A review on protective roles and potential mechanisms of metformin in diabetic patients diagnosed with COVID-19

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ABSTRACT
The novel coronavirus disease 2019 (COVID-19), is currently the leading threat to public health and a huge challenge to the healthcare systems across the globe and caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Obesity, a state of chronic inflammation, and diabetes mellitus are risk factors for severe SARS-CoV-2. Metformin is one of the most commonly used antidiabetic medications that displayed immunomodulatory activity through AMP-activated protein kinase. Metformin has sex-specific immunomodulatory and cytokine-reducing activities. Therefore, this review aimed to summarize the protective roles of Metformin and its possible molecular mechanisms for use in COVID-19 patients. To include studies, publications related to Metformin and its possible molecular mechanisms for COVID-19 were searched from the databases such as Web of Science, PubMed, Medline, Elsevier, Google Scholar, and SCOPUS, via English key terms. Maintaining proper blood glucose levels using oral antidiabetic drugs like Metformin reduced the detrimental effects of COVID-19 by different possible mechanisms such as Metformin-mediated anti-inflammatory and immunomodulatory activities; effect on viral entry and ACE2 stability; inhibition of virus infection; alters virus survival and endosomal pH; mTOR inhibition; and influence on gut microbiota. Fascinatingly, in diabetic patients with COVID-19, treatment with Metformin was associated with a noticeable reduction in mortality rates and disease severity among infected patients. Metformin was comprehensively investigated for its anti-inflammatory, antiviral capabilities, immunomodulatory, and antioxidant, which would elucidate its capability to confer vascular and cardiopulmonary protection in COVID-19.

1. Introduction
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused a worldwide respiratory illness pandemic known as COVID-19 that was reported in December 2019 [1,2]. Death rates have wide-ranging among patient cohorts and nations [3,4]. There are numerous risk factors of COVID-19 such as cardiovascular disease [3], old age [1], chronic kidney disease [3], malignancies [5], diabetes mellitus and obesity [6], chronic pulmonary disease and smoking [3], and chronic HIV infection [7].

As of September 2021, more than 218,730,353 patients are infected and about 4,537,792 deaths were reported due to COVID-19 [8]. Different therapeutic agents like tocilizumab, lopinavir/ritonavir, favipiravir, and chloroquine have been used therapeutically without any confirmed effect [9], although some medications like remdesivir have revealed some effects on decreasing time to recovery [10]. Patients presented with coronary heart disease, diabetes, chronic obstructive pulmonary disease, and hypertension are more likely to be affected by COVID-19 [10]. According to a previous study, levels of inflammation-related biomarkers and inflammatory responses are higher among diabetic patients when compared to controls, which indicates being diabetic is a major risk factor in the prognosis and progression of COVID-19 [11].

Diabetes mellitus is one of the most public comorbidities among patients with COVID-19, which predisposes to intensive care unit admission in 14–32% of the cases [12]. Several mechanisms were identified for increased risk and severity of COVID-19 among diabetic patients such as decreasing CD4-positive T-cells in diabetic patients with MERS-CoV [13], increased interleukin-6 (IL-6) expression [14], and upregulation of angiotensin-converting-enzyme-2 (ACE2) expression...
2. Diabetes mellitus increased disease severity and mortality in patients with COVID-19

Patients with diabetes mellitus are prone to severe viral and bacterial infections, have a compromised immune response, present longer-lasting adverse effects, and require more recovery time than nondiabetic people [19]. Appropriate follow-up and control of blood glucose levels are closely linked to the body’s ability to fight infections in chronic diabetic patients and regulate inflammatory and immune responses [20]. Serious immune and inflammatory responses are related to higher mortality and severe course of the disease, which was observed in diabetic patients infected with COVID-19, and could be noticeably inverted by controlling blood glucose levels [21].

