Mitochondrial metabolic manipulation by SARS-CoV-2 in peripheral blood mononuclear cells of patients with COVID-19

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Abstract

The COVID-19 pandemic has been the primary global health issue since its outbreak in December 2019. Patients with metabolic syndrome suffer from severe complications and a higher mortality rate due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. We recently proposed that SARS-CoV-2 can hijack host mitochondrial function and manipulate metabolic pathways for their own advantage. The aim of the current study was to investigate functional mitochondrial changes in live peripheral blood mononuclear cells (PBMCs) from patients with COVID-19 and to decipher the pathways of substrate utilization in these cells and corresponding changes in the inflammatory pathways. We demonstrate mitochondrial dysfunction, metabolic alterations with an increase in glycolysis, and high levels of mitokine in PBMCs from patients with COVID-19. Interestingly, we found that levels of fibroblast growth factor 21 mitokine correlate with COVID-19 disease severity and mortality. These data suggest that patients with COVID-19 have a compromised mitochondrial function and an energy deficit that is compensated by a metabolic switch to glycolysis. This metabolic manipulation by SARS-CoV-2 triggers an enhanced inflammatory response that contributes to the severity of symptoms in COVID-19. Targeting mitochondrial metabolic pathway(s) can help define novel strategies for COVID-19.

COVID-19; glycolysis; mitochondrial dysfunction; mitokines; SARS-CoV-2

INTRODUCTION

COVID-19 has spread globally at an exponential rate, making it one of the worst pandemics in the current century. It is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 activation of the host’s own immune system sets off a high level of inflammatory cytokines (“cytokine storm”) that produce systemic inflammation and multiorgan failure. Epidemiological studies have identified diabetes, hypertension, and cardiovascular and cerebrovascular diseases as risk factors of mortality in COVID-19 (18). Emerging evidence has also suggested that diabetes can increase COVID-19 severity and mortality (19, 20).

Macrophages are important tissue-resident immune cells that protect against foreign pathogens such as bacteria and virus infection via phagocytosis. Mitochondria are a major contributor to phagocytic capacity when macrophages remove virus invasion. Immunoocytes, especially macrophages and neutrophils, can produce significant amounts of reactive oxygen species (ROS). In viral diseases, the role of ROS is more complex because it includes metabolic regulation for both host metabolism and viral replication (11). Generally, a certain level of ROS is important for regulating immunological responses and for clearing viruses, but excessive ROS will oxidize cellular proteins and membrane lipids and destroy not only virus-infected cells but also normal cells in lung, heart, and other organs, resulting in multiorgan failure.

Viral infections can affect human cellular metabolism and use it to their own advantage. Recently, Singh et al. (14) described that SARS-CoV-2 RNA and proteins localize to mitochondria and hijack the host cell’s mitochondrial function. Previously, SARS-CoV-1 was shown to manipulate host cell mitochondria and mitochondrial function (13). In this current study, we performed mitochondrial functional analysis in peripheral blood mononuclear cells (PBMCs) of patients with COVID-19, compared with PBMCs of patients with chest infections and healthy individuals.

In severe COVID-19, there is a disconnect as shown by large quantities of immune cells that are defective in their function (12). The association between cellular energy metabolism and immune function depends on the flexibility of...
cells to use different substrates to produce energy (10). The three primary substrates that drive the mitochondrial activities are long-chain fatty acids (LCFAs), glucose/pyruvate, and glutamine. Using inhibitors of specific substrate oxidation pathways, we investigated substrate utilization in PBMCs of patients with COVID-19 and healthy controls (HC).

Mitochondrial dysfunction and associated oxidative stress drive the production of proinflammatory cytokines that, in turn, play an important role in the immune response. Fibroblast growth factor 21 (FGF-21), also known as a mitokine, is a hormone that is expressed in several metabolically active organs and regulates many important metabolic pathways (15). FGF-21 was proposed as a biomarker of mitochondrial dysfunction, and many diseases are characterized with alterations of mitokine secretion (5). We measured fibroblast growth factor 21 (FGF-21) and interleukin-6 (IL-6) in plasma of the patients with COVID-19, patients with chest infection, and healthy controls (HC).

