Title: Brain and Gut Plasticity in mild TBI or post-acute COVID Syndrome Following Growth Hormone Therapy

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Specific Aims

Increasing evidence suggests the gastrointestinal (GI) microbiome is altered in patients with history of mild traumatic brain injury (mTBI). In this project, we propose to characterize the GI microbiome in patients with a history of mTBI or post-acute COVID syndrome complex (PASC) in comparison to healthy controls. Furthermore, we propose to investigate the potential effects of recombinant Human Growth Hormone (rhGH, given as somatropin, a polypeptide hormone (GENOTROPIN)) on the GI microbiome, fatigue, cognition, brain activation, and brain morphology in individuals with a history of mTBI or PASC.

Specific Aim 1a. To characterize the GI microbiome in patients with a history of mTBI compared to controls. - mTBI Baseline vs. Control Baseline - Fecal samples for determination of the GI microbiome will be collected monthly from mTBI subjects and healthy controls over 6 months to examine the diversity of the microbiome within and between groups. **Outcomes**: Genus, species, q-PCR for selected marker organisms.

Specific Aim 1b. To characterize the GI microbiome in patients with a history of mTBI based on presence of *Prevotella spp*. – mTBI *Prevotella spp.* positive Baseline vs. mTBI *Prevotella spp.* negative Baseline - Fecal samples for determination of the GI microbiome will be collected monthly from mTBI subjects over 6 months to examine the diversity of the microbiome within and between groups. **Outcomes**: Genus, species, q-PCR for selected marker organisms

Specific Aim 2. To characterize the GI microbiome in patients with a history of post- COVID-19 syndrome (PASC) compared to arm 2 controls. - PASC Baseline vs. Control Baseline - Fecal samples for determination of the GI microbiome will be collected monthly from PASC subjects over 6 months to examine the diversity of the microbiome within and between groups. **Outcomes**: Genus, species, q-PCR for selected marker organisms.

Specific Aim 3. To determine whether growth hormone therapy-induced changes in fatigue and cognition correlate to changes in the GI microbiome. – mTBI/PASC Baseline (month 0) vs. mTBI/PASC Month 6 – Changes in GI microbiome of mTBI or PASC patients will be correlated to fatigue/cognition symptom resolution in response to growth hormone treatment. Healthy

controls will provide control data. Controls will not receive rhGH treatment. **Outcomes:** Changes in genus, species, Questionnaires (GSRS (GI), MoCA (cognition), BFI and MFSI (fatigue)).

Secondary Aims and Symptom-Related Outcomes and Assessment of Body Composition and Metabolism:

Specific Aim 4a. To determine whether growth hormone therapy alters mood, sleep quality, and quality of life. Mood, sleep, and quality of life will be measured at baseline (month 0) and month 6. (POMS (mood/QOL), QOL-AGHDA (QOL of growth hormone (GH) deficiency), PSQI (sleep), FPQ (food preferences)).

4b. To determine whether growth hormone therapy alters body composition and muscle function. Skeletal muscle mass, as derived from lean body mass determinations, and fat mass will be measured using dual energy x-ray absorptiometry (DEXA) at baseline (month 0) and month 6. (DEXA, Biodex, 6 min walk).

4c. To determine whether growth hormone therapy alters resting energy expenditure. Resting energy expenditure (REE) will be measured using indirect calorimetry at baseline (month 0) and month 6.

Background and Significance

Annually 1.5 million children and adults experience trauma to the head and brain that results in a traumatic brain injury (TBI). While TBI are classified as severe, moderate, or mild based upon the Glasgow Coma Scale (GCS), of the more than 1.5 million people who experience a TBI each year, as many as 90% sustain a mild TBI (mTBI) (1). mTBI can occur during organized sport (football, soccer), home recreation (bike riding, skateboarding), and as a result of a motor vehicle accident or other physical traumatic event. The term 'mild TBI' is misleading as we now know that a significant proportion of mTBI patients suffer from significant impairments or disabilities for many years following the initial brain injury. These include, "foggy brain," short-term memory loss, (1) impaired conversational abilities, and inability to organize daily activities. Together, this symptom complex renders many individuals unable to function in school or maintain a job.

Our research team has found evidence to suggest that TBI induces a chronic inflammatory process that renders a subset of the patients with pituitary dysfunction and abnormal GH secretion along with other metabolic abnormalities (impaired GI microbiome, impaired nutrient absorption). The clinical syndrome, which we have named **Brain Injury Associated Fatigue and Altered Cognition (BIAFAC),** associated with abnormal GH secretion is characterized by profound fatigue and cognitive dysfunction related to executive function, short-term memory, and processing speed index. Fatigue in abnormal GH secretion patients is profound and debilitating, results in increased sleepiness, with disturbed sleep. These patients are no longer able to maintain their usual activity levels and other hormone replacements such as testosterone do not improve their condition. While we and others are working to classify this clinical syndrome, the metabolic implications of TBI as a chronic inflammatory disease are unknown and need classifying to describe the syndrome complex.

Remarkably, our examination of the GI microbiome in patients with chronic TBI revealed significant differences in selected bacteria (both presence/absence as well as altered relative abundance in the community) suggesting a mechanism by which the gut/brain axis was impacted leading to the fatigue and cognitive dysfunction syndrome. Further, these altered bacterial

communities were associated with changes in amino acid absorption as indicated by blood levels in chronic TBI patients. These recent and exciting data suggest that TBI and its symptom complex persist for many years and with far-reaching metabolic health implications. Together, these data also suggest similar alterations in the GI microbiome in mTBI patients would be observed providing opportunities for both diagnostic and therapeutic improvements.

Our recent work has shown that cognitive and physical dysfunction are significantly improved with recombinant human growth hormone (rhGH) replacement in patients with abnormal GH secretion. The mechanisms causing fatigue and cognitive dysfunction need to be determined, but our brain imaging from functional MRI (fMRI) data suggests that brain connectivity is impaired in patients and rhGH may improve connectivity. In chronic TBI patients who have received rhGH treatments, it is not yet established if the GI microbiome is returned to a healthy profile observed in control cohorts. Our *overall hypothesis* is that baseline GI microbiome in mTBI will be significantly altered compared to household controls, and that rhGH therapy may result in symptom resolution in mTBI which may correlate with the change in GI microbiome.

Post-acute COVID Syndrome Complex (PASC)

The onset of the COVID-19 pandemic has led to a subset of patients that, once recovered from the acute infection, also experience an intractable and debilitating set of lingering symptoms termed post-COVID syndrome (PASC). The most common symptoms include anxiety, shortness of breath, continued loss of the sense of smell and taste, loss of appetite with subsequent weight loss, sleep difficulties, severe fatigue, cognitive dysfunction (foggy brain) and increased frailty. These patients frequently present to the emergency room looking for symptom management because they are unable to perform normal activities of daily living and maintain job performance. The mechanisms behind this syndrome have proven elusive because patients varied in symptom severity and treatment regimen including different requirements for hospitalization, supplemental oxygen, dexamethasone and remdesivir.

Persistent and long-lasting health problems are common in patients after COVID-19 infection. In a recent study of patients that had been hospitalized with COVID-19, two months after discharge, 87% reported at least one lingering symptom (joint pain, fatigue, breathing issues, etc), more than 50% reported more than three lingering issues, and over 40% reported a reduction in their of quality of life. Another study found that at 1-month after hospitalization for COVID-19, 74% reported persistent issues related to shortness of breath and a decrease in both physical and mental health. Preliminary data from our Post-COVID Recovery clinic agree with these two recent reports. In our study, 1 1/2 months after COVID-19 diagnosis, patients reported on average 10 of the 18 common symptoms (with 90% having chest pain, 87% dyspnea, 75% fatigue, and 90% with cognitive changes). While the previous studies examined patients that had severe COVID-19 infections, >50% of our patients were never hospitalized, yet have numerous persistent symptoms. This has serious implications for the ability of patients to return to work, downstream effects on mental health due to sometimes drastic lifestyle and work capacity changes, and the ability to engage in activities or hobbies enjoyed prior to COVID-19 illness. In a current study we aimed to characterize the baseline endocrine, metabolic, inflammatory and microbiome alterations in the post-COVID syndrome patients to better identify and manage the symptoms to

prevent potential long-term health consequences. Two post-COVID subjects have been studied and both subjects had low levels of growth hormone secretion, consistent with BIAFAC subjects. Fecal microbiome analysis of the subjects using the BIAFAC 96 well qPCR array showed striking similarity to BIAFAC subjects showing low abundance of *Prevotella spp* and an absence of *P. copri*.

Therapeutic Rationale

As described above, our research team has developed a novel therapeutic strategy using rhGH that we have shown to be efficacious at improving fatigue, skeletal muscle function, cognitive function, and now appears to be positively impacting the connection between different brain regions as measured with fMRI.