Plotting glycated hemoglobin against the risk of COVID-19-related hospitalization displays a distinguishing J-curve, which shows that DM is related to a higher risk of respiratory infections [22]. Even though pre-existing DM did not rise the risk of incidence of SARS-CoV-2 infection, there was a substantial increase in the severity of COVID-19 among patients with diabetic mellitus, thus increasing their risk of a requirement for emergency care and hospitalization [22]. Aged diabetic patients infected with COVID-19 showed an inflated inflammatory response and were more likely to need ICU support and mechanical ventilation, with a higher risk of mortality when compared to nondiabetic patients infected with COVID-19 [23]. A previous study conducted globally revealed that severe pneumonia cases, increased mortality rates, and risk of ICU admissions in diabetic patients with COVID-19.

Viral infections which are caused by mumps virus, rotavirus, and enterovirus, may lead to acute type I diabetes [24]. SARS-CoV-1 infected patients with no previous history of DM showed elevated blood glucose levels, an independent indicator for higher mortality among diabetic patients with COVID-19 [25]. This was related to the capability of the SARS-CoV-1 virus to damage the pancreatic islet through binding to the pancreatic islet ACE2 receptors [25]. Pancreatic ACE2 receptor simulates SARS-CoV-2 entry and binding, and the subsequent cellular damage could elucidate the new-onset of DM in patients infected with COVID-19 [22,26]. The binding of SARS-CoV-2 to ACE2 receptor on the pancreas creates an imbalance in RAAS and downregulates ACE2 activity [27]. The consequent over-activation of the AngII/angiotensin II type I receptor (AT1R)-axis and accumulation of Ang II, triggers NF-κB signaling and macrophage activation. This, increase the secretion and synthesis of several inflammatory cytokines, resulting in pancreatic damage, and partly clarifies new-onset DM in COVID-19 patients [27–29]. A noticeable rise in the levels of several proinflammatory cytokines/markers like tumor necrosis factor-α, IL-1β, IL-6, and IL-10 were reported in severely ill patients with COVID-19 [28,30]. An in vitro study revealed that the pancreatic cells were highly permissive to COVID-19 entry and mimicked the chemokine induction that is characteristic of patients infected with SARS-CoV-2 [24].

3. Repurposing metformin: their possible mechanism of action for COVID-19

Numerous biological possibilities can clarify the potential mechanism of metformin in patients with COVID-19 [Table 1]. Previous findings have revealed that tumor necrosis factor-α has a key role in COVID-19 pathology; it worsens the patient’s condition, increases cytokine release, and activates macrophage. However, Metformin has a significant role in decreasing thrombosis, increasing the neutrophil to lymphocyte ratio, reducing glycaemia, and decreasing cytokine release. There was a decreased level of IL-6, TNFα, and inflammatory mediators in both non-diabetes and diabetes patients while using Metformin. Furthermore, Metformin displays a noticeable positive activity in decreasing neutrophil extracellular traps and reducing the neutrophil [36,37].

Expression of ACE-2 via adenosine monophosphate-activated protein kinase is increased by Metformin, which leads to decreased cytokine response [38]. Additionally, Metformin is also important to decrease the release of inflammatory markers through affecting the NF-kappa B and MTOR pathways [39]. Other molecular mechanisms common to SARS-CoV-2 infection and diabetes can be used to elucidate the possible role of Metformin in diabetic patients with COVID-19. Viral-induced interferon-gamma secretion has been confirmed to increase circulating insulin levels and muscular insulin resistance, which, consecutively, rises the CD8 + T-cell responses.

### Table 1

| Drug       | Mechanism                                      | References |
|------------|------------------------------------------------|------------|
| Metformin  | Decrease inflammatory cytokines                | [102,103]  |
|            | Increase autophagy and Sirt1/FoxO1 and decrease | [104]      |
|            | GBM thickness                                   |            |
|            | Decrease reactive oxygen species production     | [105–107]  |
|            | Reduce inflammatory marker release              | [31]       |
|            | Protective arm of the renin angiotensin-aldosterone system (RAAS) | [62,63,108] |