Our aim was to explore the manipulation of mitochondrial function by SARS-CoV-2 and the induction of metabolic adaptations with corresponding cytokine changes in patients with COVID-19.

### METHODS

#### Study design and participants.

Patients with RT-PCR-positive COVID, clinically suspected COVID, and chest infections were recruited to the immunometabolism in sepsis, inflammation, and liver failure syndromes (I-MET) cohort observational study within 48 h of admission to an intensive care unit or a specialist ward at King’s College Hospital (REC No. 19/NW/0750REC). Healthy volunteers were also recruited as pathological controls. Written informed consent was obtained from participants with capacity. For sedated or intubated critical care participants, consent from a professional consultee was obtained with capacity. Patients under 16 yr of age, with disseminated cancer, pregnancy, or who were pregnant were excluded.

Patients with chest infection were recruited on regain of capacity. Patients under 16 yr of age, with disseminated malignancy, undertaking immunosuppressive therapy (including high dose of steroid), or who were pregnant were excluded. The samples were collected from May to July 2020 under ethical approval from the regional Research Ethics Committee (REC No. 19/NW/0750REC). The patients were studied in three groups: group 1 included healthy controls (n = 9) who had no history of any chronic disease were recruited with written informed consent; group 2 included patients with PCR-positive COVID-19 or patients with strong clinical suspicion on radiological imaging (n = 7); and group 3 included patients with chest infection who were PCR-negative for COVID-19 (n = 7). Details of sampling, ethnicities, and management are given in Supplemental Tables S5 and S6 (all Supplemental material is available at [https://doi.org/10.6084/m9.figshare.13146557](https://doi.org/10.6084/m9.figshare.13146557)).

The 14 participants with PCR-positive COVID-19 and chest infection were aged 54–80 yr (mean = 66), and the female: male ratio was 5:9. The average body mass index (kg/m²) in the group was 29 ± 6.5 kg/m². Subjects were of mixed ethnic background (43% white British, 21% Caribbean, 21% black ethnic group, and 15% others). Out of the 14 subjects, seven (50%) had type 2 diabetes mellitus (T2DM) and five (36%) were diagnosed with hypertension. Of the patients who were admitted in the intensive therapy unit (ITU; n = 9), 67% were diagnosed with T2DM. Among the subjects with COVID-19, 71% had T2DM and 29% were diagnosed with hypertension. Glycated hemoglobin (HbA1c) was the only parameter that was significantly higher in patients with COVID-19 as compared with chest infection. The ethnicities of all patients were mixed. All the subjects with COVID-19 who died (n = 3, 43%) were diagnosed with T2DM and had an average National Early Warning Score (NEWS) 2 of 8.7. Table 1 shows the baseline characteristics of the subjects used in this study.

#### Sample preparation.

Blood samples were obtained via an arterial or peripheral line, where possible, or via venipuncture. Twenty-five milliliters of blood was collected in lithium heparin tubes. PBMCs were isolated from lithium heparin blood. Blood was diluted with an equal volume of phosphate-buffered saline (PBS), before gently being layered onto Ficoll density gradient medium. Tubes were centrifuged without break at 2,000 rpm for 20 min at room temperature. The PBMC layer was transferred to a sterile tube and washed twice to remove platelets by centrifugation with break at 2,000 rpm for 10 min at room temperature. Washed cells were resuspended in 300 μL of Seahorse medium (DMEM with glucose, glutamine, and pyruvate) and cells were counted, and viability was determined using the Luna Dual Fluorescent cell counting (Logos Biosystems) protocol.

PBMCs were plated on XFp eight-well polystyrene plates designed for the Seahorse XFp analyzer (Agilent Technologies, Santa Clara, CA) within 2 h.

