

## Responses to Participants' Questions

The overarching goal of the R3 Seminars is to catalyze a shared understanding of the research being conducted by the scientific stakeholder community within the RECOVER Consortium. The R3 Seminars and the Q&As typically feature highly scientific material intended for researchers and clinicians. For other audiences interested in these topics, a link to the National Library of Medicine's MedlinePlus medical dictionary is provided at the end of the Q&As as a resource to help in understanding the scientific terminology.

This document provides responses\* to questions raised by webinar participants related to the following presentations at the R3 Seminar *Vascular Pathophysiology of PASC* held on December 13, 2022:

- **Presentation 1: *Vascular Dysfunction in Long-COVID: What We Know and What We Need to Figure Out***

Joel Trinity, PhD

- **Presentation 2: *Circulating Factors in Plasma from Patients with COVID-19 Cause Endothelial Cell Dysfunction and NAD+ Depletion: Prevention by Nicotinamide Riboside***

Katelyn Ludwig, PhD

- **Presentation 3: *Contributions of Vascular Dysfunction to Clinical Symptoms in PASC: Role of Cardiometabolic Disease, Inflammation, Vascular Aging, and Thrombosis***

Naomi Hamburg, MD, MS

- **Discussant: Janet Mullington, PhD**

\* Responses may have been edited for clarity.

## All Presenters: Questions and Responses

**Q. There is often an overreaction with the sympathetic and vasoconstriction factors leading to endothelial dysfunction in PASC. How do host defense mechanisms like these vary across sex, age, and social disparities?**

**Response:**

**Dr. Hamburg:** This is a very important question. We know there have been disparities in terms of the impact of acute COVID across our different communities. In terms of Long COVID, we've been seeing differential impact in women as compared with men. Part of what we need to know more about, and hopefully what we'll learn more from RECOVER, includes two things. First, do we think there are biological impacts and that they may be some of these underlying risk factors? Are there underlying differences in the inflammation, thrombosis, and the

endothelial activation? Are there biological differences that happen between men and women based on underlying risk factors, such as social determinants of health, that may lead to differences in incidence of Long COVID? Second, who are we seeing in our clinics as compared with what is the full picture of who is getting Long COVID? That is going to be very important to look at too; and RECOVER, with its prospective design, will provide this information. In terms of who comes to our Long COVID clinics, I tend to see more women.

**Dr. Ludwig:** I don't have much to add because unfortunately we couldn't tease out the differences in age or sex based on our data because we didn't have a big enough sample size. It wasn't dispersed enough among those groups. So, we're hoping that in our RECOVER project where we have a much larger sample size that's going to be more evenly dispersed with men and women of different ages. But that's something we can look at in terms of circulating factors. It's possible we might see a difference, especially between younger and older individuals where we would expect to see those differences. So, it'll be interesting to see if we're able to find that. But at this point in time, I just don't have any data on it.

**Q. With regard to exercise, I think it's a very interesting and delicate aspect to navigate in Long COVID, because as with ME/CFS we see that too much can potentially harm and exacerbate symptoms for a period of time. There's real opportunity to look at who may benefit and who may be at risk in terms of the different population or symptom clusters. Would the panel like to comment on exercise rehabilitation, relaxation, and sleep related to Long COVID?**

**Response:**

**Dr. Trinity:** I think the idea of exercise rehabilitation is very tricky with Long COVID patients. I noticed in the audience Q&A that for one of the attendees their lived experience is that any type of exercise rehabilitation, even if it's very low intensity, seems to exacerbate symptoms and make things worse. So, thinking physiologically about what's going on at the basic level of exercise, it's an acute stress that the body needs to deal with. I think we don't know, especially with aging or with Long COVID, what are the cellular and molecular signals that are dysfunctional or dysregulated in response to that acute stress. We know that there are increases in oxidative stress, increases in inflammation that occur. In the healthy individual, these lead to adaptation that's beneficial. So, if you can stress the system and keep stressing it, these acute bouts then lead to positive adaptation. Long COVID could be very similar to ME/CFS. It's a stress that throws everything out of whack and we don't know how to deal with it. So honestly, I don't know how to best address it. Being an exercise physiologist by training, we like to say exercise cures everything, and for most things it seems to do a great job. But this is a very interesting situation where it could make things worse. So, I'm really interested in, maybe not exercise per se, but physical activity such as getting up, moving around, standing, getting your muscles active, and increasing blood flow. Very simple things that aren't exerting yourself but might be increasing blood flow. I think that would be an interesting first step into this realm.

**Dr. Hamburg:** That's an important qualification. A lot of it is targeting appropriate recommendations to fit individual patients, and I tried to highlight in the patient case that it's helpful to have objective evidence that goes along with people's experience of how difficult it is to move. So, the reason it's difficult to move is there are real physiologic changes that are making it truly difficult to do any activity. So, it's not simply telling people to ignore it and move. That's not the recommendation. Thinking about how we're going to design interventions that help people restore their function is what's important because this is an extremely debilitating condition. Maybe there are other approaches that help induce the physiologic benefits of exercise with other safe approaches for individuals. And we need trials of all these pieces specifically with these patient groups.