GBM: Glioblastoma; FoxO1: Forkhead Box O1; NCC: Sodium-Chloride Cotransporter.
complications and are hallmarks of overt diabetes [23,40]. Moreover, the activation of receptors of advanced glycation end products (RAGE) by AGE and other ligands that trigger RAGE mediate the cell adhesion molecule coding genes and transcription of NF-κB-dependent pro-inflammatory that contribute to chronic vascular complications and coagulation and endothelial dysfunction supported by increased extravasation in diabetes, leukocyte adhesion, and vascular hyper-permeability [41–44]. Although Metformin has multiple targets, the inhibition of complex 1 of the mitochondrial electron transport chain is the most established mechanism related to, subsequent mTOR inhibition, tipping the balance toward AMPK activation, and increasing the AMP/ATP ratio. The mitochondrial electron chain inhibition also attenuates endothelial dysfunction and suppresses reactive oxygen species. Reactive oxygen species mediate the release of IL-6 that increased disease severity and mortality in diabetic patients with COVID-19 through the accumulation of intracellular Ca?+ [45,46].

Treatment with Metformin revealed the reduction of reactive oxygen species, consecutively, inhibited Ca2+ entry via CRAC and prevented the release and depletion of Ca2+ from the endoplasmic reticulum, thus inhibiting Ca2+–mediated IL-6 release [46]. The immunomodulatory activities of Metformin (as shown through the suppression of the pro-inflammatory capacity of activated macrophages, the differentiation of T cells into memory and regulatory T cells, and inhibition of monocyte-macrophage differentiation) is related to the Metformin treatment-related mTOR inhibition, reduction of oxidative stress, and activation of AMPK [47]. Moreover, Metformin constrains the RAGE-mediated NF-κB stimulation and subsequent up-regulation of cell adhesion molecules and genes that code for several proinflammatory cytokines in vascular endothelial cells, macrophages, and smooth muscle cells, thus dampening the immune and inflammatory response and consequently conferring vascular protection [48–51].

3.2. Metformin’s effect on viral entry and ACE2 stability

A previous study suggested that different virus-specific mechanisms by which virus gains entry into/infects the host cell [52]. Both receptor-dependent endocytosis and membrane fusion was reported in SARS-CoV-1 entry [53,54]. Although some viruses bind with the plasma membrane and then release the viral genome, others enter by endocytosis.

The cell surface ACE2 receptor of the respiratory tract’s target cells assisted the SARS-CoV-2 viral fusion and infection of the host cell, the transmembrane TMPRSS2 cleaved the viral spike protein and supported virion entry into the cell [3,55]. Even though the viral replication is mainly confined to the respiratory tract, previous findings revealed that replication of SARS-CoV-2 in extrapulmonary tissues like the pancreas, colon, tonsils, and ilium [56,57]. Though, it is notable that the ACE2 receptor is expressed in numerous tissues and organs such as the heart, intestine, brain, adipose tissue, pancreas, kidney, vasculature, and liver, making them potential targets for COVID-19, which may clarify the destruction reported in multi-organ systems in patients with COVID-19 [56,58,59]. Remarkably, the risk factors like obesity and DM are associated with an increased expression of ACE2 in different tissues, leading to a possible rise in the viral load [60,61]. Moreover, the flaking of ACE2 from the cell surface and its redistribution, usually detected in obese and diabetic people, supports the viral spread to various body parts [60].

Metformin which is a direct activator of AMPK, is identified to promote ACE2 phosphorylation (Ser 680), leading a conformational change, and may prevent viral binding to the host ACE2 receptor [62]. Furthermore, Metformin-mediated AMPK-dependent phosphorylation of ACE2 extends ACE2 half-life and hence potentially offers lung protection [63]. In contrast, it is theorized that Metformin could stabilize ACE2 expression in the respiratory tract and probably raise SARS-CoV-2 infection [64]. Though, it is notable that viral binding to the ACE2 declines ACE2 availability and stability, leading to the manifestation of harmful biological effects (fibrotic, vasocostrictive, proliferative, and pro-hypertrophic, induction of oxidative stress, and inflammatory effects) and an imbalance in the RAAS in various body parts [29,58]. Due to the role in the activation of AMPK and further downstream ACE2, Metformin can prevent ACE2 down-regulation mediated by SARS-CoV-2 [65].

