

Metformin Inhibits Inflammation by Targeting the NLRP3 Inflammasome: Linking NF- κ B/NEK7/AMPK Signaling to Mitochondrial Function

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Abstract: Metformin, the first-line treatment for type 2 diabetes, has significant anti-inflammatory functions in addition to controlling blood glucose levels. Although metformin has been proven to inhibit NOD-like receptor pyrin domain containing 3 (NLRP3) inflammasome, the function of mitochondria in this effect has not been fully studied. This review summarizes studies on metformin that could regulate inflammation progression through the Toll-like Receptor 4/Nuclear Factor Kappa B (TLR4/NF- κ B) pathway, NIMA-related kinase 7 (NEK7) pathway, AMPK pathway, and Janus kinase 2-Signal Transducer and Activator of Transcription (JAK2-STAT) pathway, with the involvement of mitochondria. The effects of metformin on the mitochondria block the key steps of NLRP3 inflammation activation, including NEK7 assembly, reactive oxygen species (ROS) activation, reduced thioredoxin-interacting protein (TXNIP) expression, and caspase 1-mediated IL-1 β /IL-18 maturation. Importantly, we propose that metformin improves the pathological development of common multi-organ diseases by regulating NLRP3-inflammation pathways. These findings highlight the promising treatment potential of metformin in diseases driven by inflammasomes and make it meaningful to implement clinical trials to evaluate its efficacy in NLRP3 inflammasome-driven pathologies beyond diabetes.

Keywords: metformin, inflammasome, signaling pathways, elucidation of mechanism, multi-organ diseases

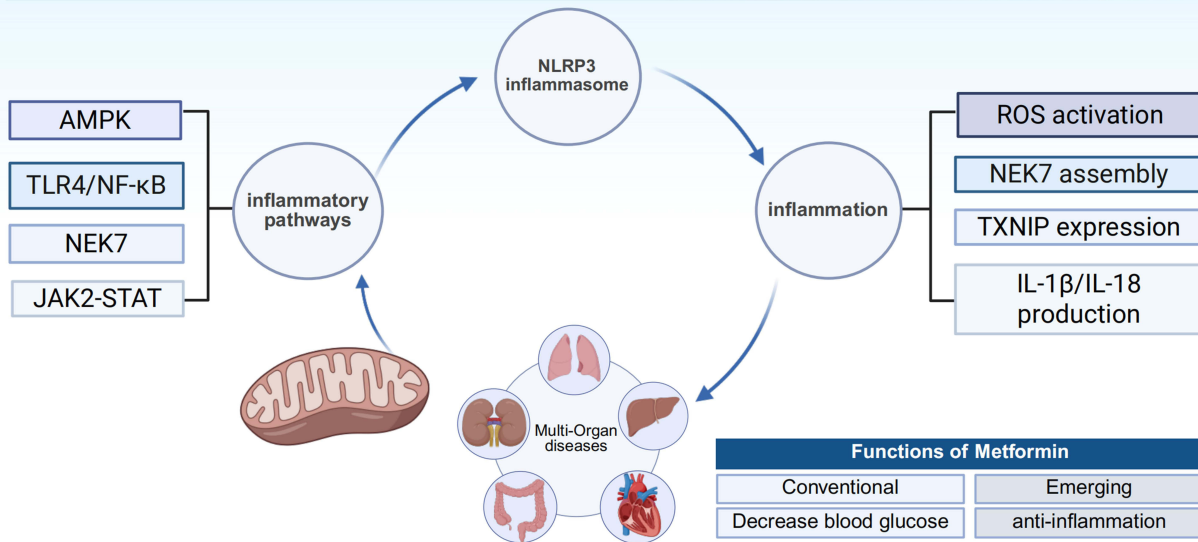
Introduction

Metformin, an antidiabetic medication, was approved by the U.S. Food and Drug Administration (FDA) in 1994 for the treatment of type 2 diabetes. This medication is available in immediate- and extended-release forms, and is frequently used with other antidiabetic drugs. Moreover, metformin is used off-label to manage gestational diabetes, address weight gain from antipsychotic medications, prevent type 2 diabetes, and treat polycystic ovarian syndrome (PCOS). Metformin is the sole antidiabetic medicine endorsed by the American Diabetes Association (ADA) for prediabetes.¹ Although metformin is widely recognized as a first-line anti-hyperglycemic drug for type 2 diabetes, emerging evidence indicates that its therapeutic potential transcends blood glucose control to improve inflammatory pathologies.² Chronic mild inflammation is a hallmark of metabolic disorders such as diabetes, where hyperglycemia and insulin resistance persistently facilitate pro-inflammatory cytokine production and endothelial dysfunction.^{3,4} The anti-inflammatory function of metformin is not merely secondary to glucose metabolism, as metformin can reduce IL-1 β and caspase-1

Graphical Abstract

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secretion.⁵ Accumulating evidence from preclinical and clinical studies has demonstrated the anti-inflammatory properties.¹

Chronic low-grade inflammation is a persistent subclinical inflammatory state characterized by a 2- to 3-fold increase in circulating inflammatory markers, such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α).⁶ It is now recognized as a key pathogenic factor in various metabolic and systemic diseases. Unlike acute inflammation, this silent process is driven by metabolic disorders, particularly in obesity, where the expansion of adipose tissue serves as a significant source of pro-inflammatory cytokines, perpetuating a vicious cycle of insulin resistance, endothelial dysfunction, and multi-organ damage.⁷ Evidence suggests that systemic inflammation is a modifiable process, emphasizing the fundamental principle of targeting inflammatory pathways.⁸ Lifestyle interventions, particularly regular physical activity, have been demonstrated to exert anti-inflammatory effects.^{9,10} Studies, including those conducted during the COVID-19 pandemic, consistently show that higher levels of physical activity, as assessed by tools such as the International Physical Activity Questionnaire (IPAQ), are associated with reduced disease severity in hospitalized patients and improved outcomes for conditions such as COVID-19, partly due to attenuated inflammatory responses.¹¹ This provides a compelling foundation for therapeutic strategies aimed at regulating the inflammatory cascade. The integration of lifestyle factors and pharmacological interventions is particularly crucial, as physical activity and metformin are often used in conjunction to treat metabolic disorders, suggesting that metformin may have a potential synergistic effect in mitigating chronic inflammation.^{12,13}

Some studies have identified that the anti-inflammatory properties of metformin may target the NLRP3 inflammasome, which has been verified to be a central hub for integrating metabolic disorders and chronic inflammation and has important functions, especially in aseptic inflammation induced by mitochondrial dysfunction in diabetes and obesity.^{14–16} NLRP3 inflammasome is a multiprotein complex that is essential for controlling inflammatory signals and the innate immune system.^{17,18} It is mainly composed of the core protein NLRP3, adaptor protein ASC, and effector protease Caspase 1.¹⁹ This review adopts the convention wherein the term “priming” denotes the initial stimulus that induces the

transcriptional upregulation of NLRP3 and pro-IL-1 β , whereas “activation” refers to the subsequent assembly of the inflammasome complex, culminating in the cleavage of caspase-1 and maturation of IL-1 β . Upstream signals from pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) can activate NLRP3, which then oligomerizes and activates caspase 1, stimulating the release of the pro-inflammatory cytokines IL-1 β and IL-18.²⁰ In this study, the TLR4/NF- κ B, Never in mitosis A-related kinase 7 (NEK7), and AMPK pathways promoted the nuclear translocation of NF- κ B, which activated the transcription of NLRP3. Diabetes, cancer, Alzheimer’s disease, and other inflammatory disorders are linked to the excessive activation of the NLRP3 inflammasome.²¹

Metformin can regulate NLRP3 activity: in colitis, metformin inhibits macrophage infiltration and decreases IL-1 β secretion in inflammatory tissues to regulate the activity of NLRP3 inflammasomes and inhibits apoptosis induced by NLRP3, which is characterized by a decrease in caspase-1 and gasdermin D (GSDMD) cleavage in lung epithelial cells.^{22,23} Although there have been some studies on this regulatory mechanism, a systematic summary is lacking. This study aimed to examine the relationship between metformin and inflammation in terms of the pathways through which metformin regulates inflammatory vesicles, the regulation of inflammatory vesicles through mitochondria, and the role of metformin in multiple organ diseases.

Regulatory Mechanisms of Metformin on NLRP3 Inflammasome Signaling Pathways

Metformin can modulate NLRP3 inflammasome signaling pathways through different mechanisms, such as the TLR4/NF- κ B signaling pathway; various molecules such as FOXO3, NEK7, and GSDMD; and key organelles such as mitochondria, endoplasmic reticulum, and Golgi (as shown in [Figure 1](#)). The regulatory mechanisms of metformin are discussed in detail below.