**Q. Is it possible to look at plasma or do some of the dish studies with samples from exercise versus in some of these affected patients as well?**

**Response:**

**Dr. Ludwig:** We certainly could do that. We haven't done any of those studies directly on exercise patients before, but we're interested in our lab in some exercise-mimetic interventions that could be very interesting in the context of Long COVID. Specifically, we look at things like heat therapy, where you're immersed in hot water for long periods of time. We also look at inspiratory muscle strength training, which is a breathing device where you breathe against resistance to act as an exercise medic. We've also done a little bit of that in the cell culture model, but it would be interesting to look at all these in the context of COVID, if it were possible.

**Q. Would any of the panelists like to share additional information about therapies that are being considered for treating vascular dysfunction after COVID?**

**Response:**

**Dr. Trinity:** We have a trial that's starting soon within the veteran population looking at a mitochondrial targeted therapy to hopefully improve the vascular function, but also to potentially improve the ability to perform an exercise rehabilitation. The mitochondrial dysfunction is definitely associated with vascular dysfunction and that could be an interesting avenue to look at.

**Dr. Hamburg:** There are also ongoing studies in the UK looking at this antithrombotic therapy question about whether there are effects in Long COVID. But again, I want to make sure that we preface any discussion about therapies to say that as of yet there are no results of any trials of therapies. But there are a lot of people treating patients with individual therapies. So just to be clear, currently there are no proven therapies.

**Dr. Ludwig:** I know there are some trials looking at NAD (nicotinamide adenine dinucleotide) boosting compounds in COVID. But again, as Dr. Hamburg said, there are no results on them. So, I don't know how they're doing in the clinic.

**Q. Since arterial stiffness is also associated with menopause vasomotor symptoms, which are generally treated with estrogen and sometimes SSRIs, are there any studies being done with hormones and PASC-related arterial stiffness?**

**Response:**

**Dr. Hamburg:** That's a two-part question. Part of it is about the hormones and then the other question is about the integrative physiology question. That's to say, I don't know of any studies specifically looking at estrogen treatment. Consequently, one may have the concern that there's some risk of estrogen therapy with thrombosis. I would think carefully about how those studies would be designed. The other question is how did the endothelial piece integrate? We all alluded in different ways about the integration with other physiology, with the autonomic nervous system, or with the neurologic systems. I'm interested in other people's thoughts on this too, but I think it would be very interesting to see whether things that may target the nervous system—like you suggested SSRIs, but that's only one of multiple therapies—would affect the vasculature. So, it would be important to make sure that the study designs look broadly at the physiologic impact of therapies for Long COVID.

**Dr. Trinity:** There's substantial evidence that dysautonomia could definitely be contributing to the vascular dysfunction. We have this balance of the basal constrictors from the sympathetic nervous system activity as compared with the vasodilators. That's certainly another avenue we could approach. Trying to turn down the sympathetic activity could benefit the vascular function, and also some of the other autonomic disorders we're seeing.

**Dr. Hamburg:** There's also a question that was raised before about whether it's the other way. We don't fully know which it is, and it could be bidirectional (microvascular dysfunction or vascular dysfunction), and all the nerves are vascularized as well. Is there some impact of the microvasculature on the autonomic nervous system as well? Certainly, it's a bit challenging. I would also caution about anecdotal reports, but I've had anecdotes where patients are on some of these therapies targeted toward autonomic and their other symptoms, such as chest pain or other pieces, get better. So, there may be pieces that go together.

**Q. Do vasospasms have a role in pathophysiology of the disease? And would any vasospastic, mitochondrial, immunological, microclots, or other pathways be potentially involved in worsening of symptoms and maybe further vascular damage?**

**Response:**

**Dr. Hamburg:** I've seen people who have manifestations of acute COVID that seemed like vasospastic disorders. This is probably a component of COVID toes that we saw early on. However, I haven't seen patients with that since the early days of COVID. I don't know if the incidence is going down with milder disease. And I think it's people who have these types of symptoms who have tried some of the treatments that we use for vasospastic disorders. But again, it's a reasonable hypothesis about which there's not a lot of evidence. I wonder about the data that

were shown with EndoPAT. It would be interesting to look at the baseline pulse amplitude, because certainly in the acute setting you might have more vasospasm than in the long-term setting.

