REVIEW ARTICLE

Targeting Mitochondrial Dysfunction for the Treatment of Diabetic Complications: Pharmacological Interventions through Natural Products

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ABSTRACT

Diabetes mellitus is a chronic hyperglycemic condition with deleterious effects on microcirculation, resulting in diabetic complications. Chronic hyperglycemia induces the generation of reactive oxygen species (ROS), which are the key pathological triggers in the development of diabetic complications. ROS are responsible for the activation of various pathways involved in the genesis of diabetic complications, mitochondrial dysfunction, as well as insulin resistance. The review describes normal mitochondrial physiology and abnormal alterations, which occur in response to hyperglycemia. Mitochondrial biogenesis is a highly regulated process mediated by several transcription factors, wherein mitochondrial fusion and fission occur in harmony in a normal healthy cell. However, this harmony is disrupted in hyperglycemic condition indicated by alteration in functions of essential transcription factors. Hyperglycemia-induced mitochondrial dysfunction plays a key role in diabetic complications, pancreatic β-cell dysfunction, as well as skeletal muscle insulin resistance as demonstrated by various *in vitro*, preclinical, and clinical studies. The review focuses on the various factors involved in mitochondrial biogenesis and maintenance of healthy mitochondrial function. Several phytoconstituents act through these pathways, either directly by stimulating biogenesis or indirectly by inhibiting or preventing dysfunction, and produce a beneficial effect on overall mitochondrial function. These phytoconstituents have enormous potential in amelioration of diabetic complications by restoring normal mitochondrial physiology and need detailed evaluation by preclinical and clinical studies. Such phytoconstituents can be included as nutraceuticals or adjuvant therapy to the mainstream treatment of diabetes.

Key words: Diabetic complications, mitochondrial dysfunction, natural products, phytoconstituents, reactive oxygen species

INTRODUCTION

Diabetes mellitus is a serious chronic noncommunicable disease that is growing at an enormous rate worldwide. It is responsible for reduced life expectancy and increased morbidity due to proliferation of diabetic vascular complications. The major concern associated with diabetes mellitus is the development of chronic complications associated with the disorder, which have been classified into two broad categories, namely, macrovascular complications (cerebrovascular diseases, peripheral vascular diseases, and cardiovascular diseases) and microvascular complications (nephropathy, neuropathy, and retinopathy).[1] Chronic hyperglycemia-induced generation of reactive oxygen species (ROS) has been identified as a central pathognomic mechanism in the development of these diabetic complications. Imbalance between overproduction of ROS and diminished antioxidant defense is responsible for end-organ damage.[2] Accumulating evidence suggests that overproduction of ROS causes mitochondrial dysfunction and insulin resistance.[3] However, the relationship between ROS, mitochondrial dysfunction, and insulin resistance represents a "catch-22" situation, wherein one factor ultimately leads to another and vice versa. Several lines of evidence suggest that "mitochondrial medicine," i.e., agents that stimulate mitochondrial metabolism and/or decrease mitochondrial dysfunction, is beneficial in ameliorating insulin resistance as well as diabetic complications. This review discusses the key players involved in the disorder: ROS, mitochondrial dysfunction, and insulin resistance as well as their pharmacological interventions through natural products.

ROLE OF MITOCHONDRIA IN THE DEVELOPMENT OF DIABETIC COMPLICATIONS

Mitochondria are multifunctional in nature playing a key role in regulating metabolic activity. So far, considerable evidence has been generated linking diabetes and mitochondria in various models. The primary function of mitochondria is to generate cellular energy, i.e., adenosine triphosphate (ATP), and they have been appropriately termed as "powerhouses" of the cell. Mitochondria not only produce cellular energy but are also major intracellular site of oxidant production as well as targets of ROS.[4] It has been observed that the normal mitochondrial physiology is altered in diabetic condition, wherein glucose overload causes generation of ROS resulting in mitochondrial dysfunction.[5] In nondiabetic conditions, glucose-derived pyruvate