Metformin-Mediated Inflammatory Action of TLR4/NF- κ B Signaling Pathway

Toll-like receptors (TLRs) are type-I transmembrane glycoproteins. They can identify both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), controlling excessive immunological reactions by activating caspase-1.^{24,25} NF- κ B is a transcription factor known to be a key downstream component of the TLR4 signaling pathway.^{26,27}

Patients with type 2 diabetes have increased TLR4 expression and activation of NF- κ B signaling pathways.²⁸ As shown in [Figure 2](#), the NF- κ B signaling pathway is activated by site-specific phosphorylation of I κ B α through the breakdown of the inhibitor κ B kinase complex (IKK). Following this process, I κ B α is ubiquitinated and degraded by the proteasome, leading to translocation of NF- κ B into the nucleus.²⁹ Phosphorylated I κ B α facilitates the translocation of NF- κ B into the nucleus and activates NLRP3. Two main pathways are involved in NLRP3 assembly regulation: transcriptional and non-transcriptional. After entering the nucleus, NF- κ B binds to the NLRP3 promoter sequence and upregulates NLRP3, pro-IL-1 β , and pro-IL-18.³⁰ Non-transcriptional regulation occurs mainly through post-translational modification. BRCC3 mediates the deubiquitination of the NLRP3 LRR region, removing its auto-inhibitory state and promoting NLRP3 oligomerization. In addition, BRCC3 acts as an upstream signal for NF- κ B to promote its expression and activate inflammasome assembly.³¹ De-ubiquitination of BRCC3 is a precondition for JNK1 phosphorylation of NLRP3 and it cooperates to promote NLRP3 assembly.³² After NF- κ B activation, A20, a deubiquitinating enzyme that removes the K63 ubiquitin chain of TRAF6 and RIP1, is upregulated and inhibits the activity of E3 ligase to reduce the ubiquitin degradation of NLRP3, thereby stabilizing NLRP3.³³

Cameron et al performed several in vitro experiments using mouse hepatocytes where only 2mM metformin was added to the experimental group. The experimental group treated with metformin had higher levels of I κ B than the control group. I κ B is a negative regulator of NF- κ B; therefore, this study showed that metformin inhibits the NF- κ B signaling pathway, indicating its anti-inflammatory effect in the body.³⁴ Firstly, Zhu et al studied the neuroprotective effects of metformin in rats with Permanent middle cerebral artery occlusion (pMCAO). pMCAO was performed on male Sprague-Dawley rats that had been administered vehicle or metformin (50 mg/kg daily) for three weeks. The levels of I κ B- α phosphorylated at Ser32 increased 4.4-fold ($P < 0.05$) 24 h after pMCAO, suggesting that NF- κ B is activated during the acute phase of ischemic stroke. Chronic metformin

Metformin related inflammation pathway

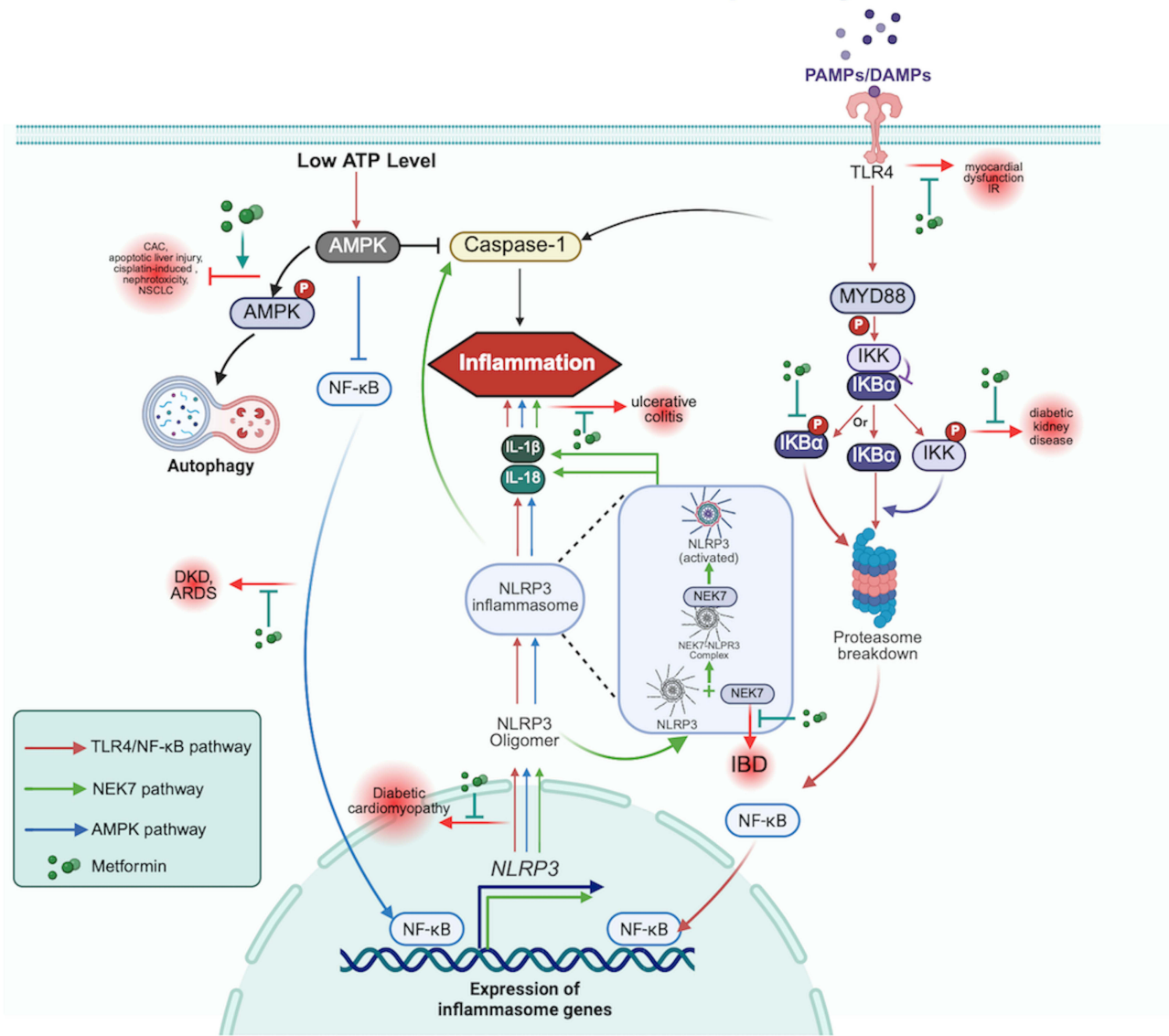


Figure 1 Metformin-related inflammatory pathways. This figure illustrates the primary molecular pathways that underlie the modulation of inflammation by metformin, with a focus on the NLRP3 inflammasome. Key upstream triggers include pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), which activate TLR4/MyD88 signaling, along with low ATP levels that activate AMPK. Both pathways converge on NF-κB, promoting the expression of pro-inflammatory genes. The assembly of the NLRP3 inflammasome, facilitated by NEK7 interaction, and subsequent activation of caspase-1 lead to the maturation of IL-1β and IL-18, thereby amplifying inflammatory responses. Metformin exerts its anti-inflammatory effects by suppressing TLR4/NF-κB signaling, enhancing AMPK-mediated autophagy, and inhibiting NLRP3 inflammasome activation through various mechanisms, as indicated by color-coded arrows: red for TLR4/NF-κB, green for NEK7, and blue for AMPK. Clinically relevant pathologies associated with these pathways include diabetic kidney disease (DKD), acute respiratory distress syndrome (ARDS), diabetic cardiomyopathy, inflammatory bowel disease (IBD), and cisplatin-induced nephrotoxicity, highlighting the therapeutic potential of metformin in alleviating inflammation-driven tissue damage.

preconditioning markedly reversed the increase in the protein levels of IκB-α phosphorylated at Ser32, 24 h after pMCAO. This shows that metformin inhibits the NF-κB pathway by decreasing the level of phosphorylated IκB-α.³⁵ Second, NF-κB and IL-1β levels in the blood were significantly decreased in people with T2D who were administered metformin daily.³⁶ In addition, powerful inhibition of NF-κB translocation from the cytoplasm to the nucleus suppresses further inflammatory cascades. The distribution of NF-κB in the nucleus was significantly decreased after treatment with metformin.³⁷ In another study, HEK293/TLR4 cells expressed TLR4, MD2, and CD14 to stimulate inflammation. The group treated with a higher dose of metformin had a larger gap than the lower-dose group, suggesting that metformin can reduce the motility of inflammatory cells.³⁸

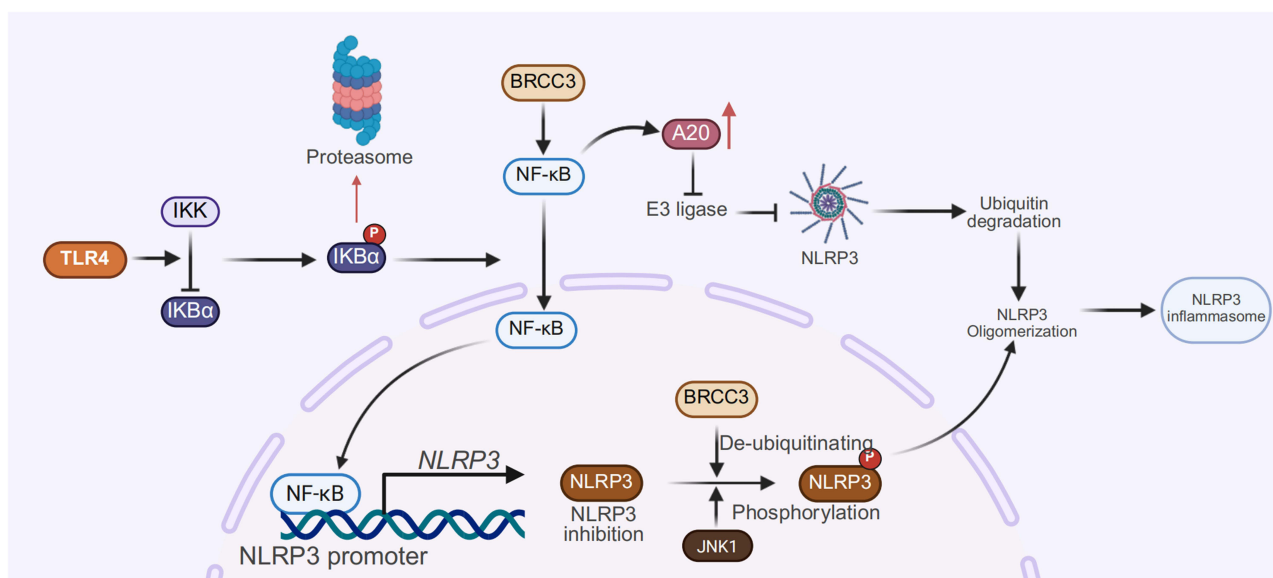


Figure 2 Inflammatory pathway of NLRP3 mediated by TLR4/NF-κB. The process is initiated by TLR4, which leads to the phosphorylation of IκBα mediated by the IKK complex, followed by the nuclear translocation of NF-κB. Nuclear NF-κB subsequently promotes the transcription of NLRP3. Post-translational regulation of NLRP3 has been demonstrated, including deubiquitination by BRCC3 and phosphorylation by JNK1. This pathway culminates in the oligomerization of NLRP3. Furthermore, A20, which is regulated by NF-κB, can inhibit the ubiquitin-mediated degradation of NLRP3, thereby promoting its oligomerization as well.

