Keystone Symposium on Long COVID, August 2023
Summary - Main Takeaway Points
Andrew Schamess

State of Research

- The work in Long COVID is in the ***early stages*** – many of the studies presented were exploratory.
- When Long COVID was first recognized, labs and scientists began by using techniques developed in other diseases to study it.
- Many participants commented that this has produced some unexpected ***cross-disciplinary collaborations*** – for example, immunologists reaching out to neuroscientists and exercise physiologists.
- There are several ***large cohorts*** of Long COVID patients for whom data is being collected for clinical status, biochemical, immunologic, and genetic profile, and other measures such as cardiopulmonary exercise testing, nerve biopsies, etc. This is a promising approach to studying Long COVID.
- The largest cohort is through the NIH-funded ***RECOVER initiative***. Other cohorts include the Long-Term Impact of Infection with COVID19 (LIINC) cohort at UCSF and the Yale COVID Recovery Study.
- The RECOVER Initiative has a large database of clinical and laboratory information which it plans to make available to researchers outside the RECOVER team in the near future.
- Several speakers made a strong case for involving Long COVID patients in research design.
    - Lisa McCorkell is the Co-Founder of the ***Patient Led Research Collaborative***, which has established priorities for future research and funded numerous groundbreaking studies.
    - Harlan Krumholtz, a Cardiology professor who directs the Center for Outcomes Research and Evaluation at Yale, talked about how his Long COVID studies have been improved in numerous ways by involving patients in research decision-making.
- There are no disease-specific therapies on the horizon, and there is still an ***urgent need to test existing rehabilitative techniques and repurposed medications*** for efficacy against Long COVID symptoms.

Emerging models of Long COVID pathophysiology

- ***Microclots***
    - Damage to the ***vascular endothelium*** (the cells that line the inside of blood vessels and help blood flow smoothly) probably plays an important role in Long COVID. Damage to the endothelium leads to generation of "microclots" that may interfere with blood flow, and embed in various body organs causing damage. One researcher (Resia Pretorius) saw improvement in her Long COVID patients after ***treatment with anticoagulants*** – but this was a non-blinded uncontrolled study. A better designed study is needed before anticoagulants can be prescribed routinely.
- ***Epstein Barr virus***
    - Epstein Barr Virus (EBV) may play an important role in causing the persistent symptoms of Long COVID. SARS-Co-V-2 infection is known to cause re-activation of latent EBV. This stimulates production of ***auto-antibodies to nerves and other "self" tissues***. In multiple sclerosis patients, and in the small number of Long COVID patients studied, presence of these autoantibodies correlates with fatigue and neurologic symptoms

    - EBV reactivation is usually difficult to detect (and thus hard to study) – but one lab has come up with a way to measure ***microRNA*** that seems reliable. This could be a useful biomarker.
    - If confirmed, EBV could be a therapeutic target.
- ***Autonomic nervous system dysfunction***
    - Several studies using cardiopulmonary exercise testing presented supported ***autonomic nervous system involvement as the cause of reduced exercise tolerance***, shortness of breath and physical fatigue. Specifically, there is a reduction in venous tone that leads to reduced blood coming into the heart and thus reduced cardiac output. There is also "chronotropic incompetence" – i.e the heart does not speed up enough to compensate for reduced stroke volume.
    - Exercise tolerance improved in patients after a does of ***pyridostigmine***, which enhances function of the parasympathetic nervous system. Potential therapy?
    - These findings could be due to small fiber neuropathy affecting the autonomic nerves, or problems with control of the autonomic nervous system at the level of the brainstem (many studies have shown that part of the brain affected in Long COVID).
- ***Mitochondrial dysfunction***
    - One small but very interesting study supported the hypothesis that mitochondrial dysfunction in the muscles leads to reduced oxygen extraction, anaerobic respiration and buildup of lactic acid, as well as reduced muscle performance and easy fatiguability.
- ***Lipid toxicity***
    - Acute infection with SARS-CoV-2 causes high metabolic demands, which leads to rapid breakdown of fat to produce sugar the body can use for energy. The process of generating these fats produces free radicals that can damage mitochondria and other cell components. It also generates ***toxic lipid products*** that build up in arteries and other tissues. After acute infection / inflammation, there are anti-inflammatory factors that help get rid of the reactive lipids and clean up the damage. An enzyme called PON-1 that circulates attached to HDL is important in this process. Some patients have ***genetic variants in the PON-1 enzyme***. A study of Long COVID patients showed that this enzyme does not "come back" after infection, and this corresponds to the presence of defined genetic variants (i.e. a particular genetic defect).
- Sex bias – see notes on Akiko Iwasaki's talk , below. Briefly –relative levels of testosterone in both men and women seem to correspond to different clinical and immunological phenotypes, with higher levels associated with less severe disease.
- Persistence of COVID19 antigens in the gut – please see my more detailed note on Michael Peluso's presentations, below.

