DIET AND FASTING FOR LONG COVID; A CROSSOVER TRIAL

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NCT06214455

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Trial Protocol and Statistical Analysis Plan

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Clinical trial protocol

SYNOPSIS

TITLE	**Diet and Fasting for Long COVID**
RESPONSIBILITY	**Jeffery Novack, PhD** Principal Investigator Pacific Northwest University - Health Sciences College of Medicine, Department of biomedical Sciences 200 University Way, Yakima WA 98901 **Thomas Bunker, PhD** Co-Principal Investigator Unaffiliated Patient Researcher 7665 OConnor Rd, Boulder CO 80303
STUDY CENTER	Pacific Northwest University - Health Sciences College of Medicine, Department of Biomedical Sciences 200 University Way, Yakima, WA 98901
PRIMARY OBJECTIVE	Change in Long COVID Symptom Severity Scores and Symptoms during Treatment A (diet, not-fasting) vs Treatment B (diet, fasting).
STUDY DESIGN	Interventional, randomized, all remote, two-arm crossover study, not blinded
STUDY ARMS	Experimental: **Arm AB (Diet then Diet and Fasting)** 1. 2 week baseline with a low sugar diet and 10-12 hour eating window 2. Treatment A: Eat a low sugar diet, 10-12 hour eating window for four weeks

	3. Treatment B: Eat a low sugar diet, 8 hour eating window, 23 to 60 hour fast once a week for four weeks Experimental: **Arm BA (Diet and Fasting then Diet)** 1. 2 week baseline with a low sugar diet and 10-12 hour eating window 2. Treatment B: Eat a low sugar diet, 8 hour eating window, 23 to 60 hour fast once a week for four weeks 3. Treatment A: Eat a low sugar diet, 10-12 hour eating window for four weeks
SAMPLE SIZE	96 Long Covid subjects (approved for 180)
INCLUSION CRITERIA	• Adult (18-69 years old) • **Five or more common Long Covid symptoms** • Free from fever > 100F and known bacterial and parasitic infections • Must indicate willingness to limit certain supplements and report all medications. • Must indicate willingness to make significant dietary changes - and limit daily eating to an 8 or 10 hour window. • Must indicate willingness to attempt 36 hr or 60 hr water fasts each week for 4 weeks. • Have a valid email address and phone number • Reside in the United States • Be able to read and to communicate in English • Must indicate willingness to avoid "extra" supplements such as Fish Yes Oil, Cod liver Oil, Krill Oil, MCT oil, Coconut Oil, Turmeric/Curcumin, Berberine, Quercetin (> 500 mg), Red Yeast Rice, French Marine Bark extract, Red Sage, Ginkgo biloba, Oregano Oil, Peppermint Oil, Black seed oil, Cinnamon bark extract, Elderberry, Stinging Nettle, Milk Thistle, Monolaurin, Vendicinals 9, Tollovid, QuadraMune and all Probiotics. • Must indicate willingness to avoid certain nutraceuticals such as Zinc (more than 25 mg), Arginine, Glutamine, Palmitoylethanolamide (PEA), Alpha Lipoic Acid, L Carnitine and Taurine during the study period. • Must indicate willingness to avoid longevity supplements such as NAD+, Niacin, NMN, Nicotinamide Riboside, Spermidine and Fisetin during the study period. • Must indicate willingness to halt Olive oil consumption greater than 1 tsp daily during the study period.
EXCLUSION CRITERIA	• Likely COVID-19 or SARS-CoV-2 infection < 45 days before enrollment • Body Mass Index (BMI) must be 20 or greater

	• Past history of an eating disorder. • Previously fasted more than 18 hours with Long COVID • Currently doing intermittent fasting • Pregnant or breast-feeding • Severe pulmonary disease requiring supplemental oxygen • Partial loss of vision due to macular degeneration • Any recent (90 days) history of malignancies, fractures, surgery, radiation, chemo, anesthesia, or traumas • Diagnosed with Type I or Type II Diabetes • Previous Autoimmune condition • Heart condition (Coronary artery disease, Heart valve disease, Heart No failure, Stroke) • Pre-pandemic Arrhythmia • Liver disease • Previous Chronic Health Conditions that did not fully resolve (Includes ME/CFS, Lyme Disease, and Fibromyalgia) • Prescription anticoagulation medication that cannot be halted during the study period. Brilinta (ticagrelor), Plavix (clopidogrel), Coumadin, Warfarin etc. • High Cholesterol medications that cannot be halted or reduced during the study period (Fenofibrate, Statins > 10 mg) • Anti-inflammatories or immunosuppressants such as Steroids, Low dose Naltrexone (LDN), Maraviroc, Remicade/Infliximab or Colchicine that cannot be halted during the study period • Metformin, Ivermectin, or peptides such as BPC-157 that cannot be halted during the study period
SCHEDULE	Recruiting first participant: November 2022 Recruiting last participant: April 2023 Study planned completion: Nov 2024

