Metabolism Open 12 (2021) 100137

Contents lists available at ScienceDirect

Metabolism Open

journal homepage: www.sciencedirect.com/journal/metabolism-open

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

Zemene Demelash Kifle [a,*], Alem Endeshaw Woldeyohanis [b], Chilot Abiyu Demeke [c]

[a] Department of Pharmacology, School of Pharmacy, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia
[b] Department of Social Pharmacy, School of Pharmacy, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia
[c] Department of Pharmaceutics, School of Pharmacy, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia

ARTICLE INFO

Keywords:
COVID-19
Metformin
Diabetes
Molecular mechanism

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

* Corresponding author.
E-mail address: zeme2010@gmail.com (Z.D. Kifle).

https://doi.org/10.1016/j.metop.2021.100137
Received 2 September 2021; Accepted 12 October 2021
Available online 14 October 2021

[15], which could have comparable mechanism with COVID-19.

Metformin is one of the most commonly used antidiabetic medications, that displayed immunomodulatory activity through AMP-activated protein kinase in *in-vivo* models [16]. An observational study revealed that mortality of chronic lower respiratory diseases was significantly reduced by metformin as compared to the overall population [17]. In addition to its antidiabetic activity, metformin has, for instance, anti-inflammatory activity and can decrease the creation of reactive oxygen species [18]. Further epidemiological investigations on the activities of metformin in patients with COVID-19 may help to ascertain whether there is a correlation between clinical outcomes and metformin use. Therefore, the present study aimed to summarize the association between metformin use and its possible molecular mechanisms in diabetic patients with COVID-19.

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 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 nod diabetic 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 simplifies 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-alpha, 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.

3.1. Metformin-mediated anti-inflammatory and immunomodulatory activities

In patients with COVID-19, decrease in ACE2 availability and SARS-CoV-2 binding to its ACE2 receptor creates an imbalance in the RAAS, resulting in the secretion and synthesis of pro-inflammatory cytokines (IL-1β, IL-1, IL-6, and TNFα), triggering the NF-κB activation mediated inflammatory process and hyper-activation of AngII/AT1R axis, which explains the multi-organ failure, severe disease manifestations, and higher mortality in diabetic patients with COVID-19 [28,29].

The ability of the SARS-CoV-2 virus to damage and infect the endothelial cells triggering endothelial dysfunction, owing to the presence of endothelial ACE2 receptors [31–33]. A higher occurrence of hypercoagulation, microvascular complications, higher incidence of thrombotic events, and endothelial dysfunction were observed in patients with COVID-19 [31,32,34–39].

Fascinatingly, the existence of a prothrombotic state and endothelial dysfunction (mediated by reduced nitric oxide levels and diabetes-induced oxidative stress) may exacerbate COVID-19 related vascular

Table 1
The beneficial mechanism of Metformin against COVID-19.

Drug	Mechanism	References
Metformin	Decrease oxidative stress	[89]
	Improve glucose control	[90,91]
	Decrease fibrosis	[92]
	Increase insulin sensitivity	[62,93]
	Decrease renal hypoxia	[94,95]
	Improves low-grade inflammation in obesity	[96,97]
	Reduction in neutrophils	[98]
	Reduction in body weight	[99,100]
	Increased urinary sodium excretion and decrease NCC activity	[101]
	Decrease inflammatory cytokines	[102,103]
	Increase autophagy and Sirt1/FoxO1 and decrease GBM thickness	[104]
	Decrease reactive oxygen species production	[105–107]
	Reduce inflammatory marker release	[51]
	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^{2+} [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 rise 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, vasoconstrictive, 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 associates 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 inhibits the mTOR signaling pathway via the inhibition of AKT activation of AMPK, and/or directly, hence hesitant 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 like 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]. I*n 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 also currently taking it.

Funding

There is no funding to report.

CRediT authorship contribution statement

Zemene Demelash Kifle: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Roles/, Writing – review & editing. **Alem Endeshaw Woldeyohanis:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Writing – original draft, Roles/, Writing – review & editing. **Chilot Abiyu Demeke:** Software, Supervision, Validation, Visualization, Writing – original draft, Roles/, Writing – review & editing.

Declaration of competing interest

The authors declares that they have no competing interests.

Acknowledgment

We would like to acknowledge the University of Gondar.

