Review

Potentially synergistic effects of melatonin and metformin in alleviating hyperglycaemia: a comprehensive review

Adrita Banerjee[1, 2], Aindrila Chattopadhyay[2], Debasish Bandyopadhyay[1*]

[1]Oxidative Stress and Free Radical Biology Laboratory, Department of Physiology, University of Calcutta, 92, APC Road, Kolkata-700009
[2]Department of Physiology, Vidyasagar College, 39, Sankar Ghosh Lane, Kolkata-700006
*Correspondence: debasish63@gmail.com; dbphys@caluniv.ac.in, +91-9433072066

Running Title: Combination of melatonin and metformin as anti-diabetic therapy

Received: August 5, 2021; Accepted: December 9, 2021

ABSTRACT

High level of glucose is hazardous for organisms since it leads to lipid peroxidation, protein glycation and free radical generation. Insulin can lower the high blood glucose by promoting cell's glucose up-taking. Thus, the impeded insulin secretion in type 1-diabetes and insensitivity of cells to insulin in type 2-diabetes cause hyperglycaemia. Hyperglycaemia impairs mitochondrial function of pancreas to trigger ROS generation. The malfunctional mitochondria cause endoplasmic reticulum to produce misfolded non-functional insulin, finally leading to diabetes. Melatonin, the mitochondria targeted antioxidant, provides protection against diabetes by multiple ways. These include balancing cellular redox status, lowering blood glucose level by modulating metabolic pathways and, finally protecting cells/organelles from high glucose induced injury. Moreover, this indoleamine preserves pancreatic physiological normalcy to facilitate insulin secretion. Thus, melatonin can effectively mitigate diabetes and diabetic complications. Metformin, the most prescribed medicine for type 2-diabetes, has similar antidiabetic activities as melatonin. Both the molecules share similar pathways to preserve stress-stricken pancreas and other organs, whereas, melatonin also potentiates the actions of metformin. The potentially synergistic actions of melatonin and metformin are expected and we strongly recommend a combined therapeutic application of these two molecules for treatment of diabetes.

Key words: diabetes, melatonin, anti-diabetic drugs, metformin, synergy

---

1. INTRODUCTION

The continuously increased diabetes over the world is an issue of concern. Oxidative stress due to high fat diet consumption has been identified as a primary factor of type 2 diabetes while impaired pancreas with deficiency of insulin secretion induced by autoimmune reaction is the cause of type 1 diabetes. Both cause hyperglycaemia and glucose toxicity (1-4). This augmented glucose level also leads to excess oxidative stress causing protein glycation and lipid peroxidation and cell death (1, 5). Oxidative stress in pancreas of diabetics further impedes insulin secretion (2). From subcellular vision, mitochondria which are the major sites of reactive oxygen species (ROS) production and glucose metabolism, became hubs of oxidative stress caused by hyperglycaemia (4, 6). To compensate mitochondrial deficiency,

endoplasmic reticulum (ER) works indefatigably causing ER overload stress with subsequent impairment in protein folding process (7). Hence, the seemingly protective action of mitochondria to maximize glucose metabolism appears doing more harm than good.

Several natural and synthetic substances are used to alleviate diabetes and/or its associated tissue damage. Metformin, a biguanide class of antidiabetics, is the first choice for diabetes due to its relatively high tolerance and low side effects (8, 9). Metformin also exhibits antioxidative and anti-inflammatory activities, thus, it protects against diabetes and its associated complications by decreasing ROS, pro-inflammatory cytokines, insulin resistance, and also by facilitating glucose uptake of cells (10-12).

Accordingly, melatonin directly scavenges ROS and also enhances the activity of antioxidant enzymes (13). It not only protects the development of diabetes but also inhibits its occurrence by increasing insulin sensitivity of cells (14). Apart from its negative regulation of pancreatic insulin secretion to lower the tissue insulin resistance (15), melatonin protects both mitochondria and ER against oxidative stress to avoid cellular glucose toxicity, especially for the pancreatic β-cells (16-18). Even though melatonin is not yet a first line recommended intervention for diabetes several current studies have observed the therapeutic effects of melatonin alone or in combination with other anti-diabetic drugs. One of them is metformin (19, 20). Here, we summarize some strategies to combine both metformin and melatonin in alleviation of hyperglycaemia. Therefore, the present review strongly suggests melatonin as an intervention for diabetics not only as a co-therapeutic agent, but also as a sole reliever of diabetes henceforth.

2. INVOLVEMENT OF OXIDATIVE STRESS IN OCCURRENCE OF DIABETES

Oxidative stress i.e., excessive ROS generation from different sources is considered as a key aetiology of diabetes and its associated complications (21-23). Glucose oxidation associated non-enzymatic protein glycation cause cellular protein and membrane lipid damage with subsequent cell death (1). Enediol radical anion and active ketoaldehyde formation by glucose autoxidation lead to superoxide anion generation that escalates the burden of stress *in vivo* (1, 24, 25). Superoxide anion promotes peroxynitrite formation and low density lipoprotein (LDL) peroxidation while reduces glucose-6-phosphate-dehydrogenase (G6PDH) activity with a result of dysfunctional pentose phosphate pathway (PPP) (26-29). Streptozotocin (STZ) and alloxan are diabetic inducers. Both of them augment oxidative stress biomarker thiobarbituric acid reactive substance (TBARS), in organs including liver, kidney, heart and brain indicating lipid peroxidation caused by hyperglycaemia (1). Not only lipids, diabetics causes the structural modifications of myriad extracellular proteins including laminin, elastin, myelin sheath etc. along with a substantial elevation in glycation and cross-linking of collagen molecules (30, 31). These cross-linking between lipids and proteins give rise to advanced glycation end products (AGE). AGE by binding to its receptor RAGE, down-regulates antioxidant system and up-regulates pro-inflammatory cytokines (32, 33). The diabetes associated oxidative stress always accompanies with the altered functions of glutathione reductase (GR), superoxide dismutase (SOD) and catalase in different organs caused by high blood glucose, which in turn makes these organs be more susceptible to ROS damage (4).

2.1. Mitochondrial stress in progression of diabetes.

When oxidative stress is considered as a foremost cause behind occurrence of hyperglycaemia, involvement of mitochondria can’t be ignored since these organelles are the major sites of ROS generation. Of note, elevated intracellular glucose level is associated with

higher mitochondrial superoxide anion generated by mitochondrial respiratory chain (29, 34, 35). Hyperglycaemia related mitochondrial stress is manifested by over-expression of mitochondrial superoxide dismutase (MnSOD) as observed in diabetic retina and endothelial cells cultured in high glucose media (33, 36, 37). Hence, a convincing correlation between these two factors renders the pathophysiology of diabetes. A crucial connection between mitochondrial anomalies and type 2 diabetes is also found in increased mitochondrial DNA mutations in the pathogenesis of diabetes and its related complications (38). High glucose induced mitochondrial ROS generation in one hand triggers conversion of glucose to sorbitol, utilizing NADPH and thus declines the level of endogenous antioxidant GSH (39) while on the other hand, it elicits reaction of glucose with free amino group by Schiff base formation to advance the process of protein glycation (40, 41). In addition, a marked decline in mitochondrial coenzyme Q9 content in diabetic rat heart, made tissues more vulnerable to stress since coenzyme Q9 is a potent mitochondrial antioxidant (42). All these evidences support the idea to target mitochondrial stress in prevention of diabetes (43).

2.2. Connection of ER stress in progression of diabetes.

After identification of oxidative stress as a fundamental factor behind hyperglycaemia, ER stress in particular becomes an independent co-factor of diabetes and its related abnormalities (44, 45). Under high glucose condition, ER stress is a prime factor of pancreatic β-cellular dysfunction and death since ER stress components are closely associated with β-cell function (46-48). The huge burden of mitochondria to carry out complete glucose oxidation for energy generation under glucotoxic condition causes the increase in misfolded or unfolded proteins in ER (49, 50). This unfolded protein ER stress in type 2 diabetes can be neutralised by chaperones, the pivotal molecules for protein folding process (51). For example, the loss of the glucose responsiveness in insulin secretory cells MIN-6 is associated with significantly down-regulated expression of ER chaperones GRP94, BiP, ERp29, and PDI (52, 53). Protein folding is an ATP-dependent process. Protein mis-folding causes futile ATP depletion and this in turn accelerates mitochondrial oxidative phosphorylation and further augments the ROS generation. ROS induces ER stress which then promotes ROS generation and this forms a vicious cycle in pancreatic secretory cells (54). This vicious cycle causes ER unfolded protein response (UPR) (55-59). When the load of misfolded polypeptide chains exceeds ER repairing process, multiple inflammatory pathways will be activated to instigate cell death (60, 61). Erroneous synthesis of pro-insulin instead of its mature form impairs insulin receptor mediated signalling process and results in hyperglycaemia (62). Additionally, accumulated mis-folded proteins trigger the leak of calcium ions from ER lumen to cytosol (63) and the elevated intracellular calcium also promotes mitochondrial ROS generation by increasing mitochondrial membrane permeability (64, 65) and blocking complex III of electron transport chain (ETC).

2.3. Pancreatic oxidative stress in development of hyperglycaemia.

Pancreas and more specifically, β-cells of islets act as the major sensor and regulator of circulated glucose level since insulin secreted by them promotes cellular glucose uptake to balance glucose load of blood, therefore, helps to avoid high level glucose toxicity. Pancreatic dysfunction results in declined insulin secretion while over-production of insulin leads to insulin resistance and β-cells exhaustion (66). Both cases cause hyperglycaemia and high amounts of ROS production (67, 68). Pancreatic β-cells with relatively low antioxidants and high content of mitochondria are prone to oxidative stress which suppresses insulin secretion (69, 70). The association between progression of diabetes and pancreatic β-cell dysfunction caused by oxidative stress are well documented (71-73). Mitochondrial dysfunction and ER

stress of pancreatic islets are also contributory to pancreatic β-cellular dysfunction and apoptosis (74, 66).

3. MELATONIN AS AN ANTIHYPERGLYCAEMIC ANTIOXIDANT

Since oxidative stress directly impacts the initiation and progression of hyperglycaemia, antioxidants can be considered as a first line of defence against diabetes. Thus, scientists have tried to use melatonin, a potent antioxidant, to target oxidative stress associated hyperglycaemia.

In Type I diabetic rats, melatonin reduced lipid peroxidation and increased glutathione peroxidase (GPx) activity in brain, kidney and liver and its protective effects are greater than that of vitamin E (75). These beneficial effects of melatonin on diabetes have been confirmed in different studies (76). Alloxan and STZ can induce diabetic status in animals by generating hydrogen peroxide and superoxide anion as well as by weakening antioxidant defence machineries (77, 78). In mice and rabbit treated by these chemicals, melatonin treatment reduced their oxidative tissue injury and maintained their redox state (79-81). In diabetic individuals, melatonin application normalized their blood glucose levels and thus protected against morphological damages of β-cells to hinder insulin leakage from these cells (81, 82). It also prevents β-cellular apoptosis caused by exposure of them to high glucose (83) (Figure. 1). In type 2 diabetic rats, melatonin application improved antioxidant features both in pancreas and heart along with a marked increase in insulin to glucose ratio (18). The alleviation of hyperglycaemia by melatonin is attributed to its antioxidant activity which mediates protection and regeneration of pancreatic islet cells (84). Furthermore, melatonin mediated slight increase in insulin secretion has also been reported as an extra-pancreatic function of this indole (85). Hence, apart from providing overall protection to hyperglycaemia by keeping homoeostasis of redox state, via inhibiting protein glycation, abating glycosylated haemoglobin production and declining glucose level, melatonin also acts as a safe guard for pancreas (86-88).

Melatonin can preserve ER in its healthy form by reducing the poorly developed rough endoplasmic reticulum (RER) in hepatic cytoplasm of diabetic rat (89, 90). Since ER stress couples to oxidative stress in occurrence of diabetes, a molecule that can provide protection against glucotoxicity induced ER stress can be a best choice as an anti-diabetic therapy. Melatonin exhibits enormous potential in hindering apoptosis of mouse osteoblastic MC3T3-E1 cell line exposed to high glucose medium in a type 2 diabetes mimicking milieu. Melatonin inhibits phosphorylation of PERK with prevention of the downstream eIF2α-CHOP pathway that ensure non-occurrence of ER UPR signalling by censoring proximal sensors of ER stress (91) (Figure 2).

4. ROLE OF MELATONIN IN GLUCOSE METABOLISM- A LINK TO MITIGATION OF DIABETES

The involvement of melatonin in glucose metabolism is linked to a surged glucose intolerance and insulin resistance in pinealectomized rats (92)**.** Accordingly, an impaired glucose uptake by adipose tissue in pinealectomized rats convinced this involvement (93). Since diabetes is a metabolic disorder, the medications must target metabolic pathways.

Gluconeogenesis is a process that synthesizes glucose from non-carbohydrate source. Overactivity of this process contributes to the blood glucose surge and glucose toxicity which may be fatal for diabetes. Melatonin is a negative regulator of gluconeogenic pathway and especially for phosphoenolpyruvate carboxykinase (PEPCK) (Figure1). Pinealectomized rats displayed uncontrolled PEPCK activity and high hepatic gluconeogenic rate even in the presence of high level of insulin (94). Melatonin is also a positive regulator of insulin involved

AKT phosphorylation, accountable for lowering PEPCK transcription via FoxO1 inhibition (94). Additionally, association of melatonin receptors (MT1 and MT2) with hypothalamic PI3K/AKT activation has been unveiled with a major role in hepatic gluconeogenesis repression (95). Melatonin stimulates Pentose Phosphate Pathway (PPP) to increase glucose utilization by enhancing the activity of G6PDH and thus reduces blood glucose accumulation in type 2 diabetic rats (96, 97). This action of melatonin helps in generating NADPH, the essential co-factor for GSH formation catalysed by GR. Melatonin not only positively affects NADPH generation, but it also reduces its degradation by inhibiting activity of NADPH oxidase (NOX) (Figure 1), which uses NADPH as an electron donor in conversion of molecular oxygen to superoxide anion. Melatonin diminished NOX4 activity and downregulated expression of $p47^{phox}$ subunit of NOX4 in kidney cortex of Zucker diabetic fatty rats with symptoms of diabetic nephropathy (98). On other hand, pinealectomy enhanced NOX subunit assembly along with excess ROS generation and insulin resistance (99). Of note, NOX4 is a factor responsible for increase in gluconeogenesis (100) and hence, the overall protective action of melatonin acts as a metabolic harmonizer in diabetes. The impaired hepatic mitochondria and low ATP level in Zucker diabetic fatty rats were alleviated by melatonin application (101) and here, melatonin regulated homoeostatic ATP level may be responsible for controlled glycolysis and Krebs cycle rate, which assists to limit ROS generation.

