Tracing mitochondrial dysfunction pathways in type 2 diabetes: The promise of mitochondria-targeted therapeutics

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ABSTRACT

The alarmingly high global prevalence of Type 2 Diabetes Mellitus (T2DM) has made novel treatment strategies imperative. One major contributing factor to the onset of T2DM is mitochondrial dysfunction, which deteriorates cellular health, interferes with energy metabolism, and reduces insulin sensitivity. Targeting mitochondrial pathways can enhance outcomes in T2DM. Promising therapeutic benefits can be derived from strategies that target and improve mitochondrial function, biogenesis, efficient turnover, and oxidative stress reduction. Through increased mitochondrial efficiency and improved insulin action, these treatments seek to address the complex problems associated with T2DM. The complexities of mitochondrial dynamics, such as their role in energy production, appropriate regulation of cell death, and creation of reactive oxygen species, highlight the necessity of comprehensive approaches in the development of medicines that target mitochondria. This strategy represents a step forward in the management of T2DM and highlights a promising area of cutting-edge research in the hunt for potent treatments that might stop or reverse the disease's development, improving patient outcomes and care.

INTRODUCTION

The International Diabetes Federation states that globally, there are 451 million type 2 diabetes mellitus (T2DM) people aged 18-99 and posits that this number will rise to 693 million by 2045, assuming the current trend continues [1]. Males are more affected by T2DM than females, with a greater incidence, especially in poor and middle-income nations. The nations with the biggest number of diabetes patients globally are India, China, and the USA, according to the IDF Diabetes Atlas, 2021 [2]. The demographic pattern of T2DM is transitioning from occurring in older individuals to affecting younger individuals with a more complex phenotype, increasing the likelihood of developing early diabetes-related comorbidities such as nephropathy, neuropathy, retinopathy, and cardiomyopathy [3]. Due to the growing prevalence of T2DM in ageing and obese individuals, it is crucial to have a profound comprehension of the molecular alterations that connect these factors [4]. The ever-expanding knowledge on modifiable risk factors for T2DM that has been underscored by recent research involving genetic and environmental influences underlines the need to encourage healthy behavior changes and early interventions. Obesity, physical inactivity, and nutrition patterns have been established as establishing factors towards the rising incidence of T2DM [5]. Furthermore, the cost implications of managing diabetes and its complications are very costly to healthcare systems across the world. That, in turn,

resulted in the shift in the approach that is now considered more and more as personalized medicine approaches based on the genetic, metabolic, and life-styling characteristics of each patient [6].

The mitochondrion is recognized as a focal point where several disrupted pathways intersect in the processes of ageing, obesity, and T2DM. Ageing is associated with mitochondrial dysfunction, including mitochondrial DNA (mtDNA) mutation and depletion, as well as mechanisms like apoptosis [7, 8]. The balance of fission, fusion, mitophagy, and mitochondrial biogenesis maintains mitochondrial homeostasis [9]. These procedures are necessary to preserve the composition and functionality of mitochondria, especially mitochondrial energetics [10]. It is worth mentioning that numerous transcriptional coactivators and corepressors control the process of mitochondrial biogenesis. Important upstream transcriptional regulators of mitochondrial biogenesis include peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), peroxisome proliferator-activated receptor γ coactivator 1β (PGC-1β), and PGC-1-related coactivator (PRC), which are members of the PGC family [11]. Mitochondrial dysfunction plays a significant role in T2DM because of its known connection with insulin resistance, as discussed in earlier studies [12-15]. It seems that the buildup of harmful lipid metabolites is caused by a malfunction in mitochondria and a decrease in the ability to metabolize fuel efficiently. A decrease in fatty acid oxidation results in ceramide and diacylglycerol accumulations that block pathways involved in insulin signal transduction [16]. The purpose of this study is to review the possible synergism between the investigated pathophysiology of T2DM and the alterations in mitochondrial function. It will give even more idea of how the improved insulin sensitivity and energy metabolism are affected by these phenomena. The current study will also seek to identify the potential interventions that can be used to increase the efficiency of mitochondrial therapy for T2DM patients. It will also determine the contribution of oxidant stress, changes in gene expression, and mutated mtDNA in the pathogenesis of the disorder. The final goal of the actual research is to present a comprehensive analysis of the new and existing treatments that need mitochondria in order to help to hinder the advancement of T2DM.

METHODS

In order to find the research articles that have been published in English and are concerned with the pathways involved in mitochondria and their relation to the treatment strategy of T2DM, the authors conducted a literature search in the relevant journals. Of the databases, PubMed, Google Scholar, Taylor & Francis, ACS, Wiley Online Library, Science Direct, and MDPI were searched. The keywords utilized were "mitochondrial dysfunction," "Type 2 Diabetes Mellitus," "Mitochondrial dysfunction in T2DM," "insulin resistance," "oxidative stress," "mitochondria-targeted therapeutics," "mitochondrial biogenesis," and "epigenetics in T2DM." Table 1 depicts the overview of the search results obtained from this procedure using the keywords. An emphasis on clinical trials, meta-analyses, and reviews that offer insights into the molecular pathways and possible therapeutic treatments was one of the inclusion criteria, which applied to the research chosen for the study. Before the data was arranged into tables and figures, Microsoft Excel (2010) was employed to structure the data. Figures were generated summarizing the studies.

