Delta University Scientific Journal
Journal home page: https://dusj.journals.ekb.eg

Cardioprotective Mechanisms of Metformin: A Review of Molecular Pathways and Therapeutic Implications Beyond Glycemic Control

Hamsa M. EL-Abasy[1], Mahmoud EA. Elsaid[1, 3], Eman M. Abdelkader[1], George S. G. Shehatou[1,2]

[1]*Department of Pharmacology and Biochemistry, Faculty of Pharmacy, Delta University for Science and Technology, International Coastal Road, Gamasa, Dakahliya, Egypt.*
[2]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura, Dakahliya, Egypt.*
[3]*Correspondence: Mahmoud EA. Elsaid, Department of Pharmacology and Biochemistry, Faculty of Pharmacy, Delta University for Science and Technology, International Coastal Road, Gamasa, Dakahliya, Egypt. Email: mahmoud.youssef@deltauniv.edu.eg Phone: 0020572078872*

ABSTRACT

Metformin (MET), a cornerstone therapy for type 2 diabetes, exhibits multifaceted therapeutic benefits extending beyond its hypoglycemic effects. This review synthesizes recent research on MET's cardioprotective mechanisms, emphasizing its modulation of critical molecular pathways. MET activates AMP-activated protein kinase (AMPK), a central energy sensor that enhances fatty acid oxidation, suppresses hepatic gluconeogenesis, and mitigates cardiac dysfunction via anti-inflammatory, anti-apoptotic, and autophagy-regulating actions. Additionally, MET inhibits the Nuclear Factor Kappa B (NF-κB) pathway, reducing pro-inflammatory cytokine production (e.g., TNF-α, IL-6, IL-1β) and attenuating endothelial dysfunction. Its suppression of the NLRP3 inflammasome further curtails oxidative stress and inflammation, pivotal contributors to diabetic cardiomyopathy and atherosclerosis. Pharmacokinetic insights underscore MET's renal clearance and safety profile, while its inhibition of mitochondrial complex I reduces reactive oxygen species (ROS), bolstering antioxidant defenses. Collectively, MET's pleiotropic effects—mediated through AMPK, NF-κB, NLRP3, and oxidative stress pathways—highlight its potential as a therapeutic agent for cardiovascular diseases. This review underscores the need for further clinical exploration of MET's repurposing in cardiometabolic disorders, leveraging its molecular mechanisms to optimize patient outcomes beyond diabetes management

Keywords: *Metformin, inflammation, NF-kB, NLRP3, Cytokines*

Mechanisms of action of MET

MET, a frequently recommended medication for the treatment of diabetes, has been shown to enhance heart function and decrease the occurrence of MI in individuals with type 2 diabetes (Zilov et al., 2019). MET has the potential to provide cardioprotective effects via many mechanisms. MET has been seen to improve cardiac dysfunctions caused by global ischemia in nondiabetic rats. This effect is achieved via the activation of the AMPK, without any significant impact on blood glucose levels {Khalaf, 2022 #57}.

Recent researches demonstrate that MET has other beneficial benefits, including anti-tumor, anti-aging, and cardioprotective properties, in addition to its well-established hypoglycemic impact (Rena et al., 2017).

MET has significant molecular biological effects via the modulation of key processes. Notably, it downregulates the activity of mitochondrial respiratory chain complex I, while concurrently upregulating the phosphorylation of AMPK. AMPK is well recognized as a crucial cellular energy sensor that plays a pivotal role in regulating many

cellular processes and functions. Numerous studies have provided evidence supporting the cardioprotective effects of MET, which may be attributed to its ability to impede inflammation, autophagy, apoptosis, and many other pathways mediated by AMPK (Hasanvand, 2022).

Pharmacokinetics and chemical structure of MET (MET)

MET, a biguanide class antidiabetic drug, has a chemical structure comprising two guanidine rings linked by a methyl group (Khan, 2024). MET imparts high polarity and water solubility, making it favorable for oral administration (Fatima et al., 2024) and it has a molecular weight of 129.16 g/mol (Abbasi et al., 2024). MET is absorbed primarily in the small intestine through active transport via organic cation transporters (OCTs), with an absolute bioavailability of approximately 50-60%. Following absorption, the drug is rapidly distributed in tissues, particularly the liver and gastrointestinal tract, where it exerts its primary effects by inhibiting hepatic gluconeogenesis and enhancing insulin sensitivity (McCreight et al., 2016).

