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Review Article

Effect of Metformin on the Expression and Action of FOXO1, RAGE, Nrf2, and SIRT1 in Diabetes-Associated Disorders: A Review Article

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Abstract
Type 2 diabetes (T2D) is one of the most prevalent, multisystemic, and chronic metabolic disorders that is characterized by the presence of oxidative stress (OS) and mitochondrial dysfunction, leading to different pathologic conditions. Various genes and their coding proteins, such as nuclear factor erythroid 2-related factor 2 (NrF2), receptor for advanced glycation end products (RAGE), silent information regulator 1 (SIRT1), and forkhead box transcription factor O1 (FOXO1), can considerably alleviate diabetes-induced OS production, mitochondrial dysfunction, and insulin resistance. In this regard, metformin is considered one of the safest and first-line oral therapies for T2D. Accordingly, this review has focused on the effect of metformin on the expression levels of NrF2, RAGE, SIRT1, and FOXO1 in diabetes-associated disorders. It is noteworthy that metformin can play an important role in the etiopathology of T2D through a variety of molecular pathways involving genes and the expression of their coding proteins, such as NrF2, RAGE, SIRT1, and FOXO1.
Keywords: SIRT1, RAGE, NrF2, FOXO1, Type 2 diabetes, Metformin

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Introduction

Type 2 diabetes (T2D) is a multisystemic, widespread, and chronic metabolic disorder that manifests by the presence of mitochondrial dysfunction and oxidative stress (OS), resulting in various complications, such as cardiovascular diseases (CVDs), nephropathy, cognitive dysfunction, and insulin resistance (1-3). The global prevalence of T2D has been rising rapidly, and the increasing number of adolescents and children with T2D is being diagnosed (4). Different genes and their coding proteins, including nuclear factor erythroid 2-related factor 2 (Nrf2), receptor for advanced glycation end products (RAGE), silent information regulator 1 (SIRT1), and forkhead box transcription factor O1 (FoxO1), have been shown to be involved in the etiology of T2D via various molecular pathways. Moreover, they are associated with a wide variety of diabetes complications. Despite the discovery of numerous new antidiabetic drugs, metformin remains the first-line approach for treating T2D, primarily owing to its function in reducing hepatic glucose production (HGP) through several mechanisms (5,6). Low concentrations of metformin activate adenosine monophosphate-activated protein kinase (AMPK), decrease glucagon-induced cyclic AMP accumulation through cyclic nucleotide phosphodiesterase-4B, and reduce endogenous HGP via inhibiting mitochondrial glycerophosphate dehydrogenase (7,8). At high concentrations, metformin also contributes to a significant increase in the ratio of AMP/adenosine triphosphate (ATP) by increasing AMP while decreasing ATP levels. The enhanced AMP/ATP ratio increases AMPK activity, thereby blocking glucagon-induced HGP (9). Therefore, metformin has a role in the suppression of hepatic gluconeogenesis via ATP depletion (8,10). Two animal studies reported the overexpression of soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins in the muscle and adipose tissues of diabetic rats (3,10).

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Forkhead Box Transcription Factor O1: Structure and Function

The forkhead transcription factors of the O class (FOXOs) family are distinguished by a winged-helix DNA binding motif and the presence of a forkhead domain (11). In mammals, the FOXO transcription factor family consists of four isoforms: FOXO1, FOXO3, FOXO4, and FOXO6. These FOXO proteins are predominantly localized in the nucleus, where they play a vital role in controlling the transcription of specific genes (12,13).

Additionally , they can be located in the cytoplasm, where they are typically considered inactive. FOXO1, FOXO3, and FOXO4 are broadly expressed across various tissues. For example, FOXO1 is predominantly present in adipose tissue but is also significantly expressed in other metabolic tissues (e.g., the liver, skeletal muscle, pancreas, and brain). In addition, FOXO3 is mainly expressed in the liver, while FOXO4 is primarily found in muscle tissue. In contrast, FOXO6 expression is largely restricted to the brain (14,15). It is noteworthy that FOXO1, FOXO3, FOXO4, and FOXO6 regulate a range of similar cellular functions by modulating genes involved in different processes, such as the cell cycle, cellular metabolism, inflammation, glucose metabolism, and autophagy.