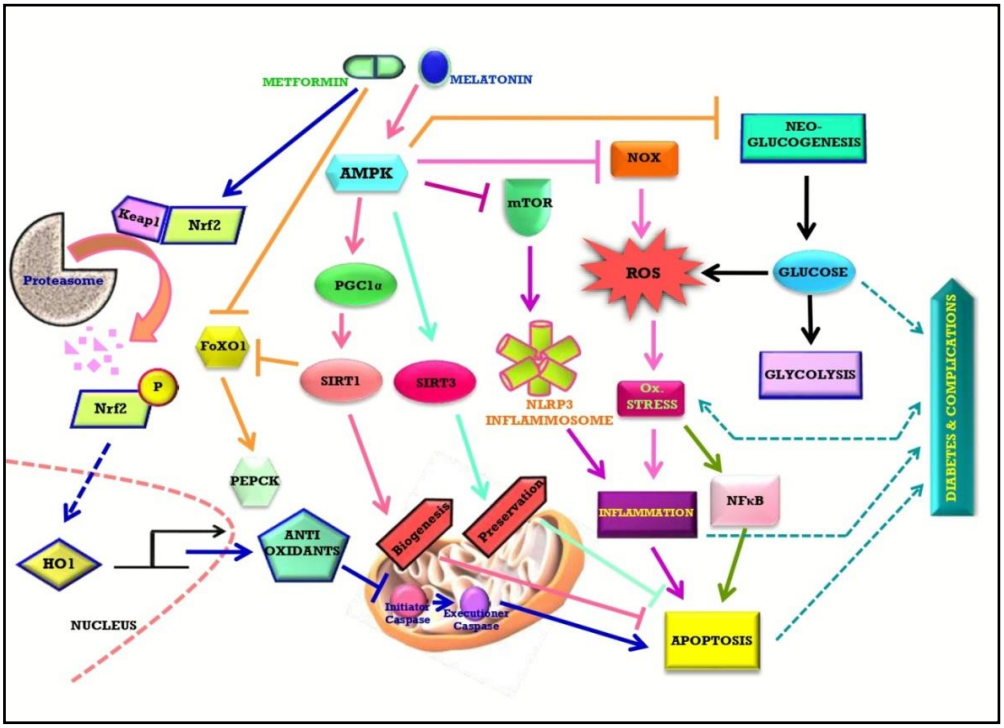

Fig.1. Potential mechanism(s) of action of melatonin and metformin in alleviation of diabetes.

Keap1: Kelch like ECH-associated protein 1, Nrf2: nuclear factor erythroid 2-related factor 2, HO1: heme oxygenase 1, FoXO1: forkhead box protein O1, PEPCK: phospho enol pyruvate carboxyKinase, AMPK: AMP-activated protein kinase, PGC1α: peroxisome proliferator-activated receptor-gamma coactivator-1 alpha, SIRT1: sirtuin1, SIRT3: sirtuin3, mTOR: mammalian target of rapamycin, NLRP3: NLR family pyrin domain containing 3, NOX: NADPH oxidase, ROS: reactive oxygen species, NFkB: nuclear factor kappa-light-chain-enhancer of activated B cells, arrows: activation, dash arrows: direction: T type bars: inhibition, colour arrows: different pathways.

5. ANTIOBESITY ROLE OF MELATONIN IN ANTIHYPERGLYCAEMIC ACTION

Melatonin has an antiobesic role in diabetic fatty mice (102). This action also contributes in alleviation of obesity associated hyperglycaemia (103, 104). Since insulin is the key hormone to maintain the rate of glucose metabolism by regulating activities and expressions of glycolytic, glycogenolytic and gluconeogenic enzymes, the hepatic resistance to insulin is primarily responsible for perturbed glucose balance in obese (105). The decreased glucose uptake by the muscle and increased glucose output by the liver (103) result in hyperglycaemia which causes macrophage infiltrated inflammation and adipocyte apoptosis (106). These are the major events responsible for occurrence of insulin insensitivity. Additionally, ER stress is a dominant factor behind obesity associated inflammation and subsequent insulin resistance in liver (107). Melatonin incurs myriad protective measures to hinder obesity related hyperglycaemia. The association of declined melatonin with increased obesity in diabetes (108) confirms the important roles of melatonin on obesity. Another action of melatonin targeting obesity in Zucker diabetic fatty rats is by increasing their brown adipose tissue mass and function (102, 109) to accelerate the metabolic rate of the animals. Melatonin administration protects adipose tissue as well as other organs including kidney (110) in obese associated diabetes via its anti-inflammatory activity and its regulation on mitochondrial dynamics (111).

6. ANTI-INFLAMMATORY ACTIVITY OF MELATONIN IN ALLEVIATION OF DIABETES

Inflammation has been emerged as a principal causative factor of diabetes (both Type I and Type II) (112). In type I diabetes, interferon gamma (IFN-γ), inflammatory cytokines including tumor necrosis factor alpha (TNF-α) and interleukin 1β (IL-1β) are the major factors of inflammation and ROS generation (113). ER stress and pancreatic islet cell death caused by IFN-γ have been reported (114, 115). Macrophages are the local inflammatory cytokine generator to cause pancreatic islet injury (116) while natural killer cells, natural killer T cells and dendritic cells, all participate in the inflammation induced diabetic progression (117). Involvement of inflammatory pathways is more relevant in advancement of type 2 diabetes. The inflammation activated transcription factor NFκB and Jun N-terminal kinase (JNK) have been found in various metabolic disorders (118-121). For example, TNF-α induces insulin resistance in obesity (122). Elevated IL-1β, IL-6 and CRP (C Reactive Protein) are common factors to diagnose type 2 diabetics with associated cardiac anomalies (123-125) while elevated serum IL-1 emerges as an indicator of inflammation in hyperglycaemia (126, 127). Adipose tissue in type 2 diabetes generates TNF-α, IL-1, IL-6, IL-10, leptin, adiponectin, chemokines etc. and their levels are further augmented by several folds when adipose tissue is infiltrated with macrophage and other immune cells (128-131).

Melatonin displays strong anti-inflammatory actions by balancing both pro and anti-inflammatory cytokines (132, 133), especially by blocking the translocation of NFκB into nucleus or by impeding the actions of NLRP3 inflammasome (134, 135) (Figure 1). Additionally, melatonin quenches deleterious hydroxyl radical, hypochlorus acid, peroxynitrite anion which are involved in production of pro-inflammatory cytokines (134, 136). Type 2 diabetic patients with insulin resistance have a strong positive correlation with upregulated IL6 and activated NLRP3 (125, 137). Melatonin, in diabetic rats rejuvenates pancreatic islet cells by escalating anti-inflammatory cytokine level IL-10 along with diminution of pro-inflammatory cytokines TNF-α, IL-1β, IL-6 (18). Similar effects of melatonin have been observed in obese patients with a concomitant decrease in insulin sensitivity (138, 139). Not only diabetes, but also many hyperglycaemia associated complications can be mitigated by the anti-inflammatory properties of melatonin.

7. MELATONIN IN ALLEVIATING DIABETES ASSOCIATED DISORDERS

The indoleamine, with its antioxidative and anti-inflammatory mechanisms not only prevents the occurrence and progression of diabetes, but also hinders the onset of its associated complications (140, 77) including diabetic cardiomyopathy (DCM), diabetic retinopathy (DR) and diabetic nephropathy (DN), and thus, it may reduce the death rate of diabetes globally.

7.1. Diabetic Cardio-myopathy (DCM).

DCM, one of the prime complications to cause diabetes associated death is mainly characterised by oxidative stress, inflammation and cardiac hypertrophy caused by dysregulated mitochondrial metabolism (141, 142). Melatonin reduces this cardiac hypertrophy in STZ induced diabetic Wistar rats and, thus, prevents cardiac remodelling by increasing phosphorylation of vascular endothelial growth factor (VEGF-A) (143). The indoleamine also facilitates autophagy pathway by inhibiting mammalian target of rapamycin (mTOR), a factor crucially accountable for cardiomyopathy and cardiac hypertrophy (144). One of the protective mechanisms of melatonin on DCM is to balance redox status, i.e., to enhance Nrf2/HO-1 for subsequent up-regulation of antioxidant enzyme expressions. These processes retard the myocardial cell apoptosis induced by activation of caspases (caspase 3, 8, 9) (145-147). Melatonin application in type 1 diabetic rats or in high glucose exposed H9c2 cells preserves mitochondrial structure and function with reduced ROS production by targeting AMPK-PGC1α-SIRT3 cascade (148). Cardiac tissue structure and function of diabetic animal models are preserved by melatonin via Syk/COX-I/SERCA signalling pathway (149) or by inhibition of PERK/ATF-6α/CHOP pathway (150). These actions mitigate ER stress, calcium overload and apoptosis in cardiac tissue. In addition, the NLRP3 inflammasome mediated cardiac fibrosis can also be reversed by melatonin application (10mg/kg/d for 8 weeks) in DMC via inhibition of lncRNA MALAT1/miR-141 axis (151). Thus, diverse activities of melatonin involve in protecting cardiac tissue from high glucose induced injury (152).

7.2. Diabetic retinopathy (DR).

Since oxidative stress and inflammation are major pathological factors of DR (153), melatonin emerges as a suitable treatment for this disorder with its antioxidative as well as anti-inflammatory activities. Melatonin reduces oxidative stress, ER stress, inflammation in DR *per se* or indirectly stimulates antioxidant enzymes in retina (154-156). The protective effect of melatonin on DR has been reported by inhibiting pro-inflammatory cytokines IL-1β, TNF-α through NFκB pathway (157) whereas, activation of MAP kinase (MAPK) pathway by melatonin reduces ROS generation, lipid peroxidation, inflammation and apoptosis (158). When diabetic Wistar rats treated with melatonin, they exhibited improved redox state and reduced retinal dilation and deformation by impeded endothelial growth factor mediated angiogenesis and tissue injury (159). These results have been substantiated by potency of melatonin in preserving retinal function of type 2 diabetic rats with its antioxidative efficacy (160).

7.3. Diabetic nephropathy (DN).

High glucose associated renal disorders are referred as DN, where compromised antioxidant capacity and ROS overload lead to apoptosis of renal epithelial cells with progressive loss of kidney functions (161). Balance of redox status and preservation of histopathological and functional kidney in diabetic rats by melatonin alone or in combinations have been well

documented (162-164). The upsurged pro- inflammatory cytokines including TNF-α, IL-6 and declined anti-inflammatory cytokine IL-10 levels in DN are normalized with melatonin application (165). A downregulation of pro-inflammatory IL-33 and another stress bio-markers in kidneys of diabetic rats are also achieved after melatonin treatment (166). Augmented kidney injury marker (KIM-1), heat shock protein (HSP-70), macrophage infiltration induced TGF-β1 are successfully ameliorated by intraperitoneal and oral application of melatonin in diabetic rats (167, 168). Melatonin pre-treated mesenchymal stem cell (MSC) transplantation significantly improves DN compared to untreated MSC by enhancing expression of autophagy mediator Beclin-1 as well as by retarding actions of TGF-β1 (169, 170).

8. SYNERGISTIC EFFECTS OF MELATONIN AND OTHER ANTI-DIABETIC DRUGS

Though the efficiency of melatonin in alleviating diabetes and associated complications have been reported in several studies, its use in diabetes remains as an adjuvant with other medications. Type I diabetes is an autoimmune disorder and it is difficult for prevention (171). In contrast, the onset and progression of type 2 diabetes can be delayed with the assistance of medications. Despite of having several treatment options for diabetes e.g., vildagliptin (172), metformin is still the first choice for type 2 diabetes (173). Its effectiveness, relatively low side effects made it be more endurable and much tolerable medicine for type 2 diabetes (174). Since, both melatonin and metformin have enormous potentials to curb diabetes and its associated complications, melatonin and metformin can be a combination of interest in treatment of diabetes.

Since obesity linked high glucose level is considered as a factor behind development of insulin resistance and subsequent diabetes, lifestyle change has been suggested for alleviating this disease. High fat diet fed Sprague-Dawley rats have a perturbed glucose metabolism, lipid profile and insulin resistance, all these alterations are improved by melatonin plus metformin treatment via modification of melatonin-leptin axis (19). It seems that melatonin acts as an amplifier for metformin and the combination achieves better outcomes comparing to metformin alone. Melatonin-metformin combination also potentiates the recovery of renal function in high glucose affected diabetic patients while metformin alone is not sufficient to provide requisite protection (175). Melatonin and metformin co-application at pharmacological doses for chronic diabetic patients significantly reduces their glycated haemoglobin (HbA1C) level whereas, metformin alone failed to do so (176). Hence, the synergistic effects of the melatonin and metformin to mitigate diabetes are suggested, i.e., melatonin potentiates the effects of metformin to amplify its therapeutic activities on diabetes.

9. MECHNISMS OF METFORMIN SHARED WITH MELATONIN ON DIABETES

Metformin, the first line oral drug of diabetes, possesses several mechanisms to protect against hyperglycaemia. It shares some mechanisms with melatonin in alleviation of the metabolic disorder. The details will be discussed following.

9.1. Antioxidative effects of metformin to restrict diabetes.

Metformin can preserve the cellular redox balance, especially by bringing homoeostasis in mitochondrial function in diabetic patients (Figure 1) (177).