Metformin-Mediated Inflammatory Action of NEK7 Signaling Pathway

NEK7 is a kinase related to NIMA that acts as an upstream regulator of NLRP3 inflammasome formation. The interaction between NEK7 and NLRP3 is strengthened in response to inflammasome activation. As shown in Figure 3, the activation of NLRP3 involves two steps. NLRP3 binds to NEK7, which is enhanced by NLRP3 triggers. In addition, NLRP3 activation requires NACHT transformation from inactivation to activation, which is achieved by binding of ATP to the NACHT domain of Apaf-1.³⁹ The oligomers are crucial for the subsequent oligomerization of ASC and the activation of caspase-1.^{39,40} The connection between the C-terminal lobe of NEK7, curved LRR, and globular NACHT domains of NLRP3 is important for activating NLRP3 inflammasome.³⁹

The relationship between metformin and NEK7 has also been investigated. Metformin may contribute to reduce NEK7 expression and inhibit inflammation, leading to decreased NLRP3 inflammasome activity. This reduction in NEK7 levels may be associated with metformin-induced cell cycle arrest. Metformin may decrease NEK7 levels by arresting the

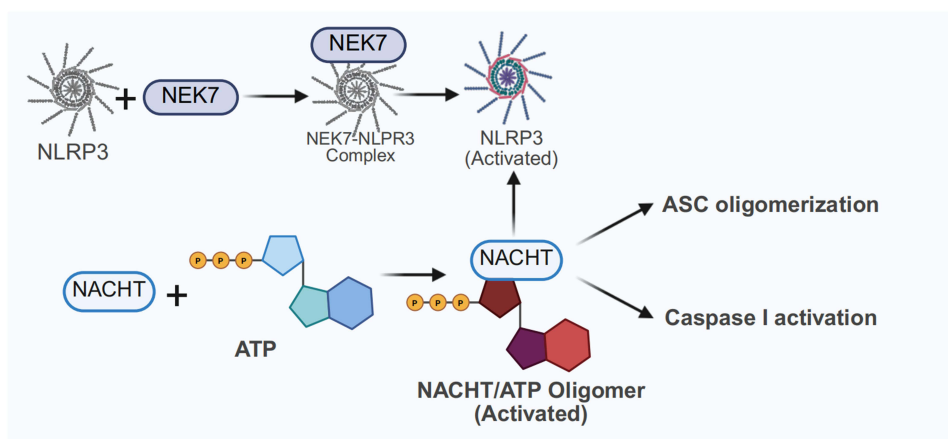


Figure 3 Inflammatory pathway of NLRP3 mediated by NEK7. The diagram illustrates the sequential interactions between NLRP3 and NEK7, culminating in the formation of an activation complex. Following this, the NACHT domain of NLRP3 binds to ATP, resulting in the formation of an activated NACHT/ATP oligomer. This oligomerization event subsequently initiates downstream NLRP3 activation, along with ASC oligomerization and Caspase-1 activation.

cell cycle.⁴¹ However, the interrelationship between metformin and the NEK7 pathway is not yet fully clear and additional studies are required to obtain more data to support this current topic.

Metformin-Mediated Inflammatory Action of AMPK Signaling Pathway

AMP-activated protein kinase (AMPK), a crucial cellular energy sensor, is a key target of metformin and plays a significant role in modulating NLRP3 inflammasome. As shown in Figure 4, metformin activates AMPK primarily through mechanisms that increase the cellular AMP/ATP ratio (eg., via mild inhibition of mitochondrial respiratory chain complex I at higher concentrations) or, more prominently, at clinically relevant doses by inhibiting the lysosomal proton pump vacuolar adenosine triphosphatase (v-ATPase) through interaction with presenilin enhancer 2 (PEN2), which activates AMPK without altering bulk AMP levels.^{42–44}

Activated AMPK mitigates NLRP3 inflammasome activity through several interconnected mechanisms. It suppresses NF- κ B signaling, a critical priming step for NLRP3, by reducing NLRP3 protein and pro-interleukin-1 β (pro-IL-1 β) availability.⁴⁵ For instance, metformin-induced AMPK activation can increase I κ B levels or decrease I κ B α phosphorylation, thus preventing NF- κ B nuclear translocation and expression of genes necessary for inflammasome priming.^{46,47} Consequently, this reduces the availability of NLRP3 and pro-IL-1 β . AMPK also inhibits the mammalian target of rapamycin (mTOR) pathway, which promotes NLRP3 activity.⁴⁸ This is achieved by directly phosphorylating and inhibiting mTOR complex 1 (mTORC1) components, such as Raptor, or by activating the mTORC1 inhibitor tuberous sclerosis complex (TSC2).⁴⁹ This AMPK/mTOR-mediated suppression of NLRP3 is evident in conditions such as diabetic cardiomyopathy.⁵⁰

Furthermore, damaged mitochondria pose an inflammatory threat by activating NLRP3 through reactive oxidative species (ROS) and mitochondrial DNA (mtDNA).⁵¹ Metformin counters this by enhancing AMPK-driven autophagy and mitophagy, processes vital for clearing these compromised organelles.^{23,52} This action effectively removes inflammasome triggers. Clinical studies involving T2D patients and preclinical models have validated this mechanism.⁵³ These studies demonstrate that metformin-enhanced mitophagy improves mitochondrial function and reduces NLRP3-related inflammation.⁵⁴

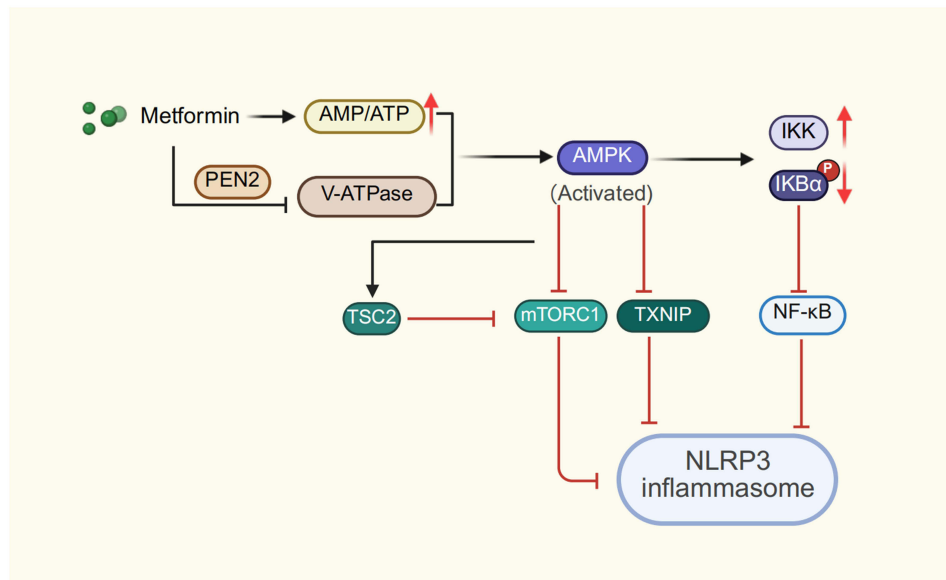


Figure 4 Metformin-mediated AMPK signaling pathway. Metformin increases the AMP/ATP ratio, thereby activating AMP-activated protein kinase (AMPK) and inhibiting vacuolar ATPase (V-ATPase) via PEN2. The activation of AMPK leads to the stabilization of tuberous sclerosis complex 2 (TSC2) and the inhibition of mechanistic target of rapamycin complex 1 (mTORC1). Concurrently, AMPK activation downregulates thioredoxin-interacting protein (TXNIP), which attenuates the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B). Additionally, AMPK may inhibit NLRP3 by suppressing NF- κ B, resulting in impaired transcription of NLRP3 and pro-inflammatory cytokines.

AMPK activation also mitigates oxidative stress by improving mitochondrial health and potentially upregulating antioxidant defenses (eg., via NRF2, discussed later), thereby reducing another critical NLRP3 activation signal.^{55–58} Some evidence also suggests that AMPK influences proteins such as thioredoxin-interacting protein (TXNIP), which directly activates NLRP3, and that metformin can reduce TXNIP expression partly via AMPK.⁵⁹ Consequently, in models like ischemia/reperfusion injury, metformin's AMPK stimulation correlates with suppressed NLRP3, ASC, cleaved caspase-1, IL-1 β , and IL-18 levels, underscoring AMPK's central role in metformin's anti-inflammatory effects on the NLRP3 inflammasome.^{50,60}

Metformin-Mediated Inflammatory Action of JAK2-STAT Signaling Pathway

The Janus kinase 2 (JAK2) Signal Transducer and Activator of Transcription (STAT) pathway transduces cytokines and growth signals via a phosphorylation cascade.⁶¹ The Tyr705 site of STAT3 is phosphorylated by activated JAK2 kinase, which drives dimerization, nuclear translocation, and transcription of target genes to regulate cell proliferation, apoptosis, and inflammation (shown in Figure 5).⁶² Dysregulated JAK2-STAT signaling, especially involving JAK2V617F mutation in myeloproliferative neoplasms (MPNs), is pathogenic.^{63,64} In type 2 diabetes, chronic hyperglycemia and insulin resistance abnormally activate JAK2-STAT3 in vascular endothelial cells and the myocardium, which upregulates ICAM-1 and VEGF to promote endothelial dysfunction.⁶² Metformin modulates JAK2-STAT signaling through AMPK-dependent and AMPK-independent mechanisms in specific contexts, AMPK-independent mechanisms.^{63,65} As a special regulator of the JAK-STAT pathway, the therapeutic potential of metformin extends beyond blood glucose control to the prevention and treatment of cardiovascular complications in diabetes.