The activity of all FOXO proteins is modulated by post-translational modifications, particularly phosphorylation and acetylation. FOXO-inactivating kinases, such as protein kinase B (AKT), extracellular signal-regulated kinase (ERK), and cyclin-dependent kinases 1/2, and phosphorylated FOXO proteins, lead to their translocation from the nucleus to the cytoplasm, where they become inactive (16). Contrarily, phosphorylation by FOXO-activating kinases, including AMPK, c-Jun N-terminal kinases (JNK), mammalian STE20-like kinase 1, and protein kinase R-like endoplasmic reticulum kinase, promotes the nuclear localization and retention of FOXO proteins, thereby enhancing their transcriptional activity. The activation and inactivation of FOXO kinases are regulated by various physiological conditions, including those implicated in the development and progression of T2D (17).

Role of Forkhead Box Transcription Factor O in Type 2 Diabetes

FOXO1, a member of the O-class forkhead/winged helix transcription factors, plays a key role in regulating gluconeogenesis. Studies have shown that the activation of FOXO1 elevates plasma glucose levels and contributes to insulin resistance. However, conflicting research exists, as FOXO1 is also recognized for its role in mitigating OS and promoting cell survival (18). FOXO1 enhances HGP in the liver, modulates hepatic lipogenesis (either increasing or decreasing it), and influences adipogenesis in white adipose tissue. Additionally, FOXO1 reduces insulin production and secretion in the pancreas. The elevated levels of FOXO1 in the hypothalamus have been linked to an increased risk of developing T2D (19).

Effects of Metformin on Forkhead Box Transcription Factor O in Type 2 Diabetes

Recent studies have demonstrated that metformin, at various concentrations, suppresses glucagon-induced HGP and blood glucose levels by inhibiting PKA activity, reducing FOXO1-S273 phosphorylation, and attenuating FOXO1 activity. It achieves this suppression through the PKA-FOXO1 pathway in hepatocytes (1). According to experimental evidence, the inactivation of FOXO1 and FOXO3 in diabetic mouse models results in decreased HGP and increased hepatic lipid synthesis. Metformin has been shown to lower blood glucose and HGP by targeting hepatic FOXO1 in mouse models (20). Conversely, hepatic FOXO1 is essential for the ability of metformin to suppress blood glucose and glucose output in mice. However, hepatic FOXO1 deficiency diminishes the suppressive effect of metformin on fasting blood glucose in *FOXO1* gene knockout mice (1). Moreover, metformin can reduce mitochondrial reactive oxygen species (ROS) levels by upregulating the expression of the antioxidant protein thioredoxin via the AMPK-FOXO3 pathway (21).

Metformin, Forkhead Box Transcription Factor O1, and Vascular Complications

Despite optimized therapeutic interventions, vascular complications arising from diabetes frequently result in poor patient outcomes (22). FOXO1 plays a critical role in the formation and maturation of blood vessels (23). The majority of patients with T2D experience cardiovascular complications (cardiac contractility variability), primarily driven by accelerated atherosclerosis and endothelial dysfunction, which contribute to the high prevalence of cardiac contractility variability (24,25). Metformin modulates the activities of carbohydrate response element-binding protein and FOXO1 in endothelial cells (26). Inhibiting FOXO1 presents a potential therapeutic strategy for managing diabetes-associated cardiovascular complications (23). Additionally, metformin can regulate macrophage functions in atherosclerosis by promoting macrophage apoptosis, reducing inflammation, and inhibiting monocyte differentiation through mechanisms such as the FOXO1/FABP4 pathway (27).

Lipid accumulation in macrophages contributes to the progression of atherosclerosis. Metformin decreases lipid buildup in both adipocytes and macrophages. Specifically, it reduces palmitate-induced lipid accumulation in macrophages by suppressing the FOXO1-mediated transcription of the fatty acid-binding protein (28). In addition, this medicine increases the population of cells expressing platelet endothelial cell adhesion molecule. The FOXO family regulates a broad range of cellular functions in cardiovascular tissues, with FOXO1 being expressed in platelet endothelial cell adhesion molecule-1-positive alveolar endothelial cells (29).