#### Measurement of bioenergetics in PBMCs.

The Seahorse XFp analyzer (Agilent Technologies) was used to measure basal, ATP-linked, and maximal oxygen consumption rate (OCR); reserve capacity; and extracellular acidification rate (ECAR). Cellular bioenergetics was performed using XFp Cell Mito Stress Test Kit (Cat. No. 103010-100; Agilent Technologies), XFp Cell Energy Phenotype Test Kit (Cat. No. 103275-100; Agilent Technologies), XFp Mito Fuel Flex Test Kit (Cat. No. 103270-100; Agilent Technologies), and Substrate Oxidation Stress Tests (Cat. No. 103672-100 for Seahorse XF Long Chain Fatty Acid Oxidation Stress Test Kit, Cat. No. 103673-100 for Seahorse XF Glucose/Pyruvate Oxidation Stress Test, Cat. No. 103674-100 for Seahorse XF Glutamine Oxidation Stress Test Kit; Agilent Technologies) in a Seahorse XFp analyzer (Agilent Technologies). PBMCs were suspended in XF medium (Cat. No. 103680-100; Agilent Technologies), and 300,000 cells/well were seeded to Cell-Tak (Cat. No. 354240; Becton Dickinson Ltd.)-coated XFp plates (Agilent Technologies). All experiments were performed with three replicate wells in the Seahorse XFp analyzer.

OCR, a measurement of mitochondrial respiration, and ECAR, which correlates to the number of protons released from the cell with potential contribution from glycolysis and the Krebs cycle, were measured in the presence of specific mitochondrial activators and inhibitors. Oligomycin (ATP synthase blocker) was used to measure ATP turnover and to determine proton leak; the mitochondrial uncoupler carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) was used to measure maximum respiratory function.
MITOCHONDRIAL METABOLIC MANIPULATION BY SARS-CoV-2

RESULTS

Mitochondrial reprogramming in PBMCs of patients with COVID-19.

We used freshly purified PBMCs within 2 h of collection from 1) patients with COVID-19 (PCR-positive COVID-19 and strong clinical suspicion on radiological imaging; \( n = 7 \)), 2) patients with chest infection (PCR-negative for COVID-19; \( n = 7 \)), and 3) healthy individuals (HC; \( n = 7 \)). Basal respiration was less, although not statistically significant, but there was a trend for significance (\( P = 0.05 \)) in patients positive for COVID-19 compared with HC (Fig. 1A). ATP-linked respiration was also significantly reduced in patients positive for COVID-19 compared with HC (\( P = 0.02 \); Fig. 1B), and reserve capacity was significantly reduced in patients positive for COVID-19 compared with HC (\( P = 0.001 \)) and patients with chest infection (\( P = 0.004 \); Fig. 1D). Maximal respiration was also reduced in COVID-19-positive patients compared with HC (\( P = 0.002 \)) and patients with chest infection (\( P = 0.004 \); Fig. 1C). Proton leak (Fig. 1E) and nonmitochondrial respiration (Fig. 1F) were similar in the three groups.
(Supplemental Table S1). Significantly reduced ATP-linked respiration, reserve capacity, and maximal respiration are indicative of a compromised mitochondrial functional response to SARS-CoV-2 infection.

**Increased glycolysis in patients with COVID-19.** OCR as a measure of mitochondrial respiration and ECAR as a measure of glycolysis were determined under baseline and stressed conditions, to reveal the baseline and stressed phenotype of PBMCs in patients with COVID-19. There was a lack of significant difference in basal and stressed OCR among COVID-19-positive individuals, patients with chest infection, and HC (Fig. 2, A and B). Interestingly, the basal ECAR was significantly higher in patients with COVID-19 as compared with in HC ($P = 0.009$; Fig. 2C). Stressed ECAR was also higher as compared with HC ($P = 0.03$; Fig. 2D; Supplemental Table S2). We conclude that the high basal and stress ECAR suggest that the PBMCs of patients with COVID-19 depend on glycolysis for energy.