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is metabolized through tricarboxylic acid (TCA) cycle and generates electron donors; NADH and $FADH_2$. NADH donates electrons to Complex I and $FADH_2$ donates electrons to Complex II of mitochondrial electron transport chain (ETC). Electrons from both these complexes are transported to coenzyme Q, which are then further transported through series of electron acceptors, namely, Complex III, cytochrome-C, Complex IV, and finally to molecular oxygen which is eventually reduced to water. As the electrons are transferred from left to right of ETC, energy of these electrons is used to pump protons across the membrane at Complex I, III, and IV generating a voltage gradient across the mitochondrial membrane. This voltage gradient is the driving force for ATP synthesis by ATP synthase.[4] However, in diabetic conditions, increased intracellular glucose flux is observed in tissues independent of insulin regulation, such as retinal cells, epithelial cells, and renal cells. In these cases, high levels of glucose enter the TCA cycle generating elevated levels of electron donors; NADH and $FADH_2$ that are pushed into the ETC. As a result, the voltage gradient across the mitochondrial membrane increases until a critical threshold is achieved. This blocks the electron transfer at Complex III causing the electrons to accumulate at coenzyme Q, which donates the electrons one at a time to molecular oxygen, thereby generating superoxide anions. Mitochondria initially generates superoxide (O_2^-) free radical which is then converted to more reactive species, such as hydroxyl (OH^-), peroxyl (RO_2^-), alkoxyl (RO), and hydroperoxyl (HO_2^-) that cause cellular damage in numerous ways. Overproduction of superoxide by mitochondrial ETC has been identified as the basis of hyperglycemia-induced diabetic complications. Hyperglycemia-induced mitochondrial superoxide causes DNA strand breakage, which in turn activates poly-ADP-ribose polymerase (PARP). PARP inhibits the activity of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a key glycolytic enzyme, thereby increasing levels of all glycolytic intermediates that are upstream of glyceraldehyde 3-phosphate. Subsequently, these accumulated glycolytic intermediates enter one of the four pathways, namely, polyol pathway, advanced glycation end product (AGE) formation and RAGE ligand binding, hexosamine shunt pathway, and protein kinase C (PKC) pathway. The triggering of these alternate signaling pathways causes deleterious irreversible changes in the biological system leading to end-organ damage.[5,6] Therefore, agents which inhibit the generation or scavenge mitochondrial superoxide and/or inhibit PARP may prove to be beneficial in preventing the development of hyperglycemia-induced diabetic complications, which is further discussed in detail.

Mitochondrial biogenesis

To completely recognize the involvement of mitochondria in diabetes, it is important to first understand the physiology of mitochondrial biogenesis. Mitochondrial biogenesis is defined as regeneration of mitochondria, a process essential in maintaining its integrity. New mitochondria are synthesized from existing mitochondria by two highly regulated processes, namely, fusion and fission. Mitochondrial fusion involves joining of membranes and compartments to yield a single larger mitochondrion. Mitochondrial fission is a process identical to cell division, wherein a mitochondrion elongates and increases in volume before septation giving rise to two physically distinct mitochondria.[7] Under normal physiological conditions, there is a dynamic balance between fission and fusion in mammalian cells. Disruption of this fission–fusion balance alters mitochondrial morphology; excessive fission produces fragmented mitochondria, whereas excessive fusion results in elongated mitochondrial tubules.[8] Nonetheless, these processes occur in a harmonic manner in a healthy cell. The mammalian mitochondrial fusion machinery comprises mitofusin 1 (Mfn1) and Mfn 2, a class of nuclear-encoded dynamin-related guanosine triphosphate (GTPase). Mitofusins are proteins responsible for tethering and fusion of

outer mitochondrial membrane. Fusion of inner mitochondrial membrane (IMM) is regulated by another dynamic family GTPase, namely, optic atrophy 1 (OPA1). Mitochondrial fission is regulated by mitochondrial fission machinery comprising dynamin-related protein 1 (Drp1), a type of dynamin-related GTPase, and fission protein 1(Fis1). Fis1 recruits Drp1, a cytosolic protein, to scission sites on outer mitochondrial membrane to induce mitochondrial fission.[9] Mitochondrial fission is not only vital for mitochondrial biogenesis but also operates as mitochondrial waste disposal system; a process vital for maintaining mitochondrial respiration. Several studies indicate that mitochondrial fission produces mitochondria with abnormal membrane potential, and these damaged organelles are removed through autophagy; a process termed as mitophagy for mitochondria. The Pink1 protein, a mitochondrial serine-threonine kinase, phosphorylates ubiquitin and Parkin to activate Parkin's E3 ligase activity, thus tagging mitochondria with abnormal membrane potential for ubiquitination by Parkin. Parkin ubiquitinates substrate on outer mitochondrial membrane and recruits an autophagosomal membrane to the mitochondrion. Ultimately, the autophagosome fuses with lysome resulting in degradation and recycling of the contents. Mitophagy is also one of the pathways which suppress mitochondrial DNA (mtDNA) mutations.[7] Interestingly, in hyperglycemic conditions, the expression and protein content of mitochondrial fission proteins, Drp1 and Fis1, are increased, whereas that of fusion proteins, Mfn1, Mfn2, and OPA1 are decreased suggesting that normal mitochondrial biogenesis is disrupted in hyperglycemic conditions.[10] Several lines of evidence suggest that peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) is a major regulator of mitochondrial biogenesis. Sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide (NAD^+)-dependent histone deacetylase, deacetylates and activates PGC-1α. PGC-1α, in conjunction with a network of transcription factors, helps coordinate the expression of genes involved in aerobic metabolism. PGC-1α regulates the expression of several nuclear genes coding for mitochondrial enzymes. These in turn stimulate the expression of other transcription factors involved in the coordinated expression of mitochondrial genes, such as nuclear respiratory factor-1 (NRF1) and NRF2. NRF1 and NRF2 trigger the expression of nuclear genes coding for polypeptides of the respiratory chain and proteins involved in transcription and replication of mtDNA. In addition, the expression of nuclear genes coding for subunits of the oxidative phosphorylation (OXPHOS) system is also transcriptionally regulated by NRF1 and NRF2. They also regulate the expression of many other genes involved in mtDNA replication through binding to the consensus sequences in the promoters of the OXPHOS genes in the nucleus. Mitochondrial transcription factor A (mtTFA) is a transcription factor that regulates the replication and transcription of mitochondrial genome by acting on the promoters within the D-loop region of mtDNA. It has been evinced that the mtTFA gene carries consensus binding sites for both NRF1 and NRF2, which provide a unique mechanism for the cell to integrate the expression of nuclear DNA-encoded proteins with the transcription of genes encoded by mtDNA.[11-14]

