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Samson Mathews Samuel, Elizabeth Varghese, Dietrich Büsselberg

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Therapeutic Potential of Metformin in COVID-19: Reasoning for its Protective Role

Samson Mathews Samuel[†,] *, Elizabeth Varghese[†], Dietrich Büsselberg*

Department of Physiology and Biophysics, Weill Cornell Medicine-Qatar, Education City, Qatar Foundation, 24144, Doha, Qatar

† These authors contributed equally.

***Correspondence:**
Prof. Dr. Dietrich Büsselberg
Department of Physiology and Biophysics
Weill Cornell Medicine-Qatar
Education City, Qatar Foundation,
P.O.Box: 24144, Doha, Qatar.
email: dib2015@qatar-med.cornell.edu
Phone: +974-4492 8334
Fax: +974-4492 8333

AND

Dr. Samson Mathews Samuel
Department of Physiology and Biophysics
Weill Cornell Medicine-Qatar
Education City, Qatar Foundation,
P.O.Box: 24144, Doha, Qatar.
email: sms2016@qatar-med.cornell.edu
Phone: +974-4492 8269
Fax: +974-4492 8333

Abstract

SARS-CoV-2 infections present with increased disease severity and poor clinical outcomes in diabetic patients compared with their non-diabetic counterparts. Diabetes/hyperglycemia-triggered endothelial dysfunction and hyperactive inflammatory and immune responses are correlated to two- to three-fold higher intensive care hospitalizations and more than twice the mortality among diabetic COVID-19 patients. While comorbidities such as obesity, cardiovascular disease, and hypertension worsen the prognosis of diabetic COVID-19 patients, COVID-19 infections are also associated with new-onset diabetes, severe metabolic complications, and increased thrombotic events in the backdrop of aberrant endothelial function. While several antidiabetic medications are used to manage blood glucose levels, we discuss the multi-faceted ability of metformin to control blood glucose levels, attenuate endothelial dysfunction, inhibit viral entry, and infection and modify inflammatory and immune responses during SARS-CoV-2 infections. These actions make it a viable candidate for drug repurposing and the higher ground against the SARS-CoV-2 induced tsunami in diabetic COVID-19 patients.

Key Words: Blood Glucose Control, Coronavirus, COVID-19, Diabetes, Endothelial Dysfunction, Metformin, SARS-CoV-2

Diabetes and COVID-19: Reciprocity at Play

On 7th January 2020, a novel beta-coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the causative agent of the 'atypical pneumonia', that surfaced in Wuhan, China, on 30th December 2019 [1]. After the World Health Organization (WHO) declared the SARS-CoV-2 caused coronavirus disease (COVID-19) outbreak as a pandemic on 11th March 2020, to-date (05th March 2021), over 116 million COVID-19 cases were reported, and more than 2.5 million people have lost their lives globally [1] [a]. Finding an effective way to control the rapid spread of the disease and a cure for COVID-19 remains at the forefront of the battle against this pandemic even as the vaccines, Tozinameran/BNT162b2/Comirnaty (Pfizer/BioNTech), mRNA-1273 (Moderna), AZD1222/Covishield (University of Oxford/AstraZeneca) and Ad26.COV2.S/JNJ-78436735 (Janssen Vaccines and Prevention) are being approved and authorized for emergency use in several countries [2-5]. However, the emergence of highly transmissible SARS-CoV-2 mutants has raised concerns that these variants could evade the body's immune response, threaten vaccine efficacy and may cause a resurgence of highly transmissible cases of COVID-19 [6].

The highly contagious SARS-CoV-2 can affect all individuals irrespective of age, gender, and ethnicity, but to varying degrees [7]. Most COVID-19 cases remain asymptomatic or present with relatively mild flu-like symptoms, increasing the risk of transmission and the significant spread of SARS-CoV-2 [8]. Nevertheless, the vulnerability, aggressiveness/severity of the disease, hospitalization rates, and mortality is significantly higher in men, among the elderly, and those with one or more comorbidities/pre-existing conditions such as hypertension, cardiovascular or cerebrovascular diseases, diabetes, cancers, and renal damage [1, 9]. In the face of pre-existing/comorbid conditions, severe COVID-19 cases can rapidly progress into acute respiratory distress syndrome (ARDS), septic shock, and multiple organ dysfunction syndrome (MODS), and organ failure [10].

SARS-CoV-2's ability to infect and damage multi-organ systems is dependent on the expression/distribution pattern of the host angiotensin-converting enzyme 2 receptor (ACE2; which binds to the viral spike protein) and the transmembrane serine protease 2 (TMPRSS2; which cleaves and primes the viral spike protein), in various organs and tissues, facilitating viral activation and entry in the host cell [11, 12]. Comorbidities such as diabetes and obesity upregulate ACE2, leading to an increase in viral load within these tissues [13]. Furthermore, increased ACE2 shedding from the cell surface in diabetic and obese subjects facilitates re-

distribution of ACE2 in the body and its accumulation in the lungs [13]. Conversely, the ACE2 receptor plays a crucial role in regulating the renin-angiotensin-aldosterone system (RAAS) and hence supports and protects cardiovascular and pulmonary function [14-16]. ACE2 maintains a crucial balance by downregulating the levels of AngII by activating the ACE2/Ang(1-7)/MAS axis of the RAAS, thereby conferring protective vasodilatory, vascular protective, anti-fibrotic, anti-proliferative, and anti-inflammatory effects [17]. Therefore, the depletion of ACE2 by SARS-CoV-2 binding can lead to adverse cardio-pulmonary events and multi-organ injury [12]. Interestingly, the ACE2 expressing endothelial cells of the vasculature are crucial targets for SARS-CoV-2 infection [18-20]. In diabetic and obese individuals, a decrease in the baseline expression of ACE2 in the vasculature leads to endothelial dysfunction (ED), contributing to the higher incidence of thrombotic events in COVID-19 patients [13, 19, 20].