Glucose serves as the main substrate used by mitochondria in SARS-CoV-2. The Mito Fuel Flex Test was performed using the XFp analyzer to explore the metabolic pathways activated and mitochondrial fuel usage in live cells. The dependency, capacity, and flexibility of cells to oxidize three mitochondrial fuels, namely, glucose (pyruvate), glutamine (glutamate), and long-chain fatty acids (LCFAs), were determined. Fuel flexibility, which is a difference between fuel capacity and dependency, is the ability of cells to increase oxidation of a particular fuel to compensate for inhibition of alternative fuel pathway. Two assays were performed and both showed 100% flexibility for glucose utilization in COVID-19 samples. To reveal the critical substrate dependence/reliance, we performed substrate utilization tests ($n = 2$) for glucose, glutamine, and LCFAs individually.

Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl) ethyl sulfide (BPTES) was used for inhibition of glutamine through glutaminase 1, and there was no difference in basal

![Figure 1](image-url)
FGF-21 levels were significantly higher in patients with COVID-19 as compared with in HC (Fig. 4A). FGF-2 levels were also significantly higher in patients with COVID-19 who died as compared with in HC (P = 0.001; Fig. 4B). The patients admitted to the intensive therapy unit (ITU) with severe symptoms of COVID-19 had significantly higher values of FGF-21 as compared with in HC (P < 0.0001; Fig. 4C). There is also a correlation between FGF-21 levels and mitochondrial reserve capacity (P = 0.03; Fig. 4D; Supplemental Fig. S1). The NEWS score correlation with FGF-21 for patients with COVID-19 who died was r = 0.85. These studies suggest a correlation between increased FGF-21 mitokine and COVID-19.

**High human interleukin-6 in plasma of patients with COVID-19 by ELISA.**

Human IL-6 DuoSet ELISA (R&D systems, MN) was used to measure IL-6 levels in HC (n = 5), patients with COVID-19 (n = 7), and patients with chest infection (n = 7; Supplemental Table S4). IL-6 levels were significantly higher in patients with COVID-19 (168 ± 138, P = 0.002) and chest infections (112 ± 66, P = 0.002) as compared with in HC (22 ± 5; Fig. 5A). There was a trend of higher values of IL-6 in patients admitted to the ITU (165 ± 120) with severe symptoms of COVID-19 as compared with in wards (96 ± 76); however, this was not significant. IL-6 levels were also significantly higher in patients who died due to COVID-19 (147 ± 156) as compared with in HC (22 ± 5, P = 0.007; Fig. 5B). These results indicate that high levels of IL-6 are linked to COVID-19 mortality.

**DISCUSSION**

The current study was undertaken to determine the role of mitochondrial function and associated metabolic changes that may be involved in the inflammatory response during...
COVID-19 pathogenesis. We show mitochondrial dysfunction in live PBMCs of patients with COVID-19 and demonstrate an increased rate of glycolysis and utilization of glucose as the main substrate for energy production. There was a corresponding increase in FGF-21 mitokine and IL-6 in patients with severe symptoms and higher mortality rate in COVID-19 disease. FGF-21 levels showed significant correlation with mitochondrial functional reserve capacity. Our study indicates that SARS-CoV-2-infected cells have compromised mitochondrial respiration but can use glucose for energy and cell survival. Together our studies suggest that the mitochondrial manipulation by SARS-CoV-2 may provide an advantage for viral replication and increase cytokine production.

We recently described potential hijacking of host mitochondria by SARS-CoV-2 as a mechanism underlying COVID-19 pathogenesis (14). Mitochondria function as a platform for innate immune signaling. Notably, the host responses against viral infections depend on mitochondrial functions. Mitochondrial DNA itself acts as a danger-associated molecular pattern (DAMP) (1, 2).

Previously, it has been shown that SARS-CoV-1 virus upon infection affects mitochondrial functions, influences its intracellular survival, or evades host immunity. SARS-CoV-1 ORF-9b localizes to host mitochondria, which suppresses innate immunity by manipulating mitochondrial function and the mitochondrial antiviral-signaling protein (MAVS)/tumor necrosis factor receptor-associated factor (TRAF 3 and 6) signaling pathway to host innate immunity (13). Recently, Singh et al. (14) compared mitochondria-localized CoV-1 ORFs with ORFs encoded by the CoV-2 genome. Except for ORF3b, SARS-CoV-2 encodes an amino acid sequence similar to SARS-CoV-1 ORFs (ORF7a, 8a, and 9b) that are localized to host mitochondria.