Growth hormone as a therapeutic strategy to improve the clinical syndrome and symptom complex in mTBI. The rationale behind treating mTBI and fatigue with rhGH is based upon the data that shows that individuals with a history of TBI exhibit evidence of growth hormone (GH) deficiency (2-6), including those military personnel with blast-related mTBI (2). This deficiency may be related to perceptual fatigue (2, 4-6), although not all studies show a significant relationship (7, 8). Our recently completed study will be the first that investigated, and demonstrated, the efficacy of rhGH replacement for the reduction of perceptual fatigue in mTBI patients with GH deficiency or insufficiency. Moreover, our previous data in moderate to severe GH-deficient TBI patients showed an improvement in cognitive function including processing speed index, short-term memory, and executive function (9), and our recently study in mTBI appears to improve cognition as well.

The gut-brain axis is disrupted in TBI patients, promoting alterations in the gut microbiome. There is a rapidly expanding field of study assessing the interaction between the immune system and the brain (10). One of the primary interests in this field of study is the interaction between the brain and the GI system centered on the microbiome present in the GI track (11, 12). Consistent with our ongoing hypothesis, a chronic inflammatory process in the brain could alter gut physiology and the GI microbiome in subjects with TBI resulting in abnormal amino acid absorption. Furthermore, mTBI is associated with alterations in GI metabolism and loss of tight junctions contributing to increased intestinal permeability, inflammation, and malabsorption (13, 14). In addition to contributing to chronic inflammation, disruption of the autonomic nervous following neurotrauma in mTBI patients can disrupt physiology of organs throughout the body including intestinal contractility (15-17). Reductions in gut mobility in turn disturbs the existing balance of the microflora and promotes small intestinal bacterial overgrowth contributing to dysbiosis (18). Regardless of the underlying mechanisms driving alterations in the gut-brain-microbiota axis, it is yet unknown how microflora of mTBI patients compared to that of healthy individuals.

Growth hormone as a therapeutic strategy to improve the clinical syndrome and symptom complex in PASC.

Notably, the cluster of symptoms associated with post-COVID syndrome include profound fatigue and cognitive dysfunction, which are strikingly consistent with BIAFAC. While studies are underway to understand the details of the mechanism causing BIAFAC and why GH treatment alleviates symptoms in these patients, we are intrigued that the symptom phenotype with post-COVID patients overlaps with many BIAFAC symptoms. It is possible that post-COVID syndrome may be addressed through similar treatment strategies including the potential for prebiotic/probiotic enhancement of microbiome health.

Therefore, in the current study we will assess the GI microbiota in individuals with a history of mTBI or PASC and fatigue. Each participant's baseline will serve as an individual control. An additional and critical control dataset that will be used for comparisons will be provided by analysis of the GI microbiome from healthy controls. Furthermore, we will investigate whether treatment with recombinant Human Growth Hormone (rhGH) will create changes in the gut flora producing microbiomes more similar to those expected in healthy controls and whether these changes correlate with the expected improvements in fatigue, cognition, brain activation, and brain morphology in the mTBI or PASC patients.

Experimental Design and Methods

Enrollment

TBI subjects: To address **AIM 1a** and **AIM 1b** we will study subjects with a history of mild TBI enrolled into either **arm 1** (n=25) or **arm 2** (n=25) for a total of (**n=50**).

Healthy Controls: To address **AIM 1** and **AIM 3** we will study healthy control subjects enrolled into either **arm 1 (**n=25) or **arm 2** (n=25) for a total of **(n=50**).

PASC subjects: To address **AIM 3** we will study subjects with a history of COVID-19 and PASC symptoms enrolled into **arm 3** (**n=25**).

ARM 1: mTBI and Household Controls

Subjects. We will study subjects (aged 18-70 years) with a history of mild TBI (n=25) and their household controls (aged 18 years and up) (n=25). mTBI subjects will undergo a 6-month intervention of rhGH therapy (See Figure 1). Household controls will be asked to participate in questionnaires, blood draws (amino acid analysis), and fecal sampling (GI microbiome analysis). Household controls will not receive any growth hormone treatment.

mTBI group

All patients presenting with a prior mTBI will undergo a phone pre-screen including the Brief Fatigue Inventory (BFI) questions 1-3. If they score ≥ 3 on any BFI questions 1-3, and are interested in participating in the study, they will be scheduled for a formal consenting and medical screening at the UTMB Clinical Research Center (CRC). During the medical screening, eligibility will be confirmed and a glucagon stimulation test (GST) will be performed. A glucagon stimulation with a growth hormone peak of <10 ng/mL is required to qualify for enrollment. This is the range we have used in our previous studies to be inclusive of all subjects who have a less than normal (>10 ng/mL) response to GST. In our previous studies, patients with a response to the GST in the 3 – 10 ng/mL range have benefited from GH therapy as much as those with a less than 3 ng/mL response.

Household control group

All household controls will undergo a phone pre-screen and if interested in participating in the study, will be scheduled for a formal consenting and medical screening at the UTMB Clinical Research Center (CRC).

ARM 2: mTBI and Healthy Matched Controls

Subjects. We will study subjects (aged 18-70 years) with a history of mild TBI (n=25) and healthy age/sex matched controls (aged 18-70 years) (n=25). mTBI subjects will undergo a 6-month intervention of rhGH therapy (**See Figure 2**). Healthy matched controls will be asked to participate in questionnaires, blood draws (amino acid analysis), and fecal sampling (GI microbiome analysis). Healthy controls will not receive any growth hormone treatment.

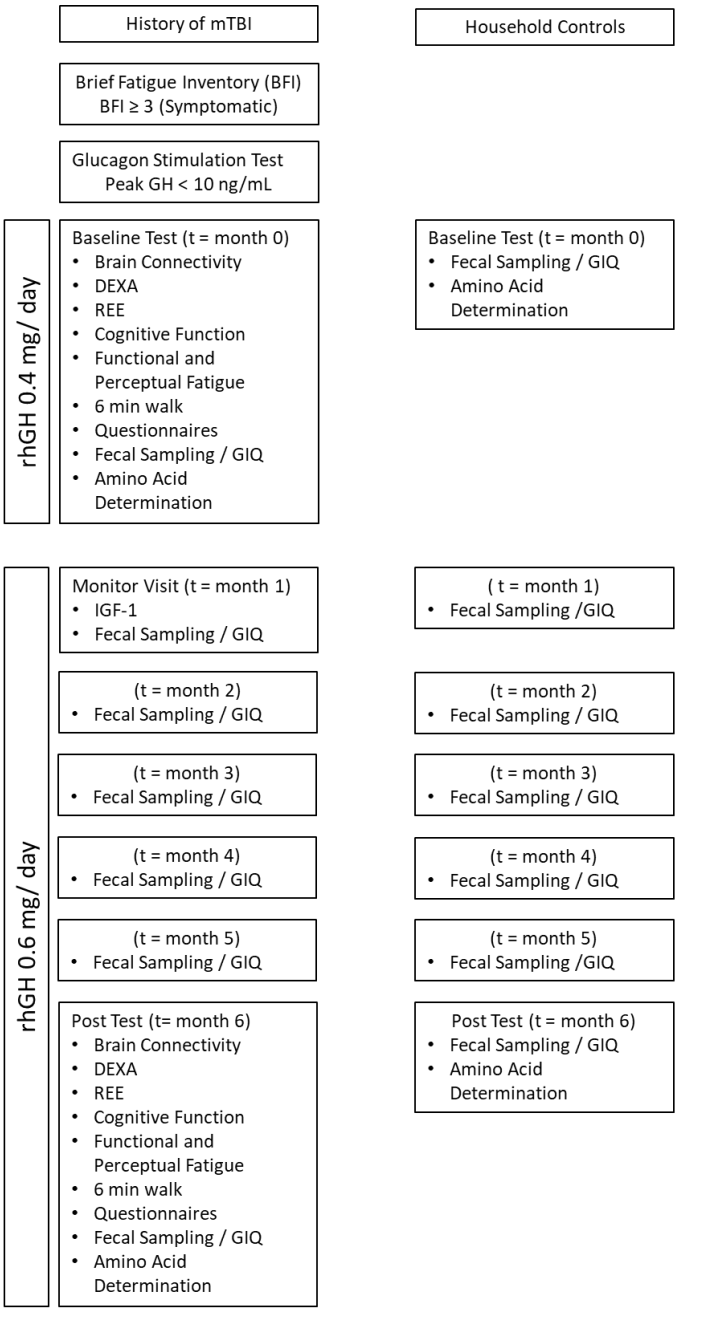

Figure 1. ARM 1: Study Design. Patients with a history of mTBI, fatigue of ≥3 on the BFI and GH <10 ng/mL will undergo 6 months of GH replacement therapy. Household controls will also be recruited for each mTBI patient. Both mTBI and control subject sets will collect fecal samples and fill out a GI questionnaire (GIQ) monthly over the study period.

mTBI group

All patients presenting with a prior mTBI will undergo a phone pre-screen including the Brief Fatigue Inventory (BFI) questions 1-3. If they score ≥ 3 on any BFI questions 1-3, and are interested in participating in the study, they will be scheduled for a formal consenting and medical screening at the UTMB Clinical Research Center (CRC). During the medical screening, eligibility will be confirmed and a glucagon stimulation test (GST) will be performed. A glucagon stimulation with a growth hormone peak of <10 ng/mL is required to qualify for enrollment. This is the range we have used in our previous studies to be inclusive of all subjects who have a less than normal (>10 ng/mL) response to GST. In our previous studies, patients with a response to the GST in the 3 – 10 ng/mL range have benefited from GH therapy as much as those with a less than 3 ng/mL response.