The results of three months clinical trial with metformin in type 2 diabetic patients showed the improved levels of advanced oxidation protein products (AOPP) and AGE along with the reduced oxidative stress, inflammation and tissue damage (178). Another similar trial with 6

months of metformin treatment has confirmed the results with reduced oxidative and nistrosative stresses in type 2 diabetic patients (179). The antioxidative potency of metformin in type 1 diabetic animal model is also observed where application of metformin and insulin reduced the free radical insults associated oxidative stress (180). The antioxidative activity of metformin may be attributed to its direct hydroxyl radical scavenging activity (181). Beside this, modulating the activity of NADPH oxidase enzyme to obstruct superoxide anion generation also contributes to antioxidant properties of metformin (182). Metformin application often exhibits reduced lipid peroxidation, protein carbonylation with concomitant surged antioxidant level in type 2 diabetes patients (183).

Since energy metabolism is a principal determinant of diabetic condition, impairments in this process are crucially involved with hyperglycaemic progression. Metformin administration seems to protect intactness of vasculature in diabetic rats with the mechanisms of lowering mitochondrial stress and enhancing activity of aconitase enzyme and thus maintain energy balance to hinder vascular dysfunction (182). Prevention of mitochondrial stress by metformin through AMP-activated protein kinase (AMPK) activation has also been evidenced in endothelial cells, exposed to high glucose (184). Metformin activates cellular nutrient sensors AMPK and sirtuin-1 (SIRT1) by phosphorylation to downregulate stress inducing and autophagy inhibiting factor FoxO1 in the *in vivo* and *in vitro* conditions (185) (Figure 2). This mechanism of metformin extends its protective arena to diabetic patients with malfunctioned kidney. Metformin can effectively suppress complex I related reverse-electron flow (39, 186, 187) to protect tissue damage, especially mitochondria (188). Metformin activates AMPK and PGC-1α pathways in diabetic mice brain. It is the core modulator of mitochondrial biogenesis, and on other hand, it is an attenuator of mitochondrial oxidative stress (189).

Noteworthy, metformin retards hyperglycaemia induced oxidative stress, inflammation and insulin resistance when offered in combination with other molecules (190, 191).

9.2. Anti-inflammatory activity of metformin.

The anti-inflammatory activity of metformin is involved in its therapeutic effects on diabetes (192). Since, oxidative stress and inflammation form a vicious cycle to accelerate progression of diabetes (193) and vascular dysfunctions, anti-inflammation targeted anti-diabetic drugs became the first choice. A major mechanism of metformin to control glycaemia is activation of AMPK pathway (194, 195), which initiates energy metabolism and inhibits NFκB expression and thus limits inflammation (196, 197). Type 2 diabetic patients treated with metformin have improved anti-inflammatory status with surge in serum IL-10 expression irrespective of its dose and duration. This treatment also reduces pro-inflammatory IL-6 and MCP-1 levels in patients' serum and urine respectively, comparing to other anti-diabetic drugs including insulin release stimulants (198). A more commendable anti-inflammatory action of metformin in diabetes suppresses formation of inflammation promoter AGE by binding to its precursor, methylglyoxal (199-201). In addition to AMPK dependent anti-inflammatory action, metformin can enhance the level of tensin homologue PTEN, responsible for NFκB inactivation via PI3K/AKT inhibition (202).

Moreover, combination of metformin and L-cysteine brings profound changes in serum MCP-1 and C-reactive protein (CRP) levels in type 2 diabetic model rats and thus, forestalls mitochondria associated apoptotic process of hepatic tissue, induced by high glucose (191).

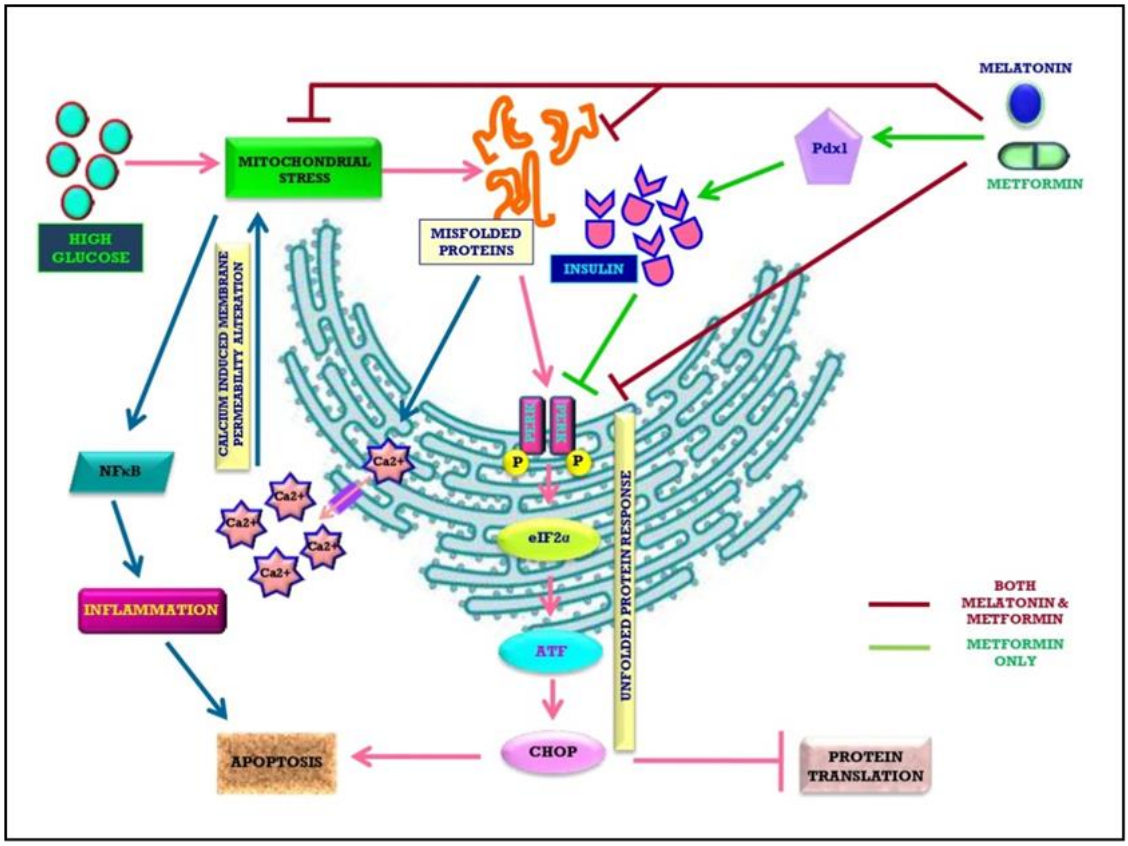

Fig.2. Protective mechanisms of melatonin and metformin on ER stress in organs and especially of pancreatic islet cells of diabetics.

Hyperglycaemia induces misfolded proteins and triggers ER stress in pancreas. Both the molecules inhibit unfolded protein response (UPR) and ER stress induced by high glucose, and thus, reduce mitochondrial oxidative stress. NFkB: nuclear factor kappa-light-chain-enhancer of activated B cells, PERK: protein kinase RNA-like endoplasmic reticulum kinase, eIF2α: eukaryotic initiation factor 2 alpha, ATF: activating transcription factor, CHOP: C/EBP homologous protein, Pdx1: pancreatic duodenal homeobox1, arrows: activation, T type bars: Inhibition, Colour arrows: different pathways.

9.3. Effects of metformin on glucose metabolic pathways.

The principal action of metformin in curbing hyperglycaemia is to lower hepatic glucose production by suppressing gluconeogenic pathway (Figure 1). The poorly controlled type 2 diabetic patients have 2 folds higher rate of glucose production and 3 folds higher gluconeogenic rate than the controls. Metformin administration reduces those rates by 24% and 33% respectively (203). In another study, a 60% reduction in glucose level has been evidenced with metformin treatment and this also contributes to its inhibitory effect on the gluconeogenesis (204). Metformin downregulates expressions of gluconeogenic rate limiting enzymes both at the mRNA and protein levels in mice liver and primary hepatocyte respectively (205-207). Growing bodies of evidence have supported the notion of anti-gluconeogenic effects of metformin while other diverse mechanisms are also present. Non-competitive inhibition of mitochondrial glycerophosphate dehydrogenase (mGPDH) by metformin is one of such mechanisms (208). It inhibits mGPDH and thus, blocks generation of dihydroxyacetonephosphate, the precursor of glucose formation from glycerol. mGPDH

inhibition also makes hepatic redox state more reductive (NADH) that suppresses the conversion of lactate/pyruvate into glucose since this required oxidised form of co-factor (NAD) dependent enzymatic actions (209). However, a redox independent mechanism of gluconeogenic inhibition by low dose of metformin has been uncovered. Metformin acts through channelizing intermediate substrates toward glycolysis to obstruct the formation of glycerol-3-phosphate, the allosteric inhibitor of glycolytic fructose-1,6 bisphosphate and/or phosphofructokinase (210). Additionally, AMPK activation is a major approach of metformin in modulation of metabolic pathways and its phosphorylation at Thr172 site is promoted by metformin (211). Metformin is also found to inhibit mitochondrial respiratory chain enzyme activities with concomitant rise of AMP/ATP ratio (212, 213). Metformin improves AMP level by inhibiting AMP deaminase (214). Correlation between loss of metformin action with deletion of liver kinase and subsequent loss of phosphorylated form of AMPK substantiates the notion of metformin's AMPK dependent antihyperglycaemic action (215). AMPK upregulation by metformin suppresses expressions of rate limiting enzymes of PEPCK and glucose-6-phosphatase in gluconeogenic pathway (216). Here, AMPK upregulates expression of small heterodimer partner (SHP) which downregulates forkhead box protein O1 (FoxO1) and A2 (FoxA2), the positive regulators of gluconeogenic gene transcription (217). Since gluconeogenesis requires ATP utilization, metformin mediated reduction in ATP level also negatively controls the fate of this pathway (218).

Metformin instigates downregulation of Akt activity via suppression of Src homology 2 domain-containing inositol-5-phosphatase 2 (SHIP2) by directly binding to its phosphate domain which is another anti-hyperglycaemic strategy of the drug (219). This property of metformin improves insulin sensitivity as well as augments glucose uptake by cells. The increase in glucose uptake by skeletal muscle cells upon metformin treatment is attributed to the fact that metformin promotes glucose transporters including their movement along cellular membrane. In addition, this cellular uptake is associated with elevated glycogen synthesis and subsequent storage along with reduction in the rate of glycogenolysis (220).

9.4. ER stress alleviation by metformin in diabetics.

Like to many other proteins, pro-insulin secretion from pancreatic β-cells requires stringent inspection and maturation by ER chaperone proteins. Under different physiological conditions, secretory β-cells use unfolded protein response (UPR) to limit the load of newly synthesized proteins that exceed beyond the ER capacity to fold proteins with accuracy (221, 222). Metformin confers extensive protections in diabetes by preserving structural and functional equilibrium of ER (223) (Figure 2). High glucose exposed rat insulinoma cells INS-1 exhibit reduction in pancreatic duodenal homeobox1 (Pdx1) expression, the protein responsible for differentiation of islet β-cells while metformin reverses Pdx1expression with improved insulin level and decreased UPR stress response from ER as indicated by diminished phosphorylated forms of PERK, eIF2α and CHOP (224). In a parallel way, oral metformin administration to diabetic male C57BL/6 mice for 15 consecutive days delays PERK phosphorylation and subsequent activation in brain tissue indicating potency of metformin in ER stress amelioration (189). The suppressive effect of metformin on ER stress may be responsible for late activation of UPR signal which by detaining eIF2α transcription assists in continuing uninterrupted protein translation (225, 189). In accordance with this study, metformin inhibits JNK phosphorylation preceded by AMPK activation and thus, prevents occurrence of inflammation and death of mouse pancreatic β cell line NIT-1 (226). In contrast, enhanced level of phosphorylated eIF2α has been reported in metformin treated peripheral blood mononuclear cells (PBMC) of type 2 diabetes as a protective act of metformin to conserve ER from entering into apoptosis by blocking ATF6 dependent pro-apoptotic pathway (222). Here, metformin

protects ER of PBMC by declining the levels of autophagy inducer becn1, atg7 and promotes adaptive branch of UPR to arrest protein translation process rather to induce apoptosis. Moreover, metformin along with resveratrol preserves ER in eustatic condition with different mechanisms. This combination alleviates excess mitochondrial ROS induced ER stress and successive NLRP3 inflammasome formation in diabetic ICR mice adipose tissue (227).

9.5. Metformin: a safe-guard for pancreas

Apart from improving insulin sensitivity in high glucose targeted tissues including muscle, adipose tissue and liver, metformin acts as an overall protector to pancreatic β-cells, particularly to preserve their insulin secretion (228, 229). In high glucose environment, metformin enhances β-cell viability and lowers insulin secretion by modulation of pancreatic translational activities to safeguard β-cells from glucose toxicity (230, 231). This molecule prevents pancreatic islet cell malfunction and thus, forestalls apoptotic pathway (232) by averting high glucose induced islet cell desensitization (233). Additionally, this drug effectively preserves morpho-functional stability of pancreatic islet cells by conserving mitochondria and ER intactness (234, 223).

10. CONCLUSION AND PROSPECTIVES

Though metformin is a well-accepted first line drug for diabetes (171), its adverse effects can't be neglected. The side effects of this biguanide class anti-diabetic drugs are relatively low as mentioned above but some unfavourable actions are still concerned by physicians and researchers (235). For example, metformin treatment, sometimes, even at low doses, causes gastrointestinal discomforts with symptoms of nausea, diarrhoea. Its safety limits have been constricted when metformin has been shown to cause complications like pancreatitis, hepatitis etc. (236, 237). Moreover, lactic acidosis is a detrimental effect since metformin blocks electron transport chain resulting in biased metabolic cycles in favour of more anaerobic glycolysis which leads to excess lactate production (238). Many clinical studies cast concerning on its long term use dependent vitamin B12 deficiency (239). Additionally, despite of having protective effects on diabetic nephropathy, metformin also can cause pernicious effects on renal function with reduced glomerular filtration rate (GFR) (240). Hence, the shortcomings of metformin have prompted the researchers to find a more tolerable molecule with antihyperglycaemic effects. Melatonin seems to be such a molecule. Melatonin has antihyperglycaemic effects with plethora of protective actions on the affected organs. For example, melatonin preserves gastrointestinal (GI) epithelium's microcirculation and thus, prevents epithelial degeneration, lesion and ulceration (241). In addition, the indole shows diverse protective mechanisms to conserve renal tissue by forestalling oxidative stress, inflammation in patients with chronic kidney disease along with safeguarding rennin-angiotensin system (242). A recent review has detailed beneficial effects of melatonin in nullifying drug induced nephrotoxicity (243) which again is in favour of melatonin as an aid for hyperglycaemia.