MET has a negligible binding to plasma proteins and does not undergo hepatic metabolism, distinguishing it from other antidiabetic agents. It is excreted unchanged in urine via renal tubular secretion and glomerular filtration, with a half-life ranging from 4 to 6 hours in healthy individuals. Impaired renal function significantly reduces its clearance, necessitating dose adjustments to prevent drug accumulation and minimize the risk of lactic acidosis. This pharmacokinetic profile underscores the importance of renal function monitoring during MET therapy (He, 2020).

Effects of MET on the adenosine monophosphate-activated protein kinase pathway

Adenosine monophosphate-activated protein kinase AMPK is a highly conserved heterotrimeric kinase. It serves as a crucial regulator of cellular metabolism by coordinating the activity of enzymes involved in the metabolism of carbohydrates and fats (Wang et al., 2018). This coordination allows for the conservation and production of Adenosine triphosphate (ATP), the primary energy currency of cells. AMPK is stimulated by circumstances that result in an elevation in the AMP:ATP ratio, such as physical activity and metabolic strain. Extensive research has been conducted to investigate the impact of stress, exercise, and several other situations that generate hypoxia and ischemia on the activation of AMPK. When the ratio of AMP to ATP rises, the activation of AMPK is facilitated by AMPK (Long & Zierath, 2006). This activation leads to a conformational change in AMPK, which occurs via its interaction with AMP. Consequently, the AMP:ATP ratio decreases as a result of the inhibition of ATP-consuming pathways and the activation of ATP-generating pathways (Dengler, 2020).

The AMPK presents itself as a potential target for facilitating the favorable metabolic impacts of MET. AMPK is a complex enzyme composed of several subunits (Viollet & Andreelli, 2011). It is well acknowledged as a significant regulator of lipid biosynthesis pathways. This recognition stems from its pivotal function in phosphorylating and subsequently deactivating crucial enzymes, including acetyl-CoA carboxylase (ACC) (Agius et al., 2020).

Recent research provides solid evidence indicating that AMPK has a strong function in the control of metabolism. This encompasses processes such as fatty acid oxidation, absorption of glucose by the muscles, and the production of genes involved in gluconeogenesis that are activated by cAMP, such as phosphoenolpyruvate carboxykinase (PEPCK) and Glucose-6-phosphatase-β (G6Pase) (Steinberg & Hardie, 2023), Additionally, it includes the activation of genes related with hepatic lipogenesis, including fatty acid synthase (FAS), Spot-14 (S14), and L-type pyruvate kinase, in response to glucose stimulation (Compe et al., 2001). The persistent activation of AMPK may also elicit the upregulation of muscle hexokinase and glucose transporters (Glut4), so replicating the physiological outcomes associated with prolonged exercise training (Szewczuk et al., 2020).

MET has been found to induce AMPK activation in primary rat hepatocytes (Stephenne et al., 2011). Additionally, it has been observed to activate muscle AMPK and enhance glucose uptake by inhibiting complex I of the mitochondrial electron transport chain in hepatocytes **(Table 1)**.

This inhibition results in a reduction in ATP levels and an increase in AMP levels, ultimately leading to the activation of AMPK through the conventional adenine-nucleotide-dependent mechanism (Hawley et al., 2010). Elevated levels of AMP further exert inhibitory effects on fructose-1,6-bisphosphatase-1 and adenylate cyclase, therefore impeding the process of gluconeogenesis (Alshawi, 2019). The assessment of the impact of MET on glucose uptake and AMPK activity in intact rat epitrochlearis muscles was conducted due to the implication of AMPK activation as a mechanism for stimulating glucose uptake in skeletal muscle (Suwa et al., 2006), The application of MET on isolated muscles led to an augmentation of the activity of both catalytic subunits of AMPK. Simultaneously, there was a notable rise in glucose absorption, which was shown to be additional to the impact of insulin stimulation (Hasanvand, 2022).

MET has been shown to stimulate the activation of AMPK in hepatocytes. Consequently, this leads to a decrease in the activity of acetyl-CoA carboxylase (ACC), an increase in fatty acid oxidation, and a suppression of the production of lipogenic enzymes (Zhou et al., 2019). The expression of Sterol regulatory element binding proteins (SREBP-1), a crucial transcription factor involved in lipogenesis, is suppressed by the activation of AMPK by MET or an adenosine analogue.

Multiple studies have shown that the activation of AMPK by various means, such as exercise, adiponectin, thiazolidinediones, and MET, has been associated with a reduction in inflammation, endothelial dysfunction, and atherosclerotic vascular disease. These findings imply that AMPK might potentially serve as a valuable therapeutic target (Mancini & Salt, 2018).