Table 1. Search result summary of the databases.

Sources	Keywords	Search Results
PubMed	Type 2 Diabetes Mellitus	202,234
	Mitochondrial dysfunction	94,876
	Mitochondrial dysfunction in T2DM	439
	Mitochondria-targeted therapeutics	1,042
	Insulin resistance	158,983
	Mitochondrial biogenesis	13,986
	Epigenetics in T2DM	270
Google Scholar	Type 2 Diabetes Mellitus	2,890,000
	Mitochondrial dysfunction	2,789,000
	Mitochondrial dysfunction in T2DM	74,900
	Mitochondria-targeted therapeutics	25,800
	Insulin resistance	3,290,000
	Mitochondrial biogenesis	457,000
	Epigenetics in T2DM	26,890
Science Direct	Type 2 Diabetes Mellitus	342,871
	Mitochondrial dysfunction	164,121
	Mitochondrial dysfunction in T2DM	4,712
	Mitochondria-targeted therapeutics	22,712
	Insulin resistance	277,112
	Mitochondrial biogenesis	46,872
	Epigenetics in T2DM	2,123

T2DM AND MITOCHONDRIAL DYSFUNCTION

Crossroads of metabolic health: Mitochondrial dysfunction in T2DM

The mitochondrion is considered a crossroads of many disrupted pathways in the course of ageing, obesity, and T2DM. The ageing process is linked with mitochondrial dysfunction, which is manifested by the mutation and depletion of mtDNA, as well as by some mechanisms such as apoptosis [7, 17]. T2DM is heavily dependent on the malfunction of mitochondria as it is associated with insulin resistance [12].

Hepatic impairment in T2DM patients has been referred to as nonalcoholic fatty liver disease (NAFLD) [18, 19]. The debate is still going on concerning whether NAFLD is a cause or a consequence of diabetic diseases. However, the alterations in hepatic energy substrate metabolism and mitochondrial function in T2DM patients with NAFLD are well characterized. The typical hepatic response to the liver of diabetic patients is reduced liver insulin sensitivity and lipid accumulation in the liver [20]. Additionally, obese, insulin-resistant individuals with nonalcoholic steatohepatitis (NASH) exhibit mitochondrial abnormalities such as reduced maximum respiration, heightened mitochondrial uncoupling, and increased proton leak [21].

Insulin resistance in skeletal muscle is a critical factor in the pathogenesis of T2DM. The study of skeletal muscle mitochondria in T2DM has been investigated extensively because of their major effects on the overall metabolism. However, many groups have agreed on the issue of skeletal muscle mitochondrial dysfunction in T2DM, but the debate goes on as to whether this is an effect or the cause of T2DM [22, 23]. This malfunction has been identified through various methodologies in living organisms and isolated tissues. In comparison studies between T2DM patients and controls matched for BMI, it was found that the phosphocreatine (PCr) recovery half-time of the vastus lateralis muscle was 45% longer in diabetic patients than in controls [24]. An increased PCr half-time in the vastus lateralis muscle of diabetics was demonstrated by Phielix and colleagues, along with decreased ADP-stimulated basal respiration and FCCP-stimulated maximum respiration in mitochondria from diabetic patients [25]. The diminished mitochondrial respiratory capacity in individuals with diabetes is

evidenced by the lower maximum ADP-stimulated respiration observed in the vastus lateralis mitochondria of diabetic patients using a pyruvate-malate combination [23]. Moreover, a more detailed study of the impact of T2DM on mitochondrial dynamics, including calcium homeostasis, fission/fusion balance, network formation, and mitochondrial reticulum alteration, in skeletal muscles is required, to understand how changes in these processes could influence metabolic dysfunction in the mitochondria of skeletal muscles.

Mitochondrial dysfunction and insulin resistance

A reduction in the oxidation of metabolic substrates constitutes the primary issue, leading to the accumulation of diacylglycerol and ceramide inside cells. This accumulation disrupts insulin signaling and contributes to insulin resistance via a series of processes. Owing to their important role in oxidative metabolism, mitochondria have been identified as the organelles within the cell responsible for the connection between disrupted fuel sources which include fatty acid oxidation, lipotoxicity, and insulin resistance [26]. Research has shown that lower mitochondrial oxidative capacity correlates with insulin resistance in people who suffer from T2DM. Studies on obese and insulin-resistant individuals indicate that there might be a link between mitochondrial dysfunction and insulin resistance according to preliminary evidence. They exhibited lower skeletal muscle mitochondria oxidative capacity and compromised lipid metabolism in contrast to the healthy controls [27].