Effect of hyperglycemia on mitochondria

Mitochondria are powerhouses of cell which generate energy for almost all cellular processes and are main oxygen consumers of the body. Therefore, optimal mitochondrial function depends on appropriate utilization of oxidative substrate and ATP generation. There exists a possibility of defective oxidative metabolism due to abnormalities in mitochondrial biogenesis in patients with diabetes mellitus. A study in nondiabetic, insulin-resistant, and prediabetic (at high risk of diabetes) individuals demonstrated that expression of gene involved in OXPHOS mainly regulated by NRF1- and NRF2-dependent transcription was significantly decreased in insulin-resistant and prediabetic

individuals as compared to nondiabetic individuals. There was also significant reduction in expression of PGC-1α, the master regulator of mitochondrial biogenesis.[15] Several studies have also provided evidence that hyperglycemia-induced ROS from mitochondria are the major cause of hyperglycemic complications. Effect of hyperglycemic environment on mitochondrial morphology was investigated by Yu *et al*. They demonstrated that exposure to hyperglycemia induces rapid mitochondrial fragmentation by activation of mitochondrial fission machinery through signals mediated by intracellular Ca^{2+} and extracellular signal-regulated kinases 1/2. Initial study involved exposure of rat liver cell line and cardiac myoblast cell line to high levels of glucose, which resulted in increased formation of mitochondrial ROS measured by difluorescein and dihydroethidium fluorescent dyes. Hyperglycemia-induced ROS production was accompanied by a marked change in mitochondrial morphology, wherein mitochondria exhibited fragmentation mediated by mitochondrial fission machinery. Furthermore, exposure of cells to stereoisomer L-glucose which cannot be transported and metabolized to pyruvate by the cells did not increase ROS production or induce mitochondrial fragmentation. This proves that glucose metabolism is essential for change in mitochondrial morphology and ROS generation. Furthermore, it was also observed that inhibition of mitochondrial pyruvate uptake that blocked ROS increase did not prevent mitochondrial fragmentation in high-glucose conditions, suggesting that mitochondrial pyruvate uptake is necessary for hyperglycemia-induced ROS production but not for mitochondrial fragmentation[16,17] Further studies demonstrated the possible mechanism of mitochondrial fragmentation in response to high glucose. Hyperglycemia causes opening of mitochondrial permeability transition pore, which induces the release of outer membrane permeabilization proteins and apoptotic factors such as cytochrome-C and caspase, which result in apoptotic cell death. The study also established that inhibition of mitochondrial fission and promotion of mitochondrial fusion prevented increased ROS formation in hyperglycemic conditions. These findings suggest that mitochondrial fission is an upstream factor that regulates mitochondrial ROS production during hyperglycemia-induced cell death.[18] Therefore, targeting mitochondrial fission machinery can be a novel target to attenuate ROS-mediated complications in hyperglycemia.

Many studies have elucidated a link between oxidative stress and pancreatic β-cell damage. It has been observed that the pancreatic β-cell mitochondrion of patients with Type 2 diabetes mellitus (T2DM) exhibits morphological abnormalities characterized by hypertrophic round shape as compared to elliptical slim shape in normal individuals. There are several mechanisms by which ROS induce pancreatic β-cell mitochondrion dysfunction. Cardiolipin, a mitochondrial phospholipid localized in the inner mitochondrial membrane (IMM) that regulates mitochondrial bioenergetics, may undergo oxidation in the presence of ROS. This in turn causes destabilization of cytochrome-C which is anchored to the outer surface of the IMM by electrostatic and hydrophobic interactions with cardiolipin. This causes detachment of cytochrome-C from the membrane which is then released into the cytoplasm through pores in the outer membrane causing activation of caspases and subsequent apoptosis.[19-21] Activation of uncoupling protein 2 (UCP2) by ROS is another mechanism of β-cell dysfunction. UCP2, a member of the mitochondrial anion carrier protein family, regulates the membrane potential of pancreatic islet β-cell mitochondria. UCP2 promotes proton leak to reduce the mitochondrial membrane potential (MMP) and thus attenuates ATP synthesis. It has been reported that UCP2 negatively regulates insulin secretion.[22,23] Obesity and chronic hyperglycemia increase mitochondrial superoxide production,[24] causing activation of UCP2 ultimately resulting in pancreatic islet β-cell dysfunction.[25-28] Pancreatic β-cell mitochondria constantly undergo fusion and fission which govern the overall morphology of the organelle. The effect of

various nutrients on pancreatic β-cell mitochondrial fusion and fission was investigated by Molina *et al*. Their results suggested that impaired mitochondrial fusion and fission plays a vital role in nutrient-induced β-cell apoptosis, which may be a key factor in pathogenesis of T2DM.[29]