BRIEF SUMMARY

This study uses a cross-over design to test a diet change plus a 10-12 hour eating window (Treatment A) compared to a diet change, an 8-hour eating window and one 36-60 hour fast per week (Treatment B) in subjects screened to have a minimum of five long COVID-19 symptoms. Fasting is for food only, but water is available. There is a two-week run-in with symptom surveys at the start (Week 0) and end (Week 2), followed by randomization to either ARM AB or ARM BA (with weekly symptom surveys). Since the water fasts are at the beginning of the week (with symptoms surveyed at the end of the week) there is a 5 day washout and then a cross-over to the other treatment for 4 weeks.

Study Hypothesis

Time-restricted eating and a no added sugars diet decreases Long Covid symptoms and symptoms scores. The addition of weekly water fasts and 8 hr time-restricted eating will result in a greater decrease of Long Covid symptoms than 10-12 hr restricted eating and a no added sugar diet alone over a four-week period.

Study Aims

This study aims to:

a) Determine if the self-treatment of 36- or 60-hour water fasting once a week for a month reduces overall long COVID severity. If so, to what degree and what is the statistical significance of the results?

b) Determine if eating in a 10-to-12-hour window daily and avoiding sugar for a month reduces overall long COVID severity. If so, to what degree and what is the statistical significance of the results?

c) Determine if the self-treatment of 36- or 60-hour water fasting once a week for a month reduces the severity of twenty-eight common long COVID symptoms. If so, to what degree and what is the statistical significance of the results?

d) Determine if eating in a 10 to 12 hour window daily and avoiding sugar for a month reduces the severity of twenty-eight common COVID symptoms. If so, to what degree and what is the statistical significance of the results?

e) Determine which is more efficacious: "time-restricted eating, no sugar diet, and a weekly short water fast" OR "time-restricted eating, no sugar diet".

Study Significance and Background:

PASC (the long-term post-acute sequelae of COVID-19), hereafter referred to as Long COVID, is a major public health crisis with no proven treatment. Estimates of the incidence of Long COVID vary from between 14 to 30% of those infected with SARS-CoV-2 in the initial waves of the pandemic. About 75% of those suffering from Long COVID are female. A large and variable constellation of symptoms characterizes Long COVID. The root cause of Long COVID is an area of active investigation. While there is growing evidence for low-level SARS-CoV-2 viral persistence, there is also some evidence for persistent microclotting, lack of oxygen utilization by mitochondria and/or lack of oxygen uptake by tissues, various autoantibodies and EBV reactivation. A large NIH autopsy study has shown that SARS-CoV-2 can infect multiple organs and tissues including the brain, heart, intestines, blood vessels, reproductive organs, adipose tissues, peripheral nervous tissue and eyes. They showed that SARS-CoV-2 is widely distributed throughout the body, even among patients who died with asymptomatic to mild COVID-19.

To date, the application of autophagy in human medicine has been largely stymied by the technical inability to measure cellular autophagy in living people. However, there is great interest in modulating cellular autophagy to prevent dementias and possibly limit the development of certain cancers.

Coronaviruses alter the two main cellular degradative processes, the ubiquitin-proteosome system and the autophagy-lysosome system for their own benefit. Some viruses are able to hide from the immune system by suppressing antigen presentation both via the ubiquitin-proteosome-MHC class I system and via the autophagy – lysosome – MHC class II system. SARS-CoV-2 does this by lysosomal targeting and degradation of MHC-I via the viral ORF8 protein. Also, the ORF3a protein has been shown to block fusion of autophagosomes with lysosomes by destabilizing the HOPS complex Additionally, the expression of viral proteins such as envelope (E), membrane (M), ORF3a, and ORF7a result in an accumulation of membrane associated LC3B, a marker of autophagosomes. Together, these perturbations in the autophagic flux likely promote SARS-CoV-2 replication and aid in evasion from immune responses.