The Metformin-mediated rise in the levels of ACE2 consequently controls RAAS offering mitigates pulmonary hypertension, stability to the pulmonary endothelium, and cardiopulmonary protection [62,66]. Especially, Metformin treatment-associated ACE2 stability and expression can prevent pancreatic damage and new-onset DM in patients with COVID-19 and positively modulate the beneficial arm (ACE2/Ang 1–7) of the RAAS and by maintaining and protecting the usual function of the pancreas [67].

3.3. Reduced insulin resistance and metformin: a potential role in the inhibition of virus infection

The increment in insulin sensitivity and reduction of blood glucose noticeably reduces the disease severity and susceptibility to viral infections among affected patients [68,69]. Metformin possesses beneficial activities on pancreatic β-cells through increasing viability of β-cells, stimulating glucose metabolism, and decreasing insulin resistance [68,70]. Metformin effects on cellular mechanism include translocation of GLUT1/4 glucose transporters and AMPK activation to the plasma membrane, smoothing higher glucose uptake by cells [68].

Insulin signaling has a noticeable effect in fighting against infections and boosting the immune system. The central immune cells like T cells, B cells, or macrophages express insulin receptors [71]. Reduced T-cell function associate with inadequate vaccine response and low viral clearance, frequently related to reduced insulin signaling [71].

ACE2 has a key role in sustaining glucose homeostasis through the activation of Ang(1–7)/MAS receptor axis, which, sequentially, maintains insulin secretion and rises the survival of β-cells of the pancreas [72]. On the contrary, finding support that an altered local RAAS system or an altered function of ACE2 favors the onset of type 2 DM [72]. Furthermore, the protecting role of ACE2 against insulin resistance is improved via Ang(1–7) levels through the transcription factor myocyte enhancer factor 2A and the expression of GLUT4 [73]. Thus, ACE2 is a possible therapeutic target for where Metformin can block the associated immune response and the activated AngII/AT1R/insulin signaling pathway.

3.4. Metformin alters virus survival and endosomal pH

Viruses use numerous escape modes to gain entry into the host cell and evade host surveillance. The mechanism of viral entry is determined by the host cell type and the endocytic route of SARS-CoV-2 in lung epithelial cells. Wang et al. described caveolae- and clathrin-independent endocytic mechanisms for SARS-CoV-1 [74]. This endocytosis process was receptor- and pH-dependent and involved the binding of viral spike protein with the ACE2 receptor, then internalization of the ACE2 receptor and the virus into the endosome and reprocessing of the ACE2 receptors back to the surface of the cell surface membrane [74].

It is well known that endosomal pH is a vital aspect for virus survival inside the host cell. A reduced intracellular pH may favor endosomal virion maturation, its multiplication, and SARS-CoV-2 binding to the host cell [75]. Therefore, targeting endosomes as a potential therapeutic target via medications capable of changing the endosomal pH may inhibit survival, assembly, and viral maturation within the host cell.

Because of the various mechanisms of viral entry of SARS-CoV-2, targeting endosomal pH is not necessarily in the membrane fusion mode of viral entry but substantial in the endocytic mode of viral replication. The endosomal Na+/H+ exchangers and vacuolar ATPase as the key regulators for endosomal pH are known targets for Metformin. Thus, it is promising that Metformin treatment suppresses the virion
maturation and endocytic cycle and increases the endosomal and cellular pH [76,77].

3.5. Metformin-mediated mTOR inhibition

The latest finding showed that a reduction in mortality among diabetic patients with COVID-19 who were on Metformin to treat their hyperglycemia, and this was associated with mTOR inhibitory activity of Metformin [78]. Metformin may interrupt the communication between the viral and host proteins essential for pathogenesis, virion assembly, and viral replication [62,79,80].