Healthy control group

Healthy controls will undergo a phone pre-screen and if interested in participating in the study, will be scheduled for a formal consenting and medical screening at the UTMB Clinical Research Center (CRC).

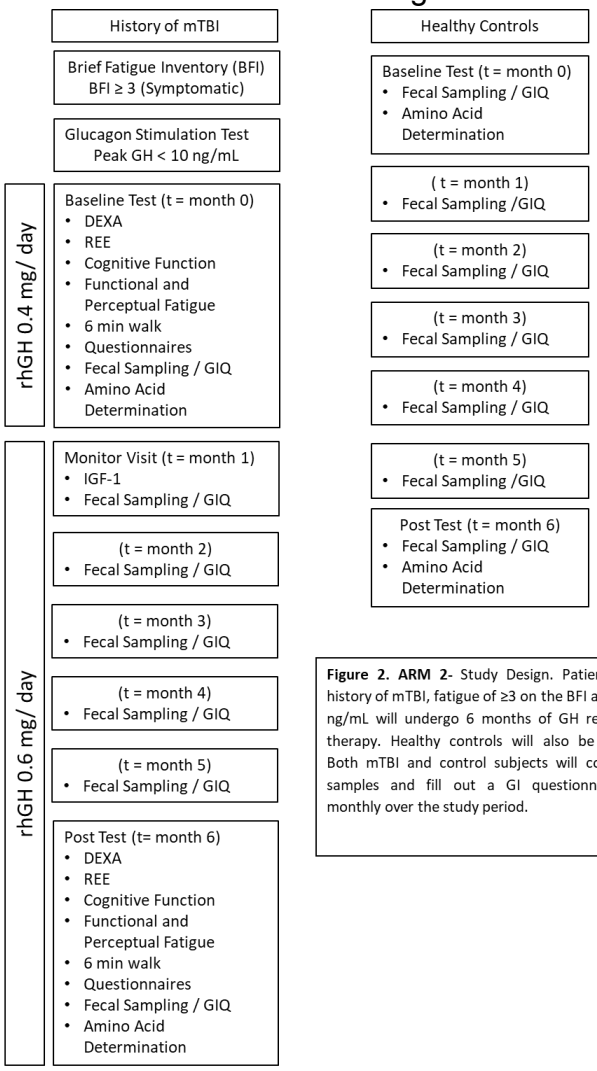

Figure 2. ARM 2- Study Design. Patients with a history of mTBI, fatigue of ≥3 on the BFI and GH <10 ng/mL will undergo 6 months of GH replacement therapy. Healthy controls will also be recruited. Both mTBI and control subjects will collect fecal samples and fill out a GI questionnaire (GIQ) monthly over the study period.

ARM 3: PASC

Subjects. We will study subjects (ages 18 - 70 years) with a history of COVID (n=25). PASC subjects will undergo a 9-month intervention of rhGH therapy (**See Figure 3**).

PASC group

All patients presenting with PASC will undergo a phone pre-screen including the Brief Fatigue Inventory (BFI) questions 1-3. If they score ≥ 3 on any BFI questions 1-3, and are interested in participating in the study, they will be scheduled for a formal consenting and medical screening at the UTMB Clinical Research Center (CRC). During the medical screening, eligibility will be confirmed and a glucagon stimulation test (GST) will be performed. A glucagon stimulation with a growth hormone peak of <10 ng/mL is required to qualify for enrollment. This is the range we have used in our previous studies to be inclusive of all subjects who have a less than normal (>10 ng/mL) response to GST. In our previous studies, patients with a response to the GST in the 3 – 10 ng/mL range have benefited from GH therapy as much as those with a less than 3 ng/mL

response. Patients with GST scores higher than 10 ng/ml will not be included in the treatment study, but may be asked to collect a single fecal sample (omnigene and viable).

See Protection of Human Subjects - Recruitment Methods and Consenting Process for detailed information regarding recruitment and consenting.

Experimental Protocol.

mTBI Group (Arm 1)

Before and at completion (month 6) of the rhGH intervention, mTBI subjects will report to the UTMB Institute for Translational Sciences (ITS) Clinical Research Center (CRC) for testing, which will consist of cognitive function assessment (MoCA), determination of brain morphology and connectivity using fMRI, determination of lean mass and fat mass using DEXA, resting energy expenditure, functional testing including leg strength and 6 minute walk test, fatigue measurements, and questionnaires of mood and quality of life, gastrointestinal health, sleep, and food preferences. Blood sampling for measurement of amino acid levels, hormones, and metabolites will be drawn before and 90 minutes (+/- 10 minutes) after a standardized meal. In addition, fecal samples for analysis of the GI microbiome will be collected monthly for the duration of the study. Insulin Growth Factor 1 (IGF-1) levels will monitored at a month 1 safety visit for all mTBI subjects. The month 1 safety visit will occur +/- 5 days from the expected date based on the baseline study visit. The monthly fecal sampling will occur +/- 7 days from the expected date based on the baseline study visit. The month 6 post study will occur +/- 7 days from the expected date based on the baseline study visit. A member of the study team will contact the subject each month to coordinate fecal sample transport to UTMB, check for adverse events and overall well-being, and ensure compliance and ongoing consent. If a mTBI subject sustains and additional TBI during the study, the study for both the mTBI and household control subject will be extended to collect monthly fecal samples and questionnaires for an additional 6 months. Genotropin will be provided for the additional 6 months.

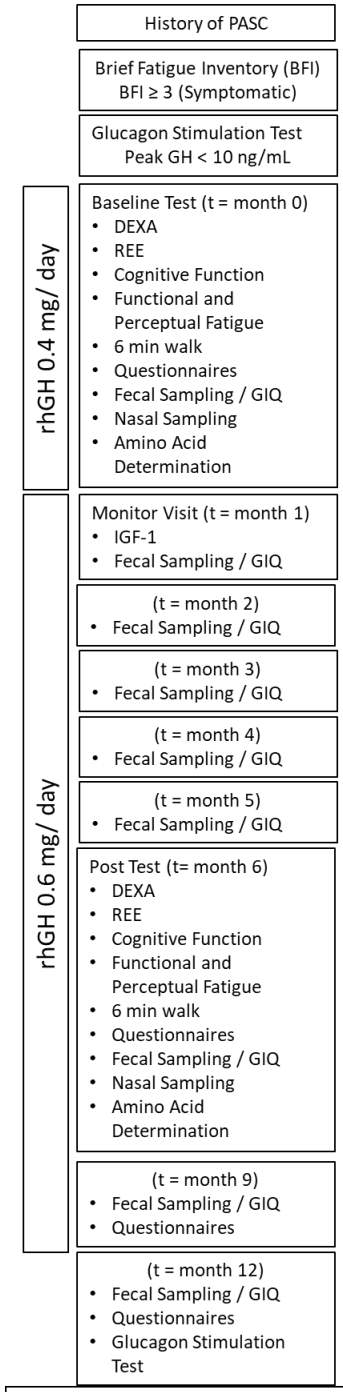

Figure 3. ARM 3: Study Design. Patients with a history of PASC, fatigue of ≥3 on the BFI and GH <10 ng/mL will undergo 9 months of GH replacement therapy. Post study measures will take place at month 6. Subjects will collect fecal samples and fill out a GI questionnaire (GIQ) at months 0, 1, 2, 3, 4, 5, 6, 9 and 12. Subjects will repeat the glucagon stimulation test at month 12. .

Household control group (Arm 1)

Before and at completion (month 6) of the rhGH intervention of their mTBI subject partners, household controls will report to the UTMB Institute for Translational Sciences (ITS) Clinical Research Center (CRC) for testing, which will consist of questionnaires of food preferences and gastrointestinal health and blood sampling for measurement of amino acid levels, hormones, and metabolites which will be drawn before and 90 minutes (+/ 10 minutes) after a standardized meal. In addition, fecal samples for the analysis of the GI microbiome will be collected monthly for the duration of the study, as well as questionnaire assessing gastrointestinal health. The monthly fecal sampling

will occur +/- 7 days from the expected date based on the baseline study visit. The post study (month 6) will occur +/- 7 days from the expected date based on the baseline study visit. A member of the study team will contact the subject each month to coordinate fecal sample transport to UTMB, check for adverse events and overall well-being, and ensure compliance and ongoing consent. If the mTBI subject sustains and additional TBI during the study, the study for both the mTBI and household control subject will be extended to collect monthly fecal samples and questionnaires for an additional 6 months.

mTBI Group (Arm 2)

Before and at completion (month 6) of the rhGH intervention, mTBI subjects will report to the UTMB Institute for Translational Sciences (ITS) Clinical Research Center (CRC) for testing, which will consist of cognitive function assessment (MoCA), determination of lean mass and fat mass using DEXA, resting energy expenditure, functional testing including leg strength and 6 minute walk test, fatigue measurements, and questionnaires of mood and quality of life, gastrointestinal health, sleep, and food preferences. Blood sampling for measurement of amino acid levels, hormones, and metabolites will be drawn before and 90 minutes (+/- 10 minutes) after a standardized meal. In addition, fecal samples for analysis of the GI microbiome will be collected monthly for the duration of the study. Insulin Growth Factor 1 (IGF-1) levels will monitored at a month 1 safety visit for all mTBI subjects. The month 1 safety visit will occur +/- 5 days from the expected date based on the baseline study visit. The monthly fecal sampling will occur +/- 7 days from the expected date based on the baseline study visit. The month 6 post study will occur +/- 7 days from the expected date based on the baseline study visit. A member of the study team will contact the subject each month to coordinate fecal sample transport to UTMB, check for adverse events and overall well-being, and ensure compliance and ongoing consent. If the mTBI subject sustains and additional TBI during the study, the study will be extended to collect monthly fecal samples and questionnaires for an additional 6 months. Genotropin will be provided for the additional 6 months.