Several studies establish link between diabetes mellitus and mitochondrial dysfunction in skeletal muscle. Impaired mitochondrial function in the skeletal muscle is implicated in the development of insulin resistance. Kelley *et al*. first demonstrated that T2DM is associated with mitochondrial dysfunction in skeletal muscle indicated by impaired activity of marker enzymes of oxidative pathways. Patients with T2DM had lower activity of NADH: O$_2$ oxidoreductase and citrate synthase compared with obese and lean control individuals. NADH: O$_2$ oxidoreductase is indicative of overall ETC function and citrate synthase reflects mitochondrial content. Further, in the same study, electron microscopy revealed that mitochondria were smaller and fractured in obese and T2DM patients compared to lean controls and mitochondrial longitudinal area correlated positively with insulin sensitivity.[30] Stump *et al*. demonstrated that mitochondrial respiration was decreased in skeletal muscle isolated from patients with T2DM as compared to controls. They studied the effect of insulin on skeletal muscle substrate metabolism and ATP production. The results demonstrated that in healthy control individuals, insulin infusion increased muscle mitochondrial ATP production capacity, muscle mitochondrial protein synthesis, cytochrome-C oxidase (COX), and citrate synthase enzyme activities along with the increase in mRNA levels of both mitochondrial (NADH dehydrogenase subunit IV) and nuclear (COX subunit IV) proteins. However, these effects were not observed in skeletal muscle of T2DM patients, indicating an impairment of muscle mitochondrial energy metabolism in diabetes. Another study demonstrated that coupling efficiency of OXPHOS in the skeletal muscle of both humans and rats was increased by insulin treatment along with the decrease in mitochondrial proton leak. Together, these findings provide evidence that insulin not only acts as a predominant postprandial anabolic hormone but also acts an important regulator of muscle mitochondrial OXPHOS.[31]

Therefore, these data support the hypothesis that impaired mitochondrial oxidative capacity and/or mitochondrial function may be early factor(s) in the pathogenesis of diabetes. One factor which might contribute to the reduced mitochondrial function in insulin-resistant skeletal muscle of T2DM is PGC-1α. As discussed previously, PGC-1α is responsible for transcription of NRF1 and 2, which regulate the transcription of genes involved in oxidative metabolism. NRF1 and 2 also stimulate mtTFA, a key transcriptional factor for mitochondrial genome. DNA microarray studies have demonstrated that PGC-1α and expression of genes involved in OXPHOS under control of PGC-1α are decreased in skeletal muscle of T2DM and offsprings of T2DM patients. This provides a link between PGC-1α polymorphisms and increased risk of diabetes. These data suggest that reduced level of PGC-1α in insulin-resistant state might decrease mitochondrial function.[15,32-34] This hypothesis was confirmed when roziglitazone, a peroxisome proliferator-activated receptor γ (PPARγ) agonist, markedly restored PGC-1α expression and improved insulin sensitivity as well as metabolic function, thus substantiating the major function of PGC-1α in Type 2 diabetes.[35]

PHARMACOLOGICAL INTERVENTIONS THROUGH NATURAL PRODUCTS

Natural products acting on mitochondrial targets

Sirtuin 1 activators

SIRT1 is a NAD$^+$-dependent histone deacetylase that is involved in the regulation of glucose and lipid metabolism through it deacetylase activity

on many substrates. It regulates insulin secretion in pancreatic cells and also positively affects the metabolic pathway through modulation in insulin signaling. SIRT1 activation serves as a calorie restriction mimetic and is considered as a potential therapeutic target. Other than glucose-lipid metabolism, SIRT1 regulates a wide array of cellular functions such as mitochondrial biogenesis, inflammation, autophagy, and circadian rhythms.[36] Resveratrol is a phytoalexin produced by many plants in response to injury and is a major constituent of red wine. Resveratrol activates SIRT1, causing deacetylation of PGC-1α at promoter regions to induce expression of genes involved in fatty acid oxidation, OXPHOS, and mitochondrial biogenesis. Resveratrol treatment protected mice from high-fat diet-induced obesity and insulin resistance.[37] A study conducted by Desquiret-Dumas *et al.* demonstrated that resveratrol mediated activation of SIRT in hepatocytes is dependent on NADH oxidation. Resveratol increases mitochondrial NAD+ level by stimulation of Complex I which further activates SIRT3.[38] UCPs are present on IMM and mediates adaptive thermogenesis. Resveratrol activates UCP in hepatocytes (UCP2) as well as skeletal muscle (UCP2, UCP3), which increases mitochondrial β-oxidation of fatty acids and OXPHOS of pyruvate, leading to increase in energy expenditure, thereby decreasing glucose and lipid levels.[39] Fisetin is a naturally occurring tetrahydroxy flavonol found in many vegetables and fruits such as strawberries and is known to have antiaging, anticancer, and antiviral activities. It has demonstrated antidiabetic effect in animal models of diabetes. Maher *et al.* demonstrated that fisetin prevents diabetic nephropathy in Akita mouse model of Type 1 diabetes. Fisetin treatment increased the level and activity of glyoxalase 1, a key enzyme involved in detoxification of AGE precursor methylglyoxal and also increased the synthesis of glutathione, an essential co-factor for glyoxalase-1, thus preventing AGE pathway-related diabetic complications.[40] Kim *et al.* also demonstrated that fisetin increases SIRT1 expression and inhibits early adipogenesis in 3T3-L1 cells.[41] Omega-3-fatty acids were found to be effective in reversing the reduction in SIRT levels in rats with mild traumatic brain injury.[42] Cohen *et al.* demonstrated the stimulatory effect of five plant polyphenols, namely, butein, piceatannol, fisetin, quercetin, and resveratrol on SIRT1 catalytic rate in yeast.[43] Apo-10'-lycopenoic acid, a metabolite of lycopene, decreased hepatic fat accumulation in ob/ob mice by increasing SIRT1 mRNA and protein levels.[44] Curcumin ameliorated the neurotoxicity of amyloid β 25-35 in rat cortical neurons and also attenuated mitochondrial oxidative damage induced by myocardial ischemia reperfusion injury by SIRT1 activation.[45] Thus, these phytoconstituents with SIRT1 activation potential can be further evaluated for their protective effect on mitochondria in animal models of diabetes mellitus.