Previously, in coronaviral infections caused by SARS-CoV-1 and the Middle East respiratory syndrome coronavirus (MERS-CoV), pre-existing diabetes was identified as an independent risk factor contributing to increased disease severity and higher mortality among affected individuals [21]. Similarly, COVID-19 patients with pre-existing diabetes with/without one or more comorbidities such as obesity, hypertension, cardiovascular disease, and chronic kidney disease (CKD) require in-hospital ICU care/treatment and tracheal intubation/mechanical ventilation and correlate with poor prognosis and increased risk of all-cause mortality when compared to their non-diabetic counterparts (Figure 1) [1]. Interestingly, the occurrence of new-onset of diabetes and related severe metabolic complications (diabetic ketoacidosis and hyperosmolarity) in COVID-19 patients indicate a bi-directional relationship between diabetes and COVID-19 (Figure 1) [22].

Reports suggest that the proper management of blood glucose levels reduced disease severity, incidence of ARDS, requirement of ICU admissions, and ventilator support and promoted recovery in COVID-19 patients with pre-existing diabetes (Figure 1) [10]. While several anti-hyperglycemic medications are available to manage blood glucose levels, it is necessary to carefully ascertain the best possible antidiabetic medication that can be safely administered to COVID-19 patients to control hyperglycemia and to suppress related adverse events. In this regard, metformin treatment was beneficial in treating diabetic COVID-19 patients since it reduced disease severity and mortality rates in COVID-19 patients [16, 23]. This opinion article highlights the multi-faceted benefits of metformin and investigates the possibility of repurposing metformin as an effective drug for the treatment of diabetic COVID-19 patients.

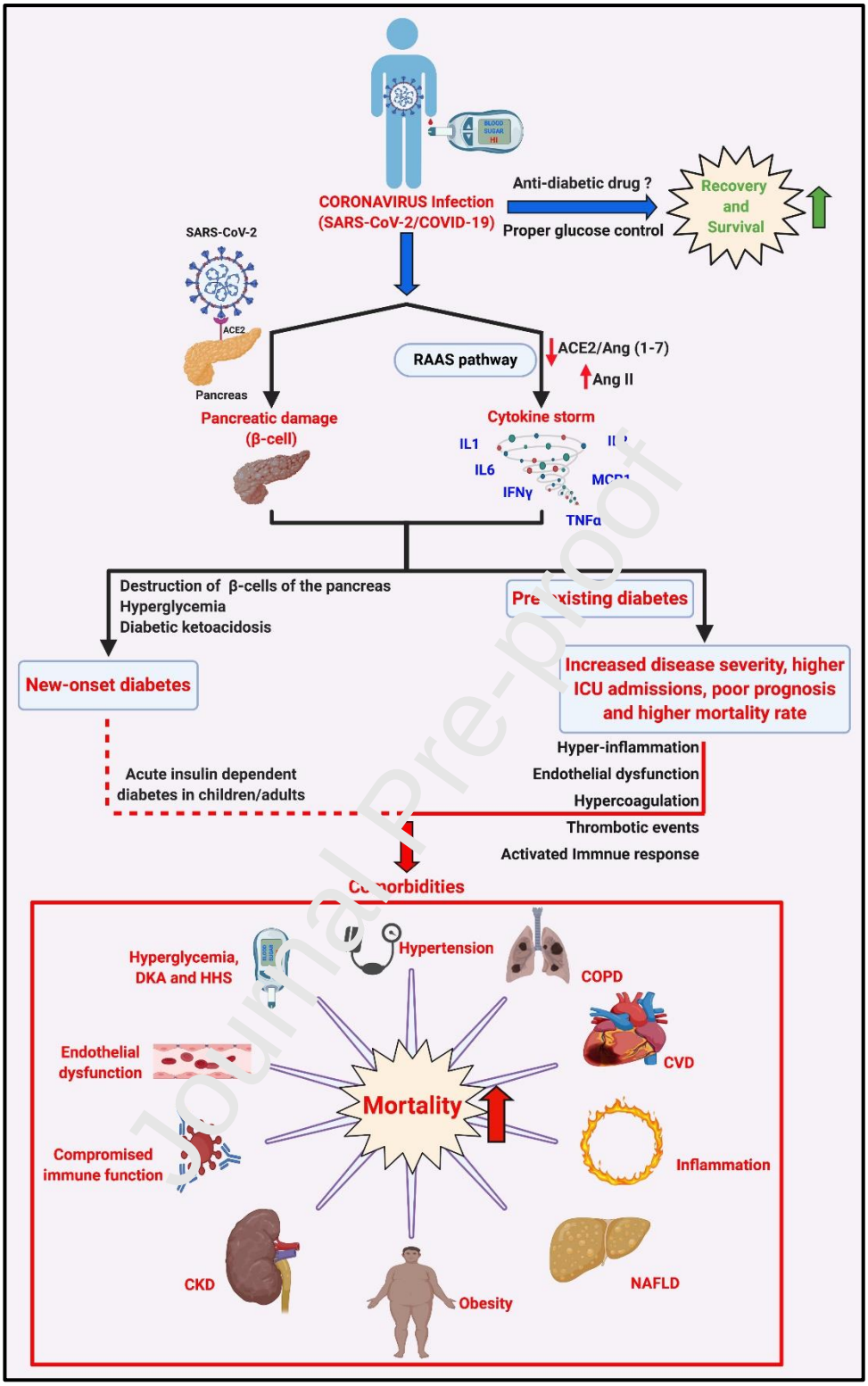

Figure 1. Diabetes/hyperglycemia and possible outcomes in COVID-19 patients. SARS-CoV-2 infection causes activation of RAAS that can result in a 'cytokine storm' via the AngII/AT1R axis resulting in the synthesis and secretion of pro-inflammatory cytokines/chemokines such as TNFα, IL1/2/6, interferon-γ (IFNγ) and monocyte chemoattractant protein-1 (MCP1). SARS-CoV-2 infection in an individual with pre-existing diabetes, the diabetes-associated pro-inflammatory status and endothelial dysfunction and the incidence of one or more comorbidities such as obesity, hypertension, CVD, NAFLD and CKD and the hyperglycemia-induced DKA and HHS can lead to increase in disease severity, higher rates of ICU admissions and may be responsible for the poor prognosis and higher mortality rates in diabetic COVID-19 patients. Interestingly, reports also suggest that SARS-CoV-2 infects the β-islets of the pancreas, causing β-cell damage and subsequent new-onset diabetes,

severe hyperglycemia and DKA in COVID-19 patients. Treatment using appropriate glucose-lowering agents and proper management of blood glucose levels aids recovery and survival among affected diabetic COVID-19 patients. Various aspects such as benefits, contra-indications, and limitations of using certain combinations of glucose-lowering agents and anti-viral treatments that could affect the outcome of the disease in a diabetic COVID-19 patient must be carefully analyzed. CKD = Chronic Kidney Disease, COPD = Chronic Obstructive Pulmonary Disorder, CVD = Cardiovascular Disease, DKA = Diabetic Ketoacidosis, HHS = Hyperglycemic Hyperosmolar Syndrome, NAFLD = Non-Alcoholic Fatty Liver Disease. Created with BioRender.com