Healthy control group (Arm 2)

Healthy control subjects will report to the UTMB Institute for Translational Sciences (ITS) Clinical Research Center (CRC) for testing at baseline and month 6. Testing will consist of questionnaires of food preferences and gastrointestinal health and blood sampling for measurement of amino acid levels, hormones, and metabolites which will be drawn before and 90 minutes (+/ 10 minutes) after a standardized meal. In addition, fecal samples for the analysis of the GI microbiome will be collected monthly for the duration of the study, as well as questionnaire assessing gastrointestinal health. The monthly fecal sampling will occur +/- 7 days from the expected date based on the baseline study visit. The post study (month 6) will occur +/- 7 days from the expected date based on the baseline study visit. A member of the study team will contact the subject each month to coordinate fecal sample transport to UTMB, check for adverse events and overall well-being, and ensure compliance and ongoing consent.

PASC Group (Arm 3)

PASC subjects will undergo 9 months of rhGH treatment. Before and at (month 6) of the rhGH intervention, PASC subjects will report to the UTMB Institute for Translational Sciences (ITS) Clinical Research Center (CRC) for testing, which will consist of cognitive function assessment (MoCA), determination of lean mass and fat mass using DEXA, resting energy expenditure, functional testing including leg strength and 6 minute walk test, fatigue measurements, and questionnaires of mood and quality of life, gastrointestinal health, sleep, and food preferences. Blood sampling for measurement of amino acid levels, hormones, and metabolites will be drawn before and 90 minutes (+/- 10 minutes) after a standardized meal. In addition, fecal samples for

analysis of the GI microbiome will be collected monthly for the duration of the study. Insulin Growth Factor 1 (IGF-1) levels will monitored at a month 1 safety visit for all PASC subjects. The month 1 safety visit will occur +/- 5 days from the expected date based on the baseline study visit. The fecal sampling will occur +/- 7 days from the expected date based on the baseline study visit at months 0, 1, 2, 3, 4, 5, 6, 9 and 12. The month 6 study will occur +/- 7 days from the expected date based on the baseline study visit. The month 9 study will consist of fecal sample and questionnaires only. At month 12, the subject will return to the ITS-CRC to repeat the glucagon stimulation test (GST). Questionnaires and a fecal sample will also be collected at this time. A member of the study team will contact the subject each month to coordinate fecal sample transport to UTMB, check for adverse events and overall well-being, and ensure compliance and ongoing consent. If a PASC subject is infected with COVID again during the study months 9 - 12, the study will be extended to collect fecal samples and questionnaires for an additional 3 months.

Therapeutic Intervention (for mTBI subjects only - Arms 1&2; PASC subjects – Arm 3). Recombinant human growth hormone (rhGH), (GENOTROPIN). Growth hormone (GH) is produced endogenously by the body and is released by the pituitary. Therapeutic rhGH is FDA approved for clinical use for replacement of endogenous growth hormone in children and adults with growth hormone deficiency. GENOTROPIN, the agent to be used in this study is manufactured by Pfizer Inc and contains somatropin, which is a polypeptide hormone of recombinant DNA origin. It contains 191 amino acid residues and is identical to human growth hormone of pituitary origin.

GENOTROPIN (5 mg) is dispensed in a two-chamber cartridge. The front chamber contains recombinant somatropin (5.8 mg), glycine (2.2 mg), mannitol (1.8 mg), sodium dihydrogen phosphate anhydrous (0.32 mg), and disodium phosphate anhydrous (0.31 mg); the rear chamber contains 0.3% m-Cresol (as a preservative) and mannitol (45 mg) in 1.14 mL water for injection. The reconstituted concentration is 5 mg/ml. GENOTROPIN will be administered utilizing the GENOTROPIN Pen 5 (manufactured by Pfizer, Inc). The GENOTROPIN Pen 5 is a reusable multi-dose pen that the two-chambered cartridge is inserted into. Once the cartridge is inserted into the pen, the two-chambers are mixed. A 31 gauge, ½ inch needle is attached to the pen for administration. The pen can then be dialed on the digital display to the correct dose and administered by the user. The needle is then carefully removed and disposed in a sharps medical waste container provided by the study team. Once the cartridge is empty, a new cartridge can be inserted into the pen, a 5 mg cartridge will last 1 – 2 weeks depending on the dose used. The GENOTROPIN Pen 5 is designed to be easily used, as most GH replacement is for pediatric use. Subjects will undergo a familiarization session with the study team to learn how to load the GENOTROPIN5 cartridges into the GENOTROPIN Pen 5 device, how to dial their dose, and how to administer injections. They will also be given a guide and the web address (**http://www.genotropin.com/genotropin-helpful-tools)** of a video prepared by the makers of GENOTROPIN to take home for reference.

In this study, single injections containing 0.4 mg of GENOTROPIN will be administered to mTBI and PASC subjects on a daily basis until the IGF-1 safety check at month 1. If the IGF-1 value is less than 400 ng/mL, the subject will be directed to increase the dose to 0.6 mg per day for the remainder of the study. 0.6 mg per day is the standard dose given in our TBI research studies. We have treated approximately 60 TBI research subjects over approximately 14 years at this dose without incident. Dr. Urban also has approximately 100 patients in his clinic being treated at 0.6

mg per day. At this dose of GH replacement, there is no evidence of insulin resistance and as long as the IGF-1 remains at or below 400 ng/mL, there is minimal risk of carpal tunnel syndrome.

GENOTROPIN and the GENOTROPIN Pen 5 will be supplied by Pfizer, with allocation managed by the Investigational Drug Service (IDS) of the UTMB Department of Pharmacy. Any unused drug will be disposed of by the IDS of the UTMB Department of Pharmacy according to standard drug destruction procedures. If a missed dose is identified on the day it is to be given, the subject will be instructed to inject the normal daily dose as soon as possible. For example, the subject usually gives their injection in the morning, but forgets and then remembers in the afternoon. If a missed dose is identified after the normal dosing day, the subject will be instructed to count that day as a missed day and resume their normal dosing schedule. For example, the subject remembers on Tuesday that they missed their dose on Monday. Subjects will be instructed not to take more than one dose a day.

Screening Procedures for mTBI subjects: All mTBI subjects consenting to be screened will undergo the following procedures. mTBI subjects will be fasted for at least 10 hours prior to blood work. Screenings will take place at the UTMB Clinical Research Center (CRC). Differences in procedures between Arm 1 and Arm 2 will be noted.

- Vital signs. Vital signs will be collected at screening. This will include height, weight, blood pressure, pulse, respirations, and temperature. Vital signs will be recorded on the screening CRF.

- Urine Pregnancy Test. Urine will be collected from female subjects for a urine pregnancy test to be performed at the UTMB CRC as POCT. Women who are post-menopausal will be exempt from this test.

- Phlebotomy. mTBI subjects will have a catheter placed into a peripheral vein in their arm (0.9% sodium chloride set at TKO) for the glucagon stimulation test and the screening labs. The IV will be discontinued when glucagon stimulation test is complete.

- Electrocardiogram (EKG). mTBI subjects will have an EKG during screening. The EKG will take place at the CRC, performed by UTMB Cardiology staff and read by UTMB Cardiology Faculty, and will be entered into the subjects UTMB medical chart.

- Screening Blood Labs. mTBI subjects will have the following labs drawn for screening. Approximately 20 ml of blood will be drawn for screening labs listed below. The labs will be sent to the UTMB clinical laboratory for analysis and entered into the subjects UTMB medical chart:

    - Complete blood count with differential (CBC w/ diff)
    - Comprehensive metabolic panel (CMP)
    - Lipid Panel
    - Hepatitis B surface antigen
    - Hepatitis C virus antibody
    - HIV screen
    - Thyroid-stimulating hormone (TSH)
    - Prolactin
    - Testosterone Total (men only)

- Insulin-like Growth Factor 1 (IGF-1)
- Cortisol

- Glucagon stimulation test. mTBI subjects will have a glucagon stimulation test. After the IV is placed, 3.5 ml of blood will be collected for the baseline (time: 0 minutes) to test for levels of human growth hormone. 1 mg glucagon (for subjects over 90 kg, 1.5 mg glucagon) will be injected IM in the deltoid muscle of the subject. Additional blood samples (3.5 mL) will be collected to test for levels of human growth hormone at time points: 90 minutes, 120 minutes, 150 minutes, and 180 minutes. All blood samples will be sent to UTMB clinical laboratory for analysis and entered into subjects UTMB medical chart.