The administration of MET effectively inhibited the decline in AMPK phosphorylation and activity, mitigated fluid accumulation in the lungs of diabetic mice, and fully restored cardiac function to a healthy state in OVE26 animals which are caring a transgene overexpressing calmodulin in pancreatic β cells, resulting in early onset of type I diabetes (Zhang et al., 2020).

Effects of MET on the Nuclear Factor Kappa B

The presence of activated NF-κB has been observed in human atherosclerotic plaques, whereas its absence or minimal presence has been noted in arteries that do not exhibit atherosclerosis (Jebari-Benslaiman et al., 2022). NF-κB regulates many genes whose products have been involved in the pathogenesis of atherosclerosis. Hence, the simultaneous activation of NF-κB-dependent genes might potentially contribute to the development of atherosclerosis (Matsumori, 2023). Studies provided evidence that MET effectively suppresses the expression of genes associated with inflammation and adhesion molecules by impeding the activation of NF-κB in vascular endothelial cells **(Table 1)**. MET exerts its effects via activating AMPK, which in turn mitigates the phosphorylation and subsequent degradation of Inhibitory kappa B kinase beta (IκB-κ) by decreasing the action of inhibitor of nuclear factor-κB (IκB) kinase (IKK) (Agius et al., 2020). This ultimately leads to the reduction of NF-κB activation produced by cytokines. While it is important to note that trials conducted on cultured cells may not fully reflect the *in vivo* condition, the findings of this study indicate that MET has potential as an antiatherogenic therapy (Poznyak et al., 2022). It seems that the modulation of NF-kB activation through AMPK activation may contribute to the vascular protective properties of MET (Agius et al., 2020). This ultimately leads to the reduction of NF-κB activation produced by cytokines **(Table 1)**.

Effects of MET on NOD-like receptor protein 3

The nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasome is a multiprotein complex consisting of NLRP3, apoptosis-associated speck-like protein containing a caspase-1 recruitment domain (ASC) and caspase-1. The NLRP3 inflammasome is a molecular complex that becomes activated in response to indications of cellular distress (Wei et al., 2019), leading to the initiation of innate immune responses through facilitating the synthesis of pro-inflammatory cytokines, including interleukin (IL). Prior research have shown that high blood sugar triggers the activation of NLRP3 (Ding et al., 2019). This activation subsequently facilitates the autocatalytic activation of pro-caspase-1, leading to the formation of cleaved caspase-1. Subsequently, the process of pro-IL-1β maturation is facilitated by cleaved caspase-1 (Sun & Scott, 2016). This biological mechanism induces programmed cell death associated with inflammation, which has the potential to advance to dilated cardiomyopathy (DCM). The restoration of cardiac function in models of (DCM) has been reported via the downregulation of the NLRP3 inflammasome (Li et al., 2014; Yang et al., 2018).

It is worth mentioning that the activation of AMPK has been seen to reduce the overexpression of NLRP3 inflammasome in several clinical conditions, including diabetes, pain, ischemic stroke, and endoplasmic reticulum stress (Li et al., 2015; Qiu et al., 2016). Moreover, prior researches have shown that autophagy has the ability to decrease the activity of the NLRP3 inflammasome via the mTOR signaling pathway (Zhong et al., 2016). Several studies indicated that MET has a role in the modulation of the NLRP3 inflammasome via the AMPK/mTOR pathway in (DCM) (Yang et al., 2019). The alleviation of (DCM) was shown to be considerable with the inhibition of the NLRP3 inflammasome and inflammatory components. Multiple studies have provided evidence supporting the notion that MET has cardioprotective and anti-inflammatory properties. These effects are believed to be mediated via the activation of AMPK/autophagy pathway, which eventually leads to the inhibition of the NLRP3 inflammasome in diabetic cardiomyopathy (DCM) (Zhong et al., 2016). (Zhang et al., 2024) **(Table 1)**.