Diabetes and COVID-19: Increased Disease Severity and Mortality

Diabetic patients have a compromised immune response and are prone to severe bacterial and viral infections/diseases, require more recovery time, and present longer-lasting adverse effects than their non-diabetic counterparts [24]. Proper management and maintenance of controlled blood glucose levels (avoiding consistent hyperglycemia) are closely related to the body's ability to regulate immune and inflammatory responses and fight infections in chronic diabetic patients [10]. An aggravated inflammatory and immune response-related severe course of the disease and higher mortality was observed during various bacterial/viral infections in hyperglycemic/diabetic COVID-19 patients that could be significantly reversed by maintaining controlled blood glucose levels.

COVID-19 and pre-existing diabetes

Plotting HbA_{1c} against the risk of SARS-CoV-2 infection/COVID-19 related hospitalization shows a characteristic J-curve, which indicates that diabetes is associated with a higher risk of infections (respiratory infections in particular) [1]. Although pre-existing diabetes did not increase the risk of occurrence of COVID-19; there was a significant increase in the severity of SARS-CoV-2 infection (COVID-19) among diabetic individuals, thereby increasing their risk of hospitalizations and requirement of emergency care [1]. Elderly severely ill diabetic COVID-19 patients exhibited an exaggerated inflammatory response and were more likely to require mechanical ventilation and ICU support with a markedly higher risk of mortality than COVID-19 patients without diabetes [25]. Worldwide studies corroborated severe pneumonia cases, increased risk of ICU admissions, and higher mortality rates in COVID-19 patients with diabetes (Table 1).

Table 1. Prevalence of pre-existing diabetes and COVID-19 outcomes (adapted from [1])

Study type	Study Origin	Study population	Prevalence of Diabetes (%)	Outcome	Reference
Retrospective	China	258	24	↑ Mortality	[26]
Meta-analysis	India	16003 (from 33 studies)	9.8	↑ Disease severity ↑ Mortality	[27]

Retrospective	China	1590	NA	↑ ICU admission, or invasive ventilation, or death	[28]
Meta-analysis	China	1527 (from 6 studies)	9.7	↑ ICU admissions	[29]
Meta-analysis	Italy	1687 (from 6 studies)	NA	↑ Disease severity	[30]
Meta-analysis	Italy	355 (from 6 studies)	35.5	↑ Mortality	[30]
Retrospective	USA	5279	22.6	↑ Hospitalization	[31]
Meta-analysis	Italy	1382 (from 4 studies)	NA	↑ ICU admissions	[32]
Meta-analysis	Italy	471 (from 4 studies)	NA	↑ Mortality	[32]
Retrospective	China	191	19	↑ Mortality	[9]
Retrospective	China	7337	13	↑ Mortality	[10]
Retrospective	China	193	25	↑ Mortality	[25]
Retrospective	Italy	59	44	↑ Disease severity ↓ Survival	[33]
Meta-analysis	China	1576 (from 7 studies)	NA	↑ Disease severity	[34]
Cohort	UK	61414470	0.4 (type 1 diabetes)	↑ Mortality	[35]
Cohort	UK	61414470	4.7 (type 2 diabetes)	↑ Mortality	[35]
Retrospective	France	1317	88.5	↑ Tracheal intubation for mechanical ventilation and/or mortality	[36]

COVID-19 and new-onset diabetes

Viral infections, including enterovirus, rotavirus, and mumps, could lead to acute type 1 diabetes [37]. Individuals with no previous diagnosis or history of diabetes developed acute hyperglycemia, upon SARS-CoV-1 infection, an independent indicator for higher mortality among such individuals [38]. This was associated with the ability of the SARS-CoV-1 virus to bind to the pancreatic islet ACE2 receptors causing acute islet damage [38].

Similarly, despite the absence of pre-existing diabetes, the occurrence of new-onset hyperglycemia in COVID-19 patients could result from SARS-CoV-2 viral infection [1, 22]. Evidence suggests that the pancreatic ACE2 receptor facilitates SARS-CoV-2 binding and entry, and the ensuing cellular damage should explain the new-onset of diabetes in COVID-19 patients [1, 39]. SARS-CoV-2 binding to its ACE2 receptor on the pancreas

downregulates ACE2 activity and creates an imbalance in the RAAS [40]. The subsequent accumulation of AngII and the over-activation of AngII/AT1R-axis triggers macrophage activation and triggers NF-κB signaling. This, in turn, leads to the excessive synthesis and secretion of several inflammatory cytokines (hyper-cytokinemia/cytokine storm), resulting in pancreatic damage, and partially explains new-onset diabetes in COVID-19 patients [17, 40, 41]. Significant increase in the levels of several pro-inflammatory cytokines/markers (IL-1β, IL-6, IL-10, and TNFα) were reported in severely ill COVID-19 patients and COVID-19 patients admitted in the ICU, while higher levels of IL-6 were correlated with higher mortality rates [41, 42]. An *in vitro* model that studied virus tropism using pseudo-viruses for SARS-CoV-2 entry in human pancreatic α- and β-cells showed that the pancreatic cells are highly permissive to SARS-CoV-2 entry and mimicked the chemokine induction that is typical of COVID-19 patients [37].

A study involving 33 children (who were previously exposed to SARS-CoV-2 or had active SARS-CoV-2 infection) reported the occurrence of new-onset type 1 diabetes in thirty of them (aged 23 months to 16.8 years) [43]. Twenty-one children (70%) developed diabetic ketoacidosis (DKA), while 11 of the 21 children reported severe DKA [43]. Despite a direct link, the study postulated that SARS-CoV-2 exposure caused an increase in new-onset type 1 diabetes in children [43]. A single-case study reported high blood glucose concentration (552 mg/dl) and HbA_{1c} (16.8%) levels in a 19-year old male who presented with DKA and tested positive for antibodies against SARS-CoV-2, indicating a possible COVID-19 infection 5-7 weeks before hospitalization [44]. Interestingly, autoimmune factors that could lead to the development of a type-1-diabetic condition were ruled out, suggesting a causal link between COVID-19 and the development of diabetes possibly due to SARS-CoV-2 infection of the β-cells via the ACE2 receptors and a direct cytolytic effect of the virus on the cells [44]. Remarkably, reports suggest a significant increase in mortality among COVID-19 patients diagnosed with new-onset hyperglycemia (without diabetes) compared to patients with pre-existing diabetes [45, 46].