The investigators propose that the viral blockage of autophagosome/lysosome fusion, lysosomal degradation and MHC-II viral antigen presentation might be overcome with the periodic induction of cellular autophagy. Further, during autophagy, viral RNA can activate Toll-Like-Receptors such as TLR7 in the lysosomes leading to the release of cytokines such as Interferons that indicate viral infection. Autophagy is also known to be integral to the process of viral antigen presentation via MHC-II, which allows CD4+ T cells to direct immune responses (Natural Killer cells and Cytotoxic CD8+ T cells) towards infected cells. Thus, it is proposed that the periodic induction of

autophagy via Intermittent fasting and short water fasts in subjects with long COVID may prime their antiviral immune responses and decrease their viral load.

Many long-haulers have symptoms similar to that of Crohn's disease and impaired autophagy in the lining of the gut could potentially be directly responsible. Daily, time-restricted eating, avoiding added sugars and processed grains is similar to diabetic diets that strive to minimize spikes in insulin and insulin-like growth factor. As both are pro-growth hormones, limiting their production may help to decrease potential viral replication. Interestingly, a study found that healthcare professionals following diets rich in vegetables, legumes, nuts and fish were less likely to have moderate or severe cases of Covid-19 than those following ketogenic or other high protein/low carbohydrate diets.

It is entirely possible that, similar to AIDS, eventually a cocktail of antiviral drugs will be developed for SARS-CoV-2. Molnupiravir and Paxlovid are the first approved antivirals but are currently indicated only for early acute covid. To date there are no studies of fasting and its effects on long COVID. This study could potentially point the way for medical researchers to study other methods of autophagy induction to treat long COVID. If positive results are obtained, this study could help people suffering from "long-haul" COVID symptoms reduce or eliminate some of these symptoms and could lead to targeted treatments for the remaining symptoms. In addition, this is a simple and inexpensive treatment that most people can try if it is found to be effective in reducing symptoms.

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Study's approach using a detailed, step-by-step plan indicating what will happen in your investigation.

Recruitment

1. Recruit subjects via social media, particularly from some of the large > 10,000 member Facebook long COVID groups.

2. The Study Advertisement with basic information about the study will include a link to the initial interest/screening survey. The Study flyer will also be available on a dedicated simple website with the url: https://longcovidtrials.info/diet-and-fasting-for-long-covid/ The website page will also contain a link to the initial screening symptom survey.

Screening

3. Potential participants indicate interest by filling out a short interest/**screening survey** in REDCap and providing their name and e-mail address. Participants must reside in the United States, be 18 or older, be younger than 70, and have at least 5 of the 28 most common long covid symptoms.

4. Have potential participants then fill out the **Current Medications and Supplements** survey and review both the **Inclusion/Exclusion Criteria** and the **Informed Consent** documents. This will allow screening of drugs and supplements that may interfere with the protocol treatments during the Investigator - Participant call.

5. Investigator contacts the potential participants to setup a 90 min video call

6. Via video call, provide information and review current meds and supplements

7. During the video call, the Investigator goes through the Inclusion/Exclusion criteria with the potential subject. If the subject meets all the criteria, then they are randomly assigned to either the 'TRE then Fasting' group or to the 'Fasting then TRE' group.

Note: Since those with long covid are predominantly female, the assignment will be random without regard to gender distribution.

If the subject does not meet all the study criteria, then they are not enrolled.

8. Next the Investigator reviews the **Participant Instructions** and **Study Diagram** with the subject. The subject is given clear instructions on what to do and when during the 10 week study. The subject is told to NOT begin their First Fast until after receiving a call from one of the study medical student volunteers. Further, subjects are asked to always take their Symptoms Surveys on the same day each week.

9. Ask subject if they have any questions

Consent and Enrollment

10. Next the Investigator reviews the **Informed Consent** with the subject within REDCap. Ask if the subject has any questions. Verify the subject's identity by driver's license or some other photo identification with birth date (passport).