Cells contain two important energy-producing pathways: mitochondrial respiration and glycolysis. Using the Seahorse XFp analyzer, we simultaneously measured both of these
pathways in live cells in a multiwell plate, interrogating key cellular functions. Our data show that peripheral cells from patients with PCR-positive patients exhibit reduced maximal respiration and reserve capacity, indicating compromised mitochondrial respiration or mitochondrial dysfunction, as compared with HC. These cells, however, have high basal and stress glycolysis, indicating the ability to use glucose for energy. We also performed the Mito Fuel Flex Test which showed that these cells have 100% fuel flexibility for glucose, which means that these cells can increase oxidation of glucose to compensate for inhibition of the alternative fuel pathway. To investigate this in detail, we performed

Figure. 4. Circulating levels of fibroblast growth factor 21 (FGF-21), a mitokine, in healthy controls (HCs), patients with COVID-19, and patients with chest infection (Chest inf), and its correlation with mitochondrial functional parameters. A: plasma concentrations of FGF-21 were measured by ELISA in HCs (n = 9), patients with PCR-positive COVID-19 (n = 6), and patients with chest infection (negative for COVID-19; n = 7). B: FGF-21 levels in plasma of HC (n = 9) vs. plasma of patients who died due to COVID-19 (n = 5). C: schematic representation of the increasing trend of FGF-21 levels with severity of disease in patients with COVID-19. D: correlation matrix of FGF-21 and mitochondrial functional parameters (reserve capacity, maximal respiration, and ATP-linked respiration). Data are represented as means ± SD, ***P < 0.001.

Figure. 5. Circulating levels of interleukin-6 (IL-6) in healthy controls (HCs), patients with COVID-19, and patients with chest infection. A: plasma concentrations of IL-6 were measured by ELISA in HCs (n = 5), patients with PCR-positive COVID-19 (n = 6), and patients with chest infection (negative for COVID-19; n = 7). B: IL-6 levels in plasma of HC vs. plasma of patients who died due to COVID-19 (n = 5). Data are represented as means ± SD, **P < 0.01.
substrate utilization tests for glutamine, long-chain fatty acids, and glucose in PBMCs of patients with COVID-19. There was no difference in basal and maximal respiration on blocking glutamine and long-chain fatty acid pathways; however, maximal respiration was reduced when the glucose pathway was blocked in cells from patients with COVID-19 as compared with from HC. This indicates dependence of SARS-CoV-2-infected cells on glucose for energy production and survival.

In our clinical cohort of subjects with COVID-19, 70% had T2DM, and of those admitted in the ITU with more severe symptoms, 80% had T2DM. All the patients with COVID-19 who died were diagnosed with diabetes. This indicates that high glucose level in T2DM favors SARS-CoV-2 replication and is associated with worse prognosis in these patients. Recently, a study of influenza A virus (IAV) infection shows activation of similar metabolic changes resulting in a cytokine storm. Authors also show that higher levels of proinflammatory cytokines correlate with higher levels of blood glucose in patients infected with IAV (16). Studies have highlighted the high mortality rate in patients with diabetes due to COVID-19 (8). Consistent with our study in patients with COVID-19, Codo et al. (4) determined that glycolysis is necessary for SARS-CoV-2 replication and monocyte response.

It is known that mitochondrial stress induces the production of stress response molecules known as mitokines (5). Many diseases are characterized by progressive mitochondrial dysfunction with alterations of mitokine secretion. It is still controversial whether altered levels of mitokines are beneficial or detrimental in humans. The function of FGF-21 mitokine is complicated due to its different sites of production and actions. Studies have previously shown high serum levels of FGF-21 in patients with metabolic disorders due to mitochondrial dysfunction (3, 6, 7, 17). Circulating levels of FGF-21 are also elevated in type 2 diabetes (T2DM) (9).