- History and Physical. mTBI subjects will undergo a medical history and physical examination by a study nurse or physician.

Screening Procedures for PASC subjects: All PASC subjects consenting to be screened will undergo the following procedures. Subjects will be fasted for at least 10 hours prior to blood work. Screenings will take place at the UTMB Clinical Research Center (CRC).

- Vital signs. Vital signs will be collected at screening. This will include height, weight, blood pressure, pulse, respirations, and temperature. Vital signs will be recorded on the screening CRF.

- Urine Pregnancy Test. Urine will be collected from female subjects for a urine pregnancy test to be performed at the UTMB CRC as POCT. Women who are post-menopausal will be exempt from this test.

- Phlebotomy. Subjects will have a catheter placed into a peripheral vein in their arm (0.9% sodium chloride set at TKO) for the glucagon stimulation test and the screening labs. The IV will be discontinued when glucagon stimulation test is complete. This may be omitted if subject has recent (within 6 months) glucagon stimulation test on file.

- Electrocardiogram (EKG). Subjects will have an EKG during screening. The EKG will take place at the CRC, performed by UTMB Cardiology staff and read by UTMB Cardiology Faculty, and will be entered into the subjects UTMB medical chart.

- Screening Blood Labs. Subjects will have the following labs drawn for screening. Approximately 20 ml of blood will be drawn for screening labs listed below. The labs will be sent to the UTMB clinical laboratory for analysis and entered into the subjects UTMB medical chart. Labs may be omitted if subject has recent (within 6 months) labs on file.

    - Complete blood count with differential (CBC w/ diff)
    - Comprehensive metabolic panel (CMP)
    - Lipid Panel
    - Hepatitis B surface antigen
    - Hepatitis C virus antibody
    - HIV screen
    - Thyroid-stimulating hormone (TSH)
    - Prolactin
    - Testosterone Total (men only)
    - Insulin-like Growth Factor 1 (IGF-1)

- Cortisol

- Glucagon stimulation test. Subjects will have a glucagon stimulation test. After the IV is placed, 3.5 ml of blood will be collected for the baseline (time: 0 minutes) to test for levels of human growth hormone. 1 mg glucagon (for subjects over 90 kg, 1.5 mg glucagon) will be injected IM in the deltoid muscle of the subject. Additional blood samples (3.5 mL) will be collected to test for levels of human growth hormone at time points: 90 minutes, 120 minutes, 150 minutes, and 180 minutes. All blood samples will be sent to UTMB clinical laboratory for analysis and entered into subjects UTMB medical chart. GST may be omitted if subject has recent (within 6 months) GST on file.

- History and Physical. Subjects will undergo a medical history and physical examination by a study nurse or physician. Recent (within 3 months) clinical history and physical may be used in place.

Screening procedures for household controls (Arm 1) and healthy controls (Arm 2): All control subjects consenting to be screened will undergo the following procedures. Subjects will be fasted for at least 10 hours prior to blood work. Screenings will take place at the UTMB Clinical Research Center (CRC).

- Vital signs. Vital signs will be collected at screening. This will include height, weight, blood pressure, pulse, respirations, and temperature. Vital signs will be recorded on the screening CRF.

- Urine Pregnancy Test. Urine will be collected from female subjects for a urine pregnancy test to be performed at the UTMB CRC as POCT. Women who are post-menopausal will be exempt from this test.

- Phlebotomy. Household control subjects will have blood taken using a 21-gauge butterfly needle.

- Screening Blood Labs. Household controls will have the following labs drawn for screening. Approximately 20 ml of blood will be taken for screening labs listed below. The labs will be sent to the UTMB clinical laboratory for analysis and will be entered into subjects UTMB medical chart:

    - Complete blood count with differential (CBC w/ diff)
    - Comprehensive metabolic panel (CMP)
    - Lipid Panel
    - Thyroid-stimulating hormone (TSH)
    - Hepatitis B surface antigen
    - Hepatitis C virus antibody
    - HIV screen

- History and Physical. Household control subjects will undergo a medical history and physical examination by a study nurse or physician.

Study Procedures for mTBI subjects (Arm 1 & 2) / PASC subjects (Arm 3):

mTBI and PASC subjects will complete all procedures listed below. Differences in procedures between Arm 1, Arm 2 and Arm 3 will be noted.

- Vital signs. Vital signs will be collected at all study visits. This will include height, weight, blood pressure, pulse, respirations, and temperature. Vital signs will be recorded on the appropriate study visit CRF.

- Blood draw. Subjects will have blood drawn at the baseline (month 0), safety check (month 1), post-study (month 6) and final study (month 12 – Arm 3 PASC only) visits. Blood will be drawn using a butterfly needle from a peripheral vein in the arm.

    - Baseline Visit (Month 0):
      Up to 15 ml of blood (1-serum separator top (5ml); 1-lithium heparin (6ml); 1-K-EDTA (4ml)) will be collected from fasted (at least 10 hours) subjects for analysis of hormones, amino acids and metabolites. In addition, up to 13ml of blood (1-serum separator top (5ml); 1-lithium heparin (6ml)) will be collected after a standardized meal for the analysis of amino acids.

    - Safety Check Visit (Month 1):
      Approximately 3.5 ml of blood for Insulin-like growth factor 1 (IGF-1) will be collected. Blood will be sent to UTMB clinical laboratory for analysis and documented in the subject's UTMB medical chart.

    - Post Visit (Month 6):
      Up to 15 ml of blood (1-serum separator top (5ml); 1-lithium heparin (6ml); 1-K-EDTA (4ml)) will be collected from fasted (at least 10 hours) subjects for analysis of hormones, amino acids and metabolites. In addition, up to 13ml of blood (1-serum separator top (5ml); 1-lithium heparin (6ml)) will be collected after a standardized meal for the analysis of amino acids.

      An additional 20 ml of blood will be collected for the following labs to be sent to the UTMB clinical laboratory for analysis (all labs run at UTMB will be documented in the subject's UTMB medical chart):
        - Complete blood count with differential (CBC w/ diff)
        - Comprehensive metabolic panel (CMP)
        - Lipid Panel
        - Insulin-like Growth Factor 1 (IGF-1)

    - Final Visit (Month 12) – Arm 3 PASC only:
      Up to 15 ml of blood (1-serum separator top (5ml); 1-lithium heparin (6ml); 1-K-EDTA (4ml)) will be collected from fasted (at least 10 hours) subjects for analysis of hormones, amino acids and metabolites.

      An additional 20 ml of blood will be collected for the following labs to be sent to the UTMB clinical laboratory for analysis (all labs run at UTMB will be documented in the subject's UTMB medical chart):
        - Complete blood count with differential (CBC w/ diff)
        - Comprehensive metabolic panel (CMP)
        - Lipid Panel
        - Insulin-like Growth Factor 1 (IGF-1)

- Urine Pregnancy Test. Urine will be collected from female subjects for a urine pregnancy test prior to DEXA scan. Urine pregnancy test will be performed at the UTMB CRC as POCT. Women who are post-menopausal will be exempt from this test.

- Body Composition. Lean body and fat mass will be measured using DEXA (GE iDEXA) in conjunction with published equations (Wang, 1999). The whole-body scan will be divided into the following sub-regions: truncal area, head, right and left arm, and right and left leg. DEXA will be measured at baseline (month 0) and post (month 6).

- Indirect calorimetry for Resting Energy Expenditure (REE). Expired gases will be collected and analyzed for O_2 and CO_2 concentrations for the determination of energy expenditure and substrate oxidation (19) in mTBI patients using the ITS-CRC indirect calorimetry system, Vmax Encore, Carefusion. The subject will be asked to lie down on a bed and the ventilated canopy hood will be placed over their head and shoulders. The room will be darkened and external noises will be eliminated, as much as possible. The subject will be asked to relax and remain awake but as still as possible for the testing period of 30 minutes. REE will be measured at baseline (month 0) and post (month 6).

- Amino Acid Levels. Circulating blood amino acid levels will be measured before (fasting) and 90 minutes (+/- 10 minutes) after a standardized meal. Amino acids in response to a meal will be measured at baseline (month 0) and post (month 6).

- Functional MRI (fMRI) – Arm 1 only. Functional MRI will be assessed at UTMB's Radiology and analyzed as in previous studies. mTBI patients will be asked to arrive fasting (at least 10 hours) for the scan and will be directed to remove all metal items from their body. They will also fill out the UTMB MRI safety checklist. Before the scan begins patients will be asked to relax and clear their minds as much as possible and to stay awake. The total time at MRI will take approximately 30 – 45 minutes. 3 scans will be performed. Scans will be read by UTMB Radiology faculty and documented in the subject's UTMB medical chart. fMRI will be measured at baseline (month 0) and post (month 6).

  Scan 1. Structural MRI scan. High-resolution anatomical images covering the whole brain including the cerebellum will be acquired using a T1-weighted gradient-echo pulse sequence with the following parameters: 3D T1 axial overlay (TR=8.9 ms, TE=1.8 ms, flip angle=15°, FOV=260 x 260 mm, slice thickness=1.4 mm, 124 slices; matrix=256 × 160).