References

[1] Grasselli G, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. Jama 2020; 323(16):1574–81.
[2] Park SE. Epidemiology, virology, and clinical features of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2; Coronavirus Disease-19). Clinic Exper Pediatr 2020;63(4):119.
[3] Guan W-j, et al. Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. Eur Respir J 2020;55(5).
[4] Choi S-H, et al. Epidemiology and clinical features of coronavirus disease 2019 in children. Clinic Exper Pediatr 2020;63(4):125.
[5] Dai M, et al. Patients with cancer appear more vulnerable to SARS-CoV-2: a multicenter study during the COVID-19 outbreak. Cancer Discov 2020;10(6): 783–91.
[6] Lighter J, Phillips M, Hochman S. Obesity in patients younger than 60 years is a risk factor for Covid-19 hospital admission [e-pub ahead of print]. accessed April Clin Infect Dis 2020. https://doi.org/10.1093/cid/ciaa415. 18.
[7] Blanco JL, et al. COVID-19 in patients with HIV: clinical case series. Lancet HIV 2020;7(5):e314–6.

[8] Worldometer. COVID-19 Coronavirus pandemic. Available from: https://www.worldometers.info/coronavirus/?%3D%3D. [Accessed 8 July 2021].
[9] Ikewaki N, et al. Hepatoprotective effects of Aureobasidium pullulans derived Beta 1, 3-1, 6 biological response modifier glucans in a STAM-animal model of non-alcoholic steatohepatitis. bioRxiv; 2021.
[10] Beigel JH, et al. Remdesivir for the treatment of Covid-19—preliminary report. N Engl J Med 2020;383(19):1813–36.
[11] Reynolds HR, et al. Renin–angiotensin–aldosterone system inhibitors and risk of Covid-19. N Engl J Med 2020;382(25):2441–8.
[12] Xie Y, et al. Estimates of all cause mortality and cause specific mortality associated with proton pump inhibitors among US veterans: cohort study. BMJ 2019:365.
[13] Woo A, et al. Incidence of cancer after asthma development: 2 independent population-based cohort studies. J Allergy Clin Immunol 2021;147(1):135–43.
[14] Li X, et al. Effects of daily treatment with acid suppressants for stress ulcer prophylaxis on risk of ventilator-associated events. Infect Control Hosp Epidemiol 2020;41(2):187–93.
[15] Ma C, et al. Interpreting reported risks associated with use of proton pump inhibitors: residual confounding in a 10-year analysis of national ambulatory data. Gastroenterology 2020;158(3):780–2. e3.
[16] Yon DK, et al. Exposure to humidifier disinfectants is associated with upper and lower airway diseases. Pediatr Allergy Immunol 2020;31(5):578–82. official publication of the European Society of Pediatric Allergy and Immunology.
[17] Ha J, Lee SW, Yon DK. Ten-Year trends and prevalence of asthma, allergic rhinitis, and atopic dermatitis among the Korean population. 2008–2017 Clinic Exper Pediatr 2020;63(7):278.
[18] Scheen AJ. Metformin and COVID-19: from cellular mechanisms to reduced mortality. Diabetes Metabol 2020;46(6):423–6.
[19] Toniolo A, et al. The diabetes pandemic and associated infections: suggestions for clinical microbiology. Rev Med Microbiol 2019;30(1):1.
[20] Zhu L, et al. Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes. Cell Metabol 2020;31(6):1068–77. e3.
[21] Samuel SM, Varghese E, Büsselberg D. Therapeutic potential of metformin in COVID-19: reasoning for its protective role. Trends Microbiol 2021;29(10):894–907.
[22] Apicella M, et al. COVID-19 in people with diabetes: understanding the reasons for worse outcomes. Lancet Diabetes & Endocrinol 2020;8(9):782–92.
[23] Yan Y, et al. Clinical characteristics and outcomes of patients with severe covid-19 with diabetes. BMJ Open Diabetes Res Care 2020;8(1):e001343.
[24] Yang L, et al. A human pluripotent stem cell-based platform to study SARS-CoV-2 tropism and model virus infection in human cells and organoids. Cell Stem Cell 2020;27(1):125–36. e7.
[25] Yang J-K, et al. Binding of SARS coronavirus to its receptor damages islets and causes acute diabetes. Acta Diabetol 2010;47(3):193–9.