Table 1: Anti-inflammatory Mechanisms of MET

Aspect	Mechanism/Target	Effect/Outcome	Pathway/Process	Clinical Relevance
Mechanism of Action	Activates AMPK; inhibits mitochondrial complex I	Reduces hepatic gluconeogenesis, enhances insulin sensitivity, improves cardiac function	AMPK activation → ATP conservation, fatty acid oxidation, glucose uptake	Cardioprotection in diabetes; reduced MI risk
Pharmacokinetics	Absorbed via OCTs in small intestine; renal excretion	Bioavailability ~50-60%; half-life 4-6 hours; no hepatic metabolism	Renal clearance; dose adjustment in renal impairment	Safe but requires monitoring in renal dysfunction to avoid lactic acidosis
AMPK Pathway	Inhibits mitochondrial complex I → ↑AMP:ATP ratio → AMPK activation	Suppresses ACC (↓lipogenesis), ↑fatty acid oxidation, ↓gluconeogenic enzymes	AMPK → metabolic regulation (glucose uptake, lipid oxidation)	Anti-inflammatory, anti-apoptotic, and anti-atherogenic effects
NF-κB Inhibition	AMPK activation → ↓IKK → ↓IκB degradation → ↓NF-κB nuclear translocation	Reduces pro-inflammatory cytokines (TNF-α, IL-6), adhesion molecules	NF-κB pathway suppression	Anti-atherogenic; mitigates endothelial dysfunction and vascular inflammation
NLRP3 Inflammasome	AMPK/mTOR → ↓NLRP3 activation; ↑autophagy → ↓caspase-1/IL-1β	Reduces inflammasome activity, oxidative stress, and pyroptosis	NLRP3 → IL-1β maturation	Protects against diabetic cardiomyopathy (DCM); anti-inflammatory effects
Cytokine Modulation	↓NF-κB and NLRP3 → ↓IL-6, TNF-α, IL-1β (dose-dependent)	Mitigates LPS-induced cytokine storms; reduces sepsis-related inflammation	AMPK-dependent suppression of cytokine production	Potential use in sepsis, acute inflammation, and cytokine-driven conditions
Oxidative Stress	Inhibits mitochondrial complex I → ↓ROS; ↑antioxidant defenses (e.g., ↓HIF-1α)	Reduces oxidative damage, ↓oxLDL-induced endothelial stress	Mitochondrial ROS suppression; ↑cellular antioxidant capacity	Prevents endothelial dysfunction, atherosclerosis, and diabetic complications
Cardiac Effects	Restores AMPK phosphorylation → ↓lung edema, ↑cardiac function in diabetic models	Improves ischemia-induced cardiac dysfunction; reverses DCM pathology	AMPK/NLRP3/NF-κB cross-talk → anti-fibrotic, anti-inflammatory	Therapeutic potential in heart failure, ischemic heart disease, and diabetic cardiomyopathy

Abbreviations: AMPK: Adenosine monophosphate-activated protein kinase, **ACC**: Acetyl-CoA carboxylase, **IL**: Interleukin, **NF-κB**: Nuclear Factor Kappa B, **NLRP3**: NOD-like receptor protein 3, **OCTs**: Organic cation transporters, **ROS**: Reactive oxygen species, **DCM**: Diabetic cardiomyopathy, **oxLDL**: Oxidized low-density lipoprotein

Effect of MET on cytokine production

MET has shown the ability to impede the activity of certain proinflammatory cytokines, including Interleukin 6 (IL-6) and Tumor Necrosis Factor α (TNF-α) (Huang et al., 2009).

It has been demonstrated that MET has a dose-dependent effect in reducing the production of IL-1, IL-6, and TNF-α at both the protein and mRNA levels. Similarly, it has been observed that the administration of MET leads to a reduction in the production of IL-6 produced by lipopolysaccharide (LPS) in the livers of mice. Furthermore, the activation of AMPK by MET has been shown to decrease the acute inflammatory response in two macrophage-like cell lines {El-Kashef, 2022 #56}. However, it does not activate HIF-1 or IL-10 (Cho et al., 2016; LIU et al., 2019).

Both oral and intraperitoneal (IP) treatment of MET showed equivalent effectiveness in reducing the considerably high levels of TNF-α in an experimental model of LPS-induced cytokine storm. No significant differences were seen between the two routes of administration (Taher et al., 2023) . Studies previously demonstrated that the oral administration of MET to mice subjected to lipopolysaccharide (LPS) treatment resulted in a decrease in the levels of TNF-α and IL-6 in the plasma, spleen, and lung tissues (Taher et al., 2023). This reduction in inflammatory markers indicates a mitigated impact of LPS-induced inflammation. The study discovered that LPS administration was associated with an increase in the levels of TNF-α and cyclooxygenase-2. Nevertheless, prior administration of MET resulted in a decrease in the synthesis of these inflammatory mediators (Lin et al., 2023). MET also showed a reduction in the generation of inflammatory cytokines, including IL-6, IL-1β, and TNF-α, hence mitigating sepsis-induced brain damage in mice (G. Tang et al., 2017) **(Table 1)**.

