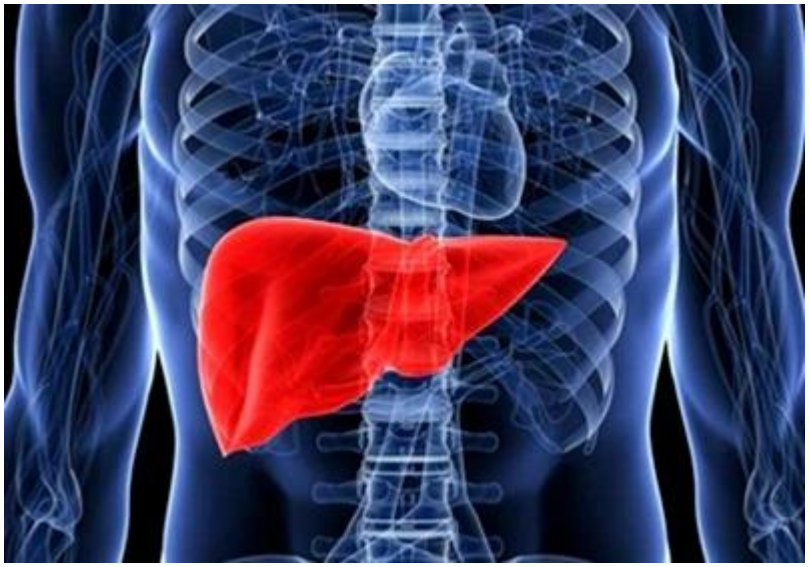
Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a systemic multiple-organ disease characterized by a broad spectrum of clinical manifestations. One of them is hyperglycemia, which could be present in COVID-19 patients, and not only in people with overt diabetes. Previous studies have shown an association between new-onset hyperglycemia and poorer clinical outcomes in COVID-19 patients admitted to the ICU. In this study, the Brazilian authors used retrospective clinical data, liver samples obtained through *postmortem* biopsies, and *in vitro* experiments to investigate the mechanism of infection of hepatocytes with SARS-CoV-2 and how SARS-CoV-2 affects hepatic glucose production.

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins, namely the spike (S), envelope (E), nucleocapsid (N), and membrane (M) protein. Two host-cell factors are important for SARS-CoV-2 viral entry into many cell types: angiotensin-converting enzyme 2 (ACE2), which is bound by S protein, and transmembrane protease serine 2 (TMPRSS2), which cleaves S protein, allowing this binding to take place.

According to previous data, other RNA viruses, such as hepatitis C and B, can induce hepatic glucose production by stimulating gluconeogenesis in hepatocytes. The SARS-CoV-2 could have a similar effect. *In vitro* studies have shown that SARS-CoV-2 can infect and damage pancreatic beta cells. Additionally, *postmortem* examination of liver samples has found that SARS-CoV-2 infects hepatocytes and damages the liver. However, the clinical relevance of these findings is still controversial.



About the study

The authors used retrospective clinical data, *postmortem* histopathological examination of liver samples, and *in vitro* experiments to investigate the mechanism of SARS-CoV-2 infection of hepatocytes.

The study included two groups: individuals who tested positive on polymerase chain reaction (PCR) test for SARS-CoV-2 (SARS-CoV-2-positive) and individuals with suspected COVID-19 based on clinical presentation but with negative PCR tests for SARS-CoV-2 (SARS-CoV-2-negative). Patients suffering from liver dysfunction, HIV infection, or cancer were excluded. A comparable proportion of SARS-CoV-2-positive and SARS-CoV-2-negative patients (85.8% and 80.3%, respectively) were admitted to the ICU.

There was no significant difference in diabetes prevalence, serum levels of alanine aminotransferase, aspartate aminotransferase, and creatinine between the two groups of participants. The SARS-CoV-2-positive group had a higher rate of obesity, a higher average body weight, and a higher body mass index than the SARS-CoV-2-negative group.

Results

The study included 647 individuals who tested positive for SARS-CoV-2 and 203 who tested negative for the virus.

Upon admission, the SARS-CoV-2-positive group had significantly higher serum concentrations of glucose and C-reactive protein, and were more likely to experience hyperglycemic episodes, regardless of their diabetes status than the SARS-CoV-2-negative group.

Upon admission, SARS-CoV-2-positive hyperglycemic participants had higher serum concentrations of C-peptide, glucagon, and glycated protein than SARS-CoV-2-negative individuals. In addition, hyperglycemic SARS-CoV-2-positive participants had higher serum levels of C-peptide and glycated serum proteins, but not glucagon than normoglycemic SARS-CoV-2-positive individuals. These findings suggest that hyperglycemic SARS-CoV-2-positive participants normally secrete insulin in response to hyperglycemia, at least at the time of admission.

Postmortem histopathological examination of liver samples obtained from four non-diabetic patients who died due to complications of COVID-19 demonstrated that approximately 40% of hepatocytes were positive for SARS-CoV-2 S protein. The S protein was co-localized with

viral double-strand RNA, indicating that SARS-CoV-2 can replicate in the liver.

The authors also performed *in vitro* experiments to investigate whether and how SARS-CoV-2 infects primary human hepatocytes. They exposed the hepatocytes to SARS-CoV-2 lineage SPBR-02 and found that the virus can infect and replicate within primary human hepatocytes, without causing significant cellular damage. These data are consistent with previous studies which have found that SARS-CoV-2 infects hepatocytes and damages the liver.

In vitro infection of human primary hepatocytes with SARS-CoV-2 lineage SPBR-02 resulted in gluconeogenesis and stimulation of phosphoenolpyruvate carboxykinase (PEPCK), an enzyme of hepatic glucose production through gluconeogenesis. 48 hours after the infection of human primary hepatocytes with SARS-CoV-2, glucose production and the activity of PEPCK increased by about two-fold. Importantly, mRNA expression of genes related to gluconeogenesis and glycogenolysis was unchanged or even reduced after the infection.

Researchers also analyzed which receptors mediate the entry of SARS-CoV-2 into the hepatocytes. The hepatocytes expressed low levels of ACE2, but highly expressed chaperone glucose-regulated protein 78 (GRP78), an endoplasmic reticulum chaperone that can be translocated to the cell surface and serve as an entry factor for several viruses. The GRP78 and ACE2 were present on the cell membrane and co-localized with the S protein.

During *in vitro* infection of human primary hepatocytes with SARS-CoV-2 lineage SPBR-02, individual blockade of ACE2 or GRP78 normalized glucose production to control levels. This indicates that viral entry at least partly occurs through mechanisms dependent on ACE2- and/or GRP78. Importantly, the simultaneous blockade of ACE2 and GRP78 did not result in a synergistic blocking effect, suggesting that these receptors likely cooperate in the same pathway to internalize the virus. The administration of blockers against both, the ACE2 and GRP78 diminished the effects of individual blockades. This indicates that an alternative pathway mediates viral internalization.

Conclusion

This study showed that SARS-CoV-2 can infect, replicate, and produce infectious viral particles in primary human hepatocytes. The results also demonstrated that SARS-CoV-2 affects hepatic glucose production through gluconeogenesis.

The alterations in glucose metabolism, caused by SARS-CoV-2, have significant clinical implications.

This article was published in the Proceedings of the National Academy of Sciences (PNAS).

Journal Reference

Barreto EA. COVID-19-related hyperglycemia is associated with infection of hepatocytes and stimulation of gluconeogenesis. PNAS 2023 Vol. 120 No. 21 e2217119120 (Open Access). https://doi.org/10.1073/pnas.2217119120