[26] Alves AM, et al. SARS-CoV-2 leading to acute pancreatitis: an unusual presentation. Braz J Infect Dis 2021;24:561–4.
[27] Ni W, et al. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Crit Care 2020;24(1):1–10.
[28] Hojyo S, et al. How COVID-19 induces cytokine storm with high mortality. Inflamm Regen 2020;40(1):1–7.
[29] Banu N, et al. Protective role of ACE2 and its downregulation in SARS-CoV-2 infection leading to macrophage activation syndrome: therapeutic implications. Life Sci 2020;256:117905.
[30] Ragab D, et al. The COVID-19 cytokine storm; what we know so far. Front Immunol 2020;11:1446.
[31] Varga Z, et al. Infecção de células endoteliais e endotelite em COVID-19. Lanceta 2020;395(10234):1417–8.
[32] Goshua G, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. Lancet Haematol 2020;7(8):e575–82.
[33] Singh AK, Khunti K. Assessment of risk, severity, mortality, glycemic control and antidiabetic agents in patients with diabetes and COVID-19: a narrative review. Diabetes Res Clin Pract 2020;165:108266.
[34] Elbadawi A, et al. Incidence and outcomes of thrombotic events in symptomatic patients with COVID-19. Arterioscler Thromb Vasc Biol 2021;41(1):545–7.
[35] Gavriilaki E, et al. Endothelial dysfunction in COVID-19: lessons learned from coronaviruses. Curr Hypertens Rep 2020;22(9):1–12.
[36] Jin Y, et al. Endothelial activation and dysfunction in COVID-19: from basic mechanisms to potential therapeutic approaches. Signal Transduct Target Thera 2020;5(1):1–13.
[37] Huertas A, et al. Endothelial cell dysfunction: a major player in SARS-CoV-2 infection (COVID-19)? Eur Respiratory Soc 2020;56(1).
[38] Ribes A, et al. Thromboembolic events and covid-19. Adv Biol Regulat 2020;77:100735.
[39] Tang N, et al. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemostasis 2020;18(4):844–7.
[40] Apicella M, et al. COVID-19 in people with diabetes: understanding the reasons for worse outcomes. Lancet Diabetes & Endocrinol 2020;8(2020):782. E5-E5.
[41] Tan KC, et al. Advanced glycation end products and endothelial dysfunction in type 2 diabetes. Diabetes Care 2002;25(6):1055–9.
[42] De Francesco EM, Vella V, Belfiore A. COVID-19 and diabetes: the importance of controlling RAGE. Front Endocrinol 2020;11.
[43] Hudson BI, Lippman ME. Targeting RAGE signaling in inflammatory disease. Annu Rev Med 2018;69:349–64.
[44] van Zoelen MA, Achouiti A, van der Poll T. The role of receptor for advanced glycation endproducts (RAGE) in infection. 2011. Annual update in intensive care and emergency medicine. 2011. p. 3–15.
[45] Menendez JA. Metformin and SARS-CoV-2: mechanistic lessons on air pollution to weather the cytokine/thrombotic storm in COVID-19. Aging (N Y) 2020;12(10):8760.
[46] Kuperman Y, et al. CRFR1 in AgRP neurons modulates sympathetic nervous system activity to adapt to cold stress and fasting. Cell Metabol 2016;23(6):1185–99.
[47] Schuiveling M, et al. Metformin, a new era for an old drug in the treatment of immune mediated disease? Curr Drug Targets 2018;19(8):945–59.
[48] Adeshara KA, et al. Action of metformin therapy against advanced glycation, oxidative stress and inflammation in type 2 diabetes patients: 3 months follow-up study. Diabetes & Metabol Syndr: Clin Res Rev 2020;14(5):1449–58.
[49] Zhou Ze, et al. Metformin inhibits advanced glycation end products-induced inflammatory response in murine macrophages partly through AMPK activation and RAGE/NFκB pathway suppression. J Diabetes Res 2016;**2016**.
[50] Chen X-F, et al. Metformin corrects RAGE overexpression caused signaling dysregulation and NF-κB targeted gene change. Int J Clin Exp Med 2016;9(2):2913–20.
[51] Isoda K, et al. Metformin inhibits proinflammatory responses and nuclear factor-κB in human vascular wall cells. Arterioscler Thromb Vasc Biol 2006;26(3):611–7.
[52] Millet JK, Whittaker GR. Physiological and molecular triggers for SARS-CoV membrane fusion and entry into host cells. Virology 2018;517:3–8.