AMPK-dependent inhibition influenced by metformin is closely related to the activity of JAK1, which is suppressed following its direct phosphorylation by AMPK at Serine 515 and 518.⁶⁶ This suppression of JAK1 can, in turn, indirectly dampen overall JAK-STAT signaling, including JAK2-dependent pathways. Furthermore, the expression or function of cytokine receptors such as IL-6R can be diminished by metformin through AMPK and mTORC1 inhibition, thereby reducing the IL-6-driven JAK2-STAT3 activation.⁶⁷ STAT3 activity is also directly affected by AMPK activation via two primary effects: inhibition of STAT3 nuclear translocation and promotion of its cytoplasmic phosphorylation at serine 727.^{68,69} This serine phosphorylation event, which results in altered STAT3 function and attenuated pro-inflammatory gene expression, is notably facilitated by AMPK α 1-dependent mTORC1 activation in response to signals like IL-10.⁶⁹

In certain contexts, particularly in JAK2V617F-mutated cells, metformin may inhibit JAK2 through AMPK-independent or complementary pathways. A key mechanism involves the activation of Protein Phosphatase 2A (PP2A) complexes containing the B56 α regulatory subunit.⁷⁰ Metformin-activated PP2A dephosphorylates and inactivates JAK2V617F.^{70,71} Thus, metformin may involve in both AMPK-dependent modulation of the broader JAK-STAT

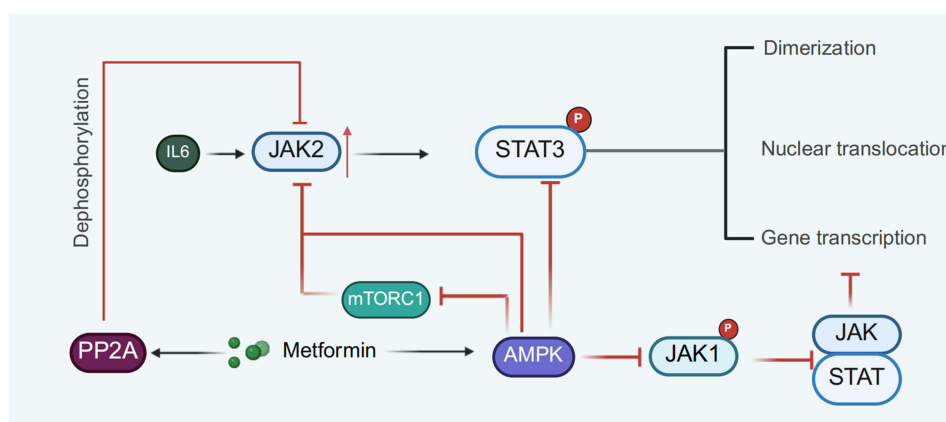


Figure 5 Metformin-mediated JAK2-STAT signaling pathway. The binding of interleukin-6 (IL-6) results in the activation of Janus kinase 2 (JAK2) and the phosphorylation of signal transducer and activator of transcription 3 (STAT3). Once phosphorylated, STAT3 dimerizes and translocates to the nucleus, where it promotes gene transcription. Metformin inhibits the activity of JAK1 through the activation of AMP-activated protein kinase (AMPK). Additionally, the phosphatase protein phosphatase 2A (PP2A) plays a crucial role in the dephosphorylation processes.

network and more direct, sometimes AMPK-independent, targeting of JAK2 activity, contributing to its anti-inflammatory and anti-proliferative effects.^{63,65} Additional studies are required to obtain more data to support this pathway.

Metformin Regulates the Inflammasome via Mitochondrion

Mitochondria play a crucial role as organelles in eukaryotic cells, and are primarily responsible for cellular energy metabolism. They are closely associated with both the maintenance of overall health and the development of various diseases such as diabetes and inflammation.⁷² In pancreatic β -cells, mitochondrial dysfunction interferes with oxidative phosphorylation, leading to decreased ATP production. Thus, ATP-sensitive potassium channels cannot be closed. Closed potassium channels hinder cell membrane depolarization by inhibiting the opening of voltage-gated calcium channels and calcium influx, thereby obstructing insulin release via exocytosis. Mutation of the mitochondrial tRNA leucine 1 (MT-TL1) gene disturbs oxidative phosphorylation and increases oxidative stress, causing maternally inherited diabetes and β -cell depletion. Mitochondrial DNA (mtDNA) released from damaged mitochondria, acting as a damage-associated molecule patterns (DAMPs), activates the NLRP3 inflammasome to promote the production of IL-1 β and TNF- α . In addition, mitochondrial ROS (mtROS) directly facilitates the assembly of the NLRP3 inflammasome by inducing potassium efflux and lysosomal instability, resulting caspase-1 dependent IL-1 β secretion from macrophages during chronic inflammation. Metformin mitigates NLRP3 inflammasome-induced inflammation in mitochondria.

As previously stated, the activation of the NLRP3 inflammasome is closely related to mitochondrial DNA and ROS. Therefore, regulation of mitochondrial homeostasis plays a crucial role in the inhibition of NLRP3 inflammasome signaling pathways.^{51,73} This section discusses the impact of metformin on the regulatory network underlying the NLRP3 inflammasome signaling pathways. The regulatory mechanism involves the interplay between various factors, including the electron transport chain (ETC), mitochondrial ROS (mtROS), oxidized mitochondrial DNA (ox-mtDNA), mitophagy, and mitochondrial antiviral signaling protein (MAVS) within the mitochondria.

The respiratory electron transport chain, mtROS and ox-DNA are shown in [Figure 6](#). ETC is a significant contributor to oxidative phosphorylation, which plays a crucial role in cellular energy.⁷⁴ Disorders affecting the mitochondrial electric potential are the main factors responsible for ROS production.⁷⁵ ROS originating from malfunctioning mitochondria plays a pivotal role in NLRP3 inflammasome activation. When the antioxidant N-acetylcysteine, which acts as a scavenger of mtROS, was introduced into THP-1 macrophages, a notable reduction in caspase-1 activation and IL-1 β production was observed.⁷⁶ This finding suggests that mtROS plays a crucial role in the activation of inflammatory responses.⁷⁷ However, recently produced mtDNA is extremely vulnerable to reactive oxygen species (ROS) within mitochondria, resulting in the formation of ox-mtDNA.⁷⁸ This ox-mtDNA then binds to NLRP3, a protein located in the cytoplasm, which subsequently initiates the assembly and activation of the NLRP3 inflammasome.^{51,73–75,77–79} Furthermore, the activation of NLRP3 can also augment mtROS production, thereby establishing a feedback loop for NLRP3 inflammasome activation. Hence, mitigation of the inflammatory response mediated by NLRP3 inflammasome requires restoration of ETC function, maintenance of mtROS homeostasis, and inhibition of ox-mtDNA generation.^{80,81}

A recent study reported that metformin effectively inhibits the synthesis of mtDNA induced by lipopolysaccharide (LPS) and ATP, as well as the production of ox-mtDNA, by specifically targeting respiratory ETC complex I. This study proposed that metformin effectively inhibits the activation of the NLRP3 inflammasome, regardless of the involvement of the AMPK and NF- κ B signaling pathways. Activation of the NLRP3 inflammasome has been postulated to be contingent upon the suppression of mtDNA synthesis induced by TLR.²³ Additionally, Yang et al⁸² reported that metformin inhibited NLRP3 inflammasome activation in diabetic mice. This inhibition was achieved through inhibition of ETC complex I, promotion of AMPK phosphorylation, and subsequent suppression of mTOR expression. Therefore, targeting respiratory ETC complex I presents a promising therapeutic approach for metformin in the treatment of NLRP3-driven diseases.

Mitophagy functions as a defence mechanism in response to diverse pathological stressors. It plays a crucial role in promoting the elimination of impaired mitochondria by guiding them to the lysosomes for degradation. This process is essential for maintaining cellular homeostasis, particularly in situations characterized by heightened oxidative stress and mitochondrial damage.^{83,84} Mitochondrial injury has the potential to disturb the cellular metabolic equilibrium, resulting

