Translational Implications of SARS-CoV-2 Mediated Loss of ACE2 in a Diabetic Obese Setting: Implications for Long COVID

Mahmoud Gheblawi[1], Mobin Khoramjoo[1], Kaiming Wang[2], Faqi Wang[2], Lakshmi Puttagunta[3], Ryan Noyce[4], Pavel Zhabyeyev[2], Ratnadeep Basu[2], Colombo Daniele[5], Franca Del Nonno[5], Monica De Gaspari[6], Cristina Basso[6], Brian Chiu[3], Ram Prasad[7], David H. Evans[4], Maria B. Grant[7], Gavin Y. Oudit [1,2]
1(1) Department of Physiology, Cardiovascular Research Centre; (2) Division of Cardiology, Department of Medicine, Mazankowski Alberta Heart Institute; (3) Department of Laboratory Medicine and Pathology; (4) Department of Medical Microbiology and Immunology, Li Ka Shing Institute of Virology; Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada.
(5) Pathology Unit, National Institute for Infectious Diseases "Lazzaro Spallanzani", IRCCS, Rome, Italy. (6) Cardiovascular Pathology Unit, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padua, Padua, Italy. (7) Department of Ophthalmology and Visual Sciences, University of Alabama at Birmingham, Birmingham, AL, USA.

1. Background: ACE2 Shedding in a Clinical Setting

- COVID-19 disproportionately affects older, male obese patients leading to a high prevalence of adverse outcomes.[1]
- SARS-CoV-2 mediated loss of angiotensin converting enzyme 2 (ACE2) may further increase the susceptibility of these patients to adverse outcomes.[2]
- Loss of the ACE2 receptor-enzyme may be a causative factor in cardiovascular and multi-organ injury independent of primary viral-mediated injury in convalescent and recovered Long COVID patients.[3]

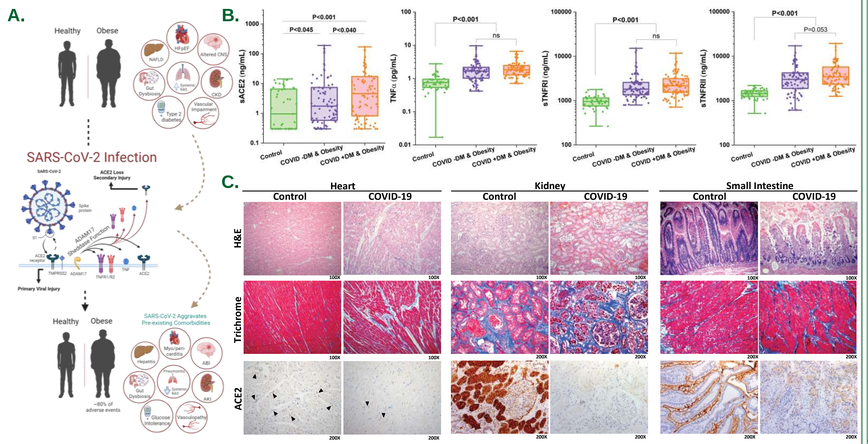


Fig 1. A) COVID-19 infection has been shown to aggravate preexisting conditions in patients and more drastically in those with diabetes & obesity leading to new onset of multi-organ injury. *B)* COVID-19 patient plasma has increased soluble ACE2 and *'a disintegrin and metalloproteinase 17'* (ADAM17) markers; tumor necrosis factor α (TNFα), TNF receptor I & II (TNFRI, TNFRII). Increase in ADAM17 markers are surrogate for its increased sheddase activity which also targets ACE2. *C)* COVID-19 patient end organ autopsy samples show decreased ACE2 +ve staining & elevated tissue fibrosis compared to age and sex matched controls.

2. Study Objectives

- Examine SARS-CoV-2 infection for loss of ACE2 in a biomedical model of COVID-19 injury.
- Determine whether loss of ACE2, independent of viral infection, can lead to cardiovascular and multi-organ injury in aged diabetic, obese animals.

3. Methods

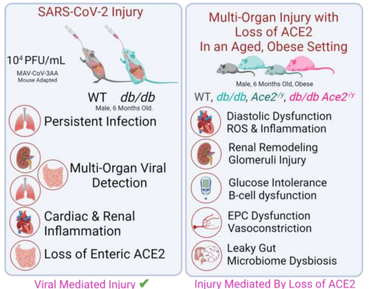


Fig 2. Overview of animal model findings.

