin resistance. FFA, free fatty acid; DAG, diacylglycerol.

Americans and Caucasians.⁴⁷ The activity and expression of PGC1 α can be regulated by histone deacetylases (HDACs), the enzymes that increase PGC1 α activity by deacetylating PGC1 α protein (e.g., SIRT1)¹⁴ or control PPARGC1A expression through deacetylating chromatin structure protein histones (e.g., HDAC3).⁴² In insulin-resistant and diabetic obese mice, SIRT1 is less active, and PGC1 α exists primarily in an acetylated form; however, activation of SIRT1 by small molecules prevents hyperacetylation

of hepatic PGC1 α protein and enhances mitochondrial biogenesis and function.¹⁴ In a study of obese diabetic mice, treatment with HDAC3 inhibitors promoted mitochondrial oxidative metabolism in skeletal muscle and adipose tissue, which increased energy expenditure and improved glucose tolerance and insulin sensitivity.⁴² These effects were associated with elevated expression and activity of PGC1 α and the downstream target Tfam, thereby increasing mitochondrial biogenesis and energy expenditure. It is known

that HDAC3 is recruited onto transcription factor MEF2 (myocyte enhancer factor 2) to form a repressive complex with HDAC4, HDAC5, and nuclear receptor corepressor (NCoR), which represses the transcriptional activation of PGC1 α by MEF2;^{42,48,49} however, treatment with HDAC3 inhibitors reduces the recruitment of HDAC3 onto MEF2, thereby removing the transcriptional repression and increasing PGC1 α expression.⁴²

Epigenetic studies have also suggested that the expression of PGC1 α may be controlled at the level of DNA methylation of PPARGC1A promoter, at both cytosine-guanine dinucleotide (CpG) sites and non-CpG sites.^{13,23-25} The increase in DNA methylation of PPARGC1A promoter appears to be a shared mechanism by multiple tissues in patients with T2DM or nonalcoholic fatty liver diseases (NAFLD), which reduces PPARGC1A expression and mitochondrial content, resulting in impaired insulin secretion from pancreatic islets²⁸ and reduced insulin sensitivity in the liver (Fig. 3).¹³ Whole-genome promoter methylation analysis of skeletal muscle biopsies reveals cytosine hypermethylation of PPARGC1A in T2DM patients compared with normal glucose-tolerant subjects, and the highest portion of non-CpG methylation levels are negatively correlated with PPARGC1A expression and mitochondrial content (Fig. 3).²³ In monozygotic twins, higher DNA methylation at CpG sites of promoters of PPARGC1A was present in skeletal muscle from type 2 diabetic vs. non-diabetic twins ($13.9 \pm 6.2\%$ vs. $9.0 \pm 4.5\%$, $P = 0.03$), which may account for the monozygotic twins discordant for T2DM.²⁵ It has also been shown that both prenatal (i.e., low birth weight) and postnatal (i.e., 5-day high-fat overfeeding) environmental factors can change PPARGC1A methylation.⁵⁰ Importantly, altered PPARGC1A methylation induced by the short-term high-fat overfeeding in normal birth weight subjects was significantly reversed by switching back to normal diet.⁵⁰ In addition, a positive correlation exists between maternal pregestational body mass index (BMI) and PPARGC1A promoter methylation in the umbilical cord of newborns ($r = 0.41$, $P = 0.0007$).²⁴ The strong