This review has been drafted to fetch attention to the use of melatonin as an anti-diabetic, having antihyperglycaemic effects along with ample protective effects for high glucose affected organs. The mechanisms of melatonin in mitigating glucose toxicity and diabetic complications appear similar to metformin. Such mimicked actions of both the molecules in diabetes may encourage prescribing melatonin alone besides its application as a co-therapeutic medicine. This recommendation requires approval from more clinical studies.

ACKNOWLEDGEMENTS

A Junior Research Fellowship (JRF) under WBDST [304(Sanc.)/STP/S&T/1G-67/2017 DATED 29.03.2018] is greatly acknowledged by AB. Dr. AC is supported by funds available to her from Department of Science and Technology, Govt. of West Bengal. Dr. DB also gratefully acknowledges the support he received from CPEPA Scheme of UGC awarded to University of Calcutta, Departmental BI Grant and DST-PURSE Program awarded to the University of Calcutta.

AUTHORSHIP

All authors have contributed to the conception. AB drafted the first version of the manuscript and figures. Dr. DB and AC critically reviewed the manuscript and approved it.

CONFLICT INTEREST

The author(s) declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

REFERENCES

1. Maritim AC, Sanders A, Watkins Iii JB (2003) Diabetes, oxidative stress, and antioxidants: a review. *J. Biochem. Mol. Toxicol.* **17** (1): 24-38. DOI: 10.1002/jbt.10058.
2. Robertson RP, Harmon JS (2006) Diabetes, glucose toxicity, and oxidative stress: a case of double jeopardy for the pancreatic islet β cell. *Free Radic. Biol. Med*. **41** (2): 177-184. DOI: 10.1016/j.freeradbiomed.2005.04.030.
3. Ozougwu JC, Obimba KC, Belonwu CD, Unakalamba CB (2013) The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *J. Physiol. Pathophysiol.* **4** (4): 46-57. DOI: 10.5897/JPAP2013.0001.
4. Asmat U, Abad K, Ismail K (2016) Diabetes mellitus and oxidative stress—A concise review. *Saudi Pharm. J.* **24** (5): 547-553. DOI: 10.1016/j.jsps.2015.03.013.
5. Pham-HuyLA, He H, Pham-Huy C (2008) Free radicals, antioxidants in disease and health. *Int. J. Biomed. Sci.* **4** (2): 89-96.PMID: 23675073.
6. Moussa SA. Oxidative stress in diabetes mellitus (2008) *Romanian J. Biophys.* **18** (3): 225-236.
7. Back SH, Kaufman RJ (2012) Endoplasmic reticulum stress and type 2 diabetes. *Annu. Rev. Biochem.* **81**: 767-793. DOI: 10.1146/annurev-biochem-072909-095555.
8. Feher MD, Al-Mrayat ME, Brake J, Leong KS (2007) Tolerability of prolonged-release metformin (Glucophage® SR) in individuals intolerant to standard metformin—results from four UK centres. *Br. J. Diabetes Vasc. Dis.* **7** (5): 225-228. DOI: 10.1177/14746514070070050501.
9. Cryer DR, Nicholas SP, Henry DH, Mills DJ, Stadel BV (2005) Comparative outcomes study of metformin intervention versus conventional approach the COSMIC Approach Study. *Diabetes Care* **28** (3): 539-543. DOI:10.2337/diacare.28.3.539.
10. Esteghamati A, Eskandari D, Mirmiranpour H, Noshad S, Mousavizadeh M, Hedayati M, Nakhjavani M (2013) Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: a randomized clinical trial. *Clin. Nutr.* **32** (2): 179-185. DOI: 10.1016/j.clnu.2012.08.006.
11. Wright AD, Cull CA, Macleod KM, Holman RR, UKPDS Group (2006) Hypoglycemia in type 2 diabetic patients randomized to and maintained on monotherapy with diet,

sulfonylurea, metformin, or insulin for 6 years from diagnosis: UKPDS73. *J. Diabetes Complicat.* **20** (6): 395-401. DOI: 10.1016/j.jdiacomp.2005.08.010.
12. Hostalek U, Gwilt M, Hildemann S (2015) Therapeutic use of metformin in prediabetes and diabetes prevention. *Drugs* **75** (10): 1071-1094. DOI: 10.1007/s40265-015-0416-8.
13. Galano A, Tan DX, Reiter RJ (2011) Melatonin as a natural ally against oxidative stress: a physicochemical examination. *J. Pineal Res.* **51** (1): 1-6. DOI: 10.1111/j.1600-079X.2011.00916.x.
14. Cagnacci A, Arangino S, Renzi A, Paoletti AM, Melis GB, Cagnacci P, Volpe A (2001) Influence of melatonin administration on glucose tolerance and insulin sensitivity of postmenopausal women. *Clin. Endocrinol.* **54** (3): 339-346. DOI: 10.1046/j.1365-2265.2001.01232.x.
15. Banerjee A, Chattopadhyay A, Bandyopadhyay D (2020) Biorhythmic and receptor mediated interplay between melatonin and insulin: its consequences on diabetic erythrocytes. *Melatonin Res.* **3** (2): 243-263. DOI: 10.32794/mr12250060.
16. Nishida S (2005) Metabolic effects of melatonin on odative stress and diabetes mellitus. *Endocrine* **27** (2): 131-135. DOI: 10.1385/endo:27:2:131.
17. Aouichat S, Navarro-Alarcon M, Alarcón-Guijo P, Salagre D, Ncir M, Zourgui L, Agil A (2021) Melatonin Improves Endoplasmic Reticulum Stress-Mediated IRE1α Pathway in Zücker Diabetic Fatty Rat. *Pharmaceuticals* **14** (3): 232. DOI: 10.3390/ph14030232.
18. Abdulwahab DA, El-Missiry MA, Shabana S, Othman AI, Amer ME (2021) Melatonin protects the heart and pancreas by improving glucose homeostasis, oxidative stress, inflammation and apoptosis in T2DM-induced rats. *Heliyon* **7** (3): e06474. DOI: 10.1016/j.heliyon.2021.e06474.
19. Dantas-Ferreira RF, Raingard H, Dumont S, Schuster-Klein C, Guardiola-Lemaitre B, Pevet P, Challet E (2018) Melatonin potentiates the effects of metformin on glucose metabolism and food intake in high-fat-fed rats. Endocrinol. *Diabetes Metab.* **1** (4): e00039. DOI: 10.1002/edm2.39.
20. Thomas AP, Hoang J, Vongbunyong K, Nguyen A, Rakshit K, Matveyenko AV (2016) Administration of melatonin and metformin prevents deleterious effects of circadian disruption and obesity in male rats. *Endocrinology* **157** (12): 4720-4731. DOI: 10.1210/en.2016-1309.
21. Ceriello A (2000) Oxidative stress and glycemic regulation. *Metabolism* **49** (2): 27-29. DOI: 10.1016/s0026-0495(00)80082-7.
22. Baynes JW, Thorpe SR (1999) Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* **48** (1): 1-9. DOI: 10.2337/diabetes.48.1.1.
23. Baynes JW (1991) Role of oxidative stress in development of complications in diabetes. *Diabetes* **40** (4): 405-412. DOI: 10.2337/diab.40.4.405.
24. Jiang ZY, Woollard AC, Wolff SP (1990) Hydrogen peroxide production during experimental protein glycation. *FEBS Lett.* **268** (1): 69-71. DOI: 10.1016/0014-5793(90)80974-n.
25. Wolff SP, Dean RT (1987) Glucose autoxidation and protein modification. The potential role of 'autoxidative glycosylation'in diabetes. *Biochem. J.* **245** (1): 243-250. DOI: 10.1042/bj2450243.
26. Hogg N, Kalyanaraman B, Joseph J, Struck A, Parthasarathy S (1993) Inhibition of low-density lipoprotein oxidation by nitric oxide Potential role in atherogenesis. *FEBS Lett.* **334** (2): 170-174. DOI: 10.1016/0014-5793(93)81706-6.
27. Tsai EC, Hirsch IB, Brunzell JD, Chait A (1994) Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. *Diabetes* **43** (8): 1010-1014. DOI: 10.2337/diab.43.8.1010.

28. Kawamura M, Heinecke JW, Chait A (1994) Pathophysiological concentrations of glucose promote oxidative modification of low density lipoprotein by a superoxide-dependent pathway. *J.Clin. Invest.* **94** (2): 771-778. DOI: 10.1172/JCI117396.
29. Nishikawa T, Edelstein D, Du XL, Yamagishi SI, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I (2000) Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* **404** (6779): 787-790. DOI: 10.1038/35008121.
30. Petrova R, Yamamoto Y, Muraki K, Yonekura H, Sakurai S, Watanabe T, Li H, Takeuchi M, Makita Z, Kato I, Takasawa S (2002) Advanced glycation endproduct-induced calcium handling impairment in mouse cardiac myocytes. *J. Mol. Cell Cardiol.* **34** (10): 1425-1431. DOI: 10.1006/jmcc.2002.2084.
31. Sugimoto K, Nishizawa Y, Horiuchi S, Yagihashi S (1997) Localization in human diabetic peripheral nerve of N (epsilon) -Carboxymethyllysine protein adducts, an advanced glycation end product. *Diabetologia* **40** (12): 1380-1387. DOI:10.1007/s001250050839.
32. Goldin A, Beckman JA, Schmidt AM, Creager MA (2006) Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation.* **114** (6): 597-605. DOI: 10.1161/CIRCULATIONAHA.106.621854.
33. Giacco F, Brownlee M (2010) Oxidative stress and diabetic complications. *Circ. Res.* **107** (9): 1058-1070. DOI: 10.1161/CIRCRESAHA.110.223545.
34. Du XL, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, Wu J, Brownlee M (2000)Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc. Natl. Acad. Sci.* **97** (22): 12222-12226. DOI: 10.1073/pnas.97.22.12222.
35. Wallace DC (1992) Diseases of the mitochondrial DNA. *Annu. Rev. Biochem.* **61** (1): 1175-1212. DOI: 10.1146/annurev.bi.61.070192.005523.
36. Kowluru RA, Kowluru V, Xiong Y, Ho YS (2006) Overexpression of mitochondrial superoxide dismutase in mice protects the retina from diabetes-induced oxidative stress. *Free Radic. Biol. Med.* **41** (8): 1191-1196. DOI: 10.1016/j.freeradbiomed.2006.01.012.
37. Ceradini DJ, Yao D, Grogan RH, Callaghan MJ, Edelstein D, Brownlee M, Gurtner GC (2008) Decreasing intracellular superoxide corrects defective ischemia-induced new vessel formation in diabetic mice. *J. Biol. Chem.* **283** (16): 10930-10938. DOI: 10.1074/jbc.M707451200.
38. Rolo AP, Palmeira CM (2006) Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicol. Appl. Pharmacol.* **212** (2): 167-178. DOI: 10.1016/j.taap.2006.01.003.
39. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature* **414** (6865): 813-820. DOI: 10.1038/414813a.
40. Ansari NA, Dash D (2013) Amadori glycated proteins: role in production of autoantibodies in diabetes mellitus and effect of inhibitors on non-enzymatic glycation. *Aging Dis.* **4** (1): 50-56. PMID: 23423609.
41. Welsh KJ, Kirkman MS, Sacks DB (2016) Role of glycated proteins in the diagnosis and management of diabetes: research gaps and future directions. *Diabetes Care* **39** (8): 1299-1306. DOI: 10.2337/dc15-2727.
42. Santos DL, Palmeira CM, Sei R, Dias J, Mesquita J, Moreno AJ, Santos MS (2003) Diabetes and mitochondrial oxidative stress: a study using heart mitochondria from the diabetic Goto-Kakizaki rat. *Mol. Cell Biochem.* **246** (1): 163-170. DOI: 10.1023/A:1023475022025.

43. Green K, Brand MD, Murphy MP (2004) Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. *Diabetes* **53** (suppl 1): S110-8. DOI: 10.2337/diabetes.53.2007.s110.
44. Mooradian AD, Haas MJ (2011) Glucose-induced endoplasmic reticulum stress is independent of oxidative stress: a mechanistic explanation for the failure of antioxidant therapy in diabetes. *Free Radic. Biol. Med* .**50** (9): 1140-1143. DOI: 10.1016/j.freeradbiomed.2011.02.002.
45. Laybutt DR, Preston AM, Åkerfeldt MC, Kench JG, Busch AK, Biankin AV, Biden TJ (2007) Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. *Diabetologia* **50** (4): 752-763. DOI: 10.1007/s00125-006-0590-z.
46. Harding HP, Ron D (2002) Endoplasmic reticulum stress and the development of diabetes: a review. *Diabetes*. **51** (suppl 3): S455-S461. DOI: 10.2337/diabetes.51.2007.s455.
47. Oyadomari S, Takeda K, Takiguchi M, Gotoh T, Matsumoto M, Wada I, Akira S, Araki E, Mori M (2001) Nitric oxide-induced apoptosis in pancreatic β cells is mediated by the endoplasmic reticulum stress pathway. *Proc. Natl. Acad. Sci.* **98** (19): 10845-10850. DOI: 10.1073/pnas.191207498.
48. Oyadomari S, Koizumi A, Takeda K, Gotoh T, Akira S, Araki E, Mori M (2002) Targeted disruption of the Chop gene delays endoplasmic reticulum stress–mediated diabetes. *J. Clin. Invest.* **109** (4): 525-532. DOI: 10.1172/JCI14550.
49. Flamment M,Hajduch E, Ferré P, Foufelle F (2012) New insights into ER stress-induced insulin resistance. *Trends Endocrinol. Metab.* **23** (8): 381-390. DOI: 10.1016/j.tem.2012.06.003.
50. Hu M, Phan F, Bourron O, Ferré P, Foufelle F (2017) Steatosis and NASH in type 2 diabetes. *Biochimie* **143**: 37-41. DOI: 10.1016/j.biochi.2017.10.019.
51. Özcan U, Yilmaz E, Özcan L, Furuhashi M, Vaillancourt E, Smith RO, Görgün CZ, Hotamisligil GS (2006) Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* **313** (5790): 1137-40. DOI: 10.1126/science.1128294.
52. Plaisance V, Brajkovic S, Tenenbaum M, Favre D, Ezanno H, Bonnefond A, Bonner C, Gmyr V, Kerr-Conte J, Gauthier BR, Widmann C (2016) Endoplasmic reticulum stress links oxidative stress to impaired pancreatic beta-cell function caused by human oxidized LDL. *PLoS One* **11**(9): e0163046. DOI: 10.1371/journal.pone.0163046.
53. Dowling P, O'Driscoll L, O'Sullivan F, Dowd A, Henry M, Jeppesen PB, Meleady P, Clynes M (2006) Proteomic screening of glucose-responsive and glucose non-responsive MIN-6 beta cells reveals differential expression of proteins involved in protein folding, secretion and oxidative stress. *Proteomics* **6** (24): 6578-6587. DOI: 10.1002/pmic.200600298.
54. Cao SS, Kaufman RJ (2014) Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. *Antioxid. Redox. Signal*. **21** (3): 396-413. DOI: 10.1089/ars.2014.5851.
55. Maamoun H, Abdelsalam SS,Zeidan A, Korashy HM, Agouni A (2019) Endoplasmic reticulum stress: a critical molecular driver of endothelial dysfunction and cardiovascular disturbances associated with diabetes. *Int. J. Mol. Sci*. **20** (7): 1658. DOI: 10.3390/ijms20071658.
56. Hasnain SZ, Prins JB, McGuckin MA (2016) Oxidative and endoplasmic reticulum stress in b-cell dysfunction in diabetes. *J. Mol. Endocrinol*. **56** (2): 33-54. DOI: 10.1530/JME-15-0232.
57. Gardner BM, Pincus D, Gotthardt K, Gallagher CM, Walter P (2013) Endoplasmic reticulum stress sensing in the unfolded protein response. *Cold Spring Harb. Perspect. Biol.* **5** (3): a013169. DOI: 10.1101/cshperspect.a013169.