We also measured FGF-21, a mitokine, in patients with COVID-19 and HC. Mitochondrial dysfunction, oxidative stress, and inflammation can lead to a compensatory increase in FGF-21 synthesis and secretion. Our study demonstrates an increase in FGF-21 levels in patients with COVID-19 as compared with in HC. There was a trend of high levels of FGF-21 and an increase in COVID-19 severity among patients who died due to COVID-19. FGF-21 levels were the highest among the patients who died. The proinflammatory cytokines IL-6 was also significantly increased in patients with COVID-19 as compared with in HC (P = 0.002). These findings strengthen the observation that mitochondrial dysfunction drives a systemic immune response in COVID-19 pathogenesis.

In summary, our study identifies the important metabolic alteration in live cells from patients with COVID-19. It paves the way to investigate whether restoring mitochondrial function and/or targeting glycolysis with new or existing drugs can potentially be used for treatment for patients with COVID-19.

ACKNOWLEDGMENTS

Special thanks to the patients and the volunteers without whose samples this work could not have been done. We are indebted to the clinical staff at King’s College Hospital who worked tirelessly during the COVID-19 pandemic looking after the patients and helping us in accessing the samples. Thanks to Nick Howe and Sofia Vikstrom at Agilent Technologies for technical support and guidance related to the Seahorse XFp analyzer. The Science and Technology Facilities Council supports I-MET, and the Biomedical Research Centre supports M. J. McPhail.

GRANTS

The study was supported by the Liver Research Fund, King’s College Hospital, London, and the Science and Technology Facilities Council (STFC) supports the I-MET study (immuno-metabolism in sepsis, inflammation, and liver failure syndromes). K. K. Singh is supported by National Cancer Institute Grant R01 CA204430.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.A. conceived and designed research; S.A., S.M., F.M.T., and S.N. performed experiments; S.A. and F.M.T. analyzed data; S.A. and M.J.M. interpreted results of experiments; S.A. prepared figures; S.A. drafted manuscript; S.A., M.J.M., K.K.S., S.M., F.M.T., S.N., and K.A. approved final version of manuscript.

REFERENCES

1. Arnoult D, Soares F, Tatello I, Girardin SE. Mitochondria in innate immunity. EMBO Rep 12: 901–910, 2011. doi:10.1038/embor.2011.157.
2. Blacchessi S, LeBerre M, Lamoureux A, Louise Y, Laurent E, Boudinot P, Bremont M. Mitochondrial antiviral signaling protein plays a major role in induction of the fish innate immune response against RNA and DNA viruses. J Virol 83: 7815–7827, 2009. doi:10.1128/JVI.00404-09.
3. Chen WW, Li L, Yang GY, Li K, Qi XY, Zhu W, Tang Y, Liu H,oden G. Circulating FGF-21 levels in normal subjects and in newly diagnose patients with Type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 116: 65–68, 2008. doi:10.1055/s-2007-985148.
4. Codo AC, Davanzo GG, Monteiro LB, de Souza GF, Muraro SP, Virgilio-da-Silva JV, et al. Elevated glucose levels favor SARS-CoV-2 infection and monocyte response through a HIF-1a/glycolysis-dependent axis. Cell Metab 32: 437–446.e5, 2020. [Erratum in Cell Metab 32: 498–499, 2020]. doi:10.1016/j.cmet.2020.07.007.
5. Conte M, Ostan R, Fabbi C, Santoro A, Guidarelli G, Vitale G, Mari D, Sevini F, Capri M, Sandri M, Monti D, Franceschi C, Salvioni S. Human aging and longevity are characterized by high levels of mitokines. J Gerontol A Biol Sci Med Sci 74: 600–607, 2019. doi:10.1093/gerona/gly153.
6. Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski VJ, Li DS, Mehrbod F, Jaskunas SR, Shanafelt AB. FGF-21 as a novel metabolic regulator. J Clin Invest 115: 1627–1635, 2005. doi:10.1172/JCI23606.
7. Lakhanl I, Gong M, Wong WT, Bazoukis G, Lampropolous K, Wong SH, Wu WKK, Wong MCS, Ong KL, Liu T, Tse G; International Health Informatics Study (IHS) Network. Fibroblast growth factor 21 in cardio-metabolic disorders: a systematic review and meta-analysis. Metabolism 83: 11–17, 2018. doi:10.1016/j.metabol.2018.01.017.
8. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, Shi J, Zhou M, Wu B, Yang Z, Zhang C, Yue J, Zhang Z, Renz H, Liu X, Xie J, Xie M, Zhao J. Risk factors for severity and mortality in adult COVID-19 inpatients in
9. Liu JJ, Foo JP, Liu S, Lim SC. The role of fibroblast growth factor 21 in diabetes and its complications: A review from clinical perspective. *Diabetes Res Clin Pract* 108: 382–389, 2015. doi:10.1016/j.diabres.2015.02.032.