Notes on each presentation

Gary Gibbons, MD

- Director, National Heart, Lung, and Blood Institute
- Senior Oversight Committee Co-Chair for RECOVER initiative (the major Federally funded Long COVID study)

Strengths of the RECOVER Initiative include large size and scope, trial sites throughout the U.S., diversity of study population, ability to correlate subjects' clinical status with a wide range of biochemical, immunological, genetic and radiographic tests, and the generation of an enormous database of this combined information that will soon be available to outside researchers.

Next round of RFAs should allow outside investigators to analyze of RECOVER data sets. Date of RFA not specified.

Note: we could prepare for this RFA by hiring a researcher who has skills in large database exploration and analysis; and thinking of what research questions we would like to pose.

Michael Holtzman, MD

- Professor, Department of Medicine, Washington University
- President, NuPeak Therapeutic

Chronic disease following a respiratory infection - Post-Viral Lung Disease (PVLD) - was well known before COVID19. His lab has been studying it for 20 years. It is caused by hyperplasia (overgrowth) and remodeling of basal cells in the epithelium, generating excess mucus-producing cells and eventually lung fibrosis. This process of dysfunctional basal cell response is mediated by a cellular molecule called MAPK-13, which is a kinase (a type of enzyme). Dr. Holtzman's lab has developed a new drug, NuP4A, a small molecule kinase inhibitor, which blocks MAPK13 expression and activation and mucus production and corrects PVLD in mice. Human trials will start soon.

Note: preliminary data show that the same basal cell hypertrophy process takes place in muscle cells after SARS-CoV-2 infection, and is also blocked by NuP4A.

Andrea Lynn Cox, Johns Hopkins University

In this cohort of subjects enrolled within 48 hours of positive test for SARS-CoV-2, 33% of had persistent symptoms at 3 months. Most of them showed peak symptoms at one year, followed by decline; but some had persistent symptoms. Different symptom clusters were associated with expansions in different immune cell populations. Anxiety, muscle ache, brain fog, memory problems: associated with increased immune-suppressive neutrophils (PMN-MSDC cells) expressing LOX-1, which plays a role in endothelial dysfunction. Anxiety, headaches and weakness were associated with increased Memory B cells positive for CD21 and negative for CD27.

Akiko Iwasaki – Yale University

In a prospective study of patients after COVID 19, looking at multiple parameters, the important findings were lower cortisol in all Long COVID subjects compared with controls (though not below the standard

lab threshold for normal). There were important sex differences, with women showing more T-cell abnormalities (reduced CD4 memory T-cells, more "exhausted" CD4 and CD8 T-cells) and men showing increased NK (natural killer) cells and higher TGF-alpha. These differences correlated with testosterone levels. Testosterone may be determinative of distinct immune profiles leading to a different clinical phenotypes. There was no difference in autoantibody levels between subjects recovered from SARS-CoV-2 infection without sequelae, and those with Long COVID.

Michael Peluso University of California San Francisco

Is there a hidden reservoir of SARS-CoV-2 virus that persists after acute infection and drives ongoing immune response?

Study 1: compared Long COVID patients to pre-pandemic controls. Multiple blood samples collected over time from each subject. About 25% of Long COVID patients had one SARS-CoV-2 antigen detected in at least one blood sample - mostly spike and nucleocapsid.

Study 2: Colon biopsies obtained by flexible sigmoidoscopy on 12 patients with Long COVID. In the 5 analyzed so far, all were positive for SARS-CoV-2 antigens.

Takeaway: In a small number of biopsy samples, persistent SARS-CoV-2 antigen is seen on stains of gut tissues. Tissue sampling may be more reliable than blood testing to identify antigen persistence.

Interesting question from audience member: is SARS-CoV-2 a bacteriophage (i.e. a virus that infects bacteria) – and would that account for its presence in the gut

Michael Patton – Hugh Kaul Precision Medicine Institute

1097 pts with pulmonary symptoms 1 month after acute infection

- 75% had a restrictive pattern on pulmonary function testing
- Not associated with high BMI (obesity)
- CT imaging corresponded with PFTs
- Restrictive pattern on PFTs persisted over time in those who had severe/moderate COVID19, despite radiographic resolution, and worsened in those who had mild disease.