- COVID-19 patient plasma was assessed for markers of ACE2 loss and corroborated with immunohistological staining of patient autopsy samples.
- Male, 6-month-old, *db/db* and WT control mice on the B6 background were examined for susceptibility and extent of multi-organ injury to a murine-adapted SARS-CoV-2 strain (MAV-CoV-3AA, at 10^4 PFU/mL inoculation) and. Mice were euthanized at day 2 & 7 post-inoculation and organs collected for examination.
- Male, 6-month-old, diabetic, obese *db/db Ace2-/y* (double mutant, DMT) mice and respective WT, *Ace2-/y*, and *db/db* controls were assessed for injury across the gut-heart axis.
- Cardiovascular parameters were evaluated by echocardiography, pressure-volume loops, and histology.
- Alterations in gut permeability were determined by measuring plasma peptidoglycan (PGN) levels and assessing microvilli structure.
- Fecal metatranscriptomics determined functional alterations of the gut microbiota.
- Fecal & plasma metabolomic profiles were assessed using LC/MS-MS.

4. Multi-Organ Detection of SARS-CoV-2 Infection

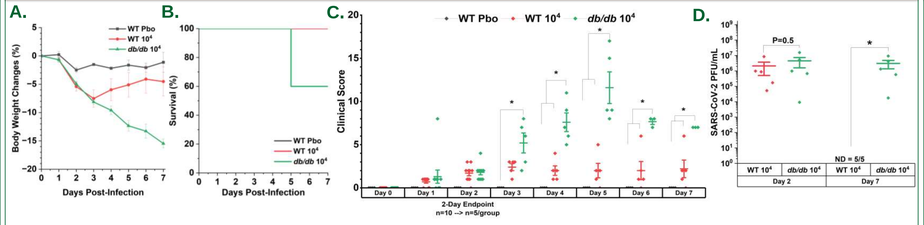


Fig 3. A-B) Intranasal inoculation with MAV-CoV-3AA @ 10^4 PFU/mL lead to transient bodyweight loss in wildtype (WT) mice and persistent weight loss and mortality in diabetic, obese *db/db* mice. *C) db/db* mice had increased clinical scores indicative of worsened outcomes. *D)* Live infectious virus was detected at Day 2 post-infection in both WT and *db/db* mice and only persisted in *db/db* mice by Day 7.

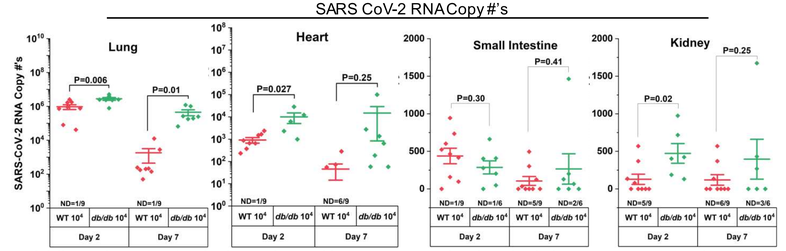
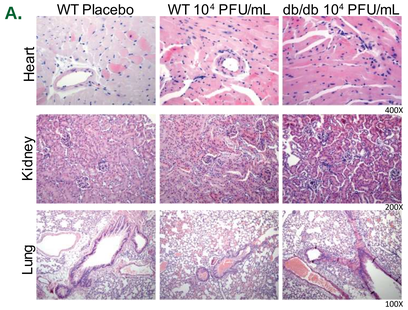


Fig 4. Intranasal delivery of MAV-CoV-3AA lead to nucleocapsid N gene copy number detection in four organs at Day 2 and Day 7 post-infection. Viral RNA was higher and incessant in *db/db* lungs and hearts compared to WT mice at both time points.

5. Infection Leads to Multi Organ Injury



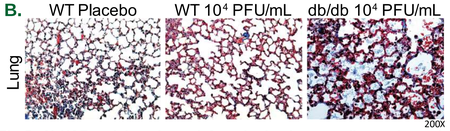
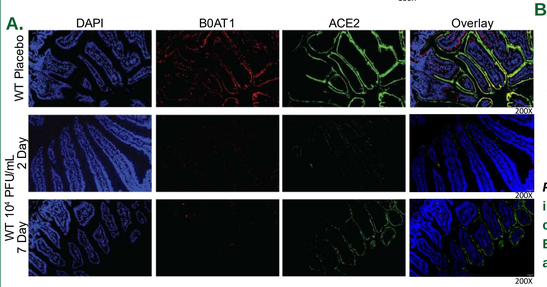


Fig 5. A) H&E staining shows infected mice have cardiac perivascular inflammation, and kidney intratubular inflammation. Infected mice lungs show interalveolar hemorrhage, bronchial epithelial cell death and profound bronchial and vascular inflammation. *B)* Diabetic, obese mouse lungs show increased edema and proteinous debris.