It has been reported by Kelley and colleagues that diabetic patients with T2DM have decreased NADH2-O2 *oxidoreductase* activity. This result supports the link between T2DM and mitochondrial dysfunction as a whole, pointing to it as an integral error in the emergence of insulin resistance [28]. Consistently, microarray studies have supported this linkage by demonstrating that genes associated with oxidative metabolism and regulated by PGC 1α are reduced in the skeletal muscle of individuals with a family history of T2DM and T2DM patients relative to healthy individuals [29]. Insulin resistance is triggered by the accumulation of intracellular fatty acyl CoA and diacylglycerol, which stimulate important signal transduction pathways and block the insulin signalling pathway (Figure 1). Among the metabolic disorders that cause the accumulation of fatty acids in the liver and/or skeletal muscle and abandons within the capacity of these organs to metabolize fatty acids, mitochondrial dysfunction appeared to be the reason for inducing insulin resistance [30].

Furthermore, in vivo, assessment of mitochondria function through measurement of phosphocreatine resynthesis after exercise has verified severe mitochondrial abnormalities at the levels of transcripts and translational in living organisms. This offers additional evidence that the oxidative capacity of muscle mitochondria is compromised in individuals with advanced T2DM [24]. A study by Phielix and colleagues found that a reduction in mitochondrial respiration was observed in the first-degree relatives of individuals with T2DM, suggesting that mitochondrial dysfunction could occur before the development of T2DM [25]. Mitochondrial failure appears to be a consequence of the inherent inadequacy of the oxidative phosphorylation (OXPHOS) system and the electron transport chain (ETC), rather than a decrease in mitochondrial content expressed by mitochondrial DNA copy number. In addition, a 35% decrease in ADP-stimulated mitochondrial respiration was observed in patients with T2DM when adjusting for mitochondrial content [25]. The decrease in metabolic fuel oxidation, which is caused by the diminishment of mitochondrial oxidative capacity, offers an explanation for the relationship between mitochondrial

dysfunction and the formation of lipotoxic lipid intermediates as the cause of insulin resistance (Table 2).

A different potential mechanism of linking mitochondrial dysfunction with insulin resistance is through the generation of reactive oxygen species (ROS) by mitochondria. ROS are unavoidable by-products of mitochondrial energy production, which are controlled by the internal antioxidant defence system of the cell. When the generation of ROS exceeds the cellular antioxidant capacity, oxidative stress develops [31]. Too much of nutrients and waste products cause an overflow of electron donors, which only improves the mitochondrial flow of electrons and consequently, there arises a great proton gradient across the inner mitochondrial membrane. If not countered by a rise in ATP generation, this can result in elevated ROS production. ROS have been known to induce oxidative damage in nuclear DNA, lipids, and proteins, but they also work as signaling molecules that may directly cause insulin resistance. The elevation in ROS generation and generated oxidative stress has a crucial impact on the oxidative metabolism of mitochondria. ROS results in oxidative injury of mitochondrial DNA, proteins, and lipids, due to which the damaged mitochondria are removed through mitophagy. Oxidative stress may disrupt the insulin signal transduction pathway, which subsequently causes insulin resistance (Table 2). It can as well impair insulin signalling by inducing mitochondrial damage and mitophagy. Table 2 provides the studies in favor of the association of mitochondrial dysfunction and insulin resistance.
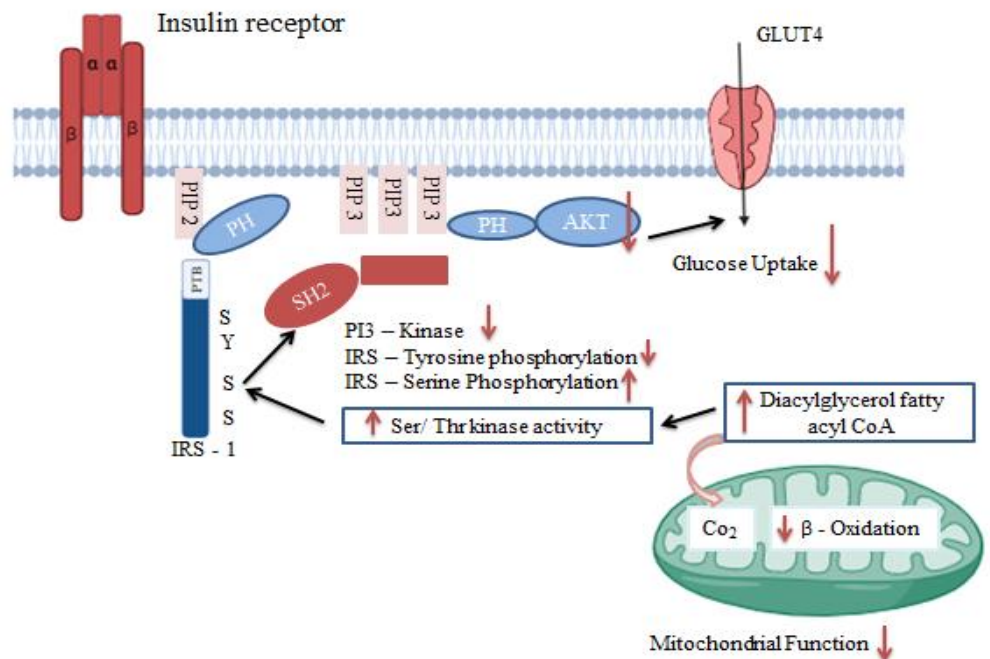