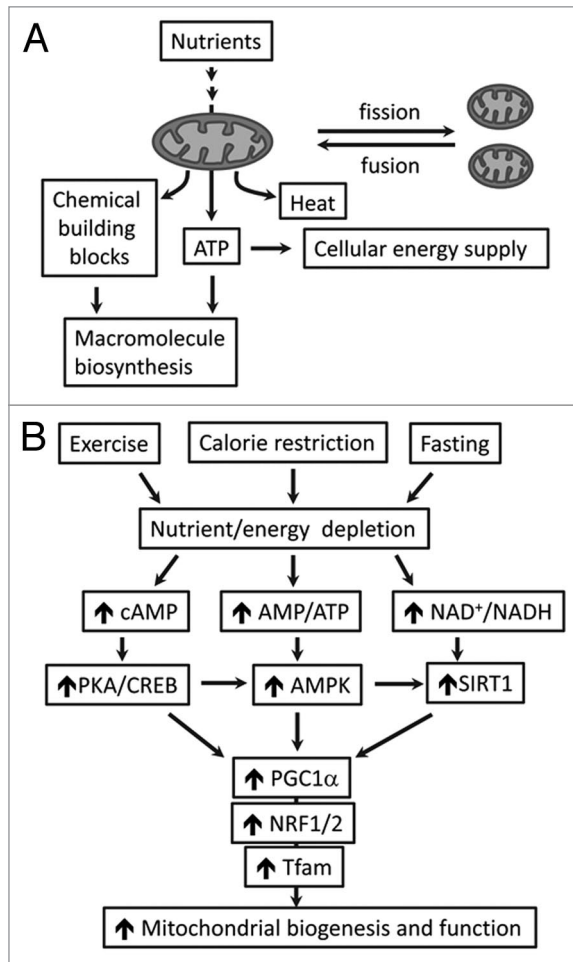


Figure 2. The role of mitochondria in energy metabolism. (A) Mitochondria undergo frequent fusion and fission (the dynamic processes that maintain mitochondrial integrity) and underpin energy homeostasis (ATP generation and thermogenesis) and macromolecule biosynthesis. (B) Energy or nutrient stimuli (e.g., exercise, fasting or calorie restriction) regulate mitochondrial biogenesis and function through pathways involving cAMP-PKA/CREB, AMP/ATP-AMPK, and NAD⁺/NADH-SIRT1. As the key regulator of mitochondria, PGC1 α funnels the signaling to NRF1/2-Tfam and upregulates the genes that are associated with mitochondrial oxidative metabolism. cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; CREB, cAMP response element-binding protein; AMP, 5'-adenosine monophosphate; AMPK, 5'-adenosine monophosphate-activated protein kinase; NAD⁺, nicotinamide adenine dinucleotide; NADH, reduced form of NAD⁺; SIRT1, sirtuin (silent mating type information regulation 2 homolog) 1; PGC1 α , peroxisome proliferator-activated receptor γ coactivator 1 α ; NRF1/2, nuclear respiratory factors 1 and 2; Tfam, mitochondrial transcription factor A.

associations of high-fat overfeeding, birth weight, and maternal pregestational BMI with PPARGC1A methylation suggest that interventions through lifestyle modification could be an effective strategy to prevent the aberrant epigenetic traits and mitochondrial alteration.

As a downstream target of PGC1 α , Tfam senses the signal of mitochondrial biogenesis and regulates mtDNA replication.^{14,51} The promoter of human Tfam has 67 CpG dinucleotides, and methylation of Tfam promoter at NRF1-binding site reduces the promoter activity by 90% (Fig. 3).⁵² According to a recent study of DNA methylation of Tfam promoter in peripheral leukocytes (white blood cells) from high-school students, the ratio of the promoter methylated DNA/unmethylated DNA was inversely correlated with fasting plasma insulin ($r = -0.26$, $P < 0.004$), homeostasis model assessment index ($r = -0.27$, $P < 0.002$), and obesity ($r = -0.27$, $P < 0.002$).²⁶ However, the mechanistic link between insulin resistance and increased methylation of Tfam promoter remains elusive.²⁶ Given that DNA hypermethylation represses Tfam promoter activity, the increased DNA methylation of Tfam promoter suggests a tendency of impaired mitochondrial biogenesis and function in the peripheral leukocytes. Indeed, leukocytes from T2DM patients were found to have compromised mitochondria and elevated ROS and pro-inflammatory cytokines, both of which have been implicated in insulin resistance and T2DM pathophysiology.⁵³ Thus, future studies designed to establish the epigenetic signature of Tfam promoter in peripheral blood and its role in T2DM pathophysiology might lead to new biomarkers for diagnosis and treatment of this disease.

