High Fat and Sugar Diet and SIV Co-Infection Enhance Nasal SARS-CoV-2 Shedding in Infected Macaques

338

Kelsie Brooks[1*], Arvind Sivanandham[2*], Quentin Le Hingrat[2], Anna J. Jasinska[2], Delmy Ruiz[1], Lilly Carson[2], Lilas Tarnus[2], Mohammed H. Daira[2] , Makheni Jean Pierre[1], Cristian Apetrei[2,3], Jason M. Brenchley[1#], Ivona Pandrea[3,4#]

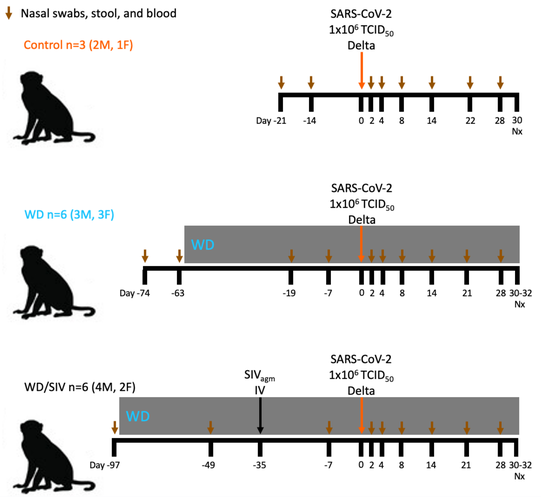
[1]Barrier Immunity Section, Laboratory of Viral Diseases, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, U.S.; [2]Division of Infectious Diseases, Department of Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, PA, U.S.; [3]Department of Infectious Diseases and Immunology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, U.S.; [4]Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, U.S. *,# Authors contributed equally

BACKGROUND

The likelihood of severe COVID-19 is increased with chronic conditions such as diabetes and obesity, which are linked to disruptions in infection response and associate with diets high in fat and sugar. The impact of diet has not been thoroughly explored in SARS-CoV-2 infection, while co-infection with SIV may disrupt the immune response to an acute viral infection. Here we investigate the impact of diet and SIV on SARS-CoV-2 infection.

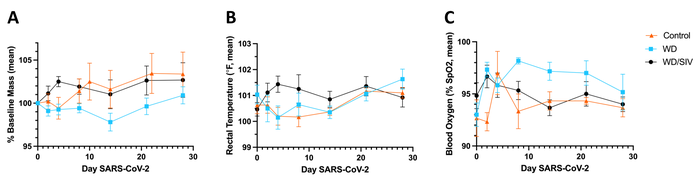
METHODS

- Three control pigtail macaques (PTM) were maintained on standard monkey chow prior to and during SARS-CoV-2 infection.
- Six PTM were fed standard monkey chow, then a high fat and sugar chow (Western diet, WD) prior to SARS-CoV-2 infection & remained on the WD throughout infection.
- Six additional PTM were fed the WD prior to SIV infection and subsequent SARS-CoV-2 infection. All animals were monitored throughout infection until euthanasia and necropsy (Nx) and sampled as below.



RESULTS

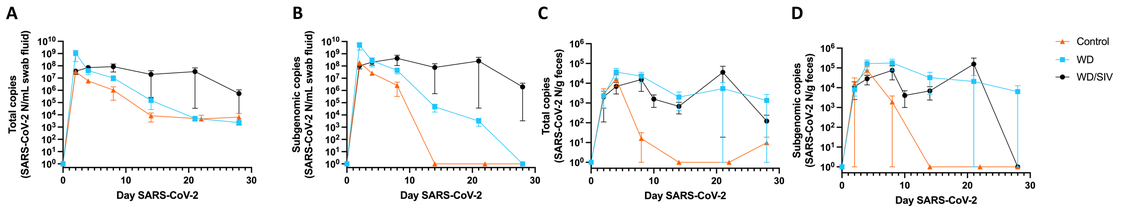
Generally Mild SARS-CoV-2 Infection Regardless of Diet, SIV



SARS-CoV-2 infection was generally mildly symptomatic regardless of diet or SIV co-infection, though one WD/SIV animal was euthanized early (Day 21 SARS-CoV-2) due to pronounced lethargy, anemia, and diarrhea. WD animals also lost more weight than controls. Mass relative to Day 0 SARS-CoV-2 is shown in **A**, and no animal experienced fever (rectal temperature >103°F, **B**) or demonstrated low blood oxygen levels (<87% SpO2, **C**). Error bars SEM.

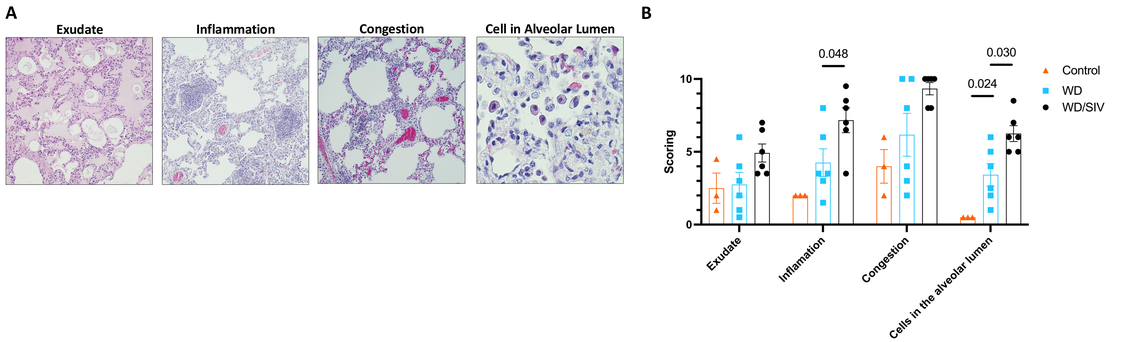
RESULTS

SARS-CoV-2 Shedding Increased in Respiratory, GI Tracts with WD and in Nasal Swabs with SIV