11. Verify that the subject has signed and dated the consent form while on the call. Note: subject consent is being done electronically via REDCap and the consent form will be maintained within REDCap

12. Investigator sends subjects a PDF copy of the completed informed consent form containing electronic signatures of both the participant and the investigator. This email may also include any agreement as to which supplements will be stopped during the 10 week study.

Study Procedures

13. After enrollment, an email for the **Demographics and Vaccination** survey is sent to the subject.

14. Upon completion of the Demographics and Vaccination survey participants are sent an e-mail notification containing a **Health Care Provider letter.** Participants are instructed to print out the letter, fill in the date and their name and either mail it to the Health Care Providers or hand deliver it to them. The letter gives information about the study and study duration to the subject's Healthcare providers.

15. The study duration is 10 weeks.

2 weeks Baseline: No added sugars diet, eat in a 10 to 12 hour window daily. Eating in a regular time window daily is called Time-Restricted Eating or TRE.

If in the **TRE then Fasting** group:

4 weeks: No added sugars diet, eat in a 10 to 12 hour window daily

4 weeks: No added sugars diet, eat in an 8 hour window daily, 36 or 60 hour water fasts weekly

If in the **Fasting then TRE** group:

4 weeks: No added sugars diet, eat in an 8 hour window daily, 36 or 60 hour water fasts weekly

4 weeks: No added sugars diet, eat in a 10 to 12 hour window daily

A no added sugars diet means avoiding all foods with added sugars including most processed and packaged foods. It means avoiding all cookies, snack/candy bars, bakery goods such as pies and donuts, breads, tortillas, chips. However, sugars naturally present in some whole fruits are OK to eat.

16. For menstrual women that experience a predictable monthly flare-up of their long covid symptoms, ask that they wait until a few days before or a few days after their flair to take their first Baseline Long Covid survey. This is to avoid a menstrual related flair-up of symptoms during the key study end-points at 4 weeks and 8 weeks.

17. Have subjects take their initial Baseline long covid symptom survey in REDCap, after 1 week they will take their 2nd Baseline symptom survey. After week 2 they take their 3rd Baseline symptom survey.

18. REDCap will send weekly reminders to subjects concerning - when to start self-treatment A, when to start self-treatment B. Treatment group A, 'TRE then Fasting', has a low sugar diet and a 10-12 hour eating window. Treatment group B, 'Fasting then TRE', will have an 8 hour eating window with either a 36 or 60 hour water fast once a week.

19. FIRST FAST Subjects will be called after they complete the appropriate survey before their first water fast by a medical student volunteer or Dr. Jeff Novack. They will be informed of what to expect and reminded to take electrolytes. They will be reminded that they can STOP FASTING at any time. They will also be reminded of how to use the symptom flare-up form (via a public link) to talk to a med student and potentially have Dr. Baldwin call them. Per the new **First Fast Call Medical Student Instructions**, the medical student volunteers are expected to talk with subjects within 24 hours of receipt of a First Fast

email notification. Meanwhile, subjects are instructed not to start their first fast until after they have received their Pre-First Fast Call. If the medical student does not reach the subject, they leave a message with their cell phone number. They then must make at least two call attempts by 4 PST the day after the First Fast Call notification was received. All call attempts and their result will be documented in the subject's First Fast Call for m. As a backup, per the crossover emails, if the participant needs to start their first fast but has not been able to talk with a medical student by 7 pm EST, they may call Dr. Bunker. Dr. Bunker will check that the subject is indeed correct to initiate their fast at this time. He will then review the Fasting Safety procedures and update the First Fast Call instrument.

20. Survey reminders will be sent using the dateDiff function comparing today's date:time to the survey date of each participant's first Baseline Survey. If a subject misses a key survey, then they are considered withdrawn from the study. The first and third baseline surveys, the survey at the end of 4 weeks water fasting and the survey at the end of the relaxed time-restricted eating are considered key surveys.

21. If a subject wants to be removed from the study or no longer qualifies from the study, the subject will be removed, but the record will be maintained.

22.

With successful completion of the 10 week study, participants will be sent a "**Participant Completion**" email offering a 30-45 min Study Review conference with an investigator. As an attachment, subjects will get a summary of their participation in the study. It will include their weekly Long Covid Symptom Survey scores and subject comments. Also included in the "Participant Completion" email will be language asking the subject to not share their study Long Covid Symptom Survey scores on social media. The letter will also request that subjects not share the study flier on social media - as recruiting for the study must be done only via investigator posts of the flier on social media.