  Scan 2. "Resting state" functional MRI scan. FMRI data will be collected using a single-shot gradient-echo (GRE) reverse spiral pulse sequence [128] to acquire 240 T2* -weighted BOLD images (TR = 2 s, TE = 30 ms, flip angle =90°, FOV = 220 x 220 mm, voxel size = 3.4 x 3.4 x 3.2 mm, 40 axial slices). Participants will be instructed to keep their eyes closed but to remain awake and to not think about anything in particular. A pressure belt will be used to record the respiratory signal and a pulse oximeter will be placed on the participant's finger to record the cardiac signal, both of which will be regressed out prior to data analyses.

  Scan 3. Diffusion tensor imaging scan. Diffusion tensor images will be collected using a single shot echo planar sequence (39 slices, slice thickness: 3 mm; TE/TR: 82.8 ms/9000 ms, field of view: 220 mm x 220 mm). Diffusion gradients will be applied in 30 directions

(b = 800 s/mm^2) (4 averages). Eight reference images (b = 0 s/mm^2) will be acquired [129] and averaged. We will calculate the diffusion tensor using the averaged images with b = 0 and b = 800 s/mm^2.

- Cognitive Assessment. Subjects will undergo a cognitive assessment at baseline (month 0) and post (month 6).

    - Montreal Cognitive Assessment (MoCA). The Montreal Cognitive Assessment (MoCA) (20) is a rapid assessment of cognition. The MoCA consists of 9 questions with the following subcategories: visuospatial/executive, naming, memory, language, abstraction, delayed recall and orientation. The MoCA has been used extensively to detect cognitive impairment in many conditions, including head trauma. Version 7.1 will be used at the Month 0 testing and Version 7.2 will be used at the Month 6 testing.

GI microbiome. The GI microbiome will be assessed using fecal samples collected at monthly time points (baseline (month 0), month 1, month 2, month 3, month 4, month 5, and month 6). Additional fecal samples will be collected for Arm 3 PASC subjects at month 9 and month 12. If a mTBI subject sustains an additional TBI during the study period, the study will be extended to collect fecal samples for an additional 6 months. If a PASC subject is infected with COVID again during the study months 9 - 12, the study will be extended to collect an additional sample at month 13 and month 15. Subjects will provide fecal samples as described below. Fecal samples will be collected +/- 7 days from specified time point based on baseline (month 0) study. Subjects will be allowed to collect monthly fecal samples at home. Samples will be stored at 4°C until transferred to UTMB (on/+ 3 days after collection). Subjects will be given a tutorial on how to properly collect the fecal sample. They will be given a handout from the manufacturer of the collection kit with step by step instructions. They will also be shown a video of the collection process supplied by the kit manufacturer. https://www.youtube.com/watch?v=ytr_hmJdHqM&feature=youtu.be&rel=0

Fecal specimens will be collected using the OMNIgene-Gut® (OMR-200) collection kit from DNA genotek (please see product inserts attached as pdf files). To assist in collection, the additional toilet accessory will be employed where appropriate (OM-AC1). The manufacturer's instructions will guide sample collection. Briefly, fecal material will be collected by catch on a kit-provided tissue or toilet hat that has been wiped with a disinfecting wipe prior to use. The study participant's fecal material will be transferred using the kit included spatula into the yellow tube top of a collection tube with the purple cap unscrewed. The tube will then be re-capped tightly and shaken for a minimum of 30 seconds to mix feces with kit included stabilization liquid. Storage recommendations as per manufacturer allow up to 60 days at room temperature or up to several months at -20°C or -80°C in the kit provided collection tube. Samples will be labeled with the subject's unique code and stored at 4°C until transported to University of Texas Medical Branch (UTMB). Samples will be processed and analyzed by qPCR as described below.

Nucleic acids will be extracted from batched fecal specimens using a DNeasy PowerMicrobiome kit (2600-50-1, Qiagen). If necessary, samples will be diluted/mixed 1:1 with sterile PBS to yield a pipettable liquid slurry. For each sample, 100 µl of the fecal liquid fraction will be mixed with 200 µl of kit-provided lysis buffer into a commercially available tube

containing 0.1 mm glass beads (2600-50-BT, Qiagen). Samples will then be heated at 55°C for 30 minutes. Subsequently, samples will be homogenized for 5 minutes at 30 Hz using a Tissue Lyser instrument (Qiagen). Samples then will be centrifuged at 13,000 x g for 1 minute at room temperature. The liquid fraction will then be placed into a sterile microtube along with 50 µl of IRS solution (2600-50-2, Qiagen) and incubated at 4°C for 5 minutes. The sample will then be centrifuged as before and the liquid fraction taken for extraction of DNA using a MagnaPure 96 automated nucleic acid extraction platform (Roche). Purified DNA will be used for next-generation sequencing and qPCR analyses to create molecular profiles for each microbiome. Residual DNA will be archived at -20°C.

An additional collection will be performed at month 0 and month 6 to collect viable fecal material. Fecal samples will be collected using the spatula included in the OMNIgene-Gut kit used in the first collection. Briefly, after the participant will transfer fecal material to the yellow top of the collection tube for the first collection, then they will scoop another small amount of fecal material with the same spatula and transfer it to a prepared cryogenic freezer tube containing sterile PBS (without Ca/Mg) and sterile glycerol. The tube will be closed and shaken for a minimum of 30 seconds to mix with the stabilization liquid. Samples will be labeled with the subject's unique code and stored at 4°C until transported to University of Texas Medical Branch. Samples will then be frozen at -80°C and stored until the molecular analysis is complete. After initial analysis, if the sample is considered useful (representative of the average BIAFAC subject), it may be thawed, diluted in saline and used either to mix in a mathematical ratio to create a synthetic community or to be directly transplanted into germ free mice. If the sample is considered not useful (not representative of the average BIAFAC subject) for fecal microbiome transplant into germ free mice, the sample will be destroyed.

- Nasal Microbiome – Arm 3 only. A nasal swab will be performed by research staff or by the subject under direction of the research staff. The sterile calcium alginate swab will be inserted into the left nare until resistance is met, rotated 5 times and then moved to the right nare and rotated 5 times. The swab will be immediately placed in a tube containing MagNAPure lysis buffer. The shaft of the swab will be snapped off and discarded leaving the head of the swab immersed in the lysis solution. The tube cap will be tightly secured and the tube will be shaken for a minimum of 5 seconds to saturate the head of the swab with the lysis buffer releasing the bacterial lysate into the fluid. Samples will be stored at -80C for subsequent batch DNA extraction on a MagNAPure96 followed by microbiome profiling by NGS and/or qPCR. Samples will be labeled with the subject's unique code. This outcome measure will be performed at baseline (month 0) and post (month 6) to address anosmia/dysosmia common in PASC subjects and to determine if exogenous GH impacts the nasal mucosa/microbiome .

- Performance fatigue. Skeletal muscle strength and fatigue will be assessed in subjects using dynamometry testing of the right knee extensors (using a Biodex Pro 4 dynamometer). If the right knee cannot be tested, the left knee will be utilized for this measurement. This outcome measure will be performed at baseline (month 0) and post (moth 6) and will include peak isometric and isokinetic (concentric) strength, fatigue index and will be performed as follows:

    - Warm up: Isokinetic (con/con/500/300); 30 contractions

- Isometric Strength: fixed 90 degrees; 15 seconds of work, 15 seconds of rest. 1 set of 3 contractions at maximal effort.
- Isokinetic Strength: (con/con/120/300); 1 contraction every 5 seconds. 1 set of 3 contractions at maximal effort.
- Fatigue Index: Isokinetic (con/con/120/300); 50 contractions at maximal effort.

- Modified 6-minute walk. Walking performance will be assessed in subjects during 6 minutes of walking in long corridor hallways. This is a standard test of walking performance that has been validated in similar studies (21-23). Here, the test will be slightly modified, with subjects asked to walk at a 25% perceived effort from minute 0 – 2, at a 50% perceived effort from minute 2 – 4, and at a 100% effort (as quickly as they can safely walk without running) from minute 4 -6. Distance traveled for each 2-minute category will be recorded. The 6-minute walking test will be completed at baseline (month 0) and post (month 6).

- Questionnaires. Questionnaires will be completed to assess perceptual fatigue, mood and quality of life, symptoms associated with growth hormone deficiency, sleep, depression, food preferences, and gastrointestinal symptoms. Time points for the collection of questionnaires are included in each description.

  - Perceptual fatigue. Perceptual fatigue will be assessed using the Brief Fatigue Inventory (BFI), a 9-item questionnaire that assesses perceptual fatigue as well as fatigue interferences (e.g. interference with enjoyment of life). The scores from all 9 questions can be average to calculate a Global Fatigue Score, with a range from 0 to 10, with a higher score indicating more fatigue. BFI will be completed at month 0 and month 6 in TBI subjects (24). PASC subjects complete the BFI at months 0, 6, 9 and 12. The Multidimensional Fatigue Symptom Inventory (MFSI) will also be used to measure perceptual fatigue. Fatigue symptoms will be measured using the 30-item Multidimensional Fatigue Symptom Inventory – Short Form, a validated measure that yields one overall score of total fatigue (range -24-96, with higher scores indicating more fatigue) and five subscales (general, physical, emotional, mental, vigor). With the exception of the vigor subscale, higher scores indicate greater fatigue. The MFSI will be measured at month 0 and month 6 in TBI subjects. PASC subjects complete the MFSI at months 0, 6, 9 and 12. If a mTBI subject sustains an additional TBI during the study period, the study will be extended for an additional 6 months. An additional questionnaire will be collected at the end of the 6 month period. If a PASC subject is infected with COVID again during the study months 9 - 12, the study will be extended to collect an additional questionnaires at month 13 and month 15.