58. Gardner BM, Walter P (2011) Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. *Science* **333** (6051): 1891-1894. DOI: 10.1126/science.1209126.
59. Walter P, Ron D (2011) The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **334** (6059): 1081-1086. DOI: 10.1126/science.1209038.
60. Marciniak SJ, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, Nagata K, Harding HP, Ron D (2004) CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev.* **18** (24): 3066-3077. DOI: 10.1101/gad.1250704.
61. Chen Y, Wang JJ, Li J, Hosoya KI, Ratan R, Townes T, Zhang SX (2012) Activating transcription factor 4 mediates hyperglycaemia-induced endothelial inflammation and retinal vascular leakage through activation of STAT3 in a mouse model of type 1 diabetes. *Diabetologia* **55** (9): 2533-2545. DOI: 10.1007/s00125-012-2594-1.
62. Sun J, Cui J, He Q, Chen Z, Arvan P, Liu M (2015) Proinsulin misfolding and endoplasmic reticulum stress during the development and progression of diabetes. *Mol. Aspects Med.* **42**: 105-118. DOI: 10.1016/j.mam.2015.01.001.
63. Hara T, Mahadevan J, Kanekura K, Hara M, Lu S, Urano F (2014) Calcium efflux from the endoplasmic reticulum leads to β-cell death. *Endocrinology* **155** (3): 758-768. DOI: 10.1210/en.2013-1519.
64. Lowell BB, Shulman GI (2005) Mitochondrial dysfunction and type 2 diabetes. *Science* **307** (5708): 384-387. DOI: 10.1126/science.1104343.
65. Supale S, Li N, Brun T, Maechler P (2012) Mitochondrial dysfunction in pancreatic β cells. *Trends Endocrinol. Metab.* **23** (9): 477-487. DOI: 10.1016/j.tem.2012.06.002. DOI: 10.1016/j.tem.2012.06.002.
66. Rolo AP, Palmeira CM (2006) Diabetes and mitochondrial function: role of hyperglycemiaand oxidative stress. *Toxicol. Appl. Pharmacol.* **212** (2): 167-178. DOI: 10.1016/j.taap.2006.01.003.
67. Acharya JD, Ghaskadbi SS (2010) Islets and their antioxidant defense. *Islets* **2** (4): 225-235. DOI: 10.4161/isl.2.4.12219.
68. Lenzen S (2008) Oxidative stress: the vulnerable β-cell. *Biochem. Soc. Trans*. **36** (3): 343-347. DOI: 10.1042/BST0360343.
69. Robertson RP (2004) Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J. Biol. Chem.* **279** (41): 42351-42354. DOI: 10.1074/jbc.R400019200.
70. Kajimoto Y, Kaneto H (2004) Role of oxidative stress in pancreatic β-cell dysfunction. *Ann. N. Y. Acad. Sci.* **1011**: 168-176. DOI: 10.1007/978-3-662-41088-2_17.
71. Kakkar R, Mantha SV, Radhi J, Prasad K, Kalra J (1998) Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. *Clin. Sci.* **94** (6): 623-632. DOI: 10.1042/cs0940623.
72. Kaneto H, Kawamori D, Matsuoka TA, Kajimoto Y, Yamasaki Y (2005) Oxidative stress and pancreatic β-cell dysfunction. *Am. J. Ther.* **12** (6): 529-533. DOI: 10.1097/01.mjt.0000178773.31525.c2.
73. Wang J, Wang H (2017) Oxidative stress in pancreatic beta cell regeneration. *Oxid. Med. Cell Longev.* **2017**: 1930261. DOI: 10.1155/2017/1930261.
74. Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, Hiai H (1999) Hyperglycaemia causes oxidative stress in pancreatic (B-Cells of GK Rats, a Model of type 2 diabetes. *Diabetes* **48** (4): 927-932. DOI: 10.2337/diabetes.48.4.927.
75. Baydas G, Canatan H, Turkoglu A (2002) Comparative analysis of the protective effects of melatonin and vitamin E on streptozocin-induced diabetes mellitus. *J. Pineal Res.* **32** (4): 225-230. DOI: 10.1034/j.1600-079x.2002.01856.x.

76. Montilla PL, Vargas JF, Túnez IF, Carmen M, de Agueda M, Valdelvira ME, Cabrera ES (1998) Oxidative stress in diabetic rats induced by streptozotocin: protective effects of melatonin. *J. Pineal Res.***25** (2): 94-100. DOI: 10.1111/j.1600-079x.1998.tb00545.x.
77. Espino J, Pariente JA, Rodríguez AB (2011) Role of melatonin on diabetes-related metabolic disorders. *World J. Diabetes* **2** (6): 82-91. DOI: 10.4239/wjd.v2.i6.82.
78. Brömme HJ, Ebelt H, Peschke D, Peschke E (1999) Alloxan acts as a prooxidant only under reducing conditions: influence of melatonin. *Cellular Mol. Life Sci.* **55** (3): 487-493. DOI: 10.1007/s000180050305.
79. Štetinová V, Smetanová L, Grossmann V, Anzenbacher P (2002) In vitro and in vivo assessment of the antioxidant activity of melatonin and related indole derivatives. *Gen. Physiol. Biophys.* **21** (2): 153-162. DOI: DOI 10.1007/s10238-004-0050-3.
80. Winiarska K, Fraczyk T, Malinska D, Drozak J, Bryla J (2006) Melatonin attenuates diabetes-induced oxidative stress in rabbits. *J. Pineal Res.* **40** (2): 168-176. DOI: 10.1111/j.1600-079X.2005.00295.x.
81. Brömme HJ, Mörke W, Peschke E, Ebelt H, Peschke D (2000) Scavenging effect of melatonin on hydroxyl radicals generated by alloxan. *J. Pineal Res.* **29** (4): 201-208. DOI: 10.1034/j.1600-0633.2002.290402.x.
82. Ebelt H, Peschke D, BrömmeHJ, Mörke W, Blume R, Peschke E (2000) Influence of melatonin on free radical-induced changes in rat pancreatic beta-cells in vitro. *J. Pineal Res.* **28** (2): 65-72. DOI: 10.1034/j.1600-079x.2001.280201.x.
83. Park JH, Shim HM, Na AY, Bae KC, Bae JH, Im SS, Cho HC, Song DK (2014) Melatonin prevents pancreatic β-cell loss due to glucotoxicity: the relationship between oxidative stress and endoplasmic reticulum stress. *J. Pineal Res.* **56** (2): 143-153. DOI: 10.1111/jpi.12106.
84. Yavuz O, Cam M, Bukan N, Guven A, Silan F (2003) Protective effect of melatonin on β-cell damage in streptozotocin-induced diabetes in rats. *Acta histochem.* **105** (3): 261-266.
85. Kanter M, Uysal H, Karaca T, Sagmanligil HO (2006) Depression of glucose levels and partial restoration of pancreatic β-cell damage by melatonin in streptozotocin-induced diabetic rats. *Arch. Toxicol.* **80** (6): 362-369. DOI: 10.1007/s00204-005-0055-z.
86. Zephy D, Ahmad J (2015) Type 2 diabetes mellitus: role of melatonin and oxidative stress. *Diabetes Metab.Syndr.* **9** (2): 127-131. DOI: 10.1016/j.dsx.2014.09.018.
87. Espino J, Rodríguez AB, Pariente JA (2019) Melatonin and oxidative stress in the diabetic state: clinical implications and potential therapeutic applications. *Curr. Med. Chem.* **26** (22): 4178-4190. DOI: 10.2174/0929867325666180410094149.
88. Doosti-Irani A, Ostadmohammadi V, Mirhosseini N, Mansournia MA, Reiter RJ, Kashanian M, Rahimi M, Razavi M, Asemi Z (2018) The effects of melatonin supplementation on glycemic control: a systematic review and meta-analysis of randomized controlled trials. *Horm. Metab. Res.* **50** (11): 783-790. DOI: 10.1055/a-0752-8462.
89. Guven A, Yavuz O, Cam M, Ercan F, Bukan N, Comunoglu C, Gokce F (2006) Effects of melatonin on streptozotocin-induced diabetic liver injury in rats. *Acta Histochem.* **108** (2): 85-93. DOI: 10.1016/j.acthis.2006.03.005.
90. Lenk SE, Bhat D, Blakeney W, Dunn Jr WA (1992) Effects of streptozotocin-induced diabetes on rough endoplasmic reticulum and lysosomes of rat liver. *Am. J. Physiol.* **263** (5): E856-E862. DOI: 10.1152/ajpendo.1992.263.5.E856.
91. Zhou R, Ma Y, Tao Z, Qiu S, Gong Z, Tao L, Zhu Y (2020) Melatonin Inhibits Glucose-Induced Apoptosis in Osteoblastic Cell Line Through PERK-eIF2α-ATF4 Pathway. *Front. Pharmacol.* **11**: 602307. DOI: 10.3389/fphar.2020.602307.
92. Lima FB, Machado UF, Bartol I, Seraphim PM, Sumida DH, Moraes SM, Hell NS, Okamoto MM, Saad MJ, Carvalho CR, Cipolla-Neto J (1998) Pinealectomy causes glucose

intolerance and decreases adipose cell responsiveness to insulin in rats. *Am. J. Physiol.* **275** (6): E934-941. DOI: 10.1152/ajpendo.1998.275.6.E934.
93. Alonso-Vale MI, Borges-Silva CN, Anhe GF, Andreotti S, Machado MA, Cipolla-Neto J, Lima FB (2004) Light/dark cycle-dependent metabolic changes in adipose tissue of pinealectomized rats. *Horm. Metab. Res.* **36** (07): 474-479. DOI: 10.1055/s-2004-825723.
94. Nogueira TC, Lellis-Santos C, Jesus DS, Taneda M, Rodrigues SC, Amaral FG, Lopes AM, Cipolla-Neto J, Bordin S, Anhê GF (2011) Absence of melatonin induces night-time hepatic insulin resistance and increased gluconeogenesis due to stimulation of nocturnal unfolded protein response. *Endocrinology* **152** (4): 1253-1263. DOI: 10.1210/en.2010-1088.
95. Faria JA, Kinote A, Ignacio-Souza LM, de Araújo TM, Razolli DS, Doneda DL, Paschoal LB, Lellis-Santos C, Bertolini GL, Velloso LA, Bordin S (2013) Melatonin acts through MT1/MT2 receptors to activate hypothalamic Akt and suppress hepatic gluconeogenesis in rats. *Am. J. Physiol.* **305** (2): E230-E242. DOI: 10.1152/ajpendo.00094.2013.
96. Sudnikovich EJ, Maksimchik YZ, Zabrodskaya SV, Kubyshin VL, Lapshina EA, Bryszewska M, Reiter RJ, Zavodnik IB (2007) Melatonin attenuates metabolic disorders due to streptozotocin-induced diabetes in rats. *Eur. J. Pharmacol.* **569** (3): 180-187. DOI: 10.1016/j.ejphar.2007.05.018
97. Pierrefiche G, Laborit H (1995) Oxygen free radicals, melatonin, and aging. *Exp. Gerontol.* **30** (3-4): 213-227. DOI: 10.1016/0531-5565(94)00036-3.
98. Winiarska K, Dzik JM, Labudda M, Focht D, Sierakowski B, Owczarek A, Komorowski L, Bielecki W (2016) Melatonin nephroprotective action in Zucker diabetic fatty rats involves its inhibitory effect on NADPH oxidase. *J. Pineal Res.* **60** (1): 109-117. DOI: 10.1111/jpi.12296.
99. Simões D, Riva P, Peliciari-Garcia RA, Cruzat VF, Graciano MF, Munhoz AC, Taneda M, Cipolla-Neto J, Carpinelli AR (2016) Melatonin modifies basal and stimulated insulin secretion via NADPH oxidase. *J Endocrinol.* **231** (3): 235-244. DOI: 10.1530/JOE-16-0259.
100. Winiarska K, Jarzyna R, Dzik JM, Jagielski AK, Grabowski M, Nowosielska A, Focht D, Sierakowski B (2015) ERK1/2 pathway is involved in renal gluconeogenesis inhibition under conditions of lowered NADPH oxidase activity. *Free Radic. Biol. Med.* **81**: 13-21. DOI: 10.1016/j.freeradbiomed.2014.12.024.
101. Agil A, El-Hammadi M, Jiménez-Aranda A, Tassi M, Abdo W, Fernández-Vázquez G, Reiter RJ (2015) Melatonin reduces hepatic mitochondrial dysfunction in diabetic obese rats. *J. Pineal Res.* **59** (1): 70-79. DOI: 10.1111/jpi.12241.
102. Agil A, Rosado I, Ruiz R, Figueroa A, Zen N, Fernández-Vázquez G (2012) Melatonin improves glucose homeostasis in young Zucker diabetic fatty rats. *J. Pineal Res.* **52** (2): 203-210. DOI: 10.1111/j.1600-079X.2011.00928.x.
103. Martyn JA, Kaneki M, Yasuhara S, Warner DS, Warner MA (2008) Obesity-induced insulin resistance and hyperglycemia: etiologic factors and molecular mechanisms. *Anesthesiology* **109** (1): 137-148. DOI: 10.1097/ALN.0b013e3181799d45.
104. Wellen KE, Hotamisligil GS (2005) Inflammation, stress, and diabetes. *J. Clin. Invest.* **115** (5): 1111-1119. DOI: 10.1172/JCI25102.
105. Saltiel AR, Kahn CR (2001) Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414** (6865): 799-806.
106. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, Wang S, Fortier M, Greenberg AS, Obin MS (2005) Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J. Lipid Res.* **46** (11): 2347-2355. DOI: 10.1194/jlr.M500294-JLR200.

107. Özcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Özdelen E, Tuncman G, Görgün C, Glimcher LH, Hotamisligil GS (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* **306** (5695): 457-461. DOI: 10.1126/science.1103160.
108. Navarro-Alarcón M, Gil-Hernández F, Sánchez-González C, Llopis J, Villalón-Mir M, Olmedo P, Alarcón-Guijo P, Salagre D, Gaona L, Paredes M, Agil A (2021) Melatonin improves levels of Zn and Cu in the muscle of diabetic obese rats. *Pharmaceutics* **13** (10): 1535. DOI: 10.3390/pharmaceutics13101535.
109. Fernández Vázquez G, Reiter RJ, Agil A (2018) Melatonin increases brown adipose tissue mass and function in Zücker diabetic fatty rats: implications for obesity control. *J. Pineal Res.* **64** (4): e12472. DOI: 10.1111/jpi.12472.
110. Promsan S, Lungkaphin A (2020) The roles of melatonin on kidney injury in obese and diabetic conditions. *BioFactors* **46** (4): 531-549. DOI: 10.1002/biof.1637.
111. Agil A, Chayah M, Visiedo L, Navarro-Alarcon M, Rodríguez Ferrer JM, Tassi M, Reiter RJ, Fernández-Vázquez G (2020) Melatonin improves mitochondrial dynamics and function in the kidney of zücker diabetic fatty rats. *J. Clin. Med* .**9** (9): 2916. DOI: 10.3390/jcm9092916.
112. Tsalamandris S, Antonopoulos AS, Oikonomou E, Papamikroulis GA, Vogiatzi G, Papaioannou S, Deftereos S, Tousoulis D (2019) The role of inflammation in diabetes: current concepts and future perspectives. *Eur. Cardiol.* **14** (1): 50. DOI: 10.15420/ecr.2018.33.1.
113. Feuerer M, Shen Y, Littman DR, Benoist C, Mathis D (2009) How punctual ablation of regulatory T cells unleashes an autoimmune lesion within the pancreatic islets. *Immunity* **31** (4): 654-664. DOI: 10.1016/j.immuni.2009.08.023.
114. Moore F, Colli ML, Cnop M, Esteve MI, Cardozo AK, Cunha DA, Bugliani M, Marchetti P, Eizirik DL (2009) PTPN2, a candidate gene for type 1 diabetes, modulates interferon-γ–induced pancreatic β-cell apoptosis. *Diabetes* **58** (6): 1283-1291. DOI: 10.2337/db08-1510.
115. Leibowitz G, Bachar E, Shaked M, Sinai A, Ketzinel-Gilad M, Cerasi E, Kaiser N (2010) Glucose regulation of β-cell stress in type 2 diabetes. *Diabetes Obes. Metab* .**12**: 66-75. DOI: 10.1111/j.1463-1326.2010.01280.x.
116. Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG (2009) Analysis of islet inflammation in human type 1 diabetes. *Clin. Exp. Immunol.* **155** (2): 173-181. DOI: 10.1111/j.1365-2249.2008.03860.x.
117. Lehuen A, Diana J, Zaccone P, Cooke A (2010) Immune cell crosstalk in type 1 diabetes. *Nat. Rev. Immunol.* **10** (7): 501-513. DOI: 10.1038/nri2787.
118. Hotamisligil GS (2006) Inflammation and metabolic disorders. *Nature* **444** (7121): 860-867. DOI: 10.1038/nature05485.
119. Olefsky JM, Glass CK (2010) Macrophages, inflammation, and insulin resistance. *Annu. Rev. Physiol.* **72**: 219-246. DOI: 10.1146/annurev-physiol-021909-135846.
120. Lumeng CN, Saltiel AR (2011) Inflammatory links between obesity and metabolic disease. *J. Clin. Invest.* **121** (6): 2111-2117. DOI: 10.1172/JCI57132.
121. Hirosumi J, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS (2002) A central role for JNK in obesity and insulin resistance. *Nature* **420** (6913): 333-336. DOI: 10.1038/nature01137.
122. Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* **259** (5091): 87-91.DOI: 10.1126/science.7678183.

123. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM (2001) C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Jama* **286** (3): 327-334. DOI: 10.1001/jama.286.3.327.
124. Donath MY, Shoelson SE (2011) Type 2 diabetes as an inflammatory disease. *Nat. Rev. Immunol.* **11** (2): 98-107. DOI: https://doi.org/10.1038/nri2925.
125. Rehman K, Akash MS, Liaqat A, Kamal S, Qadir MI, Rasul A (2017) Role of interleukin-6 in development of insulin resistance and type 2 diabetes mellitus. *Crit. Rev. Eukaryot. Gene Expr.* **27** (3): 229-236. DOI: 10.1615/CritRevEukaryotGeneExpr.2017019712.
126. Larsen CM, Faulenbach M, Vaag A, Vølund A, Ehses JA, Seifert B, Mandrup-Poulsen T, Donath MY (2007) Interleukin-1–receptor antagonist in type 2 diabetes mellitus. *N. Engl. J. Med.* **356** (15): 1517-1526. DOI: 10.1056/NEJMoa065213.
127. Ehses JA, Lacraz G, Giroix MH, Schmidlin F, Coulaud J, Kassis N, Irminger JC, Kergoat M, Portha B, Homo-Delarche F, Donath MY (2009) IL-1 antagonism reduces hyperglycemia and tissue inflammation in the type 2 diabetic GK rat. *Proc. Natl. Acad. Sci.* **106** (33): 13998-14003. DOI: 10.1073/pnas.0810087106.
128. Shoelson SE, Herrero L, Naaz A (2007) Obesity, inflammation, and insulin resistance. *Gastroenterol.* **132** (6): 2169-2180. DOI: 10.1053/j.gastro.2007.03.059.
129. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa KI, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M (2006) MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J. Clin. Invest.* **116** (6): 1494-1505. DOI: 10.1172/JCI26498.
130. Antonopoulos AS, Margaritis M, Coutinho P, Shirodaria C, Psarros C, Herdman L, Sanna F, De Silva R, Petrou M, Sayeed R, Krasopoulos G (2015) Adiponectin as a link between type 2 diabetes and vascular NADPH oxidase activity in the human arterial wall: the regulatory role of perivascular adipose tissue. *Diabetes* **64** (6): 2207-2219. DOI: 10.2337/db14-1011.
131. Nikolajczyk BS, Jagannathan-Bogdan M, Shin H, Gyurko R (2011) State of the union between metabolism and the immune system in type 2 diabetes. *Genes Immun.***12** (4): 239-250. DOI: 10.1038/gene.2011.14.
132. Hardeland R (2018) Melatonin and inflammation—Story of a double-edged blade. *J. Pineal Res.* **65** (4): e12525. DOI: 10.1111/jpi.12525.
133. Tarocco A, Caroccia N, Morciano G, Wieckowski MR, Ancora G, Garani G, Pinton P (2019) Melatonin as a master regulator of cell death and inflammation: molecular mechanisms and clinical implications for newborn care. *Cell Death Dis.* **10** (4): 1-2. DOI: 10.1038/s41419-019-1556-7.
134. Reiter RJ, Calvo JR, Karbownik M, Qi W, Tan DX (2000) Melatonin and its relation to the immune system and inflammation. *Ann. N. Y. Acad. Sci.* **917** (1): 376-386. DOI: 10.1111/j.1749-6632.2000.tb05402.x.
135. Favero G, Franceschetti L, Bonomini F, Rodella LF, Rezzani R (2017) Melatonin as an anti-inflammatory agent modulating inflammasome activation. *Int. J. Endocrinol.* **2017**: 1835195. DOI: 10.1155/2017/1835195.
136. Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L (2016) Melatonin as an antioxidant: under promises but over delivers. *J. Pineal Res.* **61** (3): 253-278. DOI: 10.1111/jpi.12360.
137. Sharma A, Tate M, Mathew G, Vince JE, Ritchie RH, De Haan JB (2018) Oxidative stress and NLRP3-inflammasome activity as significant drivers of diabetic cardiovascular complications: therapeutic implications. *Front. Physiol.* **9**: 114. DOI: 10.3389/fphys.2018.00114.

138. El-Missiry MA, El-Missiry ZM (2020) Melatonin is a potential adjuvant to improve clinical outcomes in individuals with obesity and diabetes with coexistence of Covid-19. *Eur.J. Pharmacol.* **882**: 173329. DOI: 10.1016/j.ejphar.2020.173329.
139. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest.* **112** (12): 1821-1830. DOI: 10.1172/JCI19451.
140. Pourhanifeh MH, Hosseinzadeh A, Dehdashtian E, Hemati K, Mehrzadi S (2020) Melatonin: new insights on its therapeutic properties in diabetic complications. *Diab. Metab. Syndr.* **12**: 1-20. DOI: 10.1186/s13098-020-00537-z.
141. Devereux RB, Roman MJ, Paranicas M, O'Grady MJ, Lee ET, Welty TK, Fabsitz RR, Robbins D, Rhoades ER, Howard BV (2000) Impact of diabetes on cardiac structure and function: the strong heart study. *Circulation* **101** (19): 2271-2276. DOI: 0.1161/01.cir.101.19.2271.
142. Wold LE, Ceylan-Isik AF, Ren J (2005) Oxidative stress and stress signaling: menace of diabetic cardiomyopathy. *Acta Pharmacol. Sin*. **26** (8): 908-917. DOI: 10.1111/j.1745-7254.2005.00146.x.
143. Kandemir YB, Guntekin Ü, Tosun V, Korucuk N, Bozdemir MN (2018) Melatonin protects against streptozotocin-induced diabetic cardiomyopathy by the phosphorylation of vascular endothelial growth factor-A (VEGF-A). *Cell Mol. Biol.* **64** (14): 47-52. DOI: 10.14715/cmb/2018.64.14.8.
144. Kandemir YB, Tosun V, Güntekin Ü (2019) Melatonin protects against streptozotocin-induced diabetic cardiomyopathy through the mammalian target of rapamycin (mTOR) signaling pathway. *Adv. Clin. Exp. Med.* **28** (9): 1171-1177. DOI: 10.17219/acem/103799.
145. Yu LM, Di WC, Dong X, Li Z, Zhang Y, Xue XD, Xu YL, Zhang J, Xiao X, Han JS, Liu Y (2018) Melatonin protects diabetic heart against ischemia-reperfusion injury, role of membrane receptor-dependent cGMP-PKG activation. *Biochim. Biophys. Acta Mol. Basis Dis.***1864** (2): 563-578. DOI: 10.1016/j.bbadis.2017.11.023.
146. Aksoy N, Vural H, Sabuncu T, Aksoy S (2003) Effects of melatonin on oxidative–antioxidative status of tissues in streptozotocin-induced diabetic rats. *Cell Biochem. Funct.* **21** (2): 121-125. DOI: 10.1002/cbf.1006.
147. Amin AH, El-Missiry MA, Othman AI (2015) Melatonin ameliorates metabolic risk factors, modulates apoptotic proteins, and protects the rat heart against diabetes-induced apoptosis. *Eur. J. Pharmacol.* **747**: 166-173. DOI: 10.1016/j.ejphar.2014.12.002.
148. Yu L, Gong B, Duan W, Fan C, Zhang J, Li Z, Xue X, Xu Y, Meng D, Li B, Zhang M (2017) Melatonin ameliorates myocardial ischemia/reperfusion injury in type 1 diabetic rats by preserving mitochondrial function: role of AMPK-PGC-1α-SIRT3 signaling. *Sci. Rep.* **7** (1): 1-3. DOI: 10.1038/srep41337.
149. Zhou H, Yue Y, Wang J, Ma Q, Chen Y (2018) Melatonin therapy for diabetic cardiomyopathy: a mechanism involving Syk-mitochondrial complex I-SERCA pathway. *Cell Signal.* **47**: 88-100. DOI: 10.1016/j.cellsig.2018.03.012.
150. Xiong FY, Tang ST, Su H, Tang HQ, Jiang P, Zhou Q, Wang Y, Zhu HQ (2018) Melatonin ameliorates myocardial apoptosis by suppressing endoplasmic reticulum stress in rats with long-term diabetic cardiomyopathy. *Mol. Med. Rep.* **17** (1): 374-381. DOI: 10.3892/mmr.2017.7841.
151. Che H, Wang Y, Li H, Li Y, Sahil A, Lv J, Liu Y, Yang Z, Dong R, Xue H, Wang L (2020) Melatonin alleviates cardiac fibrosis via inhibiting lncRNA MALAT1/miR-141-mediated NLRP3 inflammasome and TGF-β1/Smadssignaling in diabetic cardiomyopathy. *The FASEB Journal.* **34** (4): 5282-5298. DOI: 10.1096/fj.201902692R.