Figure 1. Potential mechanism of how skeletal muscle develops insulin resistance resulting from mitochondrial dysfunction. According to the model, intracellular fatty acyl CoA and diacylglycerol levels rise when mitochondrial fatty acid oxidation is inhibited due to dysfunctional mitochondria or decreased mitochondrial composition. These compounds trigger the activation of new *protein kinase C*, which in turn triggers a cascade of *serine kinases* that increases the serine phosphorylation (pS) of insulin receptor substrate-1 (IRS-1). The activity of *phosphatidyl inositol 3-kinase* (*PI 3-kinase*) is inhibited by increased serine phosphorylation of IRS-1, which prevents IRS-1 tyrosine phosphorylation by the insulin receptor. The suppression of insulin-stimulated glucose transport is a consequence of such inhibition. PIP: phosphatidylinositol 3, 4, 5-trisphosphate; PTB: phosphotyrosine binding domain; PH: pleckstrin homology domain; SH2: SRC homology domain; GLUT 4: Glucose transporter type 4; IRS 1: insulin receptor substrate-1.

Table 2. Link between mitochondrial dysfunction and insulin resistance.

Sample	Observation	Ref.
T2D Patients	Mitochondrial enzyme activity is reduced to 30–40%.	[32]
Obese individuals	Reduction in mitochondrial content, smaller mitochondrial size and alteration in the performance of mitochondria.	[28]
Healthy lean elderly	Decreased mitochondrial oxidative capacity and ATP synthesis, severe insulin resistance in skeletal muscle, high TG level in liver and muscle.	[33]
T2D and insulin resistant individuals	Mitochondrial density is reduced to 30%.	[34]
Young-insulin resistant offspring of parents with T2D	Decreased oxidative and glycolytic fibers in muscle, increased intramyocellular FA and reduced mitochondrial activity.	[35]
Obese Caucasians with IGT and T2D	Reduced expression of nuclear-encoded genes regulating mito biogenesis.	[29]
Obese and overweight nondiabetic Mexican-Americans	Lower expression of PGC-1α, PGC-1β and nuclear OXPHOS genes.	[36]

EPIGENETICS AND EPITRANSCRIPTOMICS IN MITOCHONDRIAL BIOLOGY

Epigenetic and epitranscriptomic regulation of mitochondria

The fields of mitochondrial epigenetics and epitranscriptomics are two of the most innovative areas of mitochondrial biology. DNA *methyltransferase 1* (DNMT1) was initially shown to be present in the mitochondria by Shock and colleagues, who also described the mitochondrial targeting sequence in this isoform [37]. They also demonstrated the existence of mtDNA alterations for 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) [37]. A follow-up investigation revealed that mtDNA had methylation at 83 distinct CpG sites [38]. The methylcytosine landscape of 39 distinct human cell and tissue types has been described by another group [39]. Ten-eleven *translocase*, the enzyme that converts 5mC hydroxylation to 5hmC, was found in the mitochondria, supporting the mtDNA 5hmC alteration [40]. There are still many unanswered concerns in the research about how T2DM affects mtDNA methylation and/or hydroxymethylation alterations and how these changes affect mitochondrial function. Many techniques have been developed to identify mtDNA methylation, and each has advantages and disadvantages of its own [41]. There are a few possible difficulties or confounding variables, such as insufficient mitochondrial purification or nuclear integration of mtDNA sequences [41].

Examining the interaction between the mitochondrion and nuclear epigenome in the context of T2DM is an interesting area of study. This relationship during both normal functioning and times of stress has been previously explored [42]. Tissue-specific variably methylated areas of the nuclear genome have been shown to exist in a healthy state, contributing to variations in function and gene expression across various tissues [43]. The tissue-specific methylation patterns account for 70–80% of all methylated CpGs in most cell types. These patterns are established through the replication of methylation patterns by DNMT1 after DNA replication and through *de novo* methylation during development, mainly carried out by DNMT3A and DNMT3B [44, 45]. DNA methylation may vary in CpG islands located in the promoter regions of certain genetic loci, leading to either increased or decreased production of certain genes, particularly in diseases like T2DM. Out of the 41 genes shown to have differing levels of activity between pancreatic β-cells of individuals with T2D and those without, 80% (34 genes) showed an opposite relationship with the methylation of their promoter region [46]. Pancreatic duodenal homeobox 1, an essential transcription factor related to the early development of the pancreas was discovered to be considerably reduced in pancreatic β-cells of individuals with T2DM. Ten CpG sites in the distal promoter and

enhancer regions of the gene were shown to have higher levels of methylation in T2DM [47].

Bisulfite sequencing of nuclear DNA from cells or tissues allows for determining the average methylation level at specific sites, which may be linked to the expression of a particular gene. The precise quantity of methylation needed at certain gene regulatory regions and inside the gene body for either repressing or activating certain genes is yet unknown. Recent developments in single-cell analysis now allow researchers to collect both methylome and transcriptome data from a single cell [48, 49]. These insightful single-cell multi-omics investigations have reinforced the role of promoter methylation in gene suppression [50].