23. If a subject schedules a Study Review conference, at that time, a participant's results, comments, and adherence to the study procedures will be reviewed. The investigator will again remind the subject to not share their study scores and to not share the study flyer on social media.

Safety Procedures

23. During water fasts, subjects are asked to drink salt water for electrolytes twice daily. The recipe has Sodium, Potassium and Magnesium in the proper ratios to replace what would normally be obtained from food. This minimizes the risk of refeeding syndrome (which is already a low risk for short water fasts). ½ tsp Morton Lite Salt in 12 ounces of water. In addition, participants are instructed to take a 200 mg Magnesium Glycinate tablet.

24. If experiencing severe symptoms, they are to fill out the Symptom Flare-up form via the link in their Participant instructions. Their symptoms will be immediately shared with two 3rd year medical students via an automated REDCap e-mail. The form also provides subjects with the medical student's names and phone numbers. They will try to provide 24-hour support for study subjects. When a medical student identifies a safety concern, they will be referred to Dr. Mark Baldwin. Dr. Baldwin will recommend if the subject should contact 911 emergency immediately and/or be removed from the study. Dr. Baldwin will then report the removal of the subject.

25.

As subjects complete their weekly Symptom Surveys, REDCap notifications will check for self-reported Serious and Concerning Adverse Events.Dr. Baldwin, Dr. Novack and Dr. Bunker will be sent all Adverse Event notifications as they are reported. The Investigator or Co-Investigator will create an Adverse Event instrument in REDCap within 24 hours. They will also contact the subject if additional

information is needed. The Investigators will leave all "medical judgment" fields blank as per the 'Dr. Baldwin REDCap Instructions'.

Dr. Baldwin will complete serious Adverse Event forms in REDCap within 48 hours of notification. He will call or text the PI/Co-PI upon completion of an Adverse Event form that he deems 'Serious'. The PI/Co-PI will then report this Serious Adverse Event in IRB Mentor as soon as possible.

For self-reported Concerning Adverse Events, Dr. Baldwin will review them weekly on Thursdays. He populates all the 'medical judgment' fields and determines if follow-up is needed or not.

Data Analysis

26. Remove identifiers from study data for any exports

27. Post study data to a secure SharePoint folder for analysis by our biostatisticians. SharePoint is HIPAA and FERPA compliant and is encrypted at rest.

28. Study records will be maintained for seven (7) years.

Study Protocol version 3.0 as of 7/21/2023

Statistical Analysis Plan

Title: Diet and Fasting for Long COVID

Principal Investigator: Jeffery Novack, PhD

Co-Principal Investigator: Thomas Bunker, PhD

Other Investigators: Mark Baldwin, DO; Robert Sorrells, PhD; Benjamin D. Horne, PhD, MStat, MPH

Study Patient Advocate: Thomas Bunker, PhD

Statistical Analysts: Lucy A. Horne; Robert Sorrells, PhD; Benjamin D. Horne, PhD, MStat, MPH

1.0 Study Overview

Introduction. Post-acute sequelae of COVID-19 (PASC), or Long COVID, is composed of a constellation of symptoms that arise after resolution of acute SARS-CoV-2 infection and constitutes an on-going crisis of international public health. Estimates of the incidence of Long COVID vary from between 6-10% in current literature, although this varies depending on definitions and estimates were higher in earlier phases of the pandemic. A majority of those reporting Long COVID are female. COVID-19 is known to directly target activated T cells and to inhibit or disable autophagy. The causes of Long COVID remain under investigation, but evidence suggests that viral persistence, persistent micro-clotting, autoantibodies, reactivation of Epstein-Barr Virus, and energy dysregulation may play a role. No proven treatment currently exists for Long COVID.

Water-only fasting is reported to activate various mechanistic pathways such as autophagy, decrease excessive inflammation, deactivate T cells and protect activated T cells, and generally strengthen the immune system. Various regimens of fasting exist, with the most popular being shorter-term durations, or intermittent fasting, in which caloric intake ceases for 2 days or less. Intermittent fasting regimens include fasting every day for 14-16 consecutive hours (also known as time-restricted eating or TRE), once per week for 24 hours, two days per week on either consecutive or non-consecutive days, and fasting every other day (also called alternate-day fasting). Whether intermittent fasting reduces the severity of acute COVID-19 or PASC/Long COVID is untested but theory suggests that it should directly counteract many of the main adverse effects of COVID-19.