Peroxisome proliferator-activated receptor gamma coactivator-1α activators

Dietary low-dose supplementation of quercetin prevented high-fat diet-induced insulin resistance in the skeletal muscle and increased PGC-1α expression in the skeletal muscle.[46] Rayamajhi *et al.* demonstrated that quercetin treatment increased the expression of mitochondrial biogenesis activators (PGC-1α, NRF1, mtTFA), mtDNA, and COX IV. These effects were mediated through activation of heme oxygenase-1 (HO-1), a cytoprotective inducible enzyme.[47] Study conducted by Shen *et al.* demonstrated that combination of α-lipoic acid (LA) and acetyl-L-carnitine (ALC) synergistically promoted mitochondrial biogenesis by the activation of PGC-1α in murine 3T3-L1 adipocytes. This was accompanied with an increase in mitochondrial mass, expression of mtDNA, mitochondrial ETC complexes, mitochondrial oxygen consumption, and fatty acid oxidation. Expression of PPARα, PPARγ, carnitine palmitoyl transferase 1α, mtTFA, and NRF1 and 2 was also increased. However, these effects were not observed with LA or ALC treatment alone at the same concentration, suggesting that the combination acts as PPAR α/γ dual agonist to synergistically promote mitochondrial biogenesis.[48] Shen *et al.* further evaluated a combination of nutrients, namely, α-LA, ALC, nicotinamide, and biotin in Type 2 diabetic Goto-Kakizaki rats. They demonstrated that the combination improved glucose tolerance and decreased basal insulin secretion and circulating levels of free fatty acids. In addition, this nutrient combination also promoted mitochondrial biogenesis through activation of PGC-1α as well as transcription factors NRF1 and mtTFA.[49] Hydroxytyrosol (HT) is a polyphenol, a potent antioxidant and the major constituent of olive oil. In 3T3-L1 adipocytes, HT stimulated mitochondrial biogenesis by activation of PGC-1α and also increased expression of its downstream targets including NRF1 and 2, mtTFA, mtDNA, and number of mitochondria. Knockdown of PGC-1α by siRNA blocked HTs stimulating effect on Complex I expression and mtDNA copy number, indicating that the ability of HT to restore mitochondrial function is indeed mediated by activation of PGC-1α. The treatment also increased mitochondrial function, including an increase in activity and protein expression of mitochondrial complexes I, II, III, and V; increased oxygen consumption; and decreased free fatty acid contents in the adipocytes. The study demonstrated that PGC-1α activation was mediated by adenosine monophosphate activated protein kinase (AMPK) pathway which is described further in detail in the following section.[50]

Natural products acting on miscellaneous targets

Poly-ADP-ribose polymerase-1 inhibitors

PARP-1 inhibitors have been widely explored as strategy for cancer treatment; however, its usefulness in the treatment of diabetic complications is an area which remains largely untraced and can be investigated.[51] As mentioned previously, hyperglycemia increases the flux of electron donors through ETC which ultimately increases mitochondrial ROS production. ROS overproduction causes DNA fragmentation and activation of nuclear enzyme PARP. PARP is a nuclear enzyme implicated in cellular response to DNA injury, and it catalyzes the transfer of ADP-ribose units from β-NAD+ to acceptor proteins. Upon binding to damaged DNA, PARP-1 gets rapidly activated and cleaves NAD+ to ADP-ribose and nicotinamide, thus promoting DNA repair and interaction with several transcription factors.[52] PARP also inhibits glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a key enzyme in glycolytic pathway, thus increasing levels of all glycolytic intermediates upstream of GAPDH. Increased level of the upstream glycolytic metabolite glyceraldehyde-3-phosphate activates the four pathways implicated in the pathogenesis of diabetic complications, namely, AGE pathway, hexosamine shunt pathway, PKC pathway, and polyol pathway.[5,6] In several disease conditions, hyperactivation of PARP-1 causes poly (ADP-ribose) accumulation and exhaustion of NAD + and ATP, which may result in caspase-independent apoptotic or necrotic cell death.[53] Interestingly, PARP-1 is also recognized as a coactivator of nuclear factor-κβ (NF-κβ) and plays a pathognomonic role in complications diabetes mellitus.[54] In a particular study, when endothelial cells and aortic rings from PARP +/+ and PARP −/− mice were incubated in high-glucose conditions, it was found that PARP +/+ mice demonstrated metabolic suppression and loss of endothelium-dependent relaxant function as compared to PARP −/− mice.[55] In streptozotocin-induced diabetic mice, *in vivo* treatment with PJ34, a phenanthridinone PARP inhibitor, prevented the genesis of diabetic endothelial dysfunction and also reversed the condition.[56] Deficiency of PARP-1 gene was capable of alleviating diabetic nephropathy in streptozotocin-induced mice.[57] These studies confirm the important role of PARP in the development