Methylation has been detected on mRNA transcripts encoded by mtDNA [51]. The enzymes TRMT10C and TRMT61B present in mitochondria assist the mRNA N1-methyladenosine (m1A) modification [52]. The T2DM-induced alterations in the mitochondrial epitranscriptome may elucidate mitochondrial dysfunction in this state due to the significant impact of the m1A mutation on translation activation or inhibition, regardless of its location on the mRNA transcript [52].

mtDNA mutation and variation

Mitochondrial DNA variations, whether fully present in a cell (homoplasmic) or partially present (heteroplasmic), may have severe consequences in cases of metabolic disorders. Various mtDNA mutations and single nucleotide polymorphisms have been linked to T2D as shown in Table 3. Some groups have categorized their study populations into mitochondrial haplogroups using mtDNA variations to investigate whether a particular haplogroup is linked to a higher or lower incidence of T2DM. A previous study demonstrated a correlation between haplogroup J in a Finnish population and maternally transmitted T2D [53]. Further research by Feder and his colleagues confirmed these findings by linking haplogroup J1 with T2DM [54]. Liou and his colleagues found that individuals belonging to mitochondrial haplogroup B4 had a higher chance of developing T2DM, whereas those in haplogroup D4 had a lower risk [55]. A prior study by Saker et al. in 1997 revealed a reduced occurrence [0.1–0.2%] of the A3243G mtDNA variation in UK white Caucasian individuals with T2DM compared to T2DM Asian patient groups (Table 3) [56]. Mitochondrial genetics have a significant influence in determining susceptibility to T2DM. An important topic that still needs to be addressed is whether metabolically active tissues that produce higher levels of mitochondrial ROS exhibit higher rates of mtDNA heteroplasmy in T2DM.

Table 3. Increased or decreased risk associated with mtDNA variation in T2DM.

mtDNA Alterations	Nature Variant	Overview	Ref.
4399–14821 Deletion	Heteroplasmic	Deletion associated with maternal inheritance of DM and deafness	[57]
A3243G	Heteroplasmic	Variant associated with T2DM	[58]
T14577C	Heteroplasmic	Variant associated with T2DM	[59]
T16189C	Mostly Homoplasmic	Variant associated with T2DM	[60]
A5178C	N/A	Variant associated with maternally inherited T2DM	[61]
C8684T	N/A	Variant associated with T2DM	[62]
T4216C, A4917G	Homoplasmic	Variants associated with T2DM	[63]
16189–16193 Polycytosine Variant	N/A	Variant associated with T2DM	[64]
G3316A, T3394C	N/A	Variants associated with T2DM	[65]

DETECTION OF T2D CONSIDERING MITOCHONDRIAL DYSFUNCTION

The predictive power of mitochondrial DNA copy number for T2DM incidence

Mitochondrial DNA copy number (mtDNA-CN) is a marker of mitochondrial malfunction and is linked to T2DM. It is unclear if mtDNA-CN can forecast the likelihood of acquiring T2DM [66]. Previous studies have shown that mtDNA-CN serves as an indicator of mitochondrial function [67]. A higher mtDNA-CN indicates improved mitochondrial function, and conversely, fluctuations in mtDNA-CN are linked to susceptibility to T2DM. However, several studies used a case-control methodology and yielded conflicting findings [68, 69]. Memon and colleagues' investigation showed that reduced mtDNA-CN was linked to both existing and new cases of T2DM [66]. The correlation between mtDNA-CN and new cases of T2DM remained statistically significant even after accounting for the other factors. With covariate adjustments, the association of mtDNA-CN and prevalent T2DM decreases. The study was adjusted for age, BMI, education, smoking status, and physical activity. The aim of these modifications was to assess the independent correlation between mtDNA-CN and the incidence of T2DM, both for newly diagnosed and pre-existing cases. The selection of these factors was made based on their established connections with the risk of T2DM and their potential impact on mitochondrial function and mtDNA-CN [66]. However, a sensitivity analysis showed that the exclusion of pre-diabetic cases strengthened the association, indicating that pre-diabetic cases might compromise the true relationship between mtDNA-CN and T2DM [66]. One possible cause of the decrease of the mtDNA-CN in diseases could be the accumulation of mutations in the mitochondrial genome leading to mitochondrial dysfunction [67].