1.1 Study Aims

1.1.5 **Primary aim:** Determine if self-treatment with 23- to 60-hour water fasting once per week in addition to eating in a daily 8-hour TRE window (i.e., daily 16-hour water fasting) and eating a low-sugar diet reduces overall Long COVID severity, as measured by sixty symptoms of Long COVID.

1.2.5 Secondary aims:

1.2.1.5 Determine if eating in a 10- to 12-hour TRE window daily (i.e., 12- to 14-hour daily fasting) and eating a low-sugar diet reduces overall Long COVID severity.

1.2.2.5 Determine if the self-treatment of 23- or 60-hour water fasting once per week for four weeks (in addition to 8-hour TRE and a low-sugar diet) reduces the severity of twenty-eight common Long COVID symptoms.

1.2.3.5 Determine if eating in a 10- to 12-hour window daily and avoiding sugar for four weeks reduces the severity of twenty-eight common COVID symptoms.

1.2.4.5 Determine which is more efficacious for reducing severity of Long COVID: a once-weekly 23- to 60-hour water fast with daily substantial 8-hour TRE (16-hour fasting) and a low-sugar diet OR a daily modestly-restricted TRE and low-sugar diet.

2.1 Study Hypotheses

2.1.5 **Primary hypothesis**: The overall Long COVID symptom severity score is lower after 4 weeks of once-per-week 23- to 60-hour water-only fasting [in addition to eating in an 8-

hour daily TRE window (16-hour water fasting) and following a low sugar diet] than after 4 weeks of 10- to 12-hour daily TRE (12- to 14-hour water fasting) and a low-sugar diet.

2.2.5 **Secondary hypotheses**:

2.2.1.5 The overall Long COVID symptom severity score is lower after 1, 2, or 3 weeks of once-per-week water-only fasting (in addition to eating in an 8-hour window daily and a low sugar diet) than after 1, 2, or 3 weeks of 10- to 12-hour daily time-restricted eating and a low-sugar diet.

2.2.2.5 The overall Long COVID symptom severity score is lower after the initial 2-week run-in phase of 10- to 12-hour daily TRE and a low-sugar diet reduces Long COVID symptom severity scores.

2.2.3.5 The count of Long COVID symptoms is lower after 1, 2, 3, or 4 weeks of once-per-week water-only fasting (in addition to eating in an 8-hour window daily and a low sugar diet) than after 1, 2, 3, or 4 weeks of 10- to 12-hour daily time-restricted eating and a low-sugar diet.

2.0 Study Population

The trial population consists of 58 individuals, including 29 randomized to 4 weeks of once-per-week 23-60 hour water-only fasting plus daily 8-hour TRE (16-hour daily water fasting) and a low-sugar diet followed by 4 weeks of 10- to 12-hour daily TRE (daily 12- to 14-hour water-only fasting) and a low-sugar diet, and the other 29 randomized to 4 weeks of daily 10-12 hour TRE (daily 12- to 14-hour water-only fasting) and a low-sugar diet followed by 4 weeks of once-per-week 23-60 hour fasting plus daily 8-hour TRE (16-hour daily water fasting) and a low-sugar diet. All participants engaged in 10 weeks of the study protocol, including a 2-week run-in period of daily 10- to 12-hour TRE (daily 12- to 14-hour water-only fasting) and a low-sugar diet.

1.1 Inclusion Criteria: participants must be adults, 18 to 69 years of age; they must have 5 or more of the 28 common Long COVID symptoms, they must reside in the United States of America, and they must be willing to stop the consumption of any nutritional supplements that may activate autophagy.

2.1 Exclusion Criteria: At baseline, participants could not have a body mass index below 20 kg/m^2, could not be pregnant or breastfeeding, and could not have had a SARS-CoV-2 infection within 45 days. Further, they could not carry a diagnosis of type 1 or type 2 diabetes and could not have a history of any eating disorder.

3.1 Data Acquisition: All participant data were collected through pragmatic electronic means in REDCap. Weekly symptom and adherence data were collected through similar REDCap forms. All data were stored in REDCap until the end of the study enrollment and follow-up period was complete for all trial participants.