Epigenetic Regulation of Mitochondrial Function

Mitochondrial function underpins insulin secretion from pancreatic β -cell to maintaining systemic glycemic and nutrient homeostasis.¹⁸ In response to elevated nutrient influx, ATP production through mitochondrial-coupled respiration (oxidative phosphorylation) is stimulated, which closes the ATP-sensitive K⁺-channels on

the plasma membrane of pancreatic β -cell and depolarizes the cell. As a result, voltage-gated Ca²⁺ channels are open, and Ca²⁺ influx raises cytosolic Ca²⁺ concentration to trigger insulin secretion.¹⁸ Uncoupling oxidative phosphorylation from mitochondrial respiration may reduce ATP production and lead to β -cell dysfunction and insulin secretory deficiency in T2DM.^{19,54} By contrast, suppression or silencing of mitochondrial uncoupling protein UCP2 in β -cell promotes insulin secretion, suggesting UCP2 can be a target to treat insulin deficiency.^{55,56} To this end, microRNA (e.g., miR-15a) was recently identified and found to promote insulin synthesis and secretion by inhibiting UCP2 (Fig. 3).⁴³ The expression of miR-15a increased significantly in mouse islets after treatment with a high concentration of glucose (33 mM) for 1 hour, accompanied with upregulated expression and biosynthesis of insulin. However, persistent high glucose (33 mM for 3 days) reduced miR-15a

level and insulin synthesis in mouse islets, suggesting dysregulation of miR-15a may account for hyperglycemia-induced compromise of insulin secretion in T2DM.⁴³ Mechanistic study indicates that miR-15a directly inhibited UCP-2 gene expression and reduced UCP2 3'-UTR luciferase reporter activity, in line with lower endogenous UCP-2 protein levels.⁴³ Another microRNA that regulates insulin secretion is miR-184, whose expression in pancreatic islets is negatively correlated with insulin secretion.⁵⁷ microRNA-184 may regulate insulin secretion through repression of Slc25a22, a mitochondrial glutamate carrier that controls cytosolic glutamate and the activities of insulin granules and secretory vesicles during insulin exocytosis (Fig. 3).⁵⁸ Transfection of MIN6 islet β -cell line with miR-184 reduces 70% of 3'-UTR luciferase reporter signal, indicative of an interaction of miR-184 with Slc25a22 3'-UTR. Ectopic expression of miR-184 in MIN6 cells suppresses