Exploring the connection: oxidative stress and its role in T2DM development

Living organisms are regularly exposed to different chemicals that lead to the production of reactive species known as free radicals (ROS/RNS). These free radicals induce oxidation in cellular machinery by transferring their unpaired electron [70]. ROS are reactive oxygen species, characterized by atoms or groups of atoms with at least one unpaired electron. Mitochondria are unavoidable producers of ROS inside the cell. Typically, around 0.2–2% of electrons bypass the conventional transfer and escape directly from the ETC under normal physiological conditions and react with oxygen to produce superoxide or hydrogen peroxide [71]. Moreover, overabundant ROS also enhances protein modifications such as carbonylation, glutathionylation, nitrosylation, and glycation. Glycated haemoglobin (HbA1c) is the most common protein modification caused by ROS and is used as a diagnostic marker for diabetes. Additionally, glutathionylation of this protein is also increased in diabetic individuals [72, 31]. Lipid peroxidation is the oxidative damage of lipids, changing their membrane fluidity and permeability. Indices of lipid peroxidation such as 8-isoprostane (8-Ip), malondialdehyde (MDA), oxidized LDL, and thiobarbituric acid reactive substances (TBARS) are elevated in individuals with T2DM and especially in the plasma and erythrocytes [73, 74]. The oxidative stress may provoke DNA damage and create mutations and lesions that can be detected by the increased levels of 8-hydroxy-2-deoxyguanosine (8-OH-G) [75]. T2DM is characterized by increased levels of both protein and lipid markers of oxidative stress. However, they are not used as reliable diagnostic markers because of their relationship with the control of the cellular antioxidant system. On the other hand, oxidative stress and ROS are becoming more linked to the pathogenesis of T2DM, mainly in relation to insulin resistance, impaired insulin production, and release. This is thought to happen through the start of an

inflammatory response and the disturbance of several molecular signaling pathways [76, 77].

MANAGING T2DM WITH MITOCHONDRIA-TARGETED THERAPEUTICS

Mitochondria-targeted gene therapy

T2DM is a complex genetic disorder significantly impacted by environmental factors. While genetic factors play a role in its development, there are also notable changes in the mitochondrial transcriptome and proteomic level in diabetic individuals [78, 79]. The increase in polynucleotide *phosphorylase* and decrease in mitochondrial heat shock protein 70 (Hsp70) in the db/db myocardium murine model may account for these findings [80, 81]. After genetically modifying these genes to their natural levels, the production of miRNA transcripts and proteins was brought closer to normal levels seen in non-diabetic individuals, leading to an improvement in mitochondrial function [80, 81]. Previous studies have shown that human antigen R and G-rich RNA sequence-binding factor 1 aid in the transportation of long non-coding RNA into the mitochondria [82]. The determination of whether the key mediators of mitochondrial transport are affected in different tissues of T2DM patients will provide important information on mitochondrial regulation. This will add to the evidence on whether gene therapy may be used to correct mitochondrial "omics" and help in the recovery of mitochondrial function in T2DM.

Mitochondria-targeted metabolic therapy

Elevated mitochondrial ROS in T2DM are closely associated with alterations in energy substrate metabolism and less effective oxidative phosphorylation. Metformin, a thiazolidinedione medication, is a frequent choice for preventing or treating T2D as a first-line therapy. Metformin is a versatile small molecule known to exhibit various modes of action across distinct tissue types. Metformin's antidiabetic impact is primarily mediated by suppression of hepatic gluconeogenesis; however, there is accumulating evidence suggests the gastrointestinal system plays a role in the drug's ability to reduce blood glucose levels. Metformin is known to target hepatic mitochondria and inhibit the first complex of the mitochondrial respiratory chain [83]. The result of this inhibition is an increase in the AMP to ATP ratio in hepatocytes and an apparent reduction in ATP generation OXPHOS. Metformin was discovered to activate *AMP-activated protein kinase* in liver cells and skeletal muscle, leading to reduced glucose synthesis by the liver and enhanced glucose uptake by skeletal muscle (Figure 2) [84]. Hence, this activation doesn't directly affect glucose production, as metformin-induced intracellular levels of AMP are mildly increased [85]. Few others also revealed a raised expression of glucose transporter 4 in the soleus muscle of STZ-induced diabetic rats upon metformin treatment, consistent with the increase in glucose uptake by skeletal muscles. In diabetic mice, metformin therapy decreased atherosclerosis by reducing endothelial mitochondrial fission and the production of mitochondrial-derived superoxide [86]. Some researchers have shown that metformin works by inhibiting ETC complex I, leading to reduced OXPHOS activity [87, 88]. It is crucial to have a systems approach when understanding how metformin improves the state of T2DM by blocking multiple targets throughout organ systems.
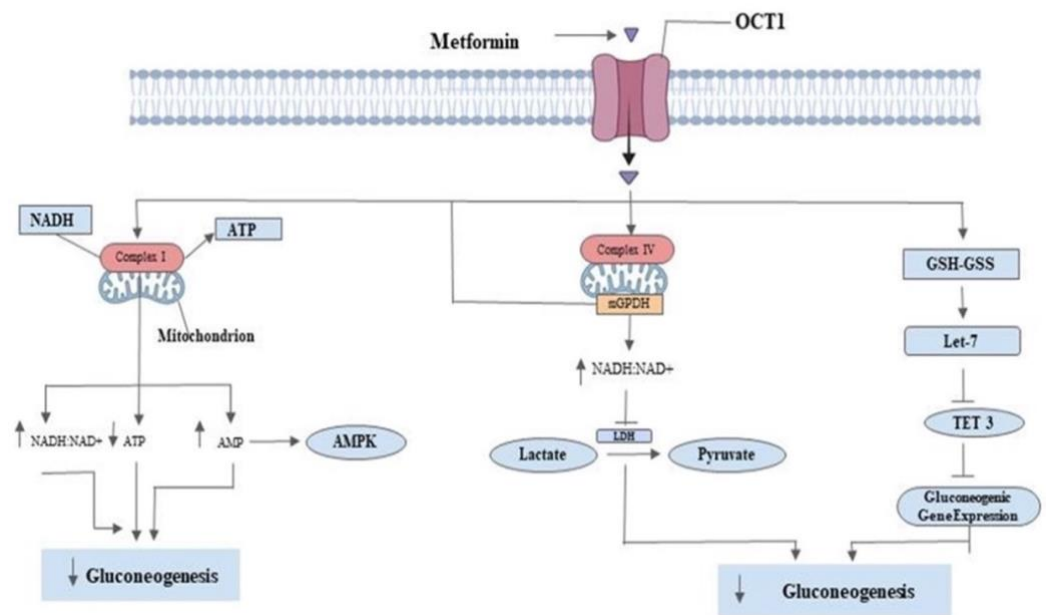
Figure 2. Potential mechanism of mitochondria-targeted metabolic therapy through metformin-induced reduction of blood glucose level. Metformin inhibits mitochondrial respiratory chain complex I in the liver, causing a decrease in ATP synthesis and an increase in cellular levels of AMP. This decrease in hepatic gluconeogenic flux is ATP-dependent and inhibits enzymes involved in gluconeogenesis. Metformin also increases cellular redox potential and inhibits *mitochondrial glycerol-3-phosphate dehydrogenase* (mGPDH)-dependent and complex IV inhibition-dependent mechanisms. Metformin directly inhibits mGPDH, resulting in an increased cytosolic redox state and reduced gluconeogenesis activity. It also raises the hepatic redox state through an increase in the glutathione to oxidized glutathione ratio (GSH -GSS), inhibiting genes encoding enzymes involved in gluconeogenesis. LDH: *lactate dehydrogenase;* OCT1: organic transporter 1; TET3: *Tet methylcytosine dioxygenase*; AMPK: *AMP-activated protein kinase*.