3.0 Outcomes, Exposures, and Other Variables

1.1 Trial Intervention and Control Arms

1.1.5 Active "Control" Arm: a low-sugar diet with a 10- to 12-hour daily TRE eating window and water-only fasting for the other daily 12 to 14 consecutive hours.

1.2.5 Intervention Arm: a low-sugar diet with a more restrictive 8-hour daily TRE eating window and 16 consecutive hours of daily fasting, along with a once-weekly 23- to 60-hour water-only fast.

2.1 Trial Endpoints

2.1.5 **Primary endpoint**: The primary trial endpoints are: 1) a composite of symptom severity scores for 28 common Long COVID symptoms and 32 less common symptoms, and 2) a sum of the presence or absence of the 60 symptoms.

2.2.5 Secondary endpoints

2.2.1.5 A composite of symptom severity scores for 28 common Long COVID symptoms.

2.2.2.5 Each of the 28 common Long COVID symptom severity scores individually.

2.2.3.5 The count of the 28 common Long COVID symptoms that are present.

2.2.4.5 The count of the 32 less common Long COVID symptoms that are present.

2.2.5.5 Adherence to the assigned trial intervention.

2.3.5 Other study data elements: percent resolution of each Long COVID symptom.

4.0 Statistical Analysis Procedures

1.1 Data Quality

1.1.5 Data are assessed by the principal investigators for data quality across the study and the investigators will keep in close contact with each participant to assess correctness of reporting and data capture.

1.2.5 Participants reporting unusual changes in symptoms will be asked to report unusual activities that may impact values.

2.1 Power Calculation

2.1.5 As no prior study involving fasting and Long COVID exist, with the exception of two case reports, this trial's sample size was estimated based on the 2022 understanding of Long COVID and on publications at that time about Long COVID that had a nascent understand of the symptoms involved in the condition. The expected effect of fasting on Long COVID symptoms was estimated roughly to require 40 to 90 individuals per trial arm, in part depending on the severity of Long COVID symptoms experienced by those who would enroll.

3.1 Analysis Plan

3.1.5 The study population will be evaluated using an intent-to-treat approach to the statistical analysis. All subjects who have data regarding symptom severity scores available at each time point will be included in the analyses.

3.2.5 Demographics, anthropometric variables, risk factors, COVID-19 vaccinations, other medications, and socioeconomic variables will be described as percentages or means with standard deviations.

3.3.5 Symptom severity scores will be compared between the two study arms using the two-sided paired T-test at each time point, as appropriate. Based on the randomized design of the study, it is expected that most if not all of these data elements will be balanced between the TRE arm and the Fasting arm of the study. Similar comparisons will also be performed to compare between the treatment arms for changes at weeks 1, 2, or 3 of each treatment to examine potential timing of the impact of those interventions.

3.4.5 The presence of individual symptoms at baseline, end of the run-in period, end of Fasting, end of TRE, and end of the 10-week trial will be summarized with percentages or means. Examination of overall changes in all participants for LC-score and numLCsym will use two-sided Student's T-test to evaluate the differences in means at baseline and the end of the 2-week run-in phase, and at baseline compared to the final week of the trial. A repeated measures general linear model will be used to test differences between the two trial arms across baseline and the 10 end-of-week measurements of the LC-score and numLCsym.

3.5.5 Adherence to the Fasting intervention will be tracked for each participant using self-reported assessment of whether the components of the intervention were followed each week of the study and will be reported as percentages.

3.6.5 Adverse events and major adverse events will be recorded for each participant and reported as percentages along with counts of events at each time point.

3.7.5 Analyses will use R Studio.

3.8.5 Exploratory analyses will examine other features of the data set

3.9.5 Subgroup analyses may be conducted using non-paired two-sided t-tests to identify statistically significant correlations between mean percent decreases in the primary measures and various baseline characteristics.

5.0 Limitations

1.1 Trial limitations include the lack of a parallel standard control arm, lack of randomization to just one treatment for each trial participant (due to the crossover design), a limited sample size, evolving definitions of Long COVID in the field, and a short duration of treatment by each intervention during the study.

2.1 Participant limitations include an open-label set of interventions conducted without blinding of participants to the treatment that they receive, self-selection for participation, and self-reporting of symptoms.

6.0 References

1.1 R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.