Health authorities must make general health recommendations that require the COVID-19 patients to monitor their blood glucose levels frequently and remain vigilant regarding possible signs/symptoms of hyperglycemia. Therapeutic recommendations must be made based on initial and continuously monitored blood glucose measurements and the patient's medical history. Evaluation of markers of inflammation, coagulation factors, acute phase reactants, hepatic and renal function will help identify hyper-cytokinemia and predict

prothrombotic events. Thus, necessary adjustments in the treatment plan will improve the prognosis and survival of high-risk diabetic COVID-19 patients.

Endothelial dysfunction: the common denominator in diabetes and COVID-19

The vasculature is most vulnerable to SARS-CoV-2 infections [18]. Endothelial cells (ECs) express ACE2 receptors through which SARS-CoV-2 enters the cells [19]. SARS-CoV-2 particles and host inflammatory cells found inside ECs with evidence of endothelial and inflammatory cell death suggest that the endothelium and alterations in its function may contribute to disease progression and outcome among COVID-19 patients [19, 20]. COVID-19 is associated with endothelial dysfunction (ED), hyperviscosity/coagulation, higher incidence of thrombotic events, and microvascular complications as evidenced by elevated levels of D-dimer, VWF, fibrinogen and soluble P-selectin and increase in activity of VWF and factor VIII activity in critically ill COVID-19 ICU patients when compared with their non-ICU counterparts [18, 47, 48]. An increase in the incidence of venous thromboembolism, microvascular lung thrombosis, arterial events, and disseminated intravascular damage was linked to higher ICU admissions, the occurrence of terminal events, and mortality among severe COVID-19 patients [1, 18, 48, 49].

In diabetic COVID-19 patients, it is still too early to precisely state whether diabetes-associated ED exacerbates COVID-19 infection or whether COVID-19 infection accentuates diabetes-associated ED. In diabetes, the endothelium is exposed to hyperinsulinemia, hyperglycemia, and an excess of free fatty acids, and a consequent array of adverse molecular events - in response to various triggers such as increased ROS and decreased endothelial nitric oxide (NO) levels - causing ED [50]. Diabetes-associated ED and consequent prothrombotic state likely increases the risk of thromboembolic events in diabetic COVID-19 patients [1, 25, 51]. Furthermore, an increase in the levels of advanced glycation end products (AGEs) and subsequent activation of the receptors of AGEs (RAGEs) contribute to ED and chronic vascular complications that promote coagulation, supported by an increase in vascular hyper-permeability, increased leukocyte adhesion, and extravasation in diabetes [52, 53]. While the lungs' type-1-alveolar epithelial cells constitutively express RAGE, its activation in the endothelial cells, smooth muscle cells, neurons, and immune cells depends on the local expression of RAGE ligands [53, 54]. Ligands (other than AGEs), such as high-mobility group box 1 protein (HMGB1), S100 calcium-binding protein A12 (S100A12), other danger-associated molecular patterns (DAMPs), and exogenous pathogen-associated molecular patterns (PAMPs), released from SARS-CoV-2 infected damaged/dying cells can

activate the innate immune system via RAGE activation [53]. The activation of RAGE, in turn, creates a feed-forward loop that exacerbates inflammatory responses via the RAGE mediated transcription of NF-κB dependent pro-inflammatory genes that code for inflammatory cytokines and cell adhesion molecules [53, 55].

Constant clinical evaluation of ED and markers of thrombotic events, radiological assessment, and thromboprophylaxis/anticoagulant therapy is recommended and encouraged as a part of standard care for all COVID-19 patients, especially those with diabetes [48, 56]. Therapeutic intervention(s) that support restoration and stabilization of the normal endothelial apparatus and function and target inflammation may improve prognosis and survival of COVID-19 patients [57]. Currently, several studies investigate the possible beneficial effects of anticoagulant therapy and potential interventions that improve endothelial function, such as RAS inhibitors, statins, and antioxidants, to decrease disease severity and mortality among COVID-19 patients [57].

Hyperglycemia and COVID-19 outcome: Need for Glycemic Control

Irrespective of whether a COVID-19 patient has pre-existing diabetes, was diagnosed with new-onset diabetes, or is non-diabetic, the available evidence points to the fact that the blood glucose level is a crucial factor that would determine 1) susceptibility to a COVID-19 infection in the event of an exposure, 2) severity of the disease, 3) treatment strategies, 4) recovery and 5) outcomes (measured in terms of disease severity/ARDS/ICU admissions/cardiac injury/renal damage/survival/mortality) among COVID-19 patients [10, 46, 58, 59]. Hyperglycemia and higher fasting blood/plasma glucose (FBG/FPG) levels (Table 2) unarguably increased disease severity, the incidence of ARDS, cardiac and renal damage and was correlated with higher ICU admissions and mortality among COVID-19 patients. Hence, blood glucose measurements are crucial and must be frequently monitored and managed efficiently under strictly supervised treatment.