of diabetic complications and recommend its inhibition to be an essential pharmacological intervention. Several natural compounds have been shown to inhibit PARP. Caffeine and its metabolites, 1,7-dimethylxanthine, 3-methylxanthine, 1-methylxanthine, as well as theobromine and theophylline showed significant PARP inhibitory activity. 1,7-dimethylxanthine, a major metabolite of caffeine, significantly inhibited PARP-1 in cultured endothelial cells and epithelial cells.[58] Effect of dietary flavonoids on PARP-1 inhibition *in vitro* was measured by ELISA assay. Dietary flavonoids such as myricetin, tricetin, gossypetin, delphinidin, quercetin, and fisetin significantly inhibited PARP *in vitro*. In the same study, treatment of A549, human pulmonary epithelial cells with N-methyl-N′-nitro-N-nitrosoguanid ine, a mutagen, enhanced formation of PARP polymers as measured by immunohistochemical staining and decreased cellular NAD+, indicating increase in cellular oxidative stress. Quercetin, fisetin, and tricetin decreased the formation of PARP polymers and increased NAD + levels in human pulmonary endothelial cells. The phytoconstituents also decreased lipopolysaccharide-stimulated release of IL-8 from the human pulmonary epithelial cells demonstrating an anti-inflammatory effect.[59] However, the role of these phytoconstituents in diabetes and its complications has not been explored and needs thorough evaluation. These phytoconstituents have enormous potential and can be subjected to detailed evaluation in cellular and animal models of diabetes.

5′adenosine monophosphate-activated protein kinase activators

Phytoconstituents activate AMPK by inhibition of mitochondrial ATP synthesis by inhibition of either Complex I or ATP synthase (Complex V) of mitochondrial ETC. Recent research has focused on inhibition of Complex I of ETC for treatment of diabetes and related complications. Inhibition of Complex I of ETC decreases proton-driven synthesis of ATP and increases AMP: ATP ratio which causes allosteric activation of 5'-AMPK, a serine-threonine kinase. AMPK is a metabolic sensor which is activated when cellular energy levels are low (i.e., intracellular AMP: ATP ratio is high).[60] Upon activation, AMPK signals to restore the normal energy levels by stimulating processes that generate ATP (such as glycolysis, fatty acid FA oxidation) and inhibiting those that use ATP (such as gluconeogenesis, triglyceride, and protein synthesis) by its action on various downstream substrates. Therefore, AMPK activation improves insulin sensitivity and glucose homeostasis, making it an attractive target for T2DM and metabolic syndrome. As previously mentioned, AMPK also regulates the activity of PGC-1α, a key regulator of mitochondrial biogenesis.[61] Several phytoconstituents activate AMPK and therefore may have a beneficial role in the treatment of T2DM. Salicylates are one of the oldest medicines to be used by humans. Salicylates are secondary metabolite produced by plants in response to pathogen infection. Hawley *et al.* reported that salicylates but not aspirin cause allosteric activation of AMPK. Salicylates also inhibit the dephosphorylation of Thr172 phosphorylation site (active site of AMPK). In addition, the beneficial effects of salicylates to increase fat uptake and lower plasma fatty acids were not observed in AMPK knockout mice, indicating that lipid-lowering effect of salicylate is indeed mediated by AMPK activation.[62] Furthermore, two randomized controlled trials demonstrated that oral salsalate treatment decreased plasma glucose levels and insulin C-peptide and increased plasma adiponectin levels in obese young adults and in patients with impaired fasting glucose and/or impaired glucose tolerance.[63,64] Berbernine, an isoquinoline alkaloid, is a major active constituent of ancient Chinese herb *Coptis chinensis* Franch, widely used in Chinese traditional medicine for treatment of diabetes. Berberine decreased body weight and lipid levels, while improving insulin sensitivity, in the animal models of insulin resistance. Studies conducted on adipocytes and myocytes demonstrated that berberine increased GLUT-4 translocation, reduced