Effect of MET on oxidative stress

Oxidative stress is a state in which there is a disparity between the generation of reactive oxygen species (ROS) and the capacity of biological systems to effectively neutralize these reactive intermediates (Pisoschi & Pop, 2015). The cellular defense against ROS encompasses enzymatic and nonenzymatic systems that function together to provide antioxidant protection. There exists a positive correlation between heightened levels of oxidative stress and the occurrence of hyperglycemia, as well as the subsequent emergence and advancement of problems related to diabetes (Pizzino et al., 2017).

MET has significant inhibitory effects on oxidative stress (Cameron et al., 2016). For instance, MET has been shown to diminish the levels of ROS and hypoxia-inducible factor-1 (HIF-1). Consequently, this reduction leads to a decrease in the expression levels of IL-1β subsequent to extended exposure to proinflammatory LPS stimuli (Dias et al., 2020; Guangming Tang et al., 2017).

Multiple investigations have shown that the principal mechanism of action of MET involves the suppression of mitochondrial complex I, also known as Nicotinamide adenine dinucleotide (NADH): ubiquinone oxidoreductase (Zhu et al., 2016). The contribution of mitochondrial complex I to the formation of ROS inside the cell may be significant (Zhu et al., 2016). There is much documentation indicating that the presence of such a complex obstruction result in a notable decline in the formation of reactive species. This decline may be attributed to the hindered transportation of electrons from NADH plus H+. Hence, the available data indicates that MET has the capacity to decrease endogenous ROS levels inside the mitochondria. The findings of the study demonstrated that the administration of MET resulted in enhancements to the antioxidant defense system (Motta et al., 2022), regardless of the level of glycemic control. Oxidative stress has been implicated in the impairment of endothelial, vascular smooth muscle, and cardiac function, hence hastening the progression of cardiovascular disease (Chang & Abe, 2016). A recent research has shown the advantageous impacts of MET on endothelial cells, as it effectively suppressed oxidative stress generated by oxidized low-density lipoprotein (oxLDL) in these cells (Hung et al., 2016).

Conclusion

MET emerges as a multifaceted therapeutic agent with profound cardioprotective benefits extending far beyond its conventional role in glycemic management. By activating AMPK, MET orchestrates a cascade of metabolic and cellular responses—enhancing fatty acid oxidation, suppressing gluconeogenesis, and mitigating cardiac dysfunction through anti-inflammatory, anti-apoptotic, and autophagy-modulating mechanisms. Its inhibition of the NF-κB pathway attenuates endothelial inflammation and atherosclerotic progression, while suppression of the NLRP3 inflammasome curtails oxidative stress and inflammatory cytokine release, pivotal drivers of diabetic cardiomyopathy and cardiovascular complications. Pharmacokinetically, MET's renal clearance profile underscores the necessity of dose adjustment in renal impairment, yet its safety and tolerability remain advantageous. Importantly, MET's ability to inhibit mitochondrial complex I reduces ROS production, fortifying

cellular antioxidant defenses and further safeguarding cardiovascular health. Clinical evidence supporting MET's role in reducing cardiovascular events in diabetic patients highlights its translational potential. However, further studies are warranted to explore its repurpose in non-diabetic cardiovascular diseases and to elucidate dose-dependent effects in diverse populations. Collectively, MET's pleiotropic actions position it as a cornerstone in cardiometabolic therapy, bridging molecular mechanisms to clinical outcomes and paving the way for innovative strategies in cardiovascular disease prevention and treatment.

Disclosure

The author reports no conflicts of interest in this work.

References

Abbasi, M., Heath, B., & McGinness, L. (2024). Advances in MET-delivery systems for diabetes and obesity management. *Diabetes, Obesity and Metabolism*, *26*(9), 3513-3529.

Agius, L., Ford, B. E., & Chachra, S. S. (2020). The metformin mechanism on gluconeogenesis and AMPK activation: the metabolite perspective. *International journal of molecular sciences*, *21*(9), 3240.

Alshawi, A. F. O. (2019). *The molecular mechanisms by which metformin inhibits gluconeogenesis* Newcastle University].

Cameron, A. R., Morrison, V. L., Levin, D., Mohan, M., Forteath, C., Beall, C., McNeilly, A. D., Balfour, D. J., Savinko, T., & Wong, A. K. (2016). Anti-inflammatory effects of metformin irrespective of diabetes status. *Circulation research*, *119*(5), 652-665.

Chang, E., & Abe, J.-i. (2016). Kinase-SUMO networks in diabetes-mediated cardiovascular disease. *Metabolism*, *65*(5), 623-633.