Ling Li, University of Minnesota

APOE3 and APOE4 are genetic variants associated with increased risk of Alzheimer's disease in humans. Mice genetically engineered to be positive for these genes were infected with SARS-CoV-2. Those with APOE4 had increased severity of SARS-CoV-2 infection. Inflammatory cytokines increased significantly in male, older and APOE4 mice despite absence of detectable virus in the brain. SARS-CoV-2 triggers inflammatory chemicals (cytokines) that cause a neuroinflammatory response in the brain.

Amber Wolabaugh - Columbia University

Human immune system mice offer a solution to studying the immune response to SARSCoV2 in vitro. Human thymus tissue was grated into mice genetically engineered to express the human ACE-2 receptor (which is the entry point for SARS-CoV-2). Comparing mice with and without T-cells, *presence of human*

immune cells markedly enhanced the spread of infection to multiple organs expressing human ACE2. Mice without T-cells show more vigorous adaptive immune response marked by high interferons. Adaptive response may damp down innate response. The mice without T-cells actually had less lung disease and overall less severe disease.

Catherine Blish, Stanford University School of Medicine

Adipose (fat) tissue is infected by SARSCoV2 – adipose cells themselves, but more importantly, a subset of macrophages found in the stroma (connective framework) of adipose tissue. These macrophages carry a large viral burden in acute infection, releasing large amounts of various cytokines, which affect nearby cells such as pre-adipocytes. There may be differences for insulin resistant adipose cells. Unclear if/how this process persists in Long COVID when viral RNA is no longer present in cells.

Resia Pretorius, Stellenbosch University, South Africa

Fibrin microclots are small clumps of activated platelets and fibrin between 2 and 200 micrometers in size. They are present in many chronic inflammatory diseases, including diabetes and rheumatoid arthritis. They cannot be detected with conventional techniques that measure clotting factors and platelets. They can be seen with special stains on light microscopy, or with electron microscopy. The composition of microclots differs between different diseases. Microclots are induced by exposure of blood to SARS-CoV-2 spike protein. The microclots seen in Long COVID patients are high in alpha-2 antiplasmin, which make them very resistant to normal fibrinolysis. The reason for the persistence of microclots after acute SARS-CoV-2 infection is not known, but may be related to viral persistence or immune factors. Microclots may cause endotheliitis that results in multi-organ damage. A group of Long COVID patients in Dr Pretorius' lab showed symptom improvement after being treated with combined antiplatelet and anti-thrombotic drugs.

Jane Mitchell – Cardiovascular pharmacologist, Imperial College London, UK

The vascular endothelium lines blood vessels. Normally it facilitates flow of blood through the vessels. Three important chemicals influence the endothelium. Nitrous oxide and prostacyclin cause relaxation of the endothelium and an anti-coagulant state. ET-1 causes constriction of the endothelium and a procoagulant state. Most evidence suggests that endothelial cells are not infected by SARS-CoV-2. However, the cytokine storm (i.e. the many pro-inflammatory chemicals) produced by cells that are infected, can influence endothelial function. In particular, the combination of tumor necrosis factor and interferon stops nitric oxide production and induces ET-1. ET-1 is a potential therapeutic target.

Avindra Nath, NINDS, National Institutes of Health

Studies the brains of young patients with history of COVID19 infection who died of other causes after recovery. Most died in their sleep of unknown cause. He found high levels of immune proteins and clots in the small blood vessels of the brain. Macrophages and astrocytes were present, but not T-Cells. There were high levels of auto-antibodies. Changes were concentrated in the brainstem. He proposes that antibody to spike protein leads to anti-idiotypic antibodies (i.e. against another antibody) that will bind to endothelial cells. This leads to activated endothelium, platelet clots, breakdown of endothelial

barrier, spillage of clots and cellular material into tissue, leading to cellular inflammatory response, which is neurotoxic.

Jean Massimo Nunes Stellenbosch University, South Africa

He studies ME/CFS, which has similarities to many symptoms seen in Long COVID. Microscopy of blood from ME/CFS patients showed tenfold increase in microclots compared with controls. Proteomic analysis showed increases in procoagulant proteins (increase in thrombospondin 1 and platelet factor 4, decrease in Protein S). ME/CFS is not the same as LC but there is overlap in symptoms, and in platelet activation, microclots and endotheliopathy.