152. Song YJ, Zhong CB, Wu W (2020) Cardioprotective effects of melatonin: Focusing on its roles against diabetic cardiomyopathy. *Biomed. Pharmacother* **128**: 110260. DOI: 10.1016/j.biopha.2020.110260.
153. Hammes HP, Feng Y, Pfister F, Brownlee M (2011) Diabetic retinopathy: targetingvasoregression. *Diabetes* **60** (1): 9-16. DOI: 10.1007/s00125-017-4435-8.
154. Mehrzadi S, Motevalian M, Rezaei Kanavi M, Fatemi I, Ghaznavi H, Shahriari M (2018) Protective effect of melatonin in the diabetic rat retina. *Fundam. Clin. Pharmacol.* **32** (4): 414-421. DOI: 10.1111/fcp.12361.
155. Jiang T, Chang Q, Zhao Z, Yan S, Wang L, Cai J, Xu G (2012) Melatonin-mediated cytoprotection against hyperglycemic injury in Müller cells. *PloS One* **7** (12): e50661. DOI: 10.1371/journal.pone.0050661.
156. Dehdashtian E, Mehrzadi S, Yousefi B, Hosseinzadeh A, Reiter RJ, Safa M, Ghaznavi H, Naseripour M (2018) Diabetic retinopathy pathogenesis and the ameliorating effects of melatonin; involvement of autophagy, inflammation and oxidative stress. *Life Sci.* **193**: 20-33. DOI: 10.1016/j.lfs.2017.12.001.
157. Jiang T, Chang Q, Cai J, Fan J, Zhang X, Xu G (2016) Protective effects of melatonin on retinal inflammation and oxidative stress in experimental diabetic retinopathy. *Oxid. Med. Cell Longev.* **2016**: 3528274 DOI:10.1155/2016/3528274.
158. Ma Y, Zhao Q, Shao Y, Cao MZ, Zhao M, Wang D (2019) Melatonin inhibits the inflammation and apoptosis in rats with diabetic retinopathy via MAPK pathway. *Eur. Rev. Med. Pharmacol. Sci.* **23** (3 Suppl): 1-8. DOI: 10.26355/eurrev_202007_22183.
159. Özdemir G, Ergün Y, Bakariş S, Kılınç M, Durdu H, Ganiyusufoğlu E (2014) Melatonin prevents retinal oxidative stress and vascular changes in diabetic rats. *Eye* **28** (8): 1020-1027. DOI: 10.1038/eye.2014.127.
160. Salido EM, Bordone M, De Laurentiis A, Chianelli M, Keller Sarmiento MI, Dorfman D, Rosenstein RE (2013) Therapeutic efficacy of melatonin in reducing retinal damage in an experimental model of early type 2 diabetes in rats. *J. Pineal Res.* **54** (2): 179-189. DOI: 10.1111/jpi.12008.
161. Allen DA, Harwood SM, Varagunam M, Raftery MJ, Yaqoob MM (2003) High glucose-induced oxidative stress causes apoptosis in proximal tubular epithelial cells and is mediated by multiple caspases. *FASEB J.* **17** (8): 1-21. DOI: 10.1096/fj.02-0130fje.
162. Alqasim AA, Eldin EE, Hammadi SH, Esheba GE (2020) Comparing the renoprotective effects of the antioxidants melatonin, vitamin D and vitamin E in diabetic rats. *J. Taibah Univ. Med. Sci.* **15** (5): 351-357. DOI: 10.1016/j.jtumed.2020.05.007.
163. Elbe H, Vardi Nİ, Esrefoglu M, Ates B, Yologlu S, Taskapan C (2015) Amelioration of streptozotocin-induced diabetic nephropathy by melatonin, quercetin, and resveratrol in rats. *Hum. Exp. Toxicol.* **34** (1): 100-113. DOI: 10.1177/0960327114531995.
164. Cam M, Yavuz Ö, GuvenA, Ercan F, Bukan N, Üstündag N (2003) Protective effects of chronic melatonin treatment against renal injury in streptozotocin-induced diabetic rats. *J. Pineal Res.***35** (3): 212-220. DOI: 10.1034/j.1600-079x.2003.00082.x.
165. Ebaid H, Bashandy SA, Abdel-Mageed AM, Al-Tamimi J, Hassan I, Alhazza IM (2020) Folic acid and melatonin mitigate diabetic nephropathy in rats via inhibition of oxidative stress. *Nutr. Metab.* **17** (1): 1-4. DOI: 10.1186/s12986-019-0419-7.
166. Onk D, Onk OA, Turkmen K, Erol HS, AyazogluTA, Keles ON, Halici M, Topal E (2016) Melatonin attenuates contrast-induced nephropathy in diabetic rats: the role of interleukin-33 and oxidative stress. *Mediators Inflamm.* **2016**: 9050828. DOI: 10.1155/2016/9050828.
167. Motawi TK, Ahmed SA, Hamed MA, El-Maraghy SA, Aziz WM (2019) Melatonin and/or rowatinex attenuate streptozotocin-induced diabetic renal injury in rats. *J. Biomed. Res.* **33** (2): 113-121. DOI: 10.7555/JBR.31.20160028.

168. Motawi TK, Ahmed SA, Hamed MA, El-Maraghy SA, Aziz WM (2016) Combination of melatonin and certain drugs for treatment of diabetic nephropathy in streptozotocin-induced diabetes in rats. *Diabetol. Int.* **7** (4): 413-424. DOI: 10.1007/s13340-016-0268-9.
169. Rashed LA, Elattar S, Eltablawy N, Ashour H, Mahmoud LM, El-Esawy Y (2018) Mesenchymal stem cells pretreated with melatonin ameliorate kidney functions in a rat model of diabetic nephropathy. *Biochem. Cell Biol.* **96** (5): 564-571. DOI: 10.1139/bcb-2017-0230.
170. Ha H, Yu MR, Kim KH (1999) Melatonin and taurine reduce early glomerulopathy in diabetic rats. *Free Radic. Biol. Med.* **26** (7-8): 944-950. DOI: 10.1016/s0891-5849(98)00276-7.
171. DeFronzo RA, Goodman AM (1995)Multicenter Metformin Study Group. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **333** (9): 541-549. DOI: 10.1056/NEJM199508313330902.
172. de Lima Ávila D, de Araújo GR, Silva M, de Amorim Miranda PH, Diniz MF, Pedrosa ML, Silva ME, de Lima WG, Costa DC (2013) Vildagliptin ameliorates oxidative stress and pancreatic beta cell destruction in type 1 diabetic rats. *Arch. Med. Res.* **44** (3): 194-202. DOI: 10.1016/j.arcmed.2013.03.004.
173. Marín-Peñalver JJ, Martín-Timón I, Sevillano-Collantes C, del Cañizo-Gómez FJ (2016) Update on the treatment of type 2 diabetes mellitus. *World J. Diabetes* **7** (17): 354-395. DOI: 10.4239/wjd.v7.i17.354.
174. Wang YW, He SJ, Feng X, Cheng J, Luo YT, Tian L, Huang Q (2017) Metformin: a review of its potential indications. *Drug Des. Devel. Ther.* **11**: 2421-2429. DOI: 10.2147/DDDT.S141675.
175. Kadhim HM, Ismail SH, Hussein KI, Bakir IH, Sahib AS, Khalaf BH, Hussain SA (2006) Effects of melatonin and zinc on lipid profile and renal function in type 2 diabetic patients poorly controlled with metformin. *J. Pineal Res.* **41** (2): 189-193. DOI: 10.1111/j.1600-079X.2006.00353.x.
176. Hussain SA, Khadim HM, Khalaf BH, Ismail SH, Hussein KI, Sahib AS (2006) Effects of melatonin and zinc on glycemic control in type 2 diabetic patients poorly controlled with metformin. *Saudi Med. J.* **27** (10): 1483-1488. PMID: 17013468.
177. Andrzejewski S, Gravel SP, Pollak M, St-Pierre J (2014) Metformin directly acts on mitochondria to alter cellular bioenergetics. *Cancer Metab.* **2** (1): 1-4. DOI: 10.1186/2049-3002-2-12.
178. Esteghamati A, Eskandari D, Mirmiranpour H, Noshad S, Mousavizadeh M, Hedayati M, Nakhjavani M (2013) Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: a randomized clinical trial. *Clin. Nutr.* **32** (2): 179-185. DOI: 10.1016/j.clnu.2012.08.006.
179. Chakraborty A, Chowdhury S, Bhattacharyya M (2011) Effect of metformin on oxidative stress, nitrosative stress and inflammatory biomarkers in type 2 diabetes patients. *Diabetes Res. Clin Pract.* **93** (1): 56-62. DOI: 10.1016/j.diabres.2010.11.030. Epub 2010 Dec 13.
180. Diniz Vilela D, Gomes Peixoto L, Teixeira RR, Belele Baptista N, Carvalho Caixeta D, Vieira de Souza A, Machado HL, Pereira MN, Sabino-Silva R, Espindola FS (2016) The role of metformin in controlling oxidative stress in muscle of diabetic rats. *Oxid. Med. Cell Longev.* **2016**: 6978625. DOI:10.1155/2016/6978625.
181. Bonnefont-Rousselot D, Raji B, Walrand S, Gardes-Albert M, Jore D, Legrand A, Peynet J, Vasson MP (2003) An intracellular modulation of free radical production could contribute to the beneficial effects of metformin towards oxidative stress. *Metabolism* **52** (5): 586-589. DOI: 10.1053/meta.2003.50093.

182. Rösen P, Wiernsperger NF (2006) Metformin delays the manifestation of diabetes and vascular dysfunction in Goto–Kakizaki rats by reduction of mitochondrial oxidative stress. *Diabetes Metab. Res. Rev.* **22** (4): 323-330. DOI: 10.1002/dmrr.623.
183. Abdulkadir AA, Thanoon IA (2012) Comparative effects of glibenclamide and metformin on C-reactive protein and oxidant/antioxidant status in patients with type II diabetes mellitus. *Sultan Qaboos Univ. Med. J.* **12** (1): 55-61. DOI: 10.12816/0003088.
184. Han X, Wang B, Sun Y, Huang J, Wang X, Ma W, Zhu Y, Xu R, Jin H, Liu N (2018) Metformin modulates high glucose-incubated human umbilical vein endothelial cells proliferation and apoptosis through AMPK/CREB/BDNF pathway. *Front. Pharmacol.* **9**: 1266. DOI: 10.3389/fphar.2018.01266.
185. Ren H, Shao Y, Wu C, Ma X, Lv C, Wang Q (2020) Metformin alleviates oxidative stress and enhances autophagy in diabetic kidney disease via AMPK/SIRT1-FoxO1 pathway. *Mol. Cell Endocrinol*. **500**: 110628. DOI: 10.1016/j.mce.2019.110628.
186. Hardie DG, Hawley SA, Scott JW (2006) AMP-activated protein kinase–development of the energy sensor concept. *J. Physiol.* **574** (1): 7-15. DOI: 10.1113/jphysiol.2006.108944.
187. Leverve XM, Guigas B, Detaille D, Batandier C, Koceir EA, Chauvin C, Fontaine E, Wiernsperger NF (2003) Mitochondrial metabolism and type-2 diabetes: a specific target of metformin. *Diabetes Metab.***29** (4): 6S88-6S94. DOI: 10.1016/s1262-3636(03)72792-x.
188. Yang M, Darwish T, Larraufie P, Rimmington D, Cimino I, Goldspink DA, Jenkins B, Koulman A, Brighton CA, Ma M, Lam BYH, Coll AP, O'Rahilly S, Reimann F, Gribble FM (Inhibition of mitochondrial function by metformin increases glucose uptake, glycolysis and GDF-15 release from intestinal cells. *Sci Rep.* **11** (1): 2529. DOI: 10.1038/s41598-021-81349-7.
189. Docrat TF, Nagiah S, Naicker N, Baijnath S, Singh S, Chuturgoon AA (2020) The protective effect of metformin on mitochondrial dysfunction and endoplasmic reticulum stress in diabetic mice brain. *Eur. J. Pharmacol.* **875**: 173059. DOI: 10.1016/j.ejphar.2020.173059.
190. Batchuluun B, Inoguchi T, Sonoda N, Sasaki S, Inoue T, Fujimura Y, Miura D, Takayanagi R (2014) Metformin and liraglutide ameliorate high glucose-induced oxidative stress via inhibition of PKC-NAD (P) H oxidase pathway in human aortic endothelial cells. *Atherosclerosis.***232** (1): 156-164. DOI: 10.1016/j.atherosclerosis.2013.10.025.
191. Salman ZK, Refaat R, Selima E, El Sarha A, Ismail MA (2013) The combined effect of metformin and L-cysteine on inflammation, oxidative stress and insulin resistance in streptozotocin-induced type 2 diabetes in rats. *Eur. J. Pharmacol.* **714** (1-3): 448-455. DOI: 10.1016/j.ejphar.2013.07.002
192. Cameron AR, Morrison VL, Levin D, Mohan M, Forteath C, Beall C, McNeilly AD, Balfour DJ, Savinko T, Wong AK, Viollet B (2016) Anti-inflammatory effects of metformin irrespective of diabetes status. *Circ. Res.* **119** (5): 652-665. DOI: 10.1161/CIRCRESAHA.116.308445.
193. Saisho Y (2015) Metformin and inflammation: its potential beyond glucose-lowering effect. *Endocr. Metab. Immune Disord. Drug Targets* **15** (3): 196-205. DOI: 10.2174/1871530315666150316124019.
194. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N (2001) Role of AMP-activated protein kinase in mechanism of metformin action. *J. Clin. Invest.* **108** (8): 1167-1174. DOI: 10.1172/JCI13505.
195. Davis BJ, Xie Z, Viollet B, Zou MH (2006) Activation of the AMP-activated kinase by antidiabetes drug metformin stimulates nitric oxide synthesis in vivo by promoting the association of heat shock protein 90 and endothelial nitric oxide synthase. *Diabetes* **55** (2): 496-505. DOI: 10.2337/diabetes.55.02.06.db05-1064.