Imbalances in OS and excessive production of ROS in tissues play a significant role in the development of various complications, such as atherosclerosis, chronic

wounds, stroke, and cancer (30).

FOXO1 is predominantly activated in the myocardium of diabetic mice. In diabetes, the overactivation of cardiac FOXO1 contributes to imbalances in myocardial oxidative metabolism, as well as mitochondrial and cardiac dysfunction (31). Metformin can suppress cardiac apoptosis and protect against myocardial hypertrophy by inhibiting FOXO1 (32). According to recent research, the inhibition of FOXO1 alleviates diastolic dysfunction associated with T2D by enhancing myocardial pyruvate dehydrogenase activity (33).

Diabetic retinopathy has been associated with increased FOXO1 transcriptional activity under hyperglycemic conditions. Elevated FOXO1 levels play a critical role in the upregulation of pro-inflammatory factors and matrix metalloproteinase expression and the phenotypic switching of smooth muscle cells, contributing to pathological vascular remodeling in diabetic patients. Inhibiting FOXO1 may serve as a promising approach to mitigate adverse vascular remodeling during the progression of diabetes (23). Based on the findings of an animal study, both curcumin and metformin exhibited potent antioxidant properties and could effectively reduce diabetes-induced OS (25).

Metformin, Forkhead Box Transcription Factor O1, and Foot Ulcer

Research has demonstrated that inhibiting the superoxide and FOXO1/inducible nitric oxide synthase pathway in diabetic mice accelerates wound healing (34). Metformin has been shown to enhance endothelial function and improve the wound healing process. By regulating FOXO1 activity in endothelial cells, metformin may also facilitate wound healing through this mechanism. Insulin promotes epithelial wound healing via FOXO (35). The overexpression of wild-type FOXO1 has been found to enhance insulin signaling, highlighting its importance in maintaining normal insulin signaling in human adipocytes (36). Additionally, metformin can increase the expression of insulin receptors (37).

Effects of Metformin on Forkhead Box Transcription Factor O1 in Renal Diseases

A significant number of diabetic patients develop renal complications, with diabetic nephropathy being the primary cause of end-stage kidney disease and one of the most severe complications globally. FOXO1 influences various cellular processes (e.g., cell cycle arrest, autophagy, and apoptosis), playing a critical role in the pathogenesis of diabetic renal disease. Recent studies suggest that correcting the dysregulation of FOXO1 activity holds therapeutic potential for mitigating diabetic renal disease (38). Metformin has been shown to effectively reduce renal damage in a T2D rat model. Mechanistically, metformin alleviates OS, enhances autophagy, and inhibits cell proliferation in high glucose-stimulated mesangial cells by modulating the AMPK/SIRT1-FOXO1 signaling pathway (39).

Roles of Metformin on Forkhead Box Transcription Factor O in Cancer

Studies have shown that FOXOs are either deleted or fully inactivated in various human cancers due to the dysregulation of the phosphatidylinositol 3 (PI3K)-AKT signaling pathway, which is commonly altered in cancer (34). The deletion or inactivation of FOXOs results in the reduced expression of genes essential for inducing cell cycle arrest, apoptosis, and senescence (40). Additionally, research has revealed that the downregulation of FOXO1 is associated with lower survival rates in breast cancer, acute myeloid leukemia, and soft tissue sarcoma (41). Similarly, the decreased expression of FOXO3 has been linked to poor prognosis in neuroblastoma, as well as breast and colorectal cancers (40).

FOXOs have been widely investigated for their role in cancer metastasis, as they are generally considered the suppressors of metastasis in primary cells. In hepatocellular carcinoma (HCC), FOXO1 expression is inversely correlated with levels of epithelial-mesenchymal transition markers. Furthermore, studies suggest that the interplay between the PI3K and ERK signaling pathways regulates FOXO1 activation and metastasis through crosstalk between the MEK1/2 and AKT pathways (42). In addition, the downregulation of FOXO3 expression plays a critical role in promoting metastasis in kidney, prostate, and colorectal cancers (43).