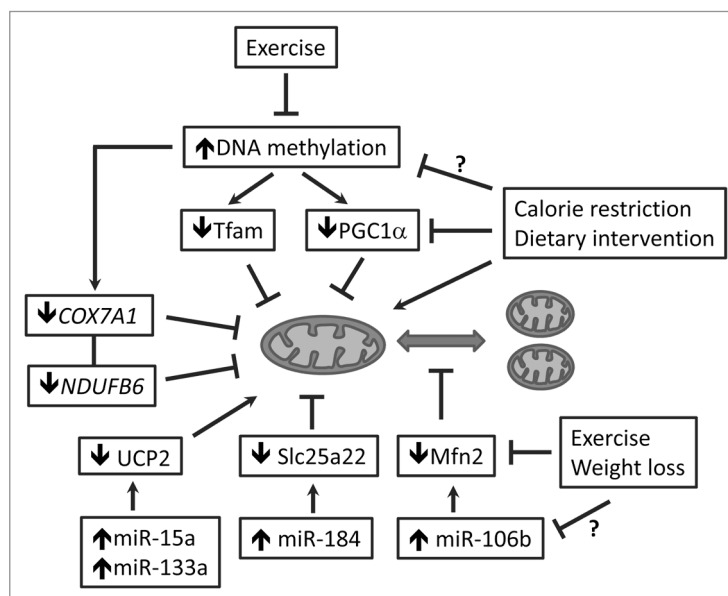


Figure 3. The epigenetic mechanisms of mitochondrial regulation in T2DM and obesity. Increased DNA methylation represses expression of PGC1 α and Tfam, the key regulators of mitochondrial biogenesis. Epigenetic regulation of mitochondrial function includes: (1) DNA hypermethylation that represses the genes of mitochondrial oxidative metabolism (e.g., COX7A1 and NDUFB6); and (2) microRNAs (miR15a, miR133a, and miR-184) that repress mitochondrial substrate carrier (Slc25a22) or uncoupling protein (UCP2). microRNA (miR-106b) was also identified to regulate mitochondrial dynamic protein (Mfn2). Behavioral intervention such as exercise has been shown to change the epigenetic signature (DNA methylation) and improve mitochondrial biogenesis and function through PGC1 α and Tfam. The mitochondria-promoting effects have also been reported for dietary intervention, calorie restriction, and weight loss; whether and how an epigenetic mechanism is involved in the mitochondrial regulation deserves further investigation. PGC1 α , peroxisome proliferator-activated receptor γ coactivator 1 α ; Tfam, mitochondrial transcription factor A; UCP2, uncoupling protein 2; Mfn2, mitofusin 2; COX7A1, cytochrome c oxidase subunit VIIa polypeptide 1; NDUFB6, NADH dehydrogenase (ubiquinone) 1 β subcomplex, 6.

Slc25a22 expression and reduces insulin secretion.⁵⁸

microRNA regulation of UCP2 appears to play a different role in skeletal muscle. It was shown that the muscle-specific microRNA, miR-133a, suppresses UCP2 expression in skeletal muscle and promotes muscle development.⁴⁴ During myogenic differentiation, MyoD can upregulate miR-133a, which, in turn, silences UCP2, the brake of muscle development.⁴⁴ In addition, epigenetic regulation of genes encoding mitochondrial proteins affects insulin responsiveness and glucose uptake in skeletal muscle. The elderly or individuals with insulin resistance and T2DM show reduced mitochondrial oxidative capacity in skeletal muscle, concomitant with downregulation of an array of genes responsible for oxidative phosphorylation, including COX7A1 (the subunit of cytochrome c oxidase, or complex IV in the respiratory chain) and NDUFB6 (subunit in complex I in the respiratory chain).^{7,45,59} Bisulfite sequencing analysis showed that DNA methylation of the COX7A1 promoter was significantly higher in muscle from elderly than young twins ($19.9 \pm 8.3\%$ vs $1.8 \pm 2.7\%$; $P = 0.035$), which was associated with a lower expression of COX7A1 in elderly than in young twins (1.00 ± 0.05 vs 1.68 ± 0.06 ; $P = 0.0005$).⁴⁵ An aging-induced epigenetic mechanism also occurs to NDUFB6 regulation: the skeletal muscles from elderly subjects have higher DNA methylation of the *NDUFB6* promoter but lower *NDUFB6* expression than from young twins.⁶⁰ Regression analysis of mitochondrial function and oxidative capacity with the expression of COX7A1 and *NDUFB6* revealed positive correlations of these 2 gene levels with total body aerobic capacity and insulin-stimulated glucose uptake.^{45,60} These findings suggest that epigenetic (DNA methylation) regulation of mitochondrial function underlies age-dependent susceptibility to insulin resistance and metabolic programming.