Table 2. Hyperglycemia and COVID-19 outcomes (adapted from [1])

Study type	Study Origin	Study population	Prevalence of Diabetes (%)	Parameter	Outcome	Reference
Retrospective	China	810	100	Median blood glucose during hospital stay (6.4 mmol/L; well controlled Vs. 10.6 mmol/L; poorly controlled)	↑ Mortality ↑ ARDS ↑ Cardiac injury ↑ Renal	[10]

					damage	
Retrospective	Italy	59	42.4	Blood glucose at the time of hospital admission (> 7.7 mmol/L)	↑ Disease severity ↑ Mortality ↓ Survival	[33]
Cohort	UK	17278392	9.9	HbA_{1c} (≥ 7.5%)	↑ Mortality	[60]
Retrospective	China	904	15	Hyperglycemia	↑ Mortality	[61]
Retrospective	China	269	19.3	Hyperglycemia	↑ Mortality	[62]
Retrospective	China	28	100	Random hyperglycemia	↑ Disease severity ↑ ICU admissions ↑ ARDS ↑ Mortality	[63]
Retrospective	USA	1122	40.2	HbA_{1c} (≥ 6.5%) and uncontrolled hyperglycemia (more than 2 blood glucose measurements ≥ 180 mg/dL within any 24-hour period)	↑ Mortality ↑ Median length of stay at hospital	[45]
Cohort	Kuwait, USA	417	23.3	Fasting blood glucose levels (1 mmol/L or 5 mmol/L increase in FBG; FBG ≥ 7 mmol/L)	↑ ICU admissions	[59]
Retrospective	China	605	No previous diagnosis of diabetes	FBG < 6.1 mmol/L FBG – 6.1 to 6.9 mmol/L FBG > 7.0 mmol/L	↑ Mortality (in patients with FBG > 7.0 mmol/L)	[58]
Retrospective	China	166	36.7 (diabetic) 12.7 (FPG > 7.0 mmol/L)	FPG > 7.0 mmol/L, but HbA_{1c} (< 6.5%)	↑ ICU admissions ↑ Mechanical ventilation ↑ Mortality	[64]

Re-purposing Metformin: Potential Impact on COVID-19 Treatment Strategy

Several anti-hyperglycemic drugs are routinely used to manage blood glucose levels in diabetic patients. The question remains as to which medication/drug a clinician would

recommend (in combination with other required drugs such as anti-virals) to effectively reduce blood glucose levels and adverse long-term post-COVID-19 effects and ultimately save the lives of SARS-CoV-2 infected diabetic patients. Glucose-lowering agents such as insulin and sitagliptin decreased disease severity and mortality rates in diabetic COVID-19 patients [33, 65]. The beneficial effects observed when using insulin could be correlated to its anti-inflammatory and immunomodulatory effects and the achievement of glycemic control in the patients [66]. Conversely, the inhibitory effect of insulin on disintegrin and metallo-proteinase domain-containing protein-17 (ADAM17) facilitates the proteolytic cleavage and shedding of the active ecto-domain of ACE2 and, in turn, increases the availability and activity of ACE2 for SARS-CoV-2 infection, which in part may explain the worsening clinical profile and poor prognosis in insulin administered COVID-19 patients [61, 67]. On the other hand, metformin, the most prescribed oral anti-hyperglycemic could be beneficial in treating COVID-19 at multiple levels. With its well-studied and documented benefits, metformin may hold a possible answer to blood glucose management and attenuation of COVID-19 complications at multiple levels.

Meta-analysis studies reported significant metformin treatment-associated reduction in COVID-19 infection-related mortality [68, 69]. Reports from retrospective studies suggest a significant metformin treatment-associated reduction in mortality among high-risk diabetic COVID-19 patients [23]. Interestingly, a retrospective cohort study, involving 6256 type 2 diabetic or obese (BMI at least 30 kg/m^2) COVID-19 participants, among whom 2333 patients were on a metformin treatment plan before their COVID-19 diagnosis, found a gender-dependent effect in which metformin treatment was associated with a reduction in disease severity and mortality among women, but not in men [70]. A retrospective study, among 1213 COVID-19 patients (678 were using metformin), showed that metformin treatment was associated with an increased incidence of acidosis (but not mortality) in type 2 diabetic COVID-19 participants, which was correlated to high metformin dosage, compromised renal function and severe COVID-19 illness [71]. However, owing to the ability of metformin to reduce inflammation and confer cardio-protection among type 2 diabetic COVID-19 patients, the researchers recommended the continuation of metformin therapy while continuously monitoring the patients for acidosis and deterioration of renal function [71]. Crouse et al. reaffirmed the role of diabetes as a prominent independent risk factor that contributed to a higher mortality rate among diabetic COVID-19 patients when compared non-diabetic COVID-19 patients [72]. They reported a three-fold decrease in

mortality among diabetic COVID-19 patients on a metformin treatment regimen before their COVID-19 diagnosis, while prior insulin use did not affect mortality [72]. This beneficial effect of metformin was observed even after correcting for other COVID-19 risk factors such as age, sex, race, obesity, and hypertension, or chronic kidney disease and heart failure [72].

In addition to lowering blood glucose levels and increasing insulin sensitivity, metformin has documented molecular effects that suggest its therapeutic efficacy against COVID-19 (Please refer to Figure 2 for details).

The use of metformin in type 2 diabetic patients is associated with a reduced risk of deep vein thrombosis, as reported by a non-randomized, pair-matched cohort study [73]. Other studies suggest that metformin prevents platelet activation and extracellular mitochondrial DNA release, thereby preventing venous and arterial thrombosis without a significantly prolonged of bleeding time [74]. Metformin confers multiple protective effects on the endothelium, improves endothelium-dependent vascular response, and attenuates ED in diabetes through several well-studied mechanisms such as the activation of AMPK, Sirt1, and eNOS [75]. Besides, metformin can protect the endothelium by reducing oxidative stress, inhibiting endothelial inflammation, and suppressing leukocyte-endothelium interactions while also attenuating endothelial cell senescence and apoptosis and preserving the endothelial glycocalyx that shields against ED [75, 76]. Furthermore, metformin attenuates RAGE overexpression, inhibits hyperglycemia-induced NF-κB activation and subsequent gene expression of several pro-inflammatory cytokines and cell adhesion molecules in vascular wall cells (endothelial and smooth muscle cells) and macrophages, and confers vascular protection [77, 78].

In chronic diabetic patients with multiple comorbidities such as obesity, hypertension and cardiovascular diseases, the aberrant activation of the RAAS, increase in oxidative stress and inflammation, and activation of the immune system can exacerbate the clinical complications during SARS-COV-2 infection and thus contribute to mortality in COVID-19 patients. Metformin may be recommended (in the absence of severe kidney ailments) as a preventive drug to protect type 2 diabetic and obese patients from severe illness and increase survival among COVID-19 patients. The protective effects of metformin go beyond its anti-hyperglycemic effect and ability to increase insulin sensitivity in chronic diabetic patients with multiple comorbidities. Studies are warranted to explore the anti-hyperglycemic, anti-viral, anti-inflammatory, immunomodulatory, and anti-thrombotic effects of metformin that

may contribute to its protective effects in diabetic COVID-19 patients in the absence/presence of one or more comorbidities.