Nuclear Factor Erythroid 2-Related Factor 2 and Metformin

Nrf2, a basic leucine zipper transcription factor belonging to the Cap-N-Collar family of proteins, plays a crucial role in maintaining ROS and redox homeostasis in the body. It is recognized as a key regulator of antioxidative responses and has been implicated in various diseases, including T2D. Nrf2 signaling is often disrupted in T2D. Metformin can noticeably modulate Nrf2 function (44). More precisely, metformin can downregulate the expression of Nrf2 target genes by enhancing glucose metabolism (45). According to previous research, metformin, particularly when combined with glibenclamide, suppresses the *RAGE* gene while activating *Nrf2* and *Sirt1* genes (46).

Effects of Metformin on Nuclear Factor Erythroid 2-Related Factor 2 in Type 2 Diabetes Osteoporosis

Recent studies have demonstrated that metformin exerts therapeutic effects on osteoporosis associated with T2D by mitigating high glucose-induced OS through the activation of the Nrf2/HO-1 signaling pathway (47). Additionally, Yang et al (48) revealed that metformin enhances osteogenic differentiation while reducing H_2O_2-induced oxidative damage in osteoblasts via the PI3K/AKT/Nrf2/HO-1 pathway.

Metformin, Nuclear Factor Erythroid 2-Related Factor 2, and Cancer

T2D is associated with an elevated risk of cancer development. The expression of Nrf2 is either upregulated or downregulated in certain cancers, including breast cancer, HCC, and pancreatic cancer (49,50). Nrf2 enhances the survival of cancer cells exposed to chemotherapeutic agents. Further, metformin exhibits anti-cancer properties when combined with other chemotherapeutic drugs through various mechanisms, such as inhibiting Nrf2-dependent transcription (49). Nrf2 is negatively regulated by Kelch-like ECH-associated protein 1 (Keap1), and the Keap1-Nrf2 axis plays a pivotal role in carcinogenesis. Metformin has been shown to counteract Nrf2-mediated chemoresistance (e.g., to cisplatin) in HepG2 HCC cells by modulating glucose metabolism and cellular glycolysis. Additionally, metformin appears to improve survival outcomes in pancreatic cancer patients with diabetes (50).

Effect of Metformin on Nuclear Factor Erythroid 2-Related Factor 2 in Kidney Diseases

T2D is independently linked to an increased risk of renal cell carcinoma (RCC) in women, though this association is not observed in men (51). The hyperactivation of Nrf2 and dysregulation of the Keap1/Nrf2 pathway have also been implicated in tumor progression and chemoresistance in kidney diseases, including RCC. Metformin can inhibit Nrf2 nuclear translocation, offering a potential therapeutic approach for treating fumarate hydratase-deficient RCC (52).

Metformin, Nuclear Factor Erythroid 2-Related Factor 2, and Stroke

Diabetes induces a range of microvascular and macrovascular alterations, making it a well-established risk factor for stroke. Adults with diabetes are 1.5 times more likely to experience a stroke compared to those without diabetes (53). Given that the Nrf2 pathway plays a central role in maintaining redox homeostasis and regulating various intermediary metabolic processes, targeting Nrf2 has emerged as a promising therapeutic strategy for managing and treating central nervous system disorders, including ischemic stroke (54). Metformin demonstrates neuroprotective effects against ischemic stroke injury by mitigating OS. It suppresses apoptosis through regulating the lncRNA-H19/miR-148a-3p/Rock2 axis and activating the HO-1/Nrf2 pathway (55).

Roles of Nuclear Factor Erythroid 2-Related Factor 2 in Cardiac and Vascular Complications

Diabetes is a significant risk factor for cardiovascular complications, such as atrial fibrillation. Recent studies have emphasized the role of Nrf2-related epigenetic regulation, including DNA and histone methylation, histone acetylation, and micro ribonucleic acid modulation, in developing diabetes-induced cardiac and vascular complications (56).

Silent Information Regulator 1

SIRT1, also called Sirtuin1, affects systemic metabolism by modulating lipid and glucose homeostasis through the deacetylation of various target proteins. Similarly, it influences glucose homeostasis through multiple mechanisms, including protecting pancreatic β-cells from OS-induced damage, regulating insulin secretion in β-cells, reducing inflammation, enhancing insulin sensitivity, promoting adiponectin secretion, facilitating lipid mobilization, controlling hepatic glucose production, and regulating mitochondrial biogenesis and fatty acid oxidation. As a result, SIRT1 represents a promising pharmacological target for the treatment of T2D (57). Reduced SIRT1 levels in diabetes are ameliorated by metformin through the activation of hepatic AMPK.