Lavanya Visvabharathy, Northwestern University, Neurology Department

Cognitive testing (using NIH Toolbox) was performed on patients aged 20-60 with Long COVID. There was a consistent deficit in attention, with normal scores for processing speed, working memory and other measures. The large majority showed improvement in scores over time; but did not show improvement in symptoms. Tested for autoantibodies in 17 NeuroPASC pts, 14 COVID convalescents, and 15 healthy controls. Multiple species of AABs found in NeuroPASC pts and (surprisingly) in healthy convalescents. Specific autoantibodies found at higher rates in NeuroPASC pts were those associated with myopathies and systemic lupus. Those antibodies correlated strongly with symptom severity, cognitive tests, and quality of life.

Jon Izquierto Pujol, IrsiCaixa AIDS Research Institute, Spain

Performed plasma inflammatory profile on children with Long COVID. Median age 13. Of note, 45% were unable to attend school due to symptoms. Findings: Increase in CD4+8 “double positive” T-cells, increased memory B cells (suggests antigen persistence), increased CD21-CD27+ B-Cells suggesting gut dysbiosis. Also did profile of inflammatory markers and found increased Eotaxin (CCL-11) (associated with brain fog), IL-15 (maintenance of cytotoxic lymphocytes), and PDGF-AB (increased in vascular complications).

Mitchell Miglis, Stanford University – autonomic specialist

One of the few clinically-oriented talks. Speaker specializes in autonomic dysfunction – POTS and related conditions. The same syndromes are also seen frequently with Long COVID (67% in one study). Common symptoms of dysautonomia include rapid heart rate, lightheadedness on standing, chest pain; also symptoms that are not cardiovascular, such as fatigue, brain fog, chronic pain and migraines. There are many theories as to the cause, including mast cell overactivation, excessive central sympathetic activation, vagus nerve dysfunction, autoimmune, small fiber neuropathy and baroreceptor reflex impairment – but there has not been sufficient research / funding to establish a cause. One study showed that dysautonomia was present in a high proportion of patients with sleep disorder, and correlated closely with skin biopsy positive for phosphorylated α-synuclein (p-syn), which may serve as a biomarker. Cerebral blood flow is abnormal in 75% of Long COVID patients with POTS. Small fiber neuropathy ahs been reported in 89% of Long COVID patients with POTS. Personal communication for author: in many patients who do not have positive tilt table or stand tests, a diagnosis can be made by home self-measurement of pulse and blood pressure with standing when symptomatic.

John Wood, University of Southern California

Brain fog in children. Cognitive testing shows deficits in concentration/attention, short-term memory and processing speed; fewer deficits in executive functioning. Prevalence: 40-50% of kids with Long COVID. Possible confounder is the social impact of the pandemic on kids: loss of social structures such as school, activities; loss of relatives to COVID19; loss of access to mental health services. Recognition of the problem of Long COVID in kids led to the creation of the RECOVER Pediatric cohort. Possible causes of brain fog / cognitive effects: during acute infection, microvascular and acute inflammation open blood brain barrier and trigger neuroinflammation. CCL-11 generated by inflammation in the lungs may be an important mediator. In mouse models, CCL-11 leads to increased microglial reactivity, decreased nerve progenitor cells (neuroblasts) and reduced white matter function. Brain autopsy studies (UK Brain Bank) show that the lower parts of the brain are most affected, particularly the olfactory cortex and the limbic system (which controls the autonomic nervous system).

Clifford J. Rosen, Maine Medical Center, Tufts University School of Medicine

Metabolic aspects of PASC and the role of HDL and oxidized lipids. Acute SARS-CoV-2 infection (like any event that causes physiologic stress) increases cellular glucose demand. To meet the demand, glucose is formed rapidly in the liver through gluconeogenesis and released into the bloodstream. This process generates reactive oxygen species ("free radicals"), which can damage mitochondria. SARS-CoV-2 infection also induces adipose tissue dysfunction, resulting in the release of large amounts of fatty acids and lipids. The high lipid levels cause glucose intolerance and hyperglycemia, which leads to more lipid release in a vicious circle. These lipids deposit in liver, muscle and blood vessels – possibly contributing to endotheliopathy. Thus, "lipid toxicity" is a cornerstone of metabolic dysregulation in acute SARS-CoV-2. The body's main defense against lipid toxicity is HDL – particularly PON-1 (Serum paraoxonase and arylesterase 1), an enzyme produced in the liver that circulates bound to HDL. PON-1 blocks lipid peroxidation and reduces production of reactive oxygen species. In a study of patients before and after COVID19 leading to Long COVID, HDL levels returned to pre-infection baseline, but PON-1 levels did not. A study of genetic risk factors showed that variants in PON-1 and antioxidant systems were associated with severe and fatigue-dominant Long COVID. Thus, in susceptible people, acute SARS-CoV-2 infection may lead to long-term reduction in antioxidant enzymes, resulting in lipid toxicity, high levels of reactive oxygen species, and mitochondrial damage in multiple tissues.