[53] Tang T, et al. Coronavirus membrane fusion mechanism offers a potential target for antiviral development. Antivir Res 2020;178:104792.
[54] Wędrowska E, et al. Coronaviruses fusion with the membrane and entry to the host cell. Ann Agric Environ Med 2020;27(2):175–83.
[55] Hoffmann M, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181(2):271–80. e8.
[56] Rockx B, et al. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. Science 2020;368(6494):1012–5.
[57] Müller JA, et al. SARS-CoV-2 infects and replicates in cells of the human endocrine and exocrine pancreas. Nature Metabol 2021;3(2):149–65.
[58] Vickers NJ. Animal communication: when i'm calling you, will you answer too? Curr Biol 2017;27(14):R713–5.
[59] Ashraf UM, et al. SARS-CoV-2, ACE2 expression, and systemic organ invasion. Physiol Genom 2021;53(2):51–60.
[60] Kruglikov IL, Shah M, Scherer PE. Obesity and diabetes as comorbidities for COVID-19: underlying mechanisms and the role of viral–bacterial interactions. Elife 2020;9:e61330.
[61] De Souza Jr MB, Pinto JC, Lima EL. Control of a chaotic polymerization reactor: a neural network based model predictive approach. Polym Eng Sci 1996;36(4):448–57.
[62] Sharma S, Ray A, Sadasivam B. Metformin in COVID-19: a possible role beyond diabetes. Diabetes Res Clin Pract 2020;164:108183.
[63] Malhotra A, et al. ACE2, metformin, and COVID-19. Iscience. 2020. p. 101425.
[64] Ursini F, et al. COVID-19 and diabetes: is metformin a friend or foe? Diabetes Res Clin Pract 2020;164:108167.
[65] Bangi S, Barve R, Qamar A. Protective effects of CVD and DM medications in SARS-CoV-2 infection. SN Comprehens Clinic Med 2020;2(9):1296–8.
[66] Zhang J, et al. AMP-activated protein kinase phosphorylation of angiotensin-converting enzyme 2 in endothelium mitigates pulmonary hypertension. Am J Respir Crit Care Med 2018;198(4):509–20.
[67] Niu M-J, et al. Loss of angiotensin-converting enzyme 2 leads to impaired glucose homeostasis in mice. Endocrine 2008;34(1):56–61.
[68] Alexopoulou O, et al. Bone density and markers of bone remodeling in type 1 male diabetic patients. Diabetes Metabol 2006;32(5):453–8.
[69] Lim S, et al. COVID-19 and diabetes mellitus: from pathophysiology to clinical management. Nat Rev Endocrinol 2021;17(1):11–30.
[70] Zimmet P, Collier G. Clinical efficacy of metformin against insulin resistance parameters. Drugs 1999;58(1):21–8.
[71] Tsai S, et al. Insulin receptor-mediated stimulation boosts T cell immunity during inflammation and infection. Cell Metabol 2018;28(6):922–34. e4.
[72] Shoemaker R, et al. ACE2 deficiency reduces β-cell mass and impairs β-cell proliferation in obese C57BL/6 mice. Am J Physiol Endocrinol Metabol 2015;309(7):E621–31.
[73] Takeda M, et al. Loss of ACE2 exaggerates high-calorie diet–induced insulin resistance by reduction of GLUT4 in mice. Diabetes 2013;62(1):223–33.
[74] Wang H, et al. SARS coronavirus entry into host cells through a novel clathrin-and caveolae-independent endocytic pathway. Cell Res 2008;18(2):290–301.
[75] Gupta S, Hrishikeshvan H, Sehajpal PK. Spirulina protects against Rosiglitazone induced osteoporosis in insulin resistance rats. Diabetes Res Clin Pract 2010;87(1):38–43.
[76] Nishimura K, et al. Development of defective and persistent Sendai virus vector: a unique gene delivery/expression system ideal for cell reprogramming. J Biol Chem 2011;286(6):4760–71.
[77] Esam Z. A proposed mechanism for the possible therapeutic potential of Metformin in COVID-19. Diabetes Res Clin Pract 2020:167.
[78] Einterz SF, et al. Development and testing of a decision aid on goals of care for advanced dementia. J Am Med Dir Assoc 2014;15(4):251–5.
[79] Samuel SM, et al. Metformin represses glucose starvation induced autophagic response in microvascular endothelial cells and promotes cell death. Biochem Pharmacol 2017;132:118–32.