10. Raud B, McGuire PJ, Jones RG, Sparwasser T, Berod L. Fatty acid metabolism in CD8+ T cell memory: Challenging current concepts. *Immunol Rev* 283: 213–231, 2018. doi:10.1111/imr.12655.

11. Reshi ML, Su YC, Hong JR. RNA viruses: ROS-mediated cell death. *Int J Cell Biol* 2014: 467452, 2014. doi:10.1155/2014/467452.

12. Schulte-Schrepping J, Reusch N, Paclik D, Baßler K, Schlickeiser S, Zhang B, et al. Severe COVID-19 is marked by a dysregulated myeloid cell compartment. *Cell* 182: 1419–1440.e23, 2020. doi:10.1016/j.cell.2020.08.001.

13. Shi CS, Qi HY, Boularan C, Huang NN, Abu-Asab M, Shelhamer JH, Kehrl JH. SARS-coronavirus open reading frame-9b suppresses innate immunity by targeting mitochondria and the MAVS/TRAF3/TRAF6 signalosome. *J Immunol* 193: 3080–3089, 2014. doi:10.4049/jimmunol.1303196.

14. Singh KK, Chaubey G, Chen JY, Suravajhala P. Decoding SARS-CoV-2 hijacking of host mitochondria in COVID-19 pathogenesis. *Am J Physiol Cell Physiol* 319: C258–C267, 2020. doi:10.1152/ajpcell.00224.2020.

15. Tezze C, Romanello V, Sandri M. FGF21 as modulator of metabolism in health and disease. *Front Physiol* 10: 419, 2019. doi:10.3389/fphys.2019.00419.

16. Wang Q, Fang P, He R, Li M, Yu H, Zhou L, Yi Y, Wang F, Rong Y, Zhang Y, Chen A, Peng N, Lin Y, Lu M, Zhu Y, Peng G, Rao L, Liu S. O-GlcNAc transferase promotes influenza A virus-induced cytokine storm by targeting interferon regulatory factor-5. *Sci Adv* 6: eaaz7086, 2020. doi:10.1126/sciadv.aaz7086.

17. Woo YC, Xu A, Wang Y, Lam KS. Fibroblast growth factor 21 as an emerging metabolic regulator: clinical perspectives. *Clin Endocrinol (Oxf)* 78: 489–496, 2013. doi:10.1111/cen.12095.

18. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 8: 475–481, 2020. doi:10.1016/S2213-2600(20)30079-5.

19. Yan Y, Yang Y, Wang F, Ren H, Zhang S, Shi X, Yu X, Dong K. Clinical characteristics and outcomes of patients with severe covid-19 with diabetes. *BMJ Open Diabetes Res Care* 8: e001343, 2020. doi:10.1136/bmjdrca-2020-001343.

20. Zhu L, She ZG, Cheng X, Qin JJ, Zhang XJ, Cai J, et al. Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes. *Cell Metab* 31: 1068–1077.e3, 2020. doi:10.1016/j.cmet.2020.04.021.