  - Mood and Quality of Life. Psychological symptoms will be measured in mTBI subjects using the 65-item Profile of Mood States (POMS) questionnaire, a validated measure that yields one overall score of total mood disturbance (range 0-168, with higher scores indicating more disturbance in mood) and six subscales (tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment). With the exception of the vigor-activity subscale, higher scores indicate greater mood disturbance or distress. POMS will be measured at month 0 and month 6 in TBI subjects. PASC subjects complete POMS at months 0, 6, 9 and 12. If a mTBI subject sustains an additional TBI during the study period, the study will be extended for an additional 6

months. An additional questionnaire will be collected at the end of the 6 month period. . If a PASC subject is infected with COVID again during the study months 9 - 12, the study will be extended to collect an additional questionnaires at month 13 and month 15.

- Growth Hormone Deficiency. Symptoms of growth hormone deficiency will be measured in mTBI subjects using the Quality of Life – Assessment of Growth Hormone Deficiency in Adults (QoL-AGHDA). This 25-item questionnaire measures specific symptoms associated with growth hormone deficiency, with a score range of 0 to 25, with a higher score indicating worse symptoms (25). The QoL-ADGHA will be measured at month 0 and month 6 in TBI subjects. PASC subjects complete the QoL-AGHDA at months 0, 6, 9 and 12. If a mTBI subject sustains an additional TBI during the study period, the study will be extended for an additional 6 months. An additional questionnaire will be collected at the end of the 6 month period. . If a PASC subject is infected with COVID again during the study months 9 – 12, the study will be extended to collect an additional questionnaires at month 13 and month 15.

- Sleep Quality. Pittsburgh Sleep Quality Index (PSQI) is a self-rated questionnaire which assesses sleep quality and disturbances over a 1 month-time interval. Minimum Score = 0 (better); Maximum Score = 21 (worse). Interpretation: Total $\leq$ 5 associated with good sleep quality. Total > 5 associated with poor sleep quality. PSQI will be measured at month 0 and month 6 in TBI subjects (26). PASC subjects complete the PSQI at months 0, 6, 9 and 12. If a mTBI subject sustains an additional TBI during the study period, the study will be extended for an additional 6 months. An additional questionnaire will be collected at the end of the 6 month period. . If a PASC subject is infected with COVID again during the study months 9 - 12, the study will be extended to collect an additional questionnaires at month 13 and month 15.

- *The Beck Depression Inventory-II* (BDI-II) is one of the most widely used self-report inventories to assess depressive symptom severity in adolescent and adult populations (clinical and non-clinical). The BDI-II is comprised of 21 individual items reflecting specific cognitive, affective, and physical symptoms of depression (27). Each item includes four statements that vary in the description of symptom of severity. Scores range from 0 to 3, with a score of "3" indicating a severe symptoms and a score of "0" indicating an absence of concern with that particular aspect of depressive symptomology. The total score is the sum of all endorsed statements. If more than one statement from a given item is chosen by the respondent, only the statement of greatest severity is scored. The maximum total score is 63. The BDI-II Manual designates the following raw score classifications depression severity: ≤13 = *minimal*; 14-19 = *mild*; 20-28 = *moderate*; ≥ 29 = *severe*. The BDI will be measured at month 0 and month 6 by TBI subjects. PASC subjects complete the BDI-II at months 0, 6, 9 and 12. If a mTBI subject sustains an additional TBI during the study period, the study will be extended for an additional 6 months. An additional questionnaire will be collected at the end of the 6 month period. . If a PASC subject is infected with COVID again during the study months 9 – 12, the study will be extended to collect an additional questionnaires at month 13 and month 15.

- Food Preference Questionnaire (FPQ). General food preferences will be assessed using a modified questionnaire. The food preference questionnaire requires participants to rate their liking of 62 individual foods on a 5-point Likert scale, ranging from "not at all" to "a lot". Participants are instructed to select 'N/A' if they are not familiar with - or have no memory of - having tried a food item. Food preference scale scores for six food categories (Vegetables, Fruit, Meat/Fish, Dairy, Snacks, and Starches) are obtained by summing the single food preference item scores within each food category and dividing this sum by the number of items on the list. FPQ will be measured at month 0 and month 6 by TBI subjects. PASC subjects complete the FPQ at months 0, 6, 9 and 12. If a mTBI subject sustains an additional TBI during the study period, the study will be extended for an additional 6 months. An additional questionnaire will be collected at the end of the 6 month period. . If a PASC subject is infected with COVID again during the study months 9 - 12, the study will be extended to collect an additional questionnaires at month 13 and month 15.

- Gastrointestinal Symptom questionnaire. Gastrointestinal symptoms will be monitored monthly using the Gastrointestinal Symptom Rating Scale (GSRS). The GSRS is a 15-item questionnaire. Each question has a seven-point Likert-type scale where 1 represents absence of symptoms and 7 represents very troublesome symptoms. The GSRS will be measured each time a fecal collection is completed. If a mTBI subject sustains an additional TBI during the study period, the study will be extended for an additional 6 months. The GSRS will be collected monthly during the 6 month extension. . If a PASC subject is infected with COVID again during the study months 9 - 12, the study will be extended to collect an additional questionnaires at month 13 and month 15.

- Recombinant Human Growth Hormone Administration. mTBI and PASC subjects will be asked to inject themselves with growth hormone daily. The starting dose will be 0.4 mg/day beginning at the baseline study and will increase to 0.6 mg/day when instructed by the study team. The dose increase will happen after the month 1 IGF-1 blood draw and the study team reviews the results and instructs the subject to increase their dose to 0.6 mg/day. TBI subjects will administer growth hormone for 6 months. PASC subjects will administer growth hormone for 9 months. If a mTBI subject sustains an additional TBI during the study period, the study will be extended for an additional 6 months. Genotropin will be provided for the additional 6 months at a dose of 0.6 mg/day.

- Glucagon stimulation test. Arm 3 PASC subjects will have a glucagon stimulation test at month 12. After the IV is placed, 3.5 ml of blood will be collected for the baseline (time: 0 minutes) to test for levels of human growth hormone. 1 mg glucagon (for subjects over 90 kg, 1.5 mg glucagon) will be injected IM in the deltoid muscle of the subject. Additional blood samples (3.5 mL) will be collected to test for levels of human growth hormone at time points: 90 minutes, 120 minutes, 150 minutes, and 180 minutes. All blood samples will be sent to UTMB clinical laboratory for analysis and entered into subjects UTMB medical chart.

Study Procedures for household and healthy control subjects.

Household controls will complete the procedures below. Differences in procedures between Arm 1 and Arm 2 will be noted.

- Vital signs. Vital signs will be collected at all study visits. This will include height, weight, blood pressure, pulse, respirations, and temperature. Vital signs will be recorded on the appropriate study visit CRF.

- Blood draw. Subjects will have blood drawn at the baseline (month 0) and post (month 6) visits. Blood will be drawn using a butterfly needle from a peripheral vein in the arm. Subjects will be fasted for at least 10 hours.

    - Baseline Visit (Month 0):
      Up to 15 ml of blood (1 serum separator top (5ml); 1 lithium heparin (6ml); 1 K-EDTA (4ml)) will be collected from fasted (at least 10 hours) subjects for analysis of hormones, amino acids and metabolites. In addition, up to 13ml of blood (1 serum separator top (5ml); 1 lithium heparin (6ml)) will be collected after a standardized meal for the analysis of amino acids.

    - Post Visit (Month 6):
      Up to 15 ml of blood (1 serum separator top (5ml); 1 lithium heparin (6ml); 1 K-EDTA (4ml)) will be collected from fasted (at least 10 hours) subjects for analysis of hormones, amino acids and metabolites. In addition, up to 13ml of blood (1 serum separator top (5ml); 1 lithium heparin (6ml)) will be collected after a standardized meal for the analysis of amino acids.
      An additional 20ml of blood will be collected for the following labs to be sent to the UTMB clinical laboratory for analysis (labs sent to UTMB lab will be entered into subjects UTMB medical chart):
        - Complete blood count with differential (CBC w/ diff)
        - Comprehensive metabolic panel (CMP)
        - Lipid Panel

- Amino Acid Levels. Circulating blood amino acid levels will be measured before (fasting) and 90 minutes (+/-10 minutes) after a standardized meal. Amino acids in response to a meal will be measured at month 0 and month 6.