Cho, J. G., Song, J. J., Choi, J., Im, G. J., Jung, H. H., & Chae, S. W. (2016). The suppressive effects of metformin on inflammatory response of otitis media model in human middle ear epithelial cells. *Int J Pediatr Otorhinolaryngol*, *89*, 28-32. https://doi.org/10.1016/j.ijporl.2016.07.025

Compe, E., de SOUSA, G., FRANCÇOIS, K., ROCHE, R., RAHMANI, R., TORRESANI, J., RAYMONDJEAN, M., & PLANELLS, R. (2001). Spot 14 protein interacts and co-operates with chicken ovalbumin upstream promoter-transcription factor 1 in the transcription of the L-type pyruvate kinase gene through a specificity protein 1 (Sp1) binding site. *Biochemical Journal*, *358*(1), 175-183.

Dengler, F. (2020). Activation of AMPK under Hypoxia: Many Roads Leading to Rome. *Int J Mol Sci*, *21*(7). https://doi.org/10.3390/ijms21072428

Dias, S. S. G., Soares, V. C., Ferreira, A. C., Sacramento, C. Q., Fintelman-Rodrigues, N., Temerozo, J. R., Teixeira, L., Nunes da Silva, M. A., Barreto, E., Mattos, M., de Freitas, C. S., Azevedo-Quintanilha, I. G., Manso, P. P. A., Miranda, M. D., Siqueira, M. M., Hottz, E. D., Pão, C. R. R., Bou-Habib, D. C., Barreto-Vieira, D. F., . . . Bozza, P. T. (2020). Lipid droplets fuel SARS-CoV-2 replication and production of inflammatory mediators. *PLoS Pathog*, *16*(12), e1009127. https://doi.org/10.1371/journal.ppat.1009127

Ding, S., Xu, S., Ma, Y., Liu, G., Jang, H., & Fang, J. (2019). Modulatory mechanisms of the NLRP3 inflammasomes in diabetes. *Biomolecules*, *9*(12), 850.

Fatima, N., Usman, M., Yusaf, A., Bokhari, T. H., Akram, N., Rehman, S., Haider, S., Siddiq, M., Bhatti, M. A., & Cheema, M. A. (2024). Unveiling the role of solubilization of metformin hydrochloride assimilated in nonionic surfactants mediated mixed micellar assemblies. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *703*, 135263.

Hasanvand, A. (2022). The role of AMPK-dependent pathways in cellular and molecular mechanisms of metformin: a new perspective for treatment and prevention of diseases. *Inflammopharmacology*, *30*(3), 775-788.

Hawley, S. A., Ross, F. A., Chevtzoff, C., Green, K. A., Evans, A., Fogarty, S., Towler, M. C., Brown, L. J., Ogunbayo, O. A., Evans, A. M., & Hardie, D. G. (2010). Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. *Cell Metab*, *11*(6), 554-565. https://doi.org/10.1016/j.cmet.2010.04.001

He, L. (2020). Metformin and systemic metabolism. *Trends in Pharmacological Sciences*, *41*(11), 868-881.

Huang, N.-L., Chiang, S.-H., Hsueh, C.-H., Liang, Y.-J., Chen, Y.-J., & Lai, L.-P. (2009). Metformin inhibits TNF-α-induced IκB kinase phosphorylation, IκB-α degradation and IL-6 production in endothelial cells through PI3K-dependent AMPK phosphorylation. *International journal of cardiology*, *134*(2), 169-175.

Hung, C.-H., Chan, S.-H., Chu, P.-M., Lin, H.-C., & Tsai, K.-L. (2016). Metformin regulates oxLDL-facilitated endothelial dysfunction by modulation of SIRT1 through repressing LOX-1-modulated oxidative signaling. *Oncotarget*, *7*(10), 10773.

Jebari-Benslaiman, S., Galicia-García, U., Larrea-Sebal, A., Olaetxea, J. R., Alloza, I., Vandenbroeck, K., Benito-Vicente, A., & Martín, C. (2022). Pathophysiology of atherosclerosis. *International journal of molecular sciences*, *23*(6), 3346.

Khan, M. F. (2024). Diabetes and Antidiabetic Drugs. In *Medicinal Chemistry for Pharmacy Students* (pp. 220-294). Bentham Science Publishers.