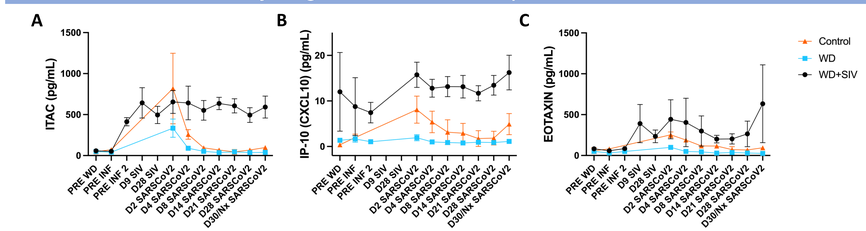
High fat and sugar diet (WD) increased shedding of SARS-CoV-2 viral RNA (vRNA) from the respiratory tract (nasal swabs, **A** and **B**) and GI tract (stool, **C** and **D**). Both total vRNA (**A** and **C**) and subgenomic vRNA indicating viral replication (sgRNA, **B** and **D**) were increased, with WD fed animals shedding higher quantities of all vRNA at peak and shedding sgRNA for longer duration than control animals. WD/SIV co-infected animals shed SARS-CoV-2 vRNA at lower peak levels than WD animals, but shedding of both total and sgRNA was prolonged in nasal swabs. Undetected samples are graphed as 10^0 and calculated as 0 in means. Viral loads mean for each group with SEM shown.

WD and SIV Enhance Lung Pathology



Lung tissue from approximately one month post-SARS-CoV-2 infection was fixed and embedded for H&E staining and evaluated for pathology (**A**). Lung lesions were generally mild and suggestive of interstitial pneumonia. Clinical scoring of lung lesions was performed (**B**), with an example of each feature evaluated shown in **A**. Significantly more cells infiltrated the alveolar lumen in WD fed animals than controls, while SIV co-infection also significantly enhanced cell infiltration and inflammation over WD fed animals. Means with SEM in **B**.

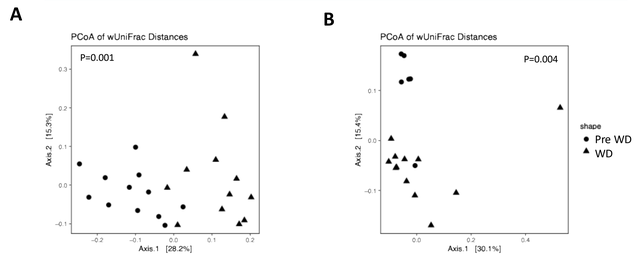
WD and SIV Dysregulate Immune Response to SARS-CoV-2 Infection



Luminex of plasma cytokines/chemokines was performed longitudinally for each group of animals. Interferon–inducible T Cell Alpha Chemoattractant (I-TAC, **A**) that is induced by IFNβ and IFNγ was elevated with SIV infection and with SARS-CoV-2, but most notably declined following Day 2 of SARS-CoV-2 in control and WD fed animals while remaining elevated in the WD/SIV group. Control animals also had higher ITAC levels at Day 2 of SARS-CoV-2 than WD animals. Interferon–gamma induced protein-10 (IP-10, **B**), a chemoattractant of myeloid cells as well as leukocytes, was also elevated with SARS-CoV-2 infection in control and WD/SIV groups, but not WD animals, suggesting a blunted response to infection. This was further evidenced with eotaxin (**C**), which was minimally induced with SARS-CoV-2 in WD animals but more clearly so in controls and WD/SIV animals. Means for each group with SEM shown.

RESULTS

Bacterial Dysbiosis with WD



Bacterial taxa from stool were identified by 16S sequencing. Beta diversity of stool bacterial communities was assessed with weighted UniFrac distances, and significantly different pre- vs. post-WD (Adonis testing, PCoA of weighted UniFrac distances) prior to infection in both the WD and WD/SIV groups. **A** is the WD group and **B** the WD/SIV animals. (all samples are pre-SIV infection)

CONCLUSIONS

A high fat and sugar diet (WD) in macaques enhanced SARS-CoV-2 shedding, suggesting worsened viral control despite mildly symptomatic infection. WD additionally enhanced lung pathology, while SIV co-infection prolonged SARS-CoV-2 shedding in nasal swabs and also enhanced lung pathology. Disruptions to the microbiome and circulating chemokine response to SARS-CoV-2 also occurred with WD, indicating the diet impacts gut microbiota and may alter immunity, predisposing to enhanced disease. SIV co-infection further altered SARS-CoV-2 infection response, with worsened viral control and greater inflammation.

FUTURE DIRECTIONS

- Explore impact of diet, SIV infection, and SARS-CoV-2 infection on:
  - short chain fatty acids in GI tract
  - biomarkers of intestinal integrity
  - microbial translocation across gut

ACKNOWLEDGEMENTS

We would like to thank the animal care teams at BIOQUAL, Inc. for their management of study animals. Funding is provided in part by the National Institute of Allergy and Infectious Diseases Division of Intramural Research; the views and conclusions expressed here do not represent NIAID, NIH, or HHS.

NIH National Institute of Allergy and Infectious Diseases

University of Pittsburgh