Matthew Durstenfeld, University of California San Francisco

Performed cardiopulmonary exercise testing (CPET), tilt table, Holter monitor and MRI on patients with Long COVID. Found decrease in exercise capacity (peak VO2) due to inability to adequately raise hart rate ("chronotropic incompetence").

William H. Robinson, Stanford University School of Medicine

Lessons from Epstein Barr Virus (EBV). Reactivation of EBV virus is seen in several autoimmune diseases that cause fatigue, brain fog and myalgias, including multiple sclerosis (MS), rheumatoid arthritis and systemic lupus. In one study, nearly 100% of patients who eventually developed MS had evidence of EBV reactivation before the onset of MS. Reactivation of MS can be difficult to detect for various reasons. The speaker looked at cerebrospinal fluid from a group of MS patients and found a high proportion of B lymphocytes making antibodies to EBV viral proteins such as EBNA-1. These antibodies also bound to a

protein (GlialCAM) in myelin, an important component of nerves. Mice injected with EBNA-1 produce antibodies to GlialCAM and demonstrate neuroinflammation on brain biopsy. We know that humans infected with SARS-CoV-2 have a prolonged, intense B-cell response, which would tend to promote EBV re-activation. It is possible that EBV reactivation results in autoantibodies to myelin, which could cause some of the symptoms of Long COVID such as fatigue, brain fog and weakness.

Braeden Charlton, Vrije Universiteit, Netherlands

Interested in studying whether muscle abnormalities could contribute to exercise intolerance, fatigue, muscle aches and post-exertional malaise in Long COVID patients. Twenty-four Long COVID patients and 15 healthy controls underwent cardiopulmonary exercise testing (CPET) with muscle biopsies before and after. CPET findings: On CPET, the Long COVID patients had impaired peak exercise capacity (measured by peak VO2). At maximum power, they had a lower gas exchange threshold, with earlier accumulation of lactate in the muscles. That means that they extracted less oxygen from the blood than controls, and switched earlier to anaerobic respiration. Theoretically, this could be due to reduced oxygen deliver to the tissues (i.e. impaired blood supply) or due to reduced oxygen use by the tissues even with an adequate blood supply (i.e. the mitochondria not using the oxygen to produce cellular energy). On biopsy, the capillary density of Long COVID patients was the same as controls, suggesting that blood supply was adequate; but Long COVID patients had severely reduced mitochondrial respiration at baseline, which was reduced after exercise. Conclusion: Reduced exercise capacity, muscle aches and post-exertional malaise in Long COVID patients could be caused by impaired mitochondrial function.

Vashi Negi, Purdue University

SARS-CoV-2 envelope protein is a viral ion channel (E protein channel) – it is a viroporin
Involved in ARDS and cytokine storm in acute infection; could be involved in chronic infection too
Fully conserved from SARS to SARS-CoV-2 and in the SARS-CoV2 variants
Their lab has developed a BSL-2 screen for ion channel inhibitors, and could also be adapted to test broad specrrum antivirals for activity against viroporins
Among other drugs, amantadine has been shown to be an effective channel inhibitor

James N. Baraniuk, Georgetown University

TRPM3 is a cellular ion receptor. It triggers degranulation in response to influx of calcium ions. It is of interest because it has been shown to be abnormal in patients with ME/CFS. Speaker's lab has developed an assay system to detect abnormalities in TRPM3 in NK cells. He demonstrated that the ion current is defective in both ME/CFS and Long COVID subjects, and not in normal controls. Administration of naltrexone corrected the receptor defect in all LC and ME/CFS subjects. Importantly, while the work was done in lymphocytes, the same receptor controls degranulation in other cell types including neurons. Speaker expressed interest in collaboration and gave his email for contact - ncned@griffeth.edu.au .