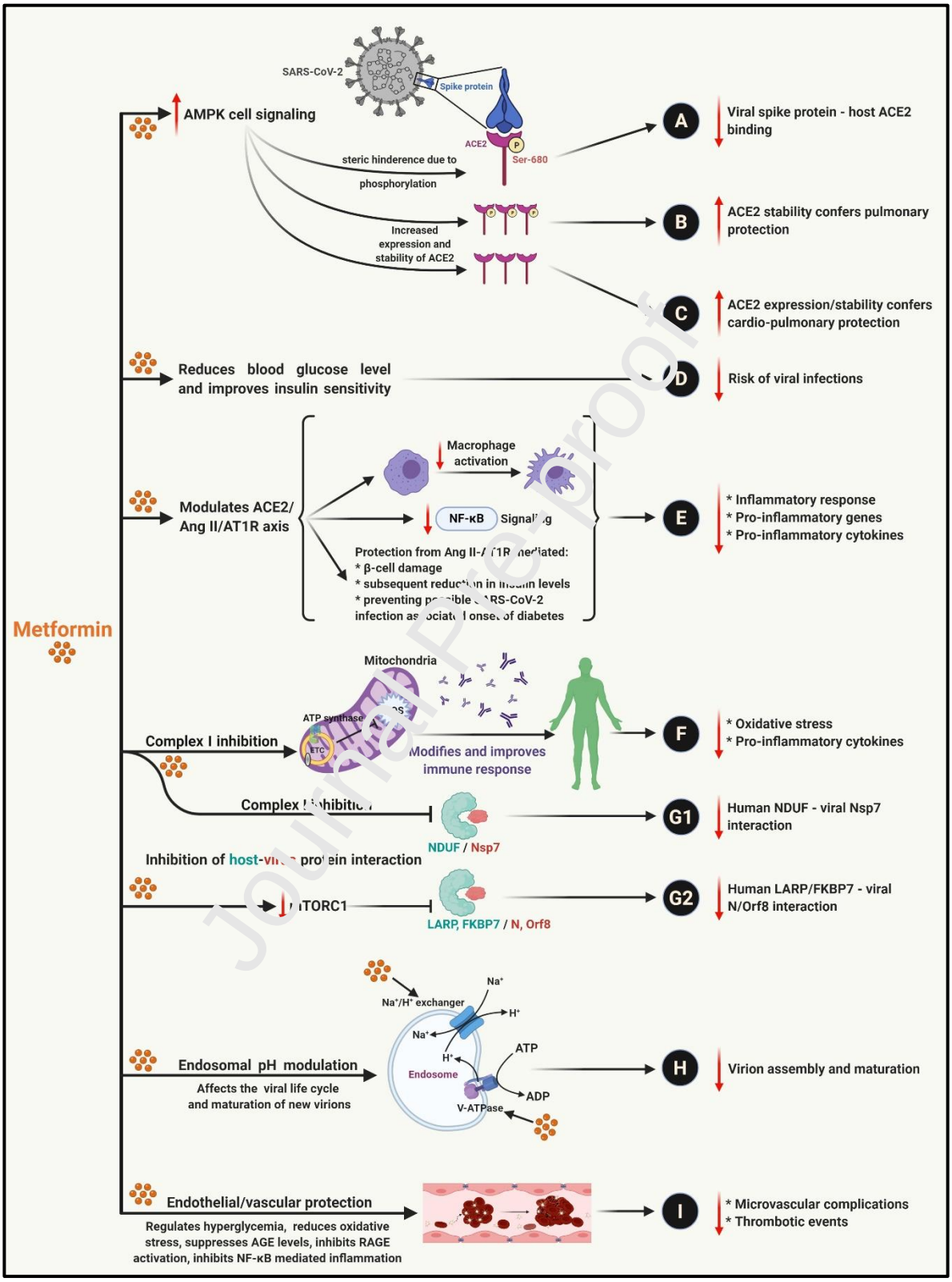

Figure 2. Multiple benefits of metformin treatment against SARS-CoV-2 infection. Metformin treatment-associated activation of AMPK mediated signalling mechanisms is well studied and documented [79]. The AMPK dependent increase in (A) ACE2 receptor phosphorylation (Ser680) causes a conformational change that inhibits ACE2 binding - viral spike protein binding and reduction of viral entry into the cell [15, 16]. AMPK

mediated increase in (B) ACE2 phosphorylation (ACE2 phosphorylation prevents poly-ubiquitination and subsequent 26-proteasome mediated degradation of ACE2) and (C) ACE2 expression, increases its half-life/stability, and offers cardio-pulmonary protection via the RAAS regulation [14-16]. The ability of metformin to reduce blood glucose levels and improve insulin stability (D) reduces the risk of SARS-CoV-2 infections [15]. Metformin treatment-associated increase in ACE2 levels and stability, in turn, regulates the ACE2/AngII/AT1R axis and suppresses (E) inflammatory response and release of pro-inflammatory cytokines by inhibiting macrophage activation and NF-κB signaling [16]. Metformin targets complex I of the mitochondrial electron transport chain (ETC), inhibits the generation of reactive oxygen species (ROS), and (F) suppresses the oxidative stress-mediated release of pro-inflammatory cytokines and attenuates inflammatory immune response [15, 80]. Inhibition of ETC and mTORC1 signalling (via AMPK or PI3K/Akt) by metformin (G1 and G2) contributes to the suppression of host-viral protein interactions, such as NDUF (human)-Nsp7 (viral) and LARP/FKBP7 (human): N/ORF8 (viral) interactions [81]. The suppression of the host-virus protein interactions inhibits host-dependent viral replication, synthesis of viral proteins, virion maturation, and release. Metformin, a strong base, targets the vacuolar ATPase (V-ATPase) and endosomal Na^+/H^+ exchangers (eNHEs) (H), increasing the cellular and endosomal pH and suppressing the endocytotic cycle and virion assembly and maturation [15, 82]. The anti-hyperglycemic, antioxidant, immunomodulatory, and anti-inflammatory effects of metformin attenuate endothelial dysfunction and confer vascular protection, thus (I) reducing microvascular complications and thrombotic events during SARS-CoV-2 infection. Created with BioRender.com

Metformin efficacy in COVID-19 treatment – clinical trials

Available data support the repurposing and use of metformin benefits as an effective therapeutic in the treatment of COVID-19. Data from ClincalTrials.gov (https://www.clinicaltrials.gov/) [as of 20th January 2020; primary search keyword (condition/disease): COVID-19; secondary search keyword (other terms): diabetes] showed 159 clinical trials that are related to COVID-19 and diabetes. However, only four of these studies were specific to metformin [secondary search keyword (other terms): metformin] in COVID-19 [b].