196. Hattori Y, Suzuki K, Hattori S, Kasai K (2006) Metformin inhibits cytokine-induced nuclear factor κB activation via AMP-activated protein kinase activation in vascular endothelial cells. *Hypertension* **47** (6): 1183-1188. DOI: 10.1161/01.HYP.0000221429.94591.72.
197. Li SN, Wang X, Zeng QT, Feng YB, Cheng X, Mao XB, Wang TH, Deng HP (2009) Metformin inhibits nuclear factor κB activation and decreases serum high-sensitivity C-reactive protein level in experimental atherogenesis of rabbits. *Heart vessels* **24** (6): 446-453. DOI: 10.1007/s00380-008-1137-7.
198. Chen W, Liu X, Ye S (2016) Effects of metformin on blood and urine pro-inflammatory mediators in patients with type 2 diabetes. *J. Inflamm.***13** (1): 1-6. DOI: 10.1186/s12950-016-0142-3.
199. Ruggiero-Lopez D, Lecomte M, Moinet G, Patereau G, Lagarde M, Wiernsperger N (1999) Reaction of metformin with dicarbonyl compounds. Possible implication in the inhibition of advanced glycation end product formation. *Biochem. Pharmacol.* **58** (11): 1765-1773. DOI: 10.1016/s0006-2952(99)00263-4.
200. Beisswenger P, Ruggiero-Lopez D (2003) Metformin inhibition of glycation processes. *Diabetes Metab.* **29** (4): 6S95-6S103. DOI: 10.1016/s1262-3636(03)72793-1.
201. Ishibashi Y, Matsui T, Takeuchi M, Yamagishi S (2012) Metformin inhibits advanced glycation end products (AGEs)-induced renal tubular cell injury by suppressing reactive oxygen species generation via reducing receptor for AGEs (RAGE) expression. *Horm. Metab. Res.* **44** (12): 891-895. DOI: 10.1055/s-0032-1321878.
202. Kim SA, Choi HC (2012) Metformin inhibits inflammatory response via AMPK–PTEN pathway in vascular smooth muscle cells. *Biochem.Biophys. Res. Commun.* **425** (4): 866-872. DOI: 10.1016/j.bbrc.2012.07.165.
203. Hundal RS, Krssak M, Dufour S, Laurent D, Lebon V, Chandramouli V, Inzucchi SE, Schumann WC, Petersen KF, Landau BR, Shulman GI (2000) Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes* **49** (12): 2063-2069. DOI: 10.2337/diabetes.49.12.2063.
204. Takashima M, Ogawa W, Hayashi K, Inoue H, Kinoshita S, Okamoto Y, Sakaue H, Wataoka Y, Emi A, Senga Y, Matsuki Y (2010) Role of KLF15 in regulation of hepatic gluconeogenesis and metformin action. *Diabetes* **59** (7): 1608-1615. DOI: 10.2337/db09-1679.
205. Cheng JT, Huang CC, Liu IM, Tzeng TF, Chang CJ (2006) Novel mechanism for plasma glucose–lowering action of metformin in streptozotocin-induced diabetic rats. *Diabetes* **55** (3): 819-825. DOI: 10.2337/diabetes.55.03.06.db05-0934.
206. Heishi M, Hayashi K, Ichihara J, Ishikawa H, Kawamura T, Kanaoka M, Taiji M, Kimura T (2008) Comparison of gene expression changes induced by biguanides in db/db mice liver. *J. Toxicol. Sci.* **33** (3): 339-347. DOI: 10.2131/jts.33.339.
207. He L, Sabet A, Djedjos S, Miller R, Sun X, Hussain MA, Radovick S, Wondisford FE (2009) Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. *Cell* **137** (4): 635-646. DOI: 10.1016/j.cell.2009.03.016.
208. Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA, Prigaro BJ, Wood JL, Bhanot S, MacDonald MJ, Jurczak MJ (2014) Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature* **510** (7506): 542-546. DOI: 10.1038/nature13270.
209. LaMoia TE, Shulman GI (2021) Cellular and molecular mechanisms of metformin action. *Endocr. Rev.* **42** (1): 77-96. DOI: 10.1210/endrev/bnaa023.
210. Alshawi A, Agius L (2019) Low metformin causes a more oxidized mitochondrial NADH/NAD redox state in hepatocytes and inhibits gluconeogenesis by a redox-

independent mechanism. *J. Biol. Chem.* **294** (8): 2839-2853. DOI:10.1074/jbc.RA118.006670.

211. Li M, Li X, Zhang H, Lu Y (2018) Molecular mechanisms of metformin for diabetes and cancer treatment. *Front. Physiol*. **9**: 1039. DOI: 10.3389/fphys.2018.01039.
212. Batandier C, Guigas B, Detaille D, El-Mir M, Fontaine E, Rigoulet M, Leverve XM (2006) The ROS production induced by a reverse-electron flux at respiratory-chain complex 1 is hampered by metformin. *J. Bioenerg. Biomembr.* **38** (1): 33-42. DOI: 10.1007/s10863-006-9003-8.
213. Owen MR, Doran E, Halestrap AP (2000) Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem. J.* **348** (3): 607-614.PMID: 10839993.
214. Ouyang J, Parakhia RA, Ochs RS (2011) Metformin activates AMP kinase through inhibition of AMP deaminase. *J. Biol. Chem.* **286** (1): 1-11. DOI:10.1074/jbc.M110.121806.
215. Shaw RJ, Lamia KA, Vasquez D, Koo SH, Bardeesy N, DePinho RA, Montminy M, Cantley LC (2005) The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science* **310** (5754): 1642-1646. DOI: 10.1126/science.1120781.
216. Kim YD, Park KG, Lee YS, Park YY, Kim DK, Nedumaran B, Jang WG, Cho WJ, Ha J, Lee IK, Lee CH (2008) Metformin inhibits hepatic gluconeogenesis through AMP-activated protein kinase–dependent regulation of the orphan nuclear receptor SHP. *Diabetes* **57** (2): 306-314. DOI: 10.2337/db07-0381.
217. Rines AK, Sharabi K, Tavares CD, Puigserver P (2016) Targeting hepatic glucose metabolism in the treatment of type 2 diabetes. *Nat. Rev. Drug Discov.* **15** (11): 786-804. DOI: 10.1038/nrd.2016.151.
218. An H, He L (2016) Current understanding of metformin effect on the control of hyperglycemia in diabetes. *J. Endocrinol.* **228** (3): R97-106. DOI:10.1530/JOE-15-0447.
219. Polianskyte-Prause Z, Tolvanen TA, Lindfors S, Dumont V, Van M, Wang H, Dash SN, Berg M, Naams JB, Hautala LC,Nisen H (2019) Metformin increases glucose uptake and acts renoprotectively by reducing SHIP2 activity. *FASEB J.* **33** (2): 2858-2869. DOI: 10.1096/fj.201800529RR.
220. Wiernsperger NF, Bailey CJ (1999) The antihyperglycaemic effect of metformin. *Drugs* **58** (1): 31-39. DOI: 10.2165/00003495-199958001-00009.
221. Diaz-Morales N, Rovira-Llopis S, Bañuls C, Lopez-Domenech S, Escribano-Lopez I, Veses S, Jover A, Rocha M, Hernandez-Mijares A, Victor VM (2017) Does metformin protect diabetic patients from oxidative stress and leukocyte-endothelium interactions?. *Antioxid. Redox. Signal* **27** (17): 1439-1445. DOI: 10.1089/ars.2017.7122.
222. Diaz-Morales N, Iannantuoni F, Escribano-Lopez I, Banuls C, Rovira-Llopis S, Sola E, Rocha M, Hernandez-Mijares A, Victor VM (2018) Does metformin modulate endoplasmic reticulum stress and autophagy in type 2 diabetic peripheral blood mononuclear cells?. *Antioxid. Redox. Signal* **28** (17): 1562-1569.DOI: 10.1089/ars.2017.7409.
223. Simon-Szabó L, Kokas M, Mandl J, Kéri G, Csala M (2014) Metformin attenuates palmitate-induced endoplasmic reticulum stress, serine phosphorylation of IRS-1 and apoptosis in rat insulinoma cells. *PLoS One* **9** (6): e97868. DOI: 10.1371/journal.pone.0097868.
224. Moon JS, Karunakaran U, Elumalai S, Lee IK, Lee HW, Kim YW, Won KC (2017) Metformin prevents glucotoxicity by alleviating oxidative and ER stress–induced CD36 expression in pancreatic beta cells. *J. Diab. Complicat.* **31** (1): 21-30. DOI: 10.1016/j.jdiacomp.2016.09.001.

225. Quentin T, Steinmetz M, Poppe A, Thoms S (2012) Metformin differentially activates ER stress signaling pathways without inducing apoptosis. *Dis. Model Mech.* **5** (2): 259-269. DOI: 10.1242/dmm.008110.
226. Jung TW, Lee MW, Lee YJ, Kim SM (2012) Metformin prevents endoplasmic reticulum stress-induced apoptosis through AMPK-PI3K-c-Jun NH2 pathway. *Biochem. Biophys. Res. Commun.* **417** (1): 147-152. DOI: 10.1016/j.bbrc.2011.11.073.
227. Li A, Zhang S, Li J, Liu K, Huang F, Liu B (2016) Metformin and resveratrol inhibit Drp1-mediated mitochondrial fission and prevent ER stress-associated NLRP3 inflammasome activation in the adipose tissue of diabetic mice. *Mol. Cell Endocrinol.* **434**: 36-47. DOI: 10.1016/j.mce.2016.06.008.
228. Kitabchi AE, Temprosa M, Knowler WC, Kahn SE, Fowler SE, Haffner SM, Andres R, Saudek C, Edelstein SL, Arakaki R, Murphy MB, Shamoon H (2005) Diabetes Prevention Program Research Group Role of insulin secretion and sensitivity in the evolution of type 2 diabetes in the diabetes prevention program: effects of lifestyle intervention and metformin. *Diabetes* **54** (8): 2404-2414. DOI: 10.2337/diabetes.54.8.2404.
229. Yang X, Xu Z, Zhang C, Cai Z, Zhang J (2017) Metformin, beyond an insulin sensitizer, targeting heart and pancreatic β cells. *Biochim. Biophys. Acta Mol. Basis Dis.***1863** (8): 1984-1990. DOI: 10.1016/j.bbadis.2016.09.019. Epub 2016 Oct 1.
230. Hashemitabar M, Bahramzadeh S, Saremy S, Nejaddehbashi F (2015) Glucose plus metformin compared with glucose alone on β cell function in mouse pancreatic islets. *Biomed. Rep.* **3** (5): 721-725. DOI: 10.3892/br.2015.476.
231. Patanè G, Piro S, Rabuazzo AM, Anello M, Vigneri R, Purrello F (2000) Metformin restores insulin secretion altered by chronic exposure to free fatty acids or high glucose: a direct metformin effect on pancreatic beta-cells. *Diabetes* **49** (5): 735-740. DOI: 10.2337/diabetes.49.5.735.
232. Marchetti P, Del Guerra S, Marselli L, Lupi R, Masini M, Pollera M, Bugliani M, Boggi U, Vistoli F, Mosca F, Del Prato S (2004) Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are ameliorated by metformin. *J. Clin. Endocrinol. Metab.* **89** (11): 5535-5541. DOI: 10.1210/jc.2004-0150.
233. Lupi R, Del Guerra S, Tellini C, Giannarelli R, Coppelli A, Lorenzetti M, Carmellini M, Mosca F, Navalesi R, Marchetti P (1999) The biguanide compound metformin prevents desensitization of human pancreatic islets induced by high glucose. *Eur. J. Pharmacol.* **364** (2-3): 205-209. DOI: 10.1016/s0014-2999(98)00807-3.
234. Lablanche S, Cottet-Rousselle C, Lamarche F, Benhamou PY, Halimi S, Leverve X, Fontaine E (2011) Protection of pancreatic INS-1 β-cells from glucose-and fructose-induced cell death by inhibiting mitochondrial permeability transition with cyclosporin A or metformin. *Cell Death Dis.***2** (3): e134. DOI: 10.1038/cddis.2011.15.
235. Sanchez-Rangel E, Inzucchi SE (2017) Metformin: clinical use in type 2 diabetes. *Diabetologia* **60** (9): 1586-1593. DOI: 10.1007/s00125-017-4336-x.
236. McCreight LJ, Bailey CJ, Pearson ER (2016) Metformin and the gastrointestinal tract. *Diabetologia* **59** (3): 426-435. DOI: 10.1007/s00125-015-3844-9.
237. Shurrab NT, Arafa ES (2020) Metformin: A review of its therapeutic efficacy and adverse effects. *Ob. Med.***17**: 100186. DOI: 10.1016/j.obmed.2020.100186.
238. DeFronzo R, Fleming GA, Chen K, Bicsak TA (2016) Metformin-associated lactic acidosis: Current perspectives on causes and risk. *Metabolism* **65** (2): 20-29. DOI: 10.1016/j.metabol.2015.10.014.
239. De Jager J, Kooy A, Lehert P, Wulffelé MG, Van der Kolk J, Bets D, Verburg J, Donker AJ, Stehouwer CD (2010) Long term treatment with metformin in patients with type 2

diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. *BMJ* **340**: c2181. DOI: 10.1136/bmj.c2181.

240. Hsu WH, Hsiao PJ, Lin PC, Chen SC, Lee MY, Shin SJ (2018) Effect of metformin on kidney function in patients with type 2 diabetes mellitus and moderate chronic kidney disease. *Oncotarget* **9** (4): 5416-5423. DOI: 10.18632/oncotarget.23387.

241. Bubenik GA (2001) Localization, physiological significance and possible clinical implication of gastrointestinal melatonin. *Neurosignals* **10** (6): 350-366. DOI: 10.1159/000046903

242. Rahman A, Hasan AU, Kobori H (2019) Melatonin in chronic kidney disease: A promising chronotherapy targeting the intrarenal renin–angiotensin system. *Hypertens. Res*. **42** (6): 920-923. DOI: 10.1038/s41440-019-0223-9.

243. Raza Z, Naureen Z (2020) Melatonin ameliorates the drug induced nephrotoxicity: Molecular insights. *Nefrología* **40** (1): 12-25. DOI: 10.1016/j.nefro.2019.06.009.

Please cite this paper as:
Banerjee, A., Chattopadhyay, A. and Bandyopadhyay, D. 2021. Potentially synergistic effects of melatonin and metformin in alleviating hyperglycemia: a comprehensive review. Melatonin Research. 4, 4 (Dec. 2021), 522-550. DOI:https://doi.org/https://doi.org/10.32794/mr112500110.