Rebecca L. Skalsky, Oregon Health & Sciences University

Epstein Barr Virus (EBV) is a herpes virus that can remain in a latent state in infected immune cells (B lymphocytes) and re-activate at a later time. It is well established that SARS-CoV-2 triggers reactivation. However, reactivation is difficult to detect. Presence of virus in the blood is transient, and the antibody profile (serology) is limited as a marker of reactivation. The speaker identified micro-RNAs associated with EBV virus. These miRMAs are produced by EBV when active, and are stable and detectable in blood. Circulating EBV miRNAs were seen in 85% of SARS-CoV-2 infected patients. Conclusions: 1)

miRNAs can be a biomarker of EBV reactivation; 2) It is clear that EBV is reactivating in SARS-CoV-2. Future directions: assess Long COVID cohorts for EBV miRNAs. Speaker: "If you're interested in working with us, please contact me." skalsky@ohsu.edu

Tongcui Ma, University of California, San Francisco

Use of a mouse model for Long COVID is challenging because many LC symptoms are complex and require verbal description. Can AI detect subtle behavioral changes in mice? Speaker's lab uses deep learning framework that identifies behavioral trends from deep variational embeddings of animal motion (VAME) (ref) (ref). On videos of mouse behavior, parts of mouse body were virtually labelled to generate data on movement and posture for computer to analyze. Findings: SARS-CoV-2 infected mice (compared with non-infected) showed deficits in habituation (tendency to explore less in familiar place), which could be indicative of memory loss, and other deficits in community motif usage.

Monika Haack, Beth Israel Deaconess Medical Center
Pain in PASC – The Role of Sleep Disturbances

Sleep disturbance can be associated with Increased spontaneous pain and sensitivity, and central pain syndromes, as well as increased inflammation and reduced recovery from inflammation. Sleep disturbance is common in Long COVID. To assess whether sleep disturbance contributes to pain in Long, COVID, the speaker studied 15 Long COVID patients and 15 health matched controls. All underwent 2 weeks of at home spontaneous pain and sleep monitoring, followed by a 1 day inpatient sleep and pain assessment. Findings: 50% of patients fulfilled criteria for insomnia and 25% met criteria for both insomnia and hypersomnia. Pain was reported on 33% of monitoring days. Inpatient sleep assessment showed difficulty with sleep onset and increased time in wake and light sleep. In-hospital pain threshold measurement showed no change in nociceptive stimulus sensitivity, but impaired central pain processing, particularly in females. Biochemical studies showed higher inflammation and lower anti-inflammatory elements (Resolvins) associated with poorer sleep. Sleep disturbance may play a role in ongoing pain and inflammation in Long COVID.

Lisa E. Gralinski, University of North Carolina

Complement signaling mediates much of the alveolar damage that eventually leads to pulmonary fibrosis. Knockout mice that do not have C3 show less lung injury on microscopic stains than mice with normal complement after infection with SARS-CoV-2. At the alveolar level, C3 knockout mice show less debris, fewer fibrin deposits, fewer alveolar macrophages, and no signs of vascular damage. The two groups are about the same at 2 days, but diverge at five days. Inflammatory signaling is also higher in the normal than the C3 knockout mice. Complement deficient mice are protected from long term complications, i.e. pulmonary fibrosis.

David M. Systrom, Harvard Medical/Brigham & Women's Hospital

Laboratory able to perform invasive cardiopulmonary exercise testing (I-CPET), which involves measuring pulmonary pressures with a pulmonary artery catheter and arterial oxygen and carbon dioxide through a radial catheter during CPET. They studied patients with ME/CFS, which has similarities to Long COVID. They found that these patients have reduced maximal exercise due to reduced right heart filling and reduced stroke volume ("preload failure"). This is usually associated with dehydration, but patients were

not dehydrated. Findings were similar when Long COVID patients were studied. Speaker “I have not yet met a patient with ME/CFS or LC who does not have preload failure.” This suggests systemic vascular dysregulation due to impaired venous constriction. Patients improved when given pyridostigmine – a drug that increased parasympathetic activity - before CPET. This suggests that autonomic nervous system dysfunction is the cause of the preload failure seen in these patients. I-CPET also showed failure of tissue oxygen extraction, which would be due to reduced blood flowing to the tissues, or mitochondrial failure to extract the oxygen delivered. See notes from Braeden Charlton’s talk (above) – his findings point to mitochondrial dysfunction as the cause.