[80] Mathews Samuel S, et al. Treatment with a combination of metformin and 2-deoxyglucose upregulates thrombospondin-1 in microvascular endothelial cells: implications in anti-angiogenic cancer therapy. Cancers 2019;11(11):1737.
[81] Varghese S, et al. High glucose represses the anti-proliferative and pro-apoptotic effect of metformin in triple negative breast cancer cells. Biomolecules 2019;9(1):16.
[82] Gordon DE, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature 2020;583(7816):459–68.
[83] Thaiss CA, et al. Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. Science 2018;359(6382):1376–83.
[84] Yeoh YK, et al. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. Gut 2021;70(4):698–706.
[85] Zhang Q, et al. Inflammation and antiviral immune response associated with severe progression of COVID-19. Front Immunol 2021;12:135.
[86] Cani PD, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet–induced obesity and diabetes in mice. Diabetes 2008;57(6):1470–81.
[87] Wu H, et al. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nat Med 2017;23(7):850–8.
[88] Organization WH. Timeline of WHO's response to COVID-19, 2020. 2020. URL, https://www. who. int/news-room/detail/29-06-2020-covidtimeline. [Accessed 14 July 2020].
[89] Borges CM, et al. Metformin arrests the progression of established kidney disease in the subtotal nephrectomy model of chronic kidney disease. Am J Physiol Ren Physiol 2020;318(5):F1229–36.
[90] Ceriello A. Hyperglycemia and the worse prognosis of COVID-19. Why a fast blood glucose control should be mandatory. Diabetes Res Clin Pract 2020;163:108186.
[91] Ceriello A, De Nigris V, Prattichizzo F. Why is hyperglycaemia worsening COVID-19 and its prognosis? Diabetes Obes Metabol 2020;22(10):1951–2.
[92] Sturmlechner I, et al. Cellular senescence in renal ageing and disease. Nat Rev Nephrol 2017;13(2):77–89.
[93] Chen Y, Gu F, Guan J-L. Metformin might inhibit virus through increasing insulin sensitivity. Chin Med J 2018;131(3):376.
[94] Christensen M, et al. Metformin attenuates renal medullary hypoxia in diabetic nephropathy through inhibition uncoupling protein-2. Diabetes/Metabol Res Rev 2019;35(2):e3091.
[95] Jiang X, et al. Metformin reduces the senescence of renal tubular epithelial cells in diabetic nephropathy via the MBNL1/miR-130a-3p/STAT3 pathway. Oxidative medicine and cellular longevity. 2020. p. 2020.
[96] Jing Y, et al. Metformin improves obesity-associated inflammation by altering macrophages polarization. Mol Cell Endocrinol 2018;461:256–64.
[97] Desai N, et al. Maternal metformin treatment decreases fetal inflammation in a rat model of obesity and metabolic syndrome. Am J Obstet Gynecol 2013;209(2).136. e1-136. e9.
[98] Dalan R. Metformin, neutrophils and COVID-19 infection. Diabetes Res Clin Pract 2020:164.
[99] El-Arabey AA, Abdalla M. Metformin and COVID-19: a novel deal of an old drug. J Med Virol 2020;92(11):2293–4.
[100] Seifarth C, Schehler B, Schneider H. Effectiveness of metformin on weight loss in non-diabetic individuals with obesity. Exp Clin Endocrinol Diabetes 2013;121:27–31. 01.
[101] Hashimoto H, et al. Metformin increases urinary sodium excretion by reducing phosphorylation of the sodium-chloride cotransporter. Metabolism 2018;85:23–31.
[102] Ansari G, et al. How does blood glucose control with metformin influence intensive insulin protocols? Evidence for involvement of oxidative stress and inflammatory cytokines. Adv Ther 2008;25(7):681–702.
[103] Chen W, Liu X, Ye S. Effects of metformin on blood and urine pro-inflammatory mediators in patients with type 2 diabetes. J Inflamm 2016;13(1):1–6.
[104] Xu J, et al. Metformin alleviates renal injury in diabetic rats by inducing Sirt1/FoxO1 autophagic signal axis. Clin Exp Pharmacol Physiol 2020;47(4):599–608.
[105] Algire C, et al. Metformin reduces endogenous reactive oxygen species and associated DNA damage. Cancer Prev Res 2012;5(4):536–43.
[106] Ouslimani N, et al. Metformin decreases intracellular production of reactive oxygen species in aortic endothelial cells. Metabolism 2005;54(6):829–34.
[107] Hou X, et al. Metformin reduces intracellular reactive oxygen species levels by upregulating expression of the antioxidant thioredoxin via the AMPK-FOXO3 pathway. Biochem Biophys Res Commun 2010;396(2):199–205.
[108] Nesti L, Natali A. Metformin effects on the heart and the cardiovascular system: a review of experimental and clinical data. Nutr Metabol Cardiovasc Dis 2017;27(8):657–69.