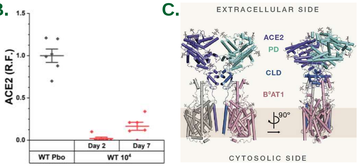
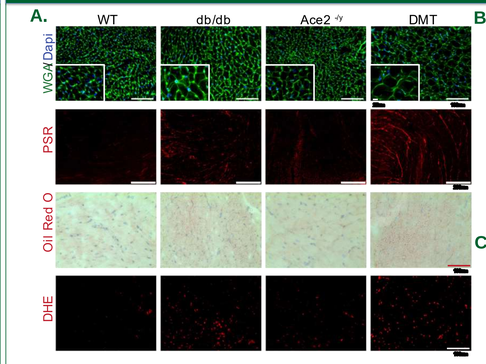


Fig 6. A-B) ACE2 is markedly decreased in the small intestine of infected mice at Day 2 and 7, with corresponding loss of the neutral amino acid transporter B0AT1 (SLC6A19). *C)* ACE2 acts as a chaperon[4] for B0AT1 at the apical surface of enterocytes in the small intestine.

6. Loss of ACE2 Exacerbates Cardiovascular Disease



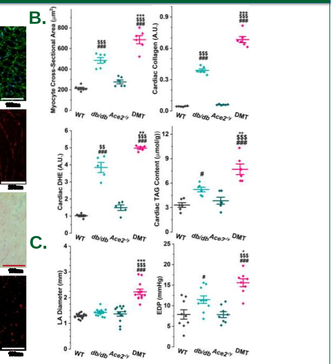
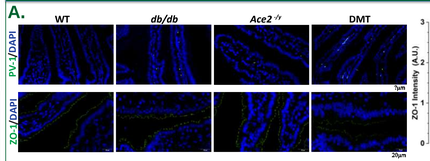


Fig 7. A-B) Diabetic, obese mice with genetic loss of ACE2 (DMT, *db/db Ace2-/y*) have cardiac hypertrophy with increased myocyte cross sectional area & collagen deposition as well as increased cardiac dyslipidemia. Loss of ACE2 in *db/db* hearts leads to increased reactive oxygen species. *C)* Transthoracic echocardiography revealed expanded left atrium diameters and elevated left ventricle end diastolic pressures determined by invasive pressure-volume loops.

7. Gut Dysbiosis & Altered Metabolomics



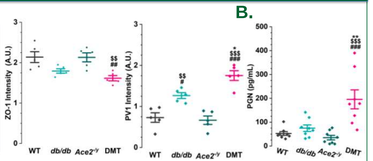


Fig 8. A-B) db/db Ace2-/y (DMT) small intestines had increased transendothelial pores (PV1 +ve) and trending decrease in zonula occluden-1 (ZO-1) indicating a leaky gut-blood barrier, which was confirmed by increased levels of plasma peptidoglycan (PGN). *C)* PLS-DA analysis of fecal microbiota metatranscriptomics showed distinct clustering across groups, and LEfSe analysis showed altered functional gene expression across groups with enrichment of amino acid metabolism pathways in DMT mice compared to *db/db*.

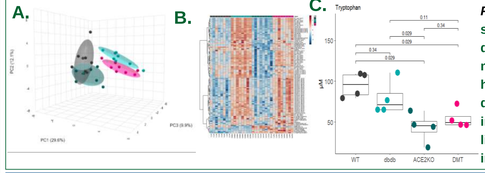


Fig 9. A) Plasma metabolomic PCA analysis shows distinct clustering of groups with a distinct ACE2 knockout phenotype. *B)* 226 metabolites were significantly altered in post-hoc analysis of 568 discrete metabolites detected. *C)* Of these, 28 are of neurological importance, such as tryptophan, which may link some of the neurocognitive disorders seen in Long COVID to loss of ACE2.

8. Conclusions & Acknowledgments

SARS-CoV-2 leads to multi-organ injury within a murine model of infection. The infection's injury and severity are worsened in diabetic, obese animals. Loss of ACE2, independent of viral-mediated injury, leads to multi-organ injury, with cardiovascular dysfunction and a leaky gut-blood barrier, leading to altered metabolomic profiles and circulating microbial toxins.

Acknowledgments

• Patients & Families • Canadian Biosample Repository • CL3 Facility Staff

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