Metformin and Silent Information Regulator 1

Metformin acts as a SIRT1 agonist, mimicking many of the metabolic effects associated with calorie restriction. Both direct and indirect roles of metformin in regulating SIRT1 and influencing the aging process have been demonstrated. Based on the evidence, metformin, both alone and in combination with glibenclamide, upregulates the *Sirt1* gene. However, the precise molecular interactions between SIRT1 and metformin, as well as the detailed mechanisms by which metformin modulates SIRT1 activity, remain to be fully elucidated.

Metformin Effects on Silent Information Regulator 1 in Nephropathy

Inflammation and OS are critical mechanisms underlying the development of microvascular complications, including diabetic nephropathy. Diabetic nephropathy is characterized by a progressive rise in albuminuria and a subsequent decline in the glomerular filtration rate (58). The activation of SIRT1 enhances the cellular antioxidant response by upregulating the transcription of antioxidant genes through pathways involving Nrf2 and FOXO3a (59). Metformin mitigates diabetic nephropathy by activating the SIRT1/FOXO1 autophagic signaling axis. It also alleviates OS in the kidney by enhancing autophagy levels and reduces glomerular pathological changes through SIRT1-mediated deacetylation and FOXO1 activation (60). Additionally, this medicine indirectly increases hepatic SIRT1 levels via the AMP-mediated induction of nicotinamide phosphoribosyl transferase. The exposure of podocytes to high glucose concentrations leads to the downregulation of SIRT1 protein expression. However, metformin activates SIRT1 in podocytes through multiple signaling pathways (61).

Role of Metformin on Silent Information Regulator 1 in Cardiovascular Protection

Metformin has demonstrated beneficial effects on mitochondrial function and endothelial nitric oxide synthase and SIRT activation, contributing to its vasoprotective actions in patients with T2D (62). It exerts

a protective effect on cardiomyocytes damaged by high glucose levels by promoting mitophagy through the AMPKα/SIRT1/Parkin pathway. The cardioprotective properties of metformin are believed to arise from its pleiotropic effects on blood vessels, including impacts on smooth muscle cells, endothelial cells, blood lipid levels, systemic inflammation, and blood pressure regulation (63).

Additionally, a reduced rate of recurrent cardiovascular events in diabetic patients treated with metformin has been observed, partly attributed to its anti-inflammatory effects mediated by SIRT1 (64).

Metformin and Silent Information Regulator 1 in Liver Injury and Cholestasis

The administration of metformin in certain patients with T2D can lead to intrahepatic cholestasis and liver injury. It significantly increases the expression of SIRT1 protein in the liver, indicating that metformin and SIRT1 exert opposing effects on hepatic gluconeogenesis. Furthermore, metformin not only suppresses hepatic gluconeogenesis but also promotes intrahepatic cholestasis by regulating SIRT1 activity (47).

Metformin, Silent Information Regulator 1, and Retinopathy

Diabetic retinopathy is a multifactorial condition that is characterized by the inhibition of retinal SIRT1 in diabetes. SIRT1 plays a regulatory role in apoptosis, OS, and inflammation, contributing to the amelioration of diabetic retinopathy. Metformin has the potential to improve diabetic retinopathy by modulating SIRT1 activity (65).

Role of Metformin on Silent Information Regulator 1 in Stroke

Experimental studies have demonstrated that the overexpression of SIRT1 exerts neuroprotective effects on neurons by modulating metabolic imbalances, including the regulation of abnormal blood glucose levels (66).

Receptor for Advanced Glycation End Products

RAGE is a multi-ligand receptor capable of binding to a variety of ligands, including S-100 calcium-binding protein, advanced glycation end products (AGEs), amyloid-β-protein, high-mobility group box 1 (HMGB1), phosphatidylserine, and Mac-1 (67). Elevated blood glucose levels, or hyperglycemia, promote the formation of irreversible AGEs. Consequently, the accumulation of AGEs in diabetic tissues and circulation plays a mediating role in the pathogenesis of diabetic complications through their interaction with the primary cellular receptor, RAGE (68). Previous research has documented the overexpression of the *RAGE* gene in diabetic rats (6).