**Q. Is there a theory as to why the vascular dysfunction in Long COVID patients isn't showing up in the acute phase but does show up weeks or months later?**

**Response:**

**Dr. Trinity:** It could be due to the tests being performed to assess vascular function. The vasculature is highly responsive to multiple signals. So, it could be that there are so many things going on in the acute phase. It could even be an overactivated immune response that's causing increases in blood flow, or something like that, that we're not seeing decreases until it resolves. And then in the long term, there's something else going on. I think it's a combination of the physiology of what's going on, controlling blood flow regulation and vascular tone. As I said earlier, we still don't have a clinically relevant test to measure vascular function. I think Dr. Hamburg and Dr. Ludwig did a great job looking at endothelial cells in these downstream, more mechanistic things. But we still don't know what our best approach should be for measuring vascular function, which is important because we need that before we can start guiding therapies that will treat and improve vascular function.

**Dr. Hamburg:** This goes along with a very interesting question given what's been shown in the acute setting; namely, that people with more severe disease have more endothelial damage. There are some questions in the comments about this paradox, such as the patient I discussed and many other patients I see in the office; they didn't necessarily have severe COVID prior to severe Long COVID. This question is possibly getting at that a bit and trying to speculate. It may be that whatever is helping you fight off having more severe infection has some lingering effects in terms of these other long-term symptoms. It's going to be very interesting to see from RECOVER how did the acute immune markers relate to the incidence of Long COVID and it may be that they're not the same as those who ended up with more severe illness.

**Q. Could enhanced external counterpulsation (EECP) potentially help endothelial dysfunction?**

**Response:**

**Dr. Hamburg:** It's possible that EECP could be a way to mimic the impact of exercise on the vasculature. I'd be interested to see the results of the study.

**Q. What is the current evidence base related to agent selection and duration of anticoagulation therapy in patients with thrombosis first diagnosed in acute phase?**

**Response:**

**Dr. Hamburg:** The trials that have been done have focused on the best way to prevent thrombosis in acute COVID. Treatment of thrombosis in COVID has generally followed the approach of treatment of thrombosis in general. So,

you would risk stratifying individual patients on the risk of recurrent thrombosis when deciding about whether longer-term therapy is needed to prevent future clots.

**Q. Has preload failure for PASC been investigated?**

**Response:**

**Dr. Hamburg:** There are studies of exercise tolerance that generally have not suggested a cardiac cause.

**Q. Do the presenters have a guess or thought as to which of the vascular biomarkers seems to be the most specific in Long COVID, and the best candidate to become a “mainstream” test?**

**Response:**

**Dr. Hamburg:** We’re still in a phase of gathering information about which vascular biomarkers are altered and at which phase of disease. We mostly have information at this point about acute illness and gathering for COVID. I would recommend to research a larger number of tests so we can evaluate the most sensitive and specific.

**Q. Is it safe to advise Long COVID patients that losing weight, quitting smoking, better diet, getting outside, and gently increasing movement might be beneficial until we know more about therapies—and at least will not make condition worse?**

**Response:**

**Dr. Hamburg:** I’d agree that all those recommendations are good for general health.

**Q. Do we know which immune pathways lead to vascular/endothelial dysfunction when triggered with acute COVID infection? Could a familial predisposition to vascular problems and/or immune dysregulation contribute, such as strong family history of large vessel disease?**

**Response:**

**Dr. Hamburg:** We don’t yet know the specific immune pathways that link SARS infection to vascular dysfunction. I haven’t seen data linking family history to risk of Long COVID. Prior studies have suggested only a modest heritability of endothelial dysfunction.

**Q. Another seminar recorded the finding of low vitamin K in many people with Long COVID. How might that affect vascular functioning? How would one be tested for that? What can be done about low vitamin K if it is found?**

**Response:**

**Dr. Hamburg:** If clotting tests are normal, I would not recommend taking vitamin K. Eating a healthy diet generally provides adequate vitamin K, unless there are other medical conditions present.

**Q. What tests can be done to evaluate vascular changes in the brain?**

**Response:**

**Dr. Hamburg:** Transcranial doppler (TCD) tests show blood flow in the brain.

**Q. Are statins/antiplatelet medications going to be studied to treat or prevent PASC? If so, when? Can they be used off label now to treat PASC patients since they are approved, safe, and cheap?**

**Response:**

**Dr. Hamburg:** As we've discussed, there are some ongoing studies, but there's no evidence yet. For statins, I'd only use them if there's another indication. Antiplatelet medications have side effects, including bleeding, so they're not recommended currently for primary prevention. In patients with established cardiovascular disease, they are used. PASC would not fall into this category.

**Q. How does BC007 fit with this pathophysiology? Do you think the vascular damage is a result of an autoimmune reaction? Are there other potential drugs being investigated that relate to the vascular pathophysiology? Is this the cause of POTS?**



**Response:**

**Dr. Hamburg:** There are individual reports with BC007, but no clinical trials have been conducted that would support its use. There's some evidence of autoimmunity linked to thrombosis in acute COVID, but whether this plays a role in Long COVID is not clear.

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