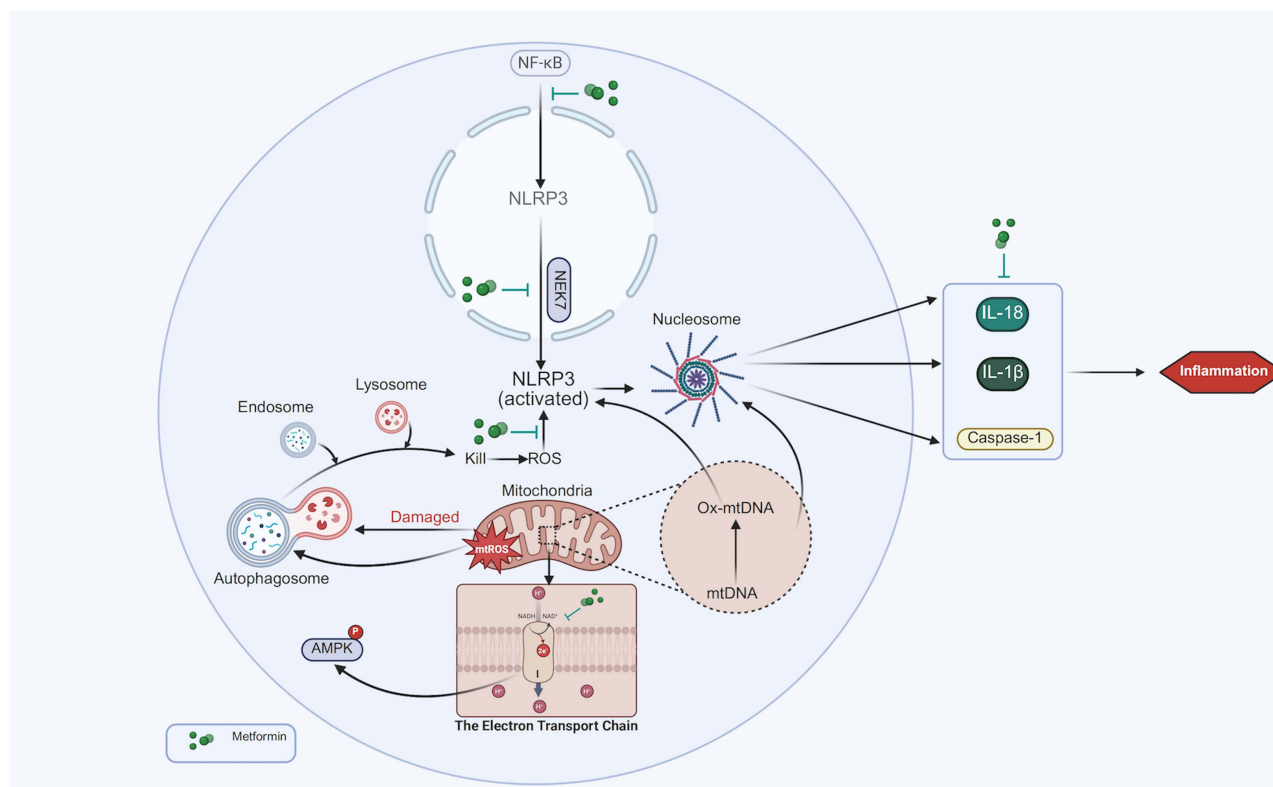


Figure 6 Metformin-mediated suppression of NLRP3 inflammasome activation via mitochondria. This figure illustrates the molecular mechanism by which metformin (depicted as green dots) inhibits NLRP3 inflammasome activation within the cellular cytoplasm. NF- κ B plays a crucial role in NLRP3 activation, which is further stimulated by reactive oxygen species (ROS) derived from mitochondrial damage and oxidized mitochondrial DNA (ox-mtDNA). The autophagosome collaborates with endosomes and lysosomes to degrade damaged mitochondria, potentially promoting the release of ROS. Nucleosomes and ox-mtDNA work synergistically to enhance NLRP3 assembly, leading to the activation of caspase-1. Subsequently, caspase-1 cleaves pro-IL-1 β and pro-IL-18 into their mature pro-inflammatory forms (IL-1 β and IL-18), thereby driving inflammation. Metformin inhibits mitochondrial electron transport chain (ETC) activity and activates AMP-activated protein kinase (AMPK), which collectively reduces mitochondrial ROS production and prevents the release of ox-mtDNA. As a result, NLRP3 inflammasome activation is suppressed. Additionally, metformin may directly obstruct NLRP3 assembly.

in ROS overproduction. Excessive ROS production subsequently initiates the activation of NLRP3 inflammasome.⁸⁵ Autophagy plays a crucial role in regulating NLRP3 inflammasome activation by eliminating dysfunctional mitochondria.^{86–89} Inhibition of the cellular process of autophagy can lead to the accumulation of dysfunctional mitochondria, which in turn can cause an increase in ROS generation and activation of the NLRP3 inflammasome.^{84,90} Autophagy functions as a buffering system that plays a crucial role in controlling ROS levels and regulating NLRP3 inflammasome activation through the clearance of damaged mitochondria.⁹¹

Metformin can promote mitophagy and inhibit NLRP3 activation by improving the abnormal morphology and function of the mitochondria, consequently reducing mtROS production.⁹² This finding aligns with a study conducted by Abad-Jiménez et al, who examined the effects of metformin treatment in obese patients with T2D.⁹³ Moreover, Fei et al demonstrated that metformin could mitigate the inflammatory response mediated by the autophagy-ROS-NLRP3 axis. This is achieved through the enhancement of autophagosome formation and autophagic flux, as well as the inhibition of ROS production in RAW264.7 cells treated with mtDNA-ATP.⁹⁴ Therefore, the activation of mitophagy could be a promising therapeutic approach for metformin, offering potential advantages in the management of diseases associated with mitochondrial dysfunction. Metformin inhibits TXNIP mRNA and protein expression in β -cells. This inhibition was thought to be mediated, at least in part, by AMPK.⁹⁵ Metformin reduces the binding of ChREBP and FOXO1 to the Thioredoxin-interacting protein (TXNIP) promoter, and reduces the nuclear entry rate.^{96,97} Metformin and resveratrol can reverse the increase in TXNIP expression and activate NLRP3 inflammasomes in the adipose tissue of diabetic mice exposed to high glucose, suggesting that metformin and resveratrol can inhibit the activation of NLRP3 inflammasomes by decreasing the expression of TXNIP.⁹⁸ Similarly, in a mouse model of ischemia-reperfusion (I/R)

injury, an increase in TXNIP expression was detected during I/R injury, and a subsequent decrease after metformin treatment.⁹⁹

Molecular Mechanisms and Clinical Transformation

The clinical effects of metformin on the NLRP3 inflammasome are substantiated by its influence on specific molecular pathways. As previously discussed, a primary mechanism involves the activation of AMP-activated protein kinase (AMPK). Additionally, metformin can reduce the production of mitochondrial reactive oxygen species (mtROS), which are potent activators of the NLRP3 inflammasome.²³

Translating these mechanisms into clinical benefits remains an active area of research. The observed association between metformin use and improved COVID-19 outcomes underscores its potential to modulate severe, dysregulated inflammation.¹⁰⁰ In the field of dermatology, the inhibitory effect on Hidradenitis Suppurativa provides a mechanistic basis for considering metformin as an adjunctive therapy for inflammatory skin diseases such as psoriasis, particularly in patients with metabolic disorders.¹⁰¹ Similarly, its role in periodontal cells suggests that it may play a significant role in controlling inflammatory responses in diabetic patients with periodontitis.¹⁰²

In summary, clinical and translational studies indicate that metformin exerts anti-inflammatory effects by targeting the NLRP3 inflammasome complex. These findings support the potential therapeutic use of metformin in treating inflammation associated with NLRP3 activation.

Metformin Affects Multiple Organs via the Inflammasome

Currently, the mechanism by which metformin affects different organ diseases via modulation of NLRP3 inflammasome-mediated inflammation remains unexplored in the existing literature. In this study, we conducted a comprehensive review of the influence of metformin on various organs that affect major physiological systems. Additionally, we summarized the impact of metformin on the NLRP3 inflammasome pathway (as shown in [Figure 7](#)). In [Tables 1](#) and [2](#), the mechanisms by which metformin acts on NLRP3 inflammasomes are respectively organized from the perspectives of diseases and pathways.

Heart

Metformin has significant cardioprotective functions in type 2 diabetes, reducing cardiovascular mortality and morbidity, as demonstrated in the UK Prospective Diabetes Study (UKPDS) where metformin therapy was associated with a 39% lower risk of myocardial infarction in overweight patients.¹¹⁷ Cardiovascular disease (CVD) is a leading cause of disability and morbidity in the majority of individuals with diabetes and lacks effective treatment.¹¹⁸ Therefore, understanding the relationship between metformin and the heart is important for the prevention and treatment of CVD. Diabetes-associated hyperglycemia leads to an increase in ROS, which triggers the activation of the NLRP3 inflammasome. This section explores the mechanism of action of metformin in regulating inflammation in the heart based on the signaling pathways by which inflammatory vesicles regulate inflammation.

TLR4/NF- κ B Signaling Pathway: Myocardial Dysfunction

Metformin inhibits the expression and activation of TLR4 and NF- κ B. Vaez et al studied the effects of metformin on myocardial dysfunction and TLR4 activity. Male rats were randomly divided into three groups: the control group was administered normal saline, the LPS group was administered lipopolysaccharide, and the metformin-treated group was administered both LPS and metformin. After nine hours, blood samples were collected and the hearts were extracted. The assessment encompassed the evaluation of the TLR4 expression rate, AMPK phosphorylation, TNF- α concentration, and MYD88 content in the heart. The result showed that the metformin group exhibited a notable increase in phosphorylated AMPK levels in the heart by 43%, whereas the concentration of the TLR adapter protein, MyD88, was diminished by 45%. This phenomenon occurred simultaneously with the significantly decreased expression of myocardial TLR4. Therefore, metformin inhibited TLR4.¹⁰⁹