- GI microbiome. The GI microbiome will be assessed using fecal samples collected at monthly time points (month 0, month 1, month 2, month 3, month 4, month 5, and month 6). Subjects will provide fecal samples as described above (see procedures for mTBI subjects – GI microbiome). Fecal samples will be collected +/- 7 days from specified time point based on baseline (month 0) study. Subjects will be allowed to collect the fecal samples at home. Samples will be stored at 4°C until transferred to UTMB (on/+ 3 days after collection). Subjects will be given a tutorial on how to properly collect the fecal sample. They will be given a handout from the manufacturer of the collection kit with step by step instructions. They will also be shown a video of the collection process supplied by the kit manufacturer.

  https://www.youtube.com/watch?v=ytr_hmJdHqM&feature=youtu.be&rel=0

  An additional collection will be performed at month 0 and month 6 to collect viable fecal material. Fecal samples will be collected using the spatula included in the OMNIgene-Gut kit used in the first collection. Briefly, after the participant transfers fecal material to the yellow top of the collection

tube for the first collection, they will scoop another small amount of fecal material with the same spatula and transfer it to a prepared cryogenic freezer tube containing sterile PBS (without Ca/Mg) and sterile glycerol. The tube will be closed and shaken for a minimum of 30 seconds to mix with the stabilization liquid. Samples will be labeled with the subject's unique code and stored at 4°C until transported to University of Texas Medical Branch. Samples will then be frozen at -80°C and stored until the molecular analysis is completed. After initial analysis, if the sample is considered useful (representative of the average control subject), it may be thawed, diluted in saline and used either to mix in a mathematical ratio to create a synthetic community or to be directly transplanted into germ free mice. If the sample is considered not useful (not representative of the average control subject) for fecal microbiome transplant into germ free mice, the sample will be destroyed.

- Questionnaires. Questionnaires will be completed to assess general food preferences and gastrointestinal symptoms. Time points for the collection of the questionnaires is included in each description.

    - Food Preference Questionnaire (FPQ). General food preferences will be assessed using a modified questionnaire. The food preference questionnaire requires participants to rate their liking of 62 individual foods on a 5-point Likert scale, ranging from "not at all" to "a lot". Participants are instructed to select "N/A" if they are not familiar with - or have no memory of - having tried a food item. Food preference scale scores for six food categories (Vegetables, Fruit, Meat/Fish. Dairy, Snacks, and Starches) are obtained by summing the single food preference item scores within each food category and dividing this sum by the number of items on the list. FPQ will be measured at month 0 and month 6.
      For Arm 1 only: If the mTBI subject sustains an additional TBI during the study period, the study will be extended for both the mTBI and household control for an additional 6 months. An additional questionnaire will be collected at the end of the 6 month period.

    - Gastrointestinal Symptom questionnaire. Gastrointestinal symptoms will be monitored monthly using the Gastrointestinal Symptom Rating Scale (GSRS). The GSRS is a 15-item questionnaire. Each question has a seven-point Likert-type scale where 1 represents absence of symptoms and 7 represents very troublesome symptoms. The GSRS will be measured monthly.
      For Arm 1 only:If the mTBI subject sustains an additional TBI during the study period, the study will be extended for both the mTBI and household control for an additional 6 months. The GSRS will be collected monthy during the 6 month extension.
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Statistical Considerations

Aim 1a:
In this protocol we intend to compare the GI microbiome of diagnosed mTBI patients against the microbiome of healthy controls (HC). Data will be organized by time (PRE vs. POST) and by treatment (mTBI vs. HC). The hypothesis is that group differences between mTBI and HC will exist at PRE but that GH treatment in the mTBI group will dissolve these differences at POST.

Power calculations using q-PCR data from 22 diagnosed TBI patients and 15 healthy control subjects suggesting that we will be able to detect group differences in genus and/or species in the intestinal flora. These calculations suggest that we would need about 6 subjects per group to have an 80% probability of detecting significant differences in at least one of the previously

identified organisms (two-sided, a=0.05). With the proposed subject numbers (25 per group) we calculate that the power would be between 91 – 100 % for at least 8 of the previously identified organisms.

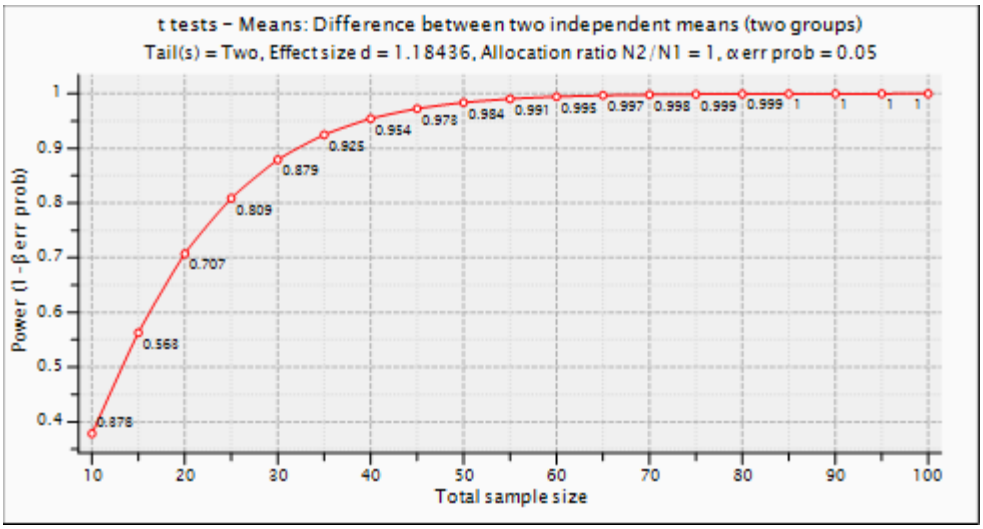
Aim 1b:
Given our recent results from mTBI patients with BIAFAC, that identified two distinct community groups, based upon the presence of specific Prevotella species, we require additional participants to achieve group sizes of each type to address differences in the individual species for the pre and post comparisons. Enrollment: We expect to enroll subjects without the need for a HHC to address these differences. These subjects will be compared to matched normal participants from the general population adding 25 TBI and 25 normals to the study creating the power needed to address Prevotella positive and negative community types.

Aim 2:
Given the data acquired from two PASC subjects under IRB 20-0361 that have shown indistinguishable profiles to those of BIAFAC communities as well as remarkably reduced GH responses to GST, we expect that the Power calculations explained in this section (above) will also apply to PASC. Because PASC is a recent consequence to the pandemic no data exist to complete Power calculations beyond those of syndromes like BIAFAC that present with similar symptoms. We therefore have proposed enrollment of at least 25 PASC subjects to compare to 25 normal controls already included in this protocol.

For quantifiable (primary) outcomes such as abundances of individual species in the GI microbiome and (secondary) symptom related outcomes, a standard repeated measure ANOVA model will be used. When possible, age and other covariates will be brought into the model to reduce variability and account for random differences between the two groups. Significance tests will be performed at the alpha=0.05 level, and multiple testing corrections will be used to account for potential Type I error rate inflation. Nonparametric, generalized linear, and transformed response models will be considered as necessary to alter the model to account for deviations from standard assumptions (normality, linearity, etc.). Current data support statistical outcomes being established with group sizes similar or slightly larger size than our previous study.

The graph below is based on group difference in *Alistipes onderdonkii.* Power calculations were performed using t-tests with G*Power (two-groups, two-sided, a=0.05, Version 3.1.9.2, Germany). Effect size in this case was 1.184.

Below is a list of organisms we identified previously and which were used to conduct the power analyses for the present study. Please note that the intestinal flora includes an incredibly diverse system of organisms and at this time it is difficult to accurately predict which organisms will be included in the biome shifts we expect to see. Therefore, additional organisms may be included in the final microbiome comparisons between mTBI patients and household control subjects. Further power analyses will be conducted using the data collected during this investigation.



Target	n = 20 (10/group)	n=30	n=40	n= 50 (25/group)
Alistipes onderdonkii	70.7%	87.9%	95.4%	98.4%
Desulfovibrio	60.4%	79.3%	89.9%	95.4%
Prevotella spp	97.5%	99.8%	100.0%	100.0%
Akkermansia	64.8%	83.2%	92.6%	96.9%
Odoribacter	52.5%	71.5%	83.8%	91.2%
Ruminococcus bicirculans	57.2%	76.3%	87.7%	93.9%
Bacteroides vulgatus	65.6%	83.9%	93.1%	97.2%
Sutterella	53.0%	72.0%	84.2%	91.5%
Lachnoclostridium	30.8%	44.4%	56.4%	66.4%
Streptococcus salivarius	27.8%	40.2%	51.4%	61.2%
16s	6.8%	7.8%	8.8%	9.8%

Study Timeline

Interventional, mechanistic studies such as this one are routinely performed by our research group and involve fine coordination between our team and the Clinical Research Center at UTMB. From experience, we know that we will be able to complete ~16 experiments of this type of trial per year. Thus, we anticipate completing all patients at the end of year 3. Therefore, a 3-year span is needed to accomplish all aims, and data analysis and manuscript writing will follow.

Protection of Human Subjects

A. Risks to Human Subjects

Human subject involvement and characteristics. Arm 1: We propose to study a total of 50 patients both males and females, with a history of mild TBI (n=25) ages 18 to 70 and their household controls (n=25) ages 18 and up. As the mTBI subjects will be selected for study based on their history and symptom complex, we expect that screening failures will be low (approximately 20). We also expect approximately 4 sets (8 total) of subjects to drop out during the course