Li, X., Du, N., Zhang, Q., Li, J., Chen, X., Liu, X., Hu, Y., Qin, W., Shen, N., Xu, C., Fang, Z., Wei, Y., Wang, R., Du, Z., Zhang, Y., & Lu, Y. (2014). MicroRNA-30d regulates cardiomyocyte pyroptosis by directly targeting foxo3a in diabetic cardiomyopathy. *Cell Death Dis*, *5*(10), e1479. https://doi.org/10.1038/cddis.2014.430

Li, Y., Li, J., Li, S., Li, Y., Wang, X., Liu, B., Fu, Q., & Ma, S. (2015). Curcumin attenuates glutamate neurotoxicity in the hippocampus by suppression of ER stress-associated TXNIP/NLRP3 inflammasome activation in a manner dependent on AMPK. *Toxicol Appl Pharmacol*, *286*(1), 53-63. https://doi.org/10.1016/j.taap.2015.03.010

Lin, H., Ao, H., Guo, G., & Liu, M. (2023). The role and mechanism of metformin in inflammatory diseases. *Journal of Inflammation Research*, 5545-5564.

LIU, C., GAO, H.-J., YANG, Q.-Q., YANG, F.-H., KONG, N., WANG, B.-K., & ZHANG, T.-Y. (2019). Anti-inflammatory effect of metformin on LPS-induced inflammation in mice. *Basic & Clinical Medicine*, *39*(9), 1248.

Long, Y. C., & Zierath, J. R. (2006). AMP-activated protein kinase signaling in metabolic regulation. *J Clin Invest*, *116*(7), 1776-1783. https://doi.org/10.1172/jci29044

Mancini, S. J., & Salt, I. P. (2018). Investigating the role of AMPK in inflammation. *AMPK: Methods and protocols*, 307-319.

Matsumori, A. (2023). Nuclear Factor-κB is a prime candidate for the diagnosis and control of inflammatory cardiovascular disease. *European Cardiology Review*, *18*.

McCreight, L. J., Bailey, C. J., & Pearson, E. R. (2016). Metformin and the gastrointestinal tract. *Diabetologia*, *59*(3), 426-435.

Motta, B. P., Pinheiro, C. G., Figueiredo, I. D., Cardoso, F. N., Oliveira, J. O., Machado, R. T. A., da Silva, P. B., Chorilli, M., Brunetti, I. L., & Baviera, A. M. (2022). Combined effects of lycopene and metformin on decreasing oxidative stress by triggering endogenous antioxidant defenses in diet-induced obese mice. *Molecules*, *27*(23), 8503.

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European journal of medicinal chemistry*, *97*, 55-74.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxid Med Cell Longev*, *2017*, 8416763. https://doi.org/10.1155/2017/8416763

Poznyak, A. V., Litvinova, L., Poggio, P., Moschetta, D., Sukhorukov, V. N., & Orekhov, A. N. (2022). From diabetes to atherosclerosis: potential of metformin for management of cardiovascular disease. *International journal of molecular sciences*, *23*(17), 9738.

Qiu, J., Wang, M., Zhang, J., Cai, Q., Lu, D., Li, Y., Dong, Y., Zhao, T., & Chen, H. (2016). The neuroprotection of Sinomenine against ischemic stroke in mice by suppressing NLRP3 inflammasome via AMPK signaling. *Int Immunopharmacol*, *40*, 492-500. https://doi.org/10.1016/j.intimp.2016.09.024

Rena, G., Hardie, D. G., & Pearson, E. R. (2017). The mechanisms of action of metformin. *Diabetologia*, *60*(9), 1577-1585.

Steinberg, G. R., & Hardie, D. G. (2023). New insights into activation and function of the AMPK. *Nature reviews Molecular cell biology*, *24*(4), 255-272.

Stephenne, X., Foretz, M., Taleux, N., Van Der Zon, G., Sokal, E., Hue, L., Viollet, B., & Guigas, B. (2011). Metformin activates AMP-activated protein kinase in primary human hepatocytes by decreasing cellular energy status. *Diabetologia*, *54*, 3101-3110.

Sun, Q., & Scott, M. J. (2016). Caspase-1 as a multifunctional inflammatory mediator: noncytokine maturation roles. *Journal of Leucocyte Biology*, *100*(5), 961-967.

Suwa, M., Egashira, T., Nakano, H., Sasaki, H., & Kumagai, S. (2006). Metformin increases the PGC-1α protein and oxidative enzyme activities possibly via AMPK phosphorylation in skeletal muscle in vivo. *Journal of Applied Physiology*, *101*(6), 1685-1692.