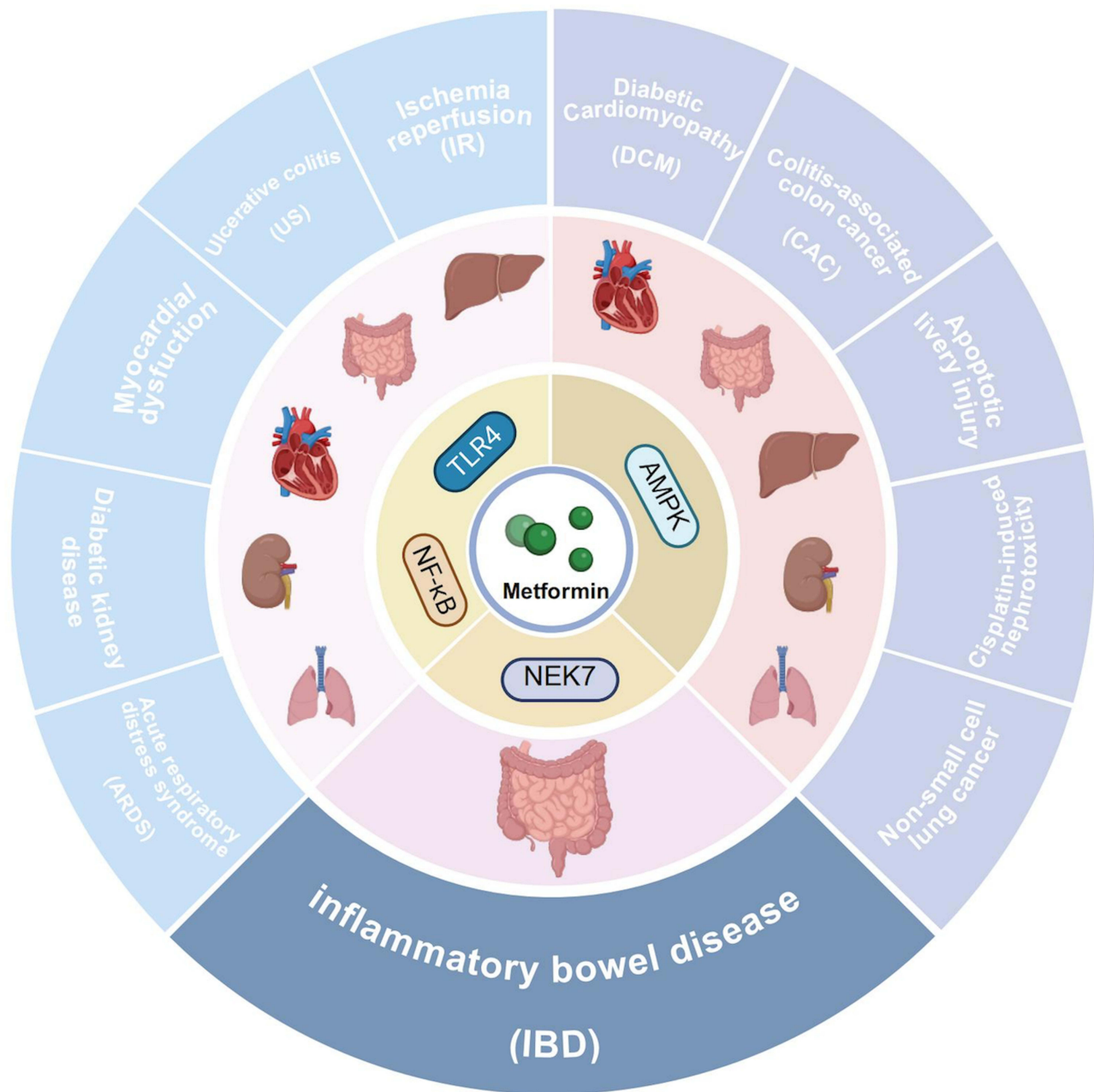


Figure 7 Schematic overview of anti-inflammatory effects of metformin across multiple diseases. This integrated illustration presents metformin as a multimodal regulator that targets key molecular pathways, including TLR4, NF- κ B, AMPK, and NEK7, to alleviate inflammation. The middle ring identifies the affected organs, while the outermost ring depicts the specific diseases modified by these actions.

AMPK Pathway: Diabetic Cardiomyopathy (DCM)

DCM is a severe complication of diabetes and is closely associated with heart failure and arrhythmia.¹¹⁶ Researchers have discovered that the potential mechanisms of DCM include mitochondrial dysfunction, excess inflammation, death of heart cells, and increased oxidative stress.¹¹⁹ NLRP3 inflammasome is important for regulating the development of inflammation and polycystic fibrosis. This mechanism may be involved in the formation of abnormal structures and functions in the heart.¹²⁰ According to Yang et al, metformin may suppress NLRP3 by activating the AMPK and mTOR pathways, which would restore the shape and function of the heart in DCM. Metformin or compound C, an AMPK inhibitor, was administered to primary myocardial cells treated with high glucose in newborn mice and streptozotocin-induced C57BL/6 mice. These results demonstrated that metformin therapy could repair the structure and function of the

Table 1 Metformin Functional Mechanisms of Action in Diseases

Diseases	Treatment	Inflammatory Markers	Mechanisms
Acute Respiratory Distress Syndrome (ARDS)	LPS + Metformin	SIRT1 ↑ NF-κB ↓	In vascular endothelial cells, Sirtuin 1 (SIRT1) can regulate NLRP3 to suppress inflammation. ^{103–105} In LPS-induced lung injury model, metformin can upregulate the expression of SIRT1 and downregulate the phosphorylation of NF-κB p65. This suggests that metformin can inhibit the NF-κB pathway by activating SIRT1, thereby reducing lung inflammation and alleviating the progression of ARDS. ¹⁰⁵
Diabetic Kidney Disease (DKD)	Streptozotocin + Metformin	TNC ↓ NF-κB ↓	Metformin can inhibit the expression of TNC to prevent inflammation and fibrosis in DKD patients. Increasing metformin doses could decrease phosphorylated NF-κB and TNC levels. ¹⁰⁶
Myocardial Dysfunction	LPS + Metformin	AMPK ↑ MYD88 ↓ TLR4 ↓	Activation of AMPK can directly phosphorylate NF-κB and inhibit its nuclear transportation, thereby reducing the expression of TLR4 pro-inflammatory cytokines. Metformin can inhibit the activation of TLR4 and inflammation, thereby reducing myocardial dysfunction. ^{94,107,108}
Ulcerative Colitis (UC)	DSS + Metformin + MCC950	NF-κB ↓ IL-6 ↓ TNF-α ↓	In UC patients, the NF-κB pathway is activated. After administration of Metformin, especially with MCC950, its level decreased significantly. This suggests that Metformin can reduce colon inflammation by inhibiting the NF-κB pathway. ¹⁰⁹
Ischemia-Reperfusion (IR)	High-fat feeding + metformin	NF-κB ↓ TLR4 ↓	Livers with excess fat produce more reactive oxygen species and are more susceptible to IR than healthy livers. The expression of phosphorylated NF-κB and TLR4 was significantly increased in fatty liver tissues. Metformin alleviates fatty liver IR by inhibiting TLR4/NF-κB signaling pathway. ¹¹⁰
Diabetic Cardiomyopathy (DCM)	Streptozotocin + Metformin	AMPK ↑ mTOR ↓ NLRP3 ↓ Caspase 1 ↓ IL-1β ↓	Metformin or AMPK inhibitor compound C treatment of neonatal mice and streptozotocin induced primary cardiomyocytes of C57BL/6 mice, the expression levels of mTOR, NLRP3, caspase-1 and IL-1β in the metformin group were significantly decreased. This decrease can be prevented using AMPK inhibitors. This finding suggests that metformin can reduce exophthalmos and enhance autophagy in DCM by activating the AMPK pathway and inhibiting the mTOR pathway. ⁹⁷
Colitis-associated Colon Cancer (CAC)	Metformin + TNF-α	p-AMPK ↑ actin ↑	In colon cancer cells, metformin inhibits tumor growth by enhancing AMPK phosphorylation in C colon cancer cells, independent of TNF-α treatment. ¹¹¹
Apoptotic Liver Injury	Compound C + Metformin or Metformin alone	Caspase 3,8,9 ↓	The caspase family can trigger apoptosis. ¹¹² Metformin inhibits caspase activation by activating AMPK, thereby reducing liver injury caused by tumor necrosis factor-induced apoptosis. ¹¹³
Cisplatin-induced Nephrotoxicity	Metformin + Cisplatin	AMPK ↑	Administration of metformin before cisplatin may enhance autophagy activation and AMPKα phosphorylation in the kidney. Metformin increased AMPKα phosphorylation, induced autophagy, and reduced cisplatin-induced cell death in rat renal tubular cells, and this effect was greatly abolished by blocking AMPKα activation or autophagy induction. ¹¹⁴
Non-small Cell Lung Cancer (NSCLC)	Metformin + ionizing radiation	AMPK ↑ mTOR ↓ p-4EBP1 ↓	Cancer cells were treated with MET (48 h) and ionizing radiation (24 h after MET) to phosphorylate AMPKα. MET pretreatment increased ionizing radiation (IR) - induced phosphorylation and total p53 amount. In irradiated cells, metformin can both enhance the sensitivity of cells to ionizing radiation and activate AMPK to arrest the cell cycle. ¹¹⁵
Inflammatory Bowel Disease (IBD)	DSS	NEK7 ↓ Caspase1 ↓ NLRP3 ↓ GSDMD ↓	The pathogenesis of IBD is related to pyroptosis induced by inflammasome formation. NEK7, an important component of NLRP3 inflammasome, may play a role in the pathogenesis of IBD. ¹¹⁶ Metformin inhibits the NEK7 pathway to alleviate IBD.

heart. Nevertheless, the effects of metformin on the heart were reduced following treatment with compound C. The metformin-treated groups showed lower levels of mTOR, NLRP3, caspase-1, and IL-1β. However, this decline may be stopped using an AMPK inhibitor, both in vivo and in vitro. This finding demonstrates that metformin can reduce proptosis in DCM and enhance autophagy by activating the AMPK pathway and inhibiting the mTOR pathway.¹²⁰

The Combination of Metformin and Other Drugs

While metformin remains the first-line therapy, recent cardiovascular outcome trials have identified sodium-glucose cotransporter-2 inhibitors (SGLT2i) and glucagon-like peptide-1 receptor agonists (GLP-1 RAs) as the preferred agents for patients with established cardiovascular disease.¹²¹ Empagliflozin, an SGLT2i, can decrease cardiovascular mortality by