Metformin Effects of the Receptor for Advanced Glycation End Products in Atherosclerotic and Cardiovascular Disease

RAGE expression has been identified in atherosclerotic lesions in both diabetic and non-diabetic individuals; however, its expression is significantly elevated in diabetic patients (69). Recent studies have confirmed a correlation between the elevated plasma levels of RAGE and an increased risk of myocardial infarction, stroke, and CVD-related mortality (70).

In a study conducted on human subjects with T2D, Ragazzi et al found that adding metformin to glimepiride or pioglitazone is effective in reducing cardiovascular risk. They further concluded that these medicines act through the AGE-RAGE axis (71).

Likewise, Mota et al reported that metformin can inhibit the inflammatory response induced by AGEs in murine macrophages. This inhibitory effect occurs through the suppression of the RAGE/nuclear factor kappa B signaling pathway, suggesting a potential therapeutic benefit of metformin in the context of diabetic atherosclerosis (72).

Metformin, Receptor for Advanced Glycation End Products, and Retinopathy

The inhibition of RAGE effectively suppresses the development and progression of diabetic retinopathy (73). Moreover, RAGE inhibition mitigates damage to retinal pericytes by reducing glial fibrillary acidic protein levels while increasing nerve growth factor expression. Additionally, it decreases neovascularization by downregulating vascular endothelial growth factor expression in diabetic retinopathy. Given that metformin, both alone and in combination with glibenclamide, exerts a more pronounced repressive effect on *RAGE* gene expression, it holds potential for alleviating diabetic retinopathy (74).

Effects of the Receptor for Advanced Glycation End Products in Diabetic Osteodystrophy

Bone microarchitecture is adversely impacted by T2D, which disrupts normal matrix structure and cellular function through reduced osteoblast differentiation, increased osteoblast apoptosis, and enhanced osteoclast-mediated bone resorption (75). AGEs promote osteoblast apoptosis and play a significant role in the pathophysiology of diabetic osteoporosis. Mitochondrial dysfunction and OS are closely associated with AGE-induced apoptosis of osteoblastic cells in diabetic patients. Studies have demonstrated that the downregulation of RAGE and modulation of RAGE-mediated mitochondrial pathways can prevent AGE-induced osteodystrophy (76).

Metformin, the Receptor for Advanced Glycation End Products, and Inflammation

Inflammation is widely recognized as a key underlying factor in the development of various chronic diseases, including CVD, diabetes, kidney disease, cancer, and neurodegenerative disorders. The interaction between HMGB1 and the RAGE triggers the activation of nuclear

factor kappa B signaling, resulting in the production of angiogenic and pro-inflammatory molecules. Metformin has been shown to mitigate complications related to inflammatory stress by reducing HMGB1 and RAGE levels (22). In a study where RAGE was overexpressed in immortalized human keratinocytes (HaCaT cells), the AGE-induced signaling dysregulation was corrected by metformin. Specifically, the dysregulated phosphorylation of p38, ERK1/2, JNK, and Akt was restored with metformin treatment (77).

The anti-inflammatory and antioxidative effects of metformin are reported in some studies. Metformin suppresses the inflammation and diminishes inflammatory factors; this mechanism may be dependent or independent of AMPK (78).

Moreover, a recently published study examined the effects of metformin in combination with ezetimibe on peroxisome proliferator-activated receptor-γ and adiponectin gene expression in type 2 diabetic patients with metabolic dysfunction-assisted fatty liver disease. It was revealed that this medicine combination showed a notable improvement in insulin sensitivity in these patients (79).

Conclusion

NrF2, *RAGE*, *SIRT1*, and FOXO1 have been the therapeutic targets of many studies since they are important genes that contribute to the pathogenesis of T2D. According to our findings, metformin can play an essential role in the etiopathology of T2D through different molecular pathways involving genes and expression of their coding proteins (e.g., NrF2, RAGE, SIRT1, and FOXO1). Further studies focusing on clinical trials in this subject can reveal more details of the mechanism of action of metformin.

Authors' Contribution

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Competing Interests

The authors declare they have no competing interests.

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