Mitochondria-targeted antioxidant therapy

Oxidative stress in mitochondria significantly contributes to mitochondrial dysfunction in several organ systems of people with T2D. The increase in ROS in diabetic mice is linked to changes in both mitochondrial structure and redox systems biology. The Clone 9 rat liver cell line and H9c2 rat myoblasts experience mitochondrial fragmentation via dynamin-like GTPase DLP1/Drp1 in response to high-glucose therapy, which is essential for and occurs before ROS overproduction [89]. Anderson and colleagues discovered a significant connection between a high-fat diet and elevated skeletal muscle mitochondria H_2O_2 emission, a more oxidized cellular redox state, and insulin resistance in their research [90]. Further studies demonstrated that mitochondrial H_2O_2 production significantly affected the intracellular redox environment. They achieved this by administering the mitochondrial H_2O_2 scavenger SS31 to rats fed standard chow and a high-fat diet. They observed that there was no alteration in oxidized glutathione levels or the ratio of reduced glutathione to oxidized glutathione after acute glucose intake. Yet, these levels were also high and low for both the high-fat and low-fat groups of rats regardless of the diet they were on [90]. The effects of mitochondria on insulin resistance in T2DM through redox biology have been investigated in another study [12]. These results have attracted the interest of several organizations to study the efficiency of mitochondria-targeted antioxidants, such as mitoquinone [MitoQ], in diabetic animals. Escribano-Lopez and his colleagues found a reduction of ROS, leukocyte-endothelium interactions, and TNFα amount in leukocytes in T2DM patients who were treated with MitoQ (Figure 3) [91]. The research showed

that it improved systemic insulin sensitivity and reduced the pancreatic islet lipid peroxide level in obese mice fed with a high-fat diet [92].
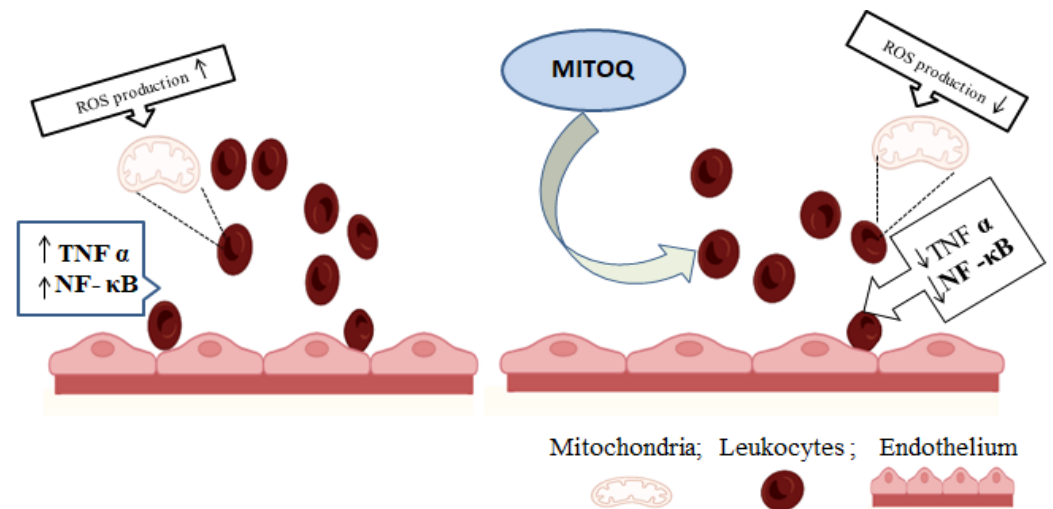