Table 2 Pleiotropic Mechanisms of Metformin

Pathway	Organ	Diseases	Mechanisms
TLR4/NF-κB	Heart	Myocardial dysfunction	AMPK↑ TLR4, MyD88↓
	Colon	UC	IL-6, TNF-α, NF-κB↓
	Liver	IR	NF-κB, TLR4, HMGB1↓
	Kidney	DKD	NF-κB, TNC↓
	Lung	ARDS	SIRT1↑ NF-κB↓
AMPK	Heart	DCM	AMPK↑ mTOR, caspase-1, IL-1β↓
	Colon	CAC	AMPK, LKBI↑
	Liver	Apoptotic liver injury	AMPK, PGC-1↑ caspase3,8,9, PDK1, HIF-1, HMGB1, TNF, IL-6↓
	Kidney	Cisplatin-induced nephrotoxicity	AMPK, autophagy↑
	Lung	NSCLC	AMPK, ionizing radiation sensitivity↑ mTORC1, S6K↓
NEK7	Colon	IBD	NEK7, caspase 1, pyroptosis↓

38% and hospitalization for heart failure by 35% compared to placebo, independent of lowering blood glucose.¹¹¹ Similarly, GLP-1 RAs such as Liraglutide and Semaglutide, could decrease the risk of major adverse cardiac events (MACE) by 14% in patients diagnosed with cardiovascular diseases. GLP-1 RAs can stabilize atherosclerotic plaques by several mechanisms: the fibrous cap is thickened by increasing collagen deposition, lipid core formation is inhibited by decreasing acetyl-CoA acetyltransferase 1 (ACAT1) expression, and semaglutide can promote the transformation of plaques from non-calcified to calcified.¹²² The current guidelines of the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) clearly recommend that patients with cardiovascular diseases, regardless of metformin use, should preferentially combine SGLT2i or GLP-1 RAs to protect the heart and kidneys. Metformin, a basic antidiabetic drug, can synergistically control blood glucose; however, it is not a prerequisite for cardiovascular protection.¹²³

Colon

High blood glucose levels can result in dysfunction of the gastrointestinal tract in patients with diabetes, which increases the risk of inflammation and damage to the intestinal mucosa. This raises the possibility of ulcerative colitis (UC). Furthermore, patients with long-term inflammatory bowel disease (IBD) are at a heightened risk of developing colitis-associated colon cancer (CAC).¹²⁴ This section examines the efficacy of metformin in improving UC and CAC symptoms.

TLR4/NF-κB Signaling Pathway: UC

UC is a chronic inflammatory bowel disease that is characterized by persistent and recurrent colonic inflammation. This condition significantly impairs the immune function of the colon mucosa.¹¹⁰ Notably, metformin has been demonstrated to protect the colon both in vivo and in vitro studies.¹²⁵ Saber et al demonstrated that metformin has the potential to reduce the levels of IL-6 and TNF-α by inhibiting the priming signal of NLRP3 activation through the suppression of the TLR4/NF-κB pathway. Saber et al examined the potential synergistic effects of metformin and MCC950 combination therapy in rodents with dextran sodium sulphate (DSS)-induced colitis. Rats treated with DSS exhibited notably elevated NF-κB levels when compared to non-treated rats, suggesting that in individuals with UC, the NF-κB pathway was activated. Nevertheless, the level decreased considerably after the administration of METF, specifically when METF and MCC950 were combined. This indicated that metformin can alleviate inflammation in the colon by limiting the NF-κB pathway.¹¹²

NEK7 Pathway: IBD

The function of NEK7 in tumors and chronic inflammatory diseases has been investigated by Wang et al They discovered that the pathogenesis of inflammatory bowel disease (IBD) is associated with pyroptosis induced by inflammasome formation, and that NEK7, a crucial component of the NLRP3 inflammasome, may play a role in IBD pathogenesis. NEK7 and proptosis-related factors (Caspase-1, NLRP3, GSDMD) were significantly elevated in tissue samples from patients with IBD. In addition, NEK7 inhibition effectively eliminated pyroptosis induced

by ATP and LPS in vitro and ameliorated chronic colitis induced by DSS in vivo.¹¹³ As previously mentioned, metformin can inhibit the NEK7 pathway, which alleviates IBD, including UC.

AMPK Pathway: CAC

The lack of AMPK decreases the phosphorylation level of tuberous sclerosis complex 2 (TSC2), which activates mammalian target of rapamycin (mTOR) and promotes protein synthesis and tumor growth under chronic inflammation.¹²⁶ In colon cancer cells, metformin suppresses cancer cell growth by stimulating the AMPK pathway.¹²⁷ Metformin induces the allostery of vacuolar proton-translocating ATPase (v-ATPase) by binding to presenilin enhancer 2 (PEN2) and recruiting liver kinase B1 (LKB1) to the lysosomal surface to directly phosphorylate the Thr172 site of AMPK.⁴⁴ In the classic AMPK pathway, AMPK activation depends on a high AMP to ATP ratio, whereas with metformin, this process is directly activated by LKB1 and provides a new pathway that is independent of the AMP/ATP ratio.¹²⁸ In an experiment conducted by Koh et al, metformin enhanced the phosphorylation level of AMPK in COLO 205 cells irrespective of TNF- α treatment.¹⁰⁶ However, the anti-tumor function of metformin relies on complete AMPK signal and AMPK α 1 knockdown abolishes its ability to inhibit mTOR and induce cell cycle arrest.¹²⁹

Liver

Following the use of vascular control methods in hepatic surgery, liver ischemia and reperfusion have been identified as significant factors contributing to postoperative morbidity and death. Reperfusion injury can lead to liver dysfunction and failure, which are significant problems that can occur after surgery. Liver dysfunction is well acknowledged as a known cause of hepatic reperfusion damage.¹¹⁴ Acute injuries, such as fulminant hepatitis and reperfusion injury, and chronic injuries, such as alcoholic liver disease, cholestatic liver disease, and viral hepatitis, can result from liver injury caused by excessive and persistent apoptosis. As a result, therapeutic approaches that block apoptosis in liver damage or target and eliminate cancer cells in tumors may be extremely effective in treating liver illnesses.¹³⁰ This section explains the protective effects of metformin against hepatic ischemia-reperfusion (IR) and apoptotic liver injury in terms of the TLR4/NF- κ B and AMPK signaling pathways.

TLR4/NF- κ B Signaling Pathway: Ischemia-Reperfusion (IR)

Liver transplantation is a crucial therapeutic intervention for severe liver conditions such as cirrhosis and hepatocellular carcinoma. Given the limited availability of liver donors, fatty liver is frequently used for liver transplantation. Nevertheless, livers with excess fat generate a higher amount of reactive oxygen species and are more prone to IR than healthy livers. The TLR4/NF- κ B pathway plays a crucial role in cytokine production and development of ischemic damage. Li et al investigated the effects of metformin on IR in fatty liver disease. The high-fat diet group exhibited a considerable increase in the expression of phosphorylated NF- κ B and TLR4. After metformin administration, the expression of the TLR4/NF- κ B signaling pathway effectively decreased, indicating that metformin reduced the damage caused by IR injury in fatty liver disease by inhibiting the TLR4/NF- κ B pathway.¹³¹ In sterile inflammation of hepatic IR, metformin decreased the release of high-mobility group box 1 (HMGB1) from hepatocytes by activating TLR4, thereby disrupting TLR4 complex formation and downstream NF- κ B signaling.¹³² These results suggest that metformin may be a promising treatment option for liver transplantation-related IR injury.

AMPK Pathway: Apoptotic Liver Injury

Inflammation can induce surrounding cells to trigger the apoptotic program, thereby expanding inflammation, which can further cause tissue and organ damage. Metformin is closely associated with inflammation and this relationship can be explored based on the connection between inflammation and apoptosis. Cai et al examined the preventive effects of metformin against liver damage caused by TNF-induced apoptosis. The mice were subjected to liver damage by intraperitoneal administration of TNF- α and D-Gal. Compound C is an AMPK inhibitor. The results showed that the activity of caspase-3,8,9 was the highest in mice treated with TNF- α /D-Gal alone, the lowest in mice treated with TNF- α /D-Gal and metformin, and intermediate in mice treated with TNF- α /D-Gal, metformin, and compound C. According to the research conducted by Fan et al, the caspase family of proteins can trigger apoptosis.¹³³ These findings suggest that

metformin inhibits caspase activation by activating AMPK, which consequently reduces the liver damage caused by TNF-induced apoptosis.¹³⁴ Metformin exerts hepatoprotective effect by activating the phosphorylation of AMP-activated protein kinase (AMPK), which then upregulates the expression of peroxisome proliferator-activated receptor gamma coactivator 1- α (PGC-1 α). The elevated PGC-1 α triggers dual regulatory pathways: PGC-1 α inhibits pyruvate dehydrogenase kinase 1 (PDK1) to recover the activity of pyruvate dehydrogenase (PDH), correcting metabolic disorder; PGC-1 α could also suppress hypoxia-inducible factor 1-alpha (HIF-1 α) to attenuate overactivation of pathological glycolysis. These two pathways significantly reduce the pro-inflammatory mediators including TNF- α , IL-6 and HMGB1, ultimately diminishing apoptosis of hepatocytes and restoring liver function.¹⁰³

Kidney

Kidney injury is an inevitable outcome of diabetes mellitus. Diabetic kidney disease (DKD) is a severe microvascular complication that can cause irreversible kidney damage. It is closely associated with DM and is considered the main cause of end-stage kidney disease.¹⁰⁴ In addition, cisplatin-based chemotherapeutic approaches have been extensively employed in clinical practice for many years to treat various solid tumors, including non-small cell lung and prostate cancer. Approximately 25–30% of individuals receiving cisplatin treatment develop nephrotoxicity.¹⁰⁵ Therefore, discovery of the role of metformin in these two diseases is of great importance for the treatment of kidney diseases in humans.