The 'COVIDOUT - Outpatient Treatment of COVID-19 with Metformin' (NCT04510194; phase 2/3; 750 participants; age 30 to 85 years) intends to investigate whether metformin (1500 mg; daily) treatment in non-hospitalized adults with SARS-CoV-2 can 1) prevent hypoxia and emergency department utilization, 2) prevent the disease progression in COVID-19, and 3) improve viral load and C-reactive protein (CRP) [c]. A phase 2 trial 'Pilot Study Into the Use of Metformin and Low Dose Naltrexone (LDN) for Patients With Coronavirus Disease 2019 (COVID-19) - Assessment of Short and Long Term Effects' (NCT04604678; 80 participants; age 30 to 70 years) aims to study the effect of a combination of metformin (1500 mg/day) and LDN (4.5 mg/day) on the attenuation of symptoms and disease severity, recovery time, hospitalization rates and mortality in COVID-19 patients at definite intervals over four weeks [d].

In a 20 participant (age 18 years and above) phase 2 trial (NCT04626089), the 'Adaptive Study for Efficacy and Safety of Metformin Glycinate for the Treatment of Patients With metabolic syndrome (MS) and type-2 diabetes mellitus (DM2), hospitalized with Severe

Acute Respiratory Syndrome Secondary to SARS-CoV-2 (randomized, double-blind)' the investigators intend to evaluate the efficacy and safety of metformin glycinate (620 mg; twice daily) plus standard treatment in COVID-19 patients (who have metabolic syndrome or type 2 diabetes) with ARDS (secondary to SARS-CoV-2 infections) in comparison to similar patients who receive the standard treatment alone [e]. A similar trial (NCT04625985; phase 2; enrolling an estimated 20 participants; 18 years and above) investigates the efficacy and safety of metformin glycinate (620 mg; twice daily) and standard treatment in hospitalized COVID-19 patients with ARDS (secondary to SARS-CoV-2 infection) [f].

Some of the clinical trials may include non-diabetic COVID-19 patients, which will help determine whether metformin would offer protection in non-diabetic SARS-CoV-2 infected patients. More studies and clinical trials are warranted to elucidate the molecular mechanisms that support metformin use in COVID-19 and answer the outstanding questions (see Outstanding Questions) regarding metformin and its therapeutic potential in COVID-19 treatment.

Metformin: adverse effects and possible contraindications in COVID-19

Most studies have outlined the beneficial effects of metformin in COVID-19 patients, while some studies have reported an increased risk of acidosis (but not mortality) and disease severity in COVID-19 patients using metformin [71, 83]. This suggests that metformin is not an appropriate choice in patients with severe respiratory distress, renal impairment, or heart failure [1], shedding light on the importance of paying attention to pre-existing conditions and comorbidities in drug selection. Furthermore, metformin-drug contraindications must be addressed before administration.

Concluding remarks and future perspectives

The world looks forward to the safety and efficacy of the approved vaccines for protection against new SARS-CoV-2 infections even as stringent measures (lockdowns/masking/social distancing) are being enforced to reduce the spread of the virus. Although crucial information regarding SARS-CoV-2/COVID-19 was collected in record time, a lot about how the viral infection contributes to disease progression and varying severity in different individuals remains unknown. It is, however, evident that hyperglycemia and diabetes-related comorbidities significantly increase disease severity and mortality in COVID-19 patients. Therefore, blood glucose levels must be properly monitored and managed to improve prognosis and survival rates in diabetic COVID-19 patients. The antidiabetic drug metformin,

in addition to its glucose-lowering effect, has potential anti-viral, cardio-protective, vasculo-protective, immuno-modulatory, and anti-inflammatory effects and hence could be repurposed for the treatment of COVID-19. Furthermore, metformin is considered safe, well-tolerated, has minimal side effects, is off-patent since 2002 (hence economical), and can be made readily available to COVID-19 patients who may benefit from metformin intervention [79].

The immediate need in this battle against COVID-19 is an efficacious drug/therapeutic that can successfully treat disease, improve recovery and prognosis and attenuate the long-term adverse effects of COVID-19 in the potential 'long-haulers' of the disease. While effective vaccine(s) and efficient vaccination programs are at the forefront in the fight against COVID-19, drugs routinely used for other pathological conditions must not be overlooked in terms of their potential efficacy to treat this disease. Host-directed therapies utilizing one or more of the currently marketed drugs with reasonable safety profiles are an excellent approach to combat this pandemic.

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Highlights

- Hyperglycemia (irrespective of whether acute in non-diabetics or due to pre-existing diabetes / new-onset diabetes) influences the severity of the COVID-19 disease, rate of hospitalizations and ICU admission, mortality among affected patients.
- Proper management of blood glucose levels reduced disease severity, the incidence of ARDS, requirement of ICU admissions, and ventilator support and promoted recovery in COVID-19 patients.
- Diabetes-associated endothelial dysfunction and related prothrombotic state increase the risk of thromboembolic events in diabetic COVID-19 patients.
- The safe, well-tolerated, and economical antidiabetic drug metformin could prove to be beneficial for COVID-19 therapy in multiple ways and efficiently improve treatment outcomes and reduce mortality in COVID-19 patients.
- Metformin decreases blood glucose levels and increases insulin sensitivity, inhibits viral infection, multiplication, and maturation, inhibits translation of viral proteins, regulates viral protein-host protein interactions, and modulates inflammation and immune response in COVID-19 patients.