Figure 3. Mitochondria-targeted antioxidants therapy. Leukocyte-endothelium interactions in T2D patients can be regulated by antioxidants that target the mitochondria, such as Mitoquinone (MitoQ). When diabetic leukocytes were exposed to MitoQ, their generation of ROS from mitochondria was decreased. Treatment with MitoQ decreased both TNFα and NFκB-p65, which had increased in T2D. ROS: Reactive Oxygen species; TNF α: Tumor necrosis factor α; NF-κB: nuclear factor kappa Beta; MitoQ: mitochondria-targeted ubiquinone.

LIMITATION AND FUTURE PERSPECTIVE

Though mitochondria-targeted therapies show great promise in the management of type 2 diabetes, there are several unresolved issues. First of all, there is a big problem with the intricacy of mitochondrial biology and how it interacts with other cellular functions [93]. In addition to producing energy, mitochondria are also involved in apoptosis and the creation of ROS. It's difficult to target these pathways without having unforeseen consequences.

An additional degree of complexity is created by the variability of T2DM individuals with regard to their genetic backgrounds, the course of their illness, and environmental variables. Tailored strategies are important to accommodate these variations; yet, their widespread implementation poses challenges. Due to the potential impact of each patient's distinct genetic composition on how well they respond to mitochondrial medicines, customized treatment regimens that take into account individual differences in nuclear and mitochondrial DNA as well as how they interact are required. In addition, lifestyle variables including nutrition, exercise, and exposure to pollutants in the environment might affect mitochondrial function and the general effectiveness of targeted treatments [94]. Consequently, it is doubtful that a one-size-fits-all strategy would work, and creating individualized treatment plans is crucial but logistically difficult.

In addition, there is still no evidence of the long-term outcome of using mitochondria-targeted treatments for patients. While it is clear that there may be some short-term benefits, there is little proof that there may be long-term detriment as well. For instance, preventing the formation of ROS in the mitochondria can have negative impacts on other cellular signaling processes. Similarly, in some conditions, increasing the level of mitochondria can lead to unwanted, undesirable hyperplasia or even carcinomas [95].

Thus, to avoid such limitations, future research should focus on a number of significant directions. Most importantly, the design of elaborate delivery systems will be compulsory to guarantee that the delivery of mitochondria-targeted compounds occurs selectively and, in a tissue-specific manner. Possible tools for increasing the effectiveness of such treatments can be extended by mitochondrial-targeting peptides and nanotechnology [9]. Second, further investigations need to be conducted to delineate the safety and efficacy of these therapies in the long run. Some examples of this include extensive preclinical investigations and carefully designed clinical trials. Furthermore, other courses of treatment could also benefit from the use of mitochondria-targeted medicine such that synergistic advantages could be obtained. For example, advocating for these treatments alongside dietary changes, exercise routines, or other lifestyle modifications could enhance the therapeutic impact of the treatments as well as metabolic well-being. In addition, examining mitochondrial genetic and epigenetic features in T2DM patients will help design patients' personalized treatments and explain differences in treatment effectiveness [96]. Thus, it can be stated that mitochondria-targeted therapies have much potential for the treatment of T2DM, but their deployment in clinical practice as a part of a patient's comprehensive treatment schedule is contingent on the existing challenges being addressed by innovative approaches and individually tailored models.

CONCLUSION

This review provides a persuasive argument for the creation and implementation of treatments that specifically target mitochondria. It emphasizes the substantial influence of mitochondrial dysfunction on the development of T2DM. The study highlights the potential of these medicines to address the numerous issues associated with T2DM by investigating the role of mitochondrial alterations in insulin resistance and metabolic dysregulation. Furthermore, the investigation of mitochondrial dynamics, epigenetics, and encouraging therapy outcomes provides a positive perspective for alleviating the impacts of this persistent ailment. It is strongly recommended that more research be conducted to further improve and expand upon these therapeutic options, with the ultimate objective of incorporating mitochondria-centric therapies into the broader spectrum of diabetes care.

AUTHOR CONTRIBUTION

SS, SMBUI, SSS, and TA conceived the idea and prepared the outline of the review. TA, AH, and NJ performed the literature search and data extraction, analysis of extracted data, and manuscript preparation. SS, SMBUI, and SSS supervised the manuscript

preparation and prepared the final draft. The authors read and accepted the final version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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