Szewczuk, M., Boguszewska, K., Kaźmierczak-Barańska, J., & Karwowski, B. T. (2020). The role of AMPK in metabolism and its influence on DNA damage repair. *Mol Biol Rep*, *47*(11), 9075-9086. https://doi.org/10.1007/s11033-020-05900-x

Taher, I., El-Masry, E., Abouelkheir, M., & Taha, A. E. (2023). Anti-inflammatory effect of metformin against an experimental model of LPS-induced cytokine storm. *Exp Ther Med*, *26*(3), 415. https://doi.org/10.3892/etm.2023.12114

Tang, G., Yang, H., Chen, J., Shi, M., Ge, L., Ge, X., & Zhu, G. (2017). Metformin ameliorates sepsis-induced brain injury by inhibiting apoptosis, oxidative stress and neuroinflammation via the PI3K/Akt signaling pathway. *Oncotarget*, *8*(58), 97977-97989. https://doi.org/10.18632/oncotarget.20105

Tang, G., Yang, H., Chen, J., Shi, M., Ge, L., Ge, X., & Zhu, G. (2017). Metformin ameliorates sepsis-induced brain injury by inhibiting apoptosis, oxidative stress and neuroinflammation via the PI3K/Akt signaling pathway. *Oncotarget*, *8*(58), 97977.

Viollet, B., & Andreelli, F. (2011). AMP-activated protein kinase and metabolic control. *Diabetes-perspectives in drug therapy*, 303-330.

Wang, Q., Liu, S., Zhai, A., Zhang, B., & Tian, G. (2018). AMPK-mediated regulation of lipid metabolism by phosphorylation. *Biological and Pharmaceutical Bulletin*, *41*(7), 985-993.

Wei, J., Wang, H., Wang, H., Wang, B., Meng, L., Xin, Y., & Jiang, X. (2019). The role of NLRP3 inflammasome activation in radiation damage. *Biomedicine & Pharmacotherapy*, *118*, 109217.

Yang, F., Qin, Y., Lv, J., Wang, Y., Che, H., Chen, X., Jiang, Y., Li, A., Sun, X., Yue, E., Ren, L., Li, Y., Bai, Y., & Wang, L. (2018). Silencing long non-coding RNA Kcnq1ot1 alleviates pyroptosis and fibrosis in diabetic cardiomyopathy. *Cell Death Dis*, *9*(10), 1000. https://doi.org/10.1038/s41419-018-1029-4

Yang, F., Qin, Y., Wang, Y., Meng, S., Xian, H., Che, H., Lv, J., Li, Y., Yu, Y., Bai, Y., & Wang, L. (2019). Metformin Inhibits the NLRP3 Inflammasome via AMPK/mTOR-dependent Effects in Diabetic Cardiomyopathy. *Int J Biol Sci*, *15*(5), 1010-1019. https://doi.org/10.7150/ijbs.29680

Zhang, C., Shi, Y., Liu, C., Sudesh, S. M., Hu, Z., Li, P., Liu, Q., Ma, Y., Shi, A., & Cai, H. (2024). Therapeutic strategies targeting mechanisms of macrophages in diabetic heart disease. *Cardiovascular Diabetology*, *23*(1), 169.

Zhang, J., Huang, L., Shi, X., Yang, L., Hua, F., Ma, J., Zhu, W., Liu, X., Xuan, R., & Shen, Y. (2020). Metformin protects against myocardial ischemia-reperfusion injury and cell pyroptosis via AMPK/NLRP3 inflammasome pathway. *Aging (Albany NY)*, *12*(23), 24270.

Zhong, Z., Sanchez-Lopez, E., & Karin, M. (2016). Autophagy, NLRP3 inflammasome and auto-inflammatory/immune diseases. *Clin Exp Rheumatol*, *34*(4 Suppl 98), 12-16.

Zhou, W., Rahimnejad, S., Tocher, D. R., Lu, K., Zhang, C., & Sun, Y. (2019). Metformin attenuates lipid accumulation in hepatocytes of blunt snout bream (Megalobrama amblycephala) via activation of AMP-activated protein kinase. *Aquaculture*, *499*, 90-100.

Zhu, J., Vinothkumar, K. R., & Hirst, J. (2016). Structure of mammalian respiratory complex I. *Nature*, *536*(7616), 354-358.

Zilov, A. V., Abdelaziz, S. I., AlShammary, A., Al Zahrani, A., Amir, A., Assaad Khalil, S. H., Brand, K., Elkafrawy, N., Hassoun, A. A., & Jahed, A. (2019). Mechanisms of action of metformin with special reference to cardiovascular protection. *Diabetes/metabolism research and reviews*, *35*(7), e3173.