lipid content in adipocytes, and increased expression of genes involved in fatty acid oxidation while decreased expression of genes involved in fatty acid synthesis.[65] Berberine also activated AMPK in the skeletal muscle, hepatocytes, and adipose tissue. Berberine stimulated glucose uptake by increased GLUT-4 translocation through activation of both AMPK and p38 MAPK.[66-68] Berberine mediates these effects through inhibition of Complex I of ETC, thereby increasing AMP: ATP ratio and causing allosteric activation of AMPK.[69] α-LA, a short-chain fatty acid, acts as a powerful antioxidant and is an essential cofactor for mitochondrial respiration. α-LA activates AMPK in the skeletal muscle,[70,71] heart,[72] and endothelium.[73] In addition, *ex vivo* incubation of rat skeletal muscle with α-LA prevented high glucose-or leucine-induced impairments in insulin signaling,[71] skeletal muscle lipid accumulation, and hepatic steatosis in obesity.[74] A study conducted by Shen *et al.* elucidated probable mechanism, by which α-lipoic activates AMPK. According to this study, α-LA activates AMPK by Ca^{2+}/calmodulin-dependent protein kinase (CaMKK)-mediated phosphorylation of Thr172. Further, treatment with α-LA also increased phosphorylation of AMPK substrate, acetyl CoA carboxylase (ACC), at Ser79. Moreover, addition of STO-609, a selective inhibitor of CaMKK, prevented α-LA -mediated AMPK activation and subsequent ACC phosphorylation, confirming that AMPK activation is mediated through stimulation of CaMKK.[75]

Nuclear factor erythroid 2–related factor 2 (Nrf2) activators

Nrf2 has gained considerable attention in recent years. Activation of Nrf2 is one of the most vital cellular defense mechanisms to cope with oxidative stress. It is an essential transactivator of genes responsible for the regulation of gene expression through the promoter antioxidant response element (ARE). It codes for a wide range of genes including NADPH: quinone oxidoreductase, glutathione S-transferases, aldo-ketoreductases, and HO-1.[76] Nrf2-linked gene expression protects the cells against damages induced by oxidative stress, carbonyl compounds, and electrophilic agents. Under normal physiological conditions, Nrf2 resides in cytoplasm and associates with its inhibitor kelch-like ECH-associated protein 1 (KEAP1). KEAP1 mediates rapid ubiquitination and subsequent degradation of Nrf2 by its proteasome. Exposure of cells to oxidative stress causes Nrf2 to dissociate from KEAP1 and translocate into nucleus where it binds to AREs in the genes encoding antioxidant enzymes. However, this redox homeostasis of cell is disrupted under conditions of hyperglycemia-associated chronic oxidative stress. There is substantial experimental evidence which indicates Nrf2 expression is transiently increased in response to acute cytotoxic insult of hyperglycemia, whereas chronic hyperglycemic environment decreases activity of Nrf2 and its downstream antioxidant products.[77,78] Chronic hyperglycemia-induced generation of ROS causes cellular dysfunction and induces mutations in mtDNA. Mutated mtDNA encodes defective subunits of the electron transport complexes, which eventually generate increased superoxide production at physiological concentrations of glucose. This indicates that diabetic complications can occur even after glucose levels are well controlled, a phenomenon termed as "hyperglycemic metabolic memory." Therefore, enhancing natural antioxidant defense system of the body by pharmacological activation of Nrf2 appears to be an attractive strategy for prevention of oxidative stress-induced mitochondrial dysfunction and associated diabetic complications. Bardoxolone methyl is a semi-synthetic triterpenoid based on natural product oleanolic acid and is a potent Nrf2 activator. In a phase 2 clinical trial, bardoxolone methyl was found to significantly increase the mean estimated glomerular filtration rate as compared to the placebo group in patients with moderate to severe kidney disease and T2DM.[79,80] However, the trial was terminated after patients treated with bardoxolone methyl showed higher rate of adverse cardiovascular events.[81] Several preclinical studies have also demonstrated the