Epigenetic Regulation of Mitochondrial Dynamics

Dysregulation of mitochondrial dynamics alters mitochondrial morphology, metabolism, and intracellular

signaling, and it has been implicated in various human diseases, including T2DM and obesity.^{14,39,40} The skeletal muscles in non-diabetic obese subjects and lean or obese T2DM patients have exhibited significantly lower expression of Mfn2, the regulator of mitochondrial dynamics and network, when compared with control groups.⁴⁰ The same study suggests that Mfn2 expression is directly proportional to insulin sensitivity, but inversely proportional to the body mass index; however, body weight loss through bariatric pancreatic diversion significantly increases Mfn2 expression in skeletal muscle.⁴⁰ Using gene chips and mRNA sequencing, Gallagher et al. identified a highly expressed microRNA, miR-106b, in the skeletal muscle of diabetes patients.⁶¹ This microRNA is also highly expressed in mice with insulin resistance induced by high-fat diet.⁶² Luciferase activity assay combined with mutational analysis revealed that the 3'-UTR of Mfn2 possesses miR-106b binding sites, and that miR-106b targets the 3'-UTR and silences Mfn2 expression.²⁷ Gain of miR-106b function impairs mitochondrial function, whereas loss of miR-106b function promotes mitochondrial function and insulin sensitivity (Fig. 3).²⁷ However, there was no in vivo evidence reported, and it would be of importance for further in vivo studies to validate the effect of silencing miR-106b on mitochondrial dynamics and function, as well as its effects on insulin sensitivity.

Conclusion and Perspectives

Epigenetic regulation of mitochondria affects the biogenesis, function, and dynamics of the organelles. DNA hypermethylation suppresses the expression of PGC1 α and Tfam, thereby reducing mitochondrial biogenesis and oxidative metabolism; microRNAs interact with the 3'-UTR of target genes (e.g., UCP2 and Mfn2) and silence the gene expression, which changes mitochondrial respiration and morphology (Fig. 3). In obese and T2DM individuals, the dysregulation of DNA methylation and microRNA has been increasingly discovered, and it is strongly associated with aberrant mitochondrial traits and loss of metabolic homeostasis. In addition, epigenetic

dysregulation of mitochondria has been reported in diabetic complications, such as diabetic cardiovascular diseases,⁶³⁻⁶⁵ diabetic retinopathy,⁶⁶ NAFLD,^{12,13} and cardiorenal dysfunction.⁶⁷

Epigenetic signatures feature the interactions between environmental factors and genes in human beings. Social and lifestyle factors (such as dietary pattern, physical activity, micro-nutrition status, socioeconomic status, and BMI) have been correlated with human global DNA methylation,⁶⁸⁻⁷³ suggesting that lifestyle modification and behavioral intervention may reverse or prevent epigenetic dysregulation of mitochondrial function and metabolism. Indeed, exercise intervention was found to reduce DNA methylation of the promoters of metabolism-regulating genes in both human and rodent skeletal muscles, including PPARGC1A (Fig. 3).^{71,74} The exercise-induced DNA hypomethylation led to a dose-dependent upregulation of PGC1 α , consistent with an enhanced mitochondrial biogenesis and metabolism.^{23,71,75} Physical activity also modulates methylation of hepatic mitochondrial NADH dehydrogenase 6 (MT-ND6) and its transcriptional activity, which ameliorates the histological severity of NAFLD.¹² By contrast, physical inactivity induced by 9-day bed rest increases PPARGC1A promoter methylation and significantly reduces the expression of PPARGC1A and other genes that are associated with mitochondrial function, accompanied with development of insulin resistance; however, these changes can be partly reversed by a 4-week retraining, underscoring the promising potential of physical activity in preventing aberrant epigenetic traits and mitochondrial alteration.⁷⁶ In addition, weight loss, calorie restriction, and dietary intervention have also been shown to improve mitochondrial biogenesis, network, and function, albeit an epigenetic mechanism remains to be defined (Fig. 3).^{20-22,40,41,77} Given that lifestyle interventions effectively delay and prevent the onset of diabetes and obesity,⁷⁸⁻⁸² it will be of interest to examine whether and how epigenetic mechanisms of mitochondrial regulation contribute to the efficacy of interventions. Future studies exploring these epigenetic signatures might lead to novel molecular diagnostic

options and interdisciplinary interventions for aberrant mitochondrial traits and metabolic disorders.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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