Outstanding Questions

- Are diabetic patients on a metformin treatment regimen more resistant to SARS-CoV-2 infection than diabetic patients on other anti-hyperglycemic medications?
- Is metformin treatment beneficial in 'non-diabetic' COVID-19 patients who develop acute new-onset hyperglycemia?
- Can the beneficial effects of metformin observed in diabetic or obese patients taking metformin before their COVID-19 diagnosis be replicated if patients are administered metformin after COVID-19 diagnosis, irrespective of their diabetic status (both non-diabetic and diabetic) and BMI (both non-obese and obese)?
- How does metformin influence endothelial function/dysfunction, inflammation, and thrombotic events in diabetic/hyperglycemic COVID-19 patients?
- Will metformin therapy attenuate pro-inflammatory and prothrombotic events in non-

diabetic COVID-19 patients?

- Does metformin have any contraindications when used in combination with other drugs used in the treatment of COVID-19?
- What are the long-term effects of metformin treatment in COVID-19 patients?

Glossary

ARDS: Acute Respiratory Distress Syndrome - a life-threatening respiratory failure following a rapidly progressing widespread inflammation of the lungs and fluid accumulation in the lungs. Symptoms of ARDS include severe shortness of breath, laborious rapid breathing, low blood pressure, confusion and extreme fatigue.

Composite outcome: The outcome of the disease in COVID-19 patients that include mechanical ventilation, ICU admissions and mortality.

COVID-19: Coronavirus Disease 2019 – The severe acute respiratory syndrome illness, first reported in December 2019 in Wuhan, China, caused by the novel SARS-CoV-2 virus.

DKA: Diabetic ketoacidosis - a serious complication associated with diabetes where there is excessive breakdown of fat and liver converts fat to ketones making the blood more acidic.

Euglycemia: The condition in which an individual has normal concentration of glucose in the blood.

Host-directed therapies: Therapies directed towards host mediated immune response rather than directly targeting the pathogen itself.

Hypercytokinema/Cytokine storm: An immune response which results in the excessive synthesis and secretion of pro-inflammatory cytokines into the blood.

MERS-CoV: Middle East Respiratory Syndrome Coronavirus, the novel virus, identified in Saudi Arabia in 2012, that causes MERS.

MODS: Multiple Organ Dysfunction Syndrome – infection or injury induced tissue/organ damage caused by unbalanced immune and unregulated systemic inflammatory response.

Repurposing (Drug): Using a drug (in the same form or modified form) for the treatment of a disease different from its original intended use OR process of identifying new

therapeutic uses for already available/existing routinely used drugs.

SARS-CoV-1: Severe Acute Respiratory Syndrome Coronavirus, the virus, identified in China in 2003, that causes SARS.

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2, the novel virus, identified in China in 2020, that causes COVID-19.

Figure Legends

Figure 1. Diabetes/hyperglycemia and possible outcomes in COVID-19 patients. SARS-CoV-2 infection causes activation of RAAS that can result in a 'cytokine storm' via the AngII/AT1R axis resulting in the synthesis and secretion of pro-inflammatory cytokines/chemokines such as TNFα, IL1/2/6, interferon-γ (IFNγ) and monocyte chemoattractant protein-1 (MCP1). SARS-CoV-2 infection in an individual with pre-existing diabetes, the diabetes-associated pro-inflammatory status and endothelial dysfunction and the incidence of one or more comorbidities such as obesity, hypertension, CVD, NAFLD and CKD and the hyperglycemia-induced DKA and HHS can lead to increase in disease severity, higher rates of ICU admissions and may be responsible for the poor prognosis and higher mortality rates in diabetic COVID-19 patients. Interestingly, reports also suggest that SARS-CoV-2 infects the β-islets of the pancreas causing β-cell damage and subsequent new-onset diabetes, severe hyperglycemia and DKA in COVID-19 patients. Treatment using appropriate glucose-lowering agents and proper management of blood glucose levels aids recovery and survival among affected diabetic COVID-19 patients. Various aspects such as benefits, contra-indications, and limitations of using certain combinations of glucose-lowering agents and anti-viral treatments that could affect the outcome of the disease in a diabetic COVID-19 patient must be carefully analyzed. CKD = Chronic Kidney Disease, COPD = Chronic Obstructive Pulmonary Disorder, CVD = Cardiovascular Disease, DKA = Diabetic Ketoacidosis, HHS = Hyperglycemic Hyperosmolar Syndrome, NAFLD = Non-Alcoholic Fatty Liver Disease

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Figure 2. Multiple benefits of metformin treatment against SARS-CoV-2 infection. Metformin treatment-associated activation of AMPK mediated signalling mechanisms is well studied and documented [79]. The AMPK dependent increase in (A) ACE2 receptor phosphorylation (Ser680) causes a conformational change that inhibits ACE2 binding - viral spike protein binding and reduction of viral entry into the cell [15, 16]. AMPK mediated increase in (B) ACE2 phosphorylation (ACE2 phosphorylation prevents poly-ubiquitination and subsequent 26-proteasome mediated degradation of ACE2) and (C) ACE2 expression, increases its half-life/stability, and offers cardio-pulmonary protection via the RAAS regulation [14-16]. The ability of metformin to reduce blood glucose levels and improve insulin stability (D) reduces the risk of SARS-CoV-2 infections [15]. Metformin treatment-associated increase in ACE2 levels and stability, in turn, regulates the ACE2/AngII/AT1R

axis and suppresses (E) inflammatory response and release of pro-inflammatory cytokines by inhibiting macrophage activation and NF-κB signaling [16]. Metformin targets complex I of the mitochondrial electron transport chain (ETC), inhibits the generation of reactive oxygen species (ROS), and (F) suppresses the oxidative stress-mediated release of pro-inflammatory cytokines and attenuates inflammatory immune response [15, 80]. Inhibition of ETC and mTORC1 signalling (via AMPK or PI3K/Akt) by metformin (G1 and G2) contributes to the suppression of host-viral protein interactions, such as NDUF (human)-Nsp7 (viral) and LARP/FKBP7 (human): N/ORF8 (viral) interactions [81]. The suppression of the host-virus protein interactions inhibits host-dependent viral replication, synthesis of viral proteins, virion maturation, and release. Metformin, a strong base, targets the vacuolar ATPase (V-ATPase) and endosomal Na^+/H^+ exchangers (eNHEs) (H), increasing the cellular and endosomal pH and suppressing the endocytotic cycle and virion assembly and maturation [15, 82]. The anti-hyperglycemic, antioxidant, immunomodulatory and anti-inflammatory effects of metformin attenuate endothelial dysfunction and confer vascular protection, thus (I) reducing microvascular complications and thrombotic events during SARS-CoV-2 infection.

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