importance of Nrf2 activation in the treatment of diabetic complications. Sulforaphane, an organosulfur compound with isothiocyanate group, has been widely explored for its Nrf2 activation potential in diabetes. Sulforaphane is a constituent of cruciferous vegetables such as broccoli, cabbage, and brussel sprouts. It is especially found in high concentrations in broccoli and broccoli sprouts, where it exists as glucosinate precursor, glucorphanin, which is cleaved to sulforaphane by plant myrosinases or microbial hydrolases in gut. Incubation of human microvascular endothelial cells with sulforaphane reversed endothelial dysfunction produced by hyperglycemic conditions. These effects of sulforaphane were mediated by activation of Nrf2. In addition, sulforaphane also prevented hyperglycemia-induced activation of hexosamine and PKC pathways and prevented accumulation of methylglyoxal (glycating agent).[82] Treatment of diabetic rats with sulforaphane improved motor nerve conduction velocity, nerve blood flow, and pain behavior, thus countering multiple manifestations of diabetic neuropathy.[83] In rat insulinoma cells, sulforaphane increased Nrf2 protein in the nuclear fraction and decreased cell death induced by cytokines.[84] Sulforaphane treatment prevented diabetes mellitus-induced endothelial, cardiac, and aortic damage[82,85,86] as well as testicular cell death,[87] by increasing nuclear Nrf2 translocation. Pretreatment with sulforaphane attenuated diabetic nephropathy by reducing glycogen synthase kinase-3β phosphorylation and its subsequent activation, leading to increased Nrf2 signaling.[88] It has been demonstrated that sulforaphane activates the Nrf2 pathway by inducing a modification in the cysteine thiols (C151, C489, C583) of Keap1, allowing nuclear translocation of Nrf2 and subsequent activation of ARE-responsive genes.[89] Curcumin, the major phytoconstituent of *Curcuma longa*, also activates Nrf2 by producing a covalent modification in the cysteine residues of Keap-1 and inhibits Nrf2–Keap1 protein–protein interaction.[90] Curcumin ameliorated high glucose-induced oxidative stress in normal rat kidney tubular cells by Nrf2 activation and upregulation of HO-1[91] and attenuated muscular oxidative stress while improving glucose intolerance in high-fat diet-fed mice.[92] Resveratrol protected pancreas against methylglyoxal-induced toxicity,[93] protected diabetic kidney by attenuating hyperglycemia-induced oxidative stress and renal inflammatory cytokines,[94] and attenuated methylglyoxal-induced insulin resistance in Hep G2 cells, by activation of Nrf2 pathway.[95] Digitoflavone demonstrated antioxidant and anti-inflammatory effects by the activation of Nrf2 pathway in STZ-induced diabetic mice.[96] Many phytoconstituents have been identified as Nrf2 activators. The review by Jiménez-Osorio *et al.* discusses the beneficial role of natural Nrf2 activators in diabetes.[97] Nrf2 has gained wide recognition as a key regulator of antioxidant genes transcription; however, research over the recent years has also revealed the role of Nrf2 in mitochondrial function. Nrf2 regulates production of ROS by mitochondria and NADPH oxidase. It has been proposed that Nrf2 deficiency causes impairment of Complex I activity of mitochondrial ETC due to decreased availability of substrates. This in turn causes increased mitochondrial ROS production due to reverse electron flow from Complex II. Nrf2 is also important in maintenance of MMP, a key indicator of mitochondrial health and metabolic state of the cell. In a healthy cell, MMP is maintained by mitochondrial respiratory chain. It has been demonstrated that OXPHOS is more efficient when Nrf2 is activated; consequently, Nrf2 deficiency results in decreased efficiency of OXPHOS. Furthermore, under conditions of oxidative stress, the increased ROS production is counteracted by Nrf2-dependent transcriptional upregulation of UCP3. UCP3 increases the proton conductance of IMM and thereby decreases ROS production. Nrf2 activation also regulates the import of long-chain fatty acids into mitochondria, thereby enhancing mitochondrial fatty acid oxidation. Several lines of evidence also indicate the involvement of Nrf2 in stimulating mitochondrial biogenesis. Nrf2 stimulates mitochondrial biogenesis by increasing the expression of critical transcription factor and coactivator of mitochondrial biogenesis, namely, NRF-1 and PGC-1α, as well as by enhancing nucleotide biosynthesis. Nrf2 also plays a role in mitophagy, the process by which dysfunctional mitochondria are selectively engulfed by autophagosomes and transported to lysosomes for degradation and subsequent recycling, thereby maintaining mitochondrial integrity, especially under conditions of oxidative stress and inflammation.[98] In addition to these effects, Nrf2 activation protects mitochondria under conditions of oxidative stress by increasing transcription of antioxidants. These data establish a causal link between oxidative stress and protective role of Nrf2 in maintaining mitochondrial function. Therefore, phytoconstituents which activate Nrf2 may promote mitochondrial function and have potential in ameliorating mitochondrial dysfunction associated with diabetes.

CONCLUSION

The history of diabetic therapeutics revolved around decreasing the blood glucose levels and this ideology is being followed even in the present times. However, as evidenced, diabetic complications develop even in the presence of tight regulation of glucose. Therefore, decades of exhausting research have been constantly urging us to break the traditional outlook and search for new avenues to tackle the new age disease. Instead of the symptomatic approach, the root cause of the disease should be alleviated. Several lines of evidence implicate the role of mitochondrial dysfunction in pathogenesis of diabetes and its complications as well as insulin resistance. The relationship between mitochondria and diabetes is complex. Reciprocal causation and interaction make a vicious cycle. Thus, it is necessary to explore mitochondria as a target for treatment as well as for understanding pathophysiology of the disease. Treatment strategies that focus on increasing mitochondrial function could represent important new approaches in the treatment of diabetes. The discovery that each of the four main mechanisms implicated in the pathogenesis of diabetic complications reflects a single hyperglycemia-induced process provides a new conceptual framework for future research, although clinical trials will be necessary to show that the results from cell culture and animal studies are applicable to humans. After witnessing the "other" side of synthetic medications, there has been a growing trend to discover and explore drugs of natural origin in all diseases. It is believed that since phytoconstituents present in plants are a part of the physiological functions of living flora, they are more compatible with human body. Nature has bestowed upon us several phytoconstituents which act through multiple pathways and have a potential in preventing or attenuating mitochondrial dysfunction-induced diabetic complications. Thus, the present data on phytoconstituents open new avenues for the prevention and treatment of diabetic complications and associated mitochondrial dysfunction. These phytoconstituents can be used as nutraceuticals or adjuvant therapy to the mainstream therapy of diabetes. Future clinical trials are warranted to determine the benefits of these phytoconstituents in the amelioration of diabetic complications.

Conflicts of interest

There are no conflicts of interest.

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