Treatment with Metformin can inhibit the mTOR signaling pathway via the inhibition of AKT activation of AMPK, and/or directly, hence resistant the cellular translational process, which is essential for the synthesis of viral and host proteins. On the other hand, treatment with Metformin can inhibit phosphorylation of Raptor and mTOR via REDD1 has also been testified [79,81]. Gordon et al. identified 332 high-confidence viral (COVID-19) protein-human protein interactions, of which 66 protein interactions may act as a potential target for Metformin [82]. Remarkably, two human proteins, FKBP7 and LARP1, which are controlled by the mTOR signaling pathway, interrelate with the COVID-19 Orf8 and N proteins, respectively [62]. Host proteins of the electron transport chain such as M viral proteins, NDUF, and NSP7 are Metformin targets [82]. Therefore, the host–viral interaction opens up the avenue of identification of new drug targets, repurposing of drugs, and co-therapies.

3.6. Metformin on gut microbiota

Metabolic syndrome such as DM is frequently related to increased systemic infection and leaky gut [83]. In addition to the involvement of the respiratory system in COVID-19, the gastrointestinal tract pays to the appearance of symptoms such as vomiting, loss of appetite, stomach pain, and diarrhea during COVID-19. A study showed that the severity of disease, as point out by inflammatory and cytokines markers, is related to gut microbiome composition, hence accentuating the immunomodulatory role of the gut microbiome [84].

Gut microbes like E. rectale, F. prausnitzii, and different bifidobacterial species are known for their immunomodulatory roles were exhausted in COVID-19 infected patients [84,85]. In vivo studies revealed that variations in the gut microbiota impact glucose tolerance, permeability, and intestinal integrity, which may partly clarify the SARS-CoV-2 progression in type II DM [86]. Moreover, ACE2, expressed in the GI tissue, controls innate immune function and gut homeostasis. Pollak M. argues Metformin’s immunomodulatory and antidiabetic activities by affecting gut microbiota [86]. Metformin’s modes of beneficial effect in patients with COVID-19 is indicated by Metformin’s capability to shift in the functional aspect of the gut microbiome and alter the composition of gut microbes as shown by the reduction of fasting blood glucose level and %HbA1c [87].

Generally, Metformin, in addition to its antidiabetic activity, displays a substantial therapeutic effect for COVID-19 such as it acts as an immunomodulatory agent, antimicrobial agent, ACE2 stabilizer, and agent that maintains gut homeostasis and regulates gut microbiota composition [87].

4. Possible contraindications and adverse effects of metformin in COVID-19 patients

Several findings have confirmed the valuable activities of metformin in patients infected with COVID-19, although some findings have revealed that Metformin-treatment can increase disease severity (but not mortality) and risk of acidosis in patients with COVID-19 [58,82]. This advocates that treatment with Metformin is not a suitable choice in patients with severe renal impairment, heart failure, and respiratory distress, and highlights the role of giving attention to pre-existing comorbidities and conditions in medication selection [88]. Moreover, contraindications and adverse effects of Metformin in COVID-19 patients should be addressed prior to administration.

5. Conclusion

The current review displays that Metformin use is linked to a reduced risk of mortality in COVID-19 patients. Several studies highlight the importance of further exploring Metformin’s role in improving host immune response through potentially targeting gut microbiota or energy metabolism or specific cell signaling pathways. Metformin possesses therapeutic benefits in conditions like cancer, polycystic ovary disease, fatty liver diseases, and cardiovascular complications. Maintaining proper blood glucose levels using oral antidiabetic drugs like Metformin reduced the detrimental effects of COVID-19 by different possible mechanisms such as Metformin-mediated anti-inflammatory and immunomodulatory activities; effect on viral entry and ACE2 stability; inhibition of virus infection; alters virus survival and endosomal pH; mTOR inhibition; and influence on gut microbiota. In the context of COVID-19, Metformin provides protection not only metabolically but also through the mitigation of complications related to exaggerated thrombotic events and immune response. Physicians also need to wisely assess the actual benefits of Metformin for patients who are at risk of COVID-19 mortality and who are currently taking it.

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Declaration of competing interest

The authors declares that they have no competing interests.

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Z.D. Kifle et al. Metabolism Open 12 (2021) 100137
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