TLR4/NF- κ B Pathway: Diabetic Kidney Disease (DKD)

A previous study found that, after fasting, rats were administered a low dose streptozotocin injection to induce diabetes. Blood glucose levels were measured 72 h later. The serum concentration of tenascin-C (TNC) is much higher in diabetic rats than in normal rats. Metformin may prevent inflammation and fibrosis in patients with DKD by suppressing TNC expression, as shown by the reduction in phosphorylated NF- κ B and TNC levels in response to increased metformin doses.¹⁰⁵ In streptozotocin-induced DKD, TLR4 knockdown relieves proteinuria and glomerular hypertrophy by blocking MyD88-dependent NF- κ B signalling and downregulating TGF- β 1 to inhibit renal interstitial fibrosis.¹¹⁵

AMPK Pathway: Cisplatin-Induced Nephrotoxicity

Administration of metformin prior to cisplatin administration may improve autophagy activation and AMPK α phosphorylation in the kidneys. Metformin increased AMPK α phosphorylation, induced autophagy, and reduced cisplatin-induced cell death in cultured rat kidney tubular cells. The ability of metformin to protect against cisplatin-induced cell death may be eliminated by blocking AMPK α activation or autophagy induction. Metformin may protect against cisplatin-induced tubular cell death and acute kidney injury by promoting AMPK α activation and autophagy induction in tubular cells.¹³⁵

The Limitations of Metformin Usage Based on Kidney Function

Renal function is a key limitation in the use of metformin given its predominant renal excretion and risk of accumulation.¹⁰⁷ In 2016, the Food and Drug Administration (FDA) revised the renal function contraindication criteria for metformin to replace serum creatinine with estimated glomerular filtration rate (eGFR), making many patients eligible for metformin while requiring mandatory renal function assessment before and after treatment.¹⁰⁸ Current guidelines of the National Institutes of Medicine prohibit the use of metformin in patients with eGFR < 30 mL/min/1.73 m² because renal impairment significantly increases the risk of metformin-associated lactic acidosis, which is characterized by elevated blood lactate levels and metformin plasma levels generally higher than 5 mcg/mL.¹⁰⁷ For patients with moderate renal impairment (eGFR 30–44 mL/min/1.73 m²), metformin treatment is not recommended. If metformin is already in use, the daily dose should be limited to less than 1000 mg, with regular monitoring of renal function.¹³⁶

Lung

Acute respiratory distress syndrome (ARDS) is characterized by the sudden onset of hypoxemia, non-cardiogenic pulmonary edema, and requirement for mechanical ventilation. Respiratory failure is a common cause in critically ill patients. Approximately 10% of patients in critical care units globally have acute respiratory distress syndrome (ARDS),

which is mostly associated with pneumonia, sepsis, aspiration of stomach contents, and severe trauma. In most studies, mortality was high, at 30–40%, despite minor improvements.¹³⁷ With more than 1.38 million fatalities globally, lung cancer is the leading cause of cancer-related mortalities. Approximately 80% of lung cancer cases are non-small cell lung cancers (NSCLC). Additionally, there were guidelines for the management of NSCLC until recently.¹³⁸ Given the current lack of targeted treatments, the anti-cancer effects of metformin are of great importance.

TLR4/NF- κ B Signaling Pathway: Acute Respiratory Distress Syndrome (ARDS)

Recent research suggests that Sirtuin 1 (SIRT1) has a role in controlling NLRP3 in vascular endothelial cells (ECs), which helps suppress the inflammatory response.^{137–141} Zhang et al evaluated the effect of metformin on the protein levels of SIRT1 in the lungs of a mouse model of acute respiratory distress syndrome (ARDS) and in pulmonary endothelial cells treated with LPS. The SIRT1 level in the lipopolysaccharide (LPS) group was reduced to 43% of the control level. However, subsequent treatment with metformin resulted in an increase of approximately 75% compared with the control level. Similarly, the protein level of SIRT1 was reduced in pulmonary ECs exposed to LPS, but metformin injection restored the expression level of SIRT1. Phosphorylation of the NF- κ B p65 subunit was elevated, whereas I κ B- α levels were reduced in the LPS group compared to those in the control group. However, metformin administration restored these effects in both living organisms and under laboratory conditions. These results demonstrated that metformin upregulated the expression of SIRT1 and downregulated NF- κ B p65 phosphorylation. This demonstrates that metformin can inhibit the NF- κ B pathway by activating SIRT1, thereby reducing lung inflammation and alleviating ARDS.¹⁴²

AMPK Pathway: Non-Small Cell Lung Cancer (NSCLC)

Metformin suppresses growth and amplifies the response to radiation in NSCLC. Storozhuk Y et al conducted a subsequent experiment to confirm this hypothesis. MET (48 h) and ionizing radiation (24 h after the start of MET treatment) were used to treat in cancer cells. MET pretreatment increased the levels of phosphorylated and total p53 induced by ionizing radiation (IR). Metformin (5 μ M) revealed a slight decrease in phosphorylated eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1), whereas in irradiated cells, MET strongly suppressed 4EBP1 phosphorylation at both 5 and 100 μ M. This indicates that metformin can enhance cellular sensitivity to ionizing radiation and activate AMPK to arrest the cell cycle.¹⁴³ Besides, metformin could activate AMPK to inhibit mammalian target of rapamycin complex 1 (mTORC1) signaling transduction in NSCLC, leading to reduced phosphorylation of ribosomal protein S6 kinase (S6K) thereby inhibiting protein synthesis and cell proliferation.^{129,143}

The Potential Adverse Effects of Metformin in Regulating Inflammation

New evidence suggests that while metformin's regulation of NLRP3 inflammasome activity is beneficial in suppressing inflammation, it may also be associated with environment-related adverse reactions. In a diabetic atherosclerosis model, metformin inhibited NLRP3 inflammasome activation in macrophages by suppressing high glucose-induced reactive oxygen species (ROS) accumulation and upregulating thioredoxin-interacting protein (TXNIP).¹⁴⁴ This effect is mediated by AMPK activation as an AMPK inhibitor reverses the anti-inflammatory effects of metformin. However, prolonged AMPK activation by metformin may disrupt mitochondrial redox balance, potentially worsening oxidative stress in cells with pre-existing mitochondrial dysfunction.¹⁴⁵

In acute respiratory distress syndrome (ARDS), metformin inhibits the assembly of NLRP3 inflammasomes and secretion of IL-1 β in alveolar macrophages by blocking mitochondrial ATP and ox-mtDNA synthesis. While alleviating lung injury, the drug reduces IL-6 production by inhibiting the p38/JNK pathway independent of NLRP3. This dual inhibition of inflammasome-dependent (IL-1 β) and inflammasome-independent (IL-6) cytokines raises concerns about potential immunosuppression, especially in infections in which balancing the cytokine response is crucial.²³

Conclusion

Metformin, a widely recognized and effective medication for diabetes management, has recently garnered renewed interest owing to its notable anti-inflammatory properties. In this review, we concluded three action pathways of

metformin (TLR4/NF- κ B signaling pathway, NEK7 pathway, and AMPK pathway) in addition to the common action of metformin on the blood glucose metabolism pathway. Through these three signaling pathways, metformin can suppress inflammatory responses, cancer, and many organ diseases. One significance of this review is that besides reducing blood glucose levels, metformin can also play a role in some diseases that have no mature treatment drugs, such as NSCLC. Our goal in investigating the regulatory mechanisms of metformin is to provide groundwork for future research. Clinical treatment methods may be improved by clarifying the underlying mechanisms by which metformin regulates the NLRP3 inflammasome pathway. In conclusion, metformin's targeting of NLRP3 inflammasome signaling pathways can be a ray of hope during hardship and open new avenues for the treatment of disorders linked to the NLRP3 inflammasome.

Despite the substantial evidence, several critical aspects of effects of metformin on inflammasomes remain inadequately explored, particularly the ambiguous definition of dose-response relationships across various disease models. While high concentrations typically demonstrate anti-inflammatory effects *in vitro*, the translation of these findings to clinically relevant doses, especially for non-diabetic indications, remains uncertain and necessitates systematic investigation. Besides, although some preclinical studies indicate that metformin exerts a protective effect in conditions such as acute respiratory distress syndrome (ARDS), robust large-scale randomized controlled trials in humans are still required to validate these findings and to delineate the specific patient populations that derive the most benefit. Furthermore, while preclinical studies suggest metformin can enhance endothelial function by increasing nitric oxide (NO) bioavailability, recent clinical trials in pre-diabetic patients have shown no significant impact of metformin on NO production, highlighting a key discrepancy between animal models and human pathophysiology that warrants further investigation.^{146–148} Future research should focus on making breakthroughs in several key areas to fully exploit the potential of metformin in treating diseases through the regulation of inflammasomes. At the molecular level, it is essential to clarify the regulatory mechanisms of metformin on various inflammasomes (such as NLRP3 and NLRC4) with greater precision, particularly regarding the specific action targets of its AMPK-dependent and non-dependent pathways across different organelles. Additionally, most current evidence stems from basic research, thus, there is an urgent need to design rigorous prospective randomized controlled trials to validate its efficacy and determine the optimal dosing regimen for inflammasome-driven diseases (including specific types of arthritis, neurodegenerative diseases, and preeclampsia) within particular patient populations. Considering the pleiotropic effects of metformin in organ protection, the development of targeted delivery systems for specific organs or cell types, such as those utilizing nanotechnology, is anticipated to enhance its local efficacy while minimizing systemic side effects. Collectively, these efforts will facilitate the transformation of metformin from a classic hypoglycemic agent to a mechanism-based anti-inflammatory treatment.

Data Sharing Statement

Data availability is not applicable to this review as no new data were created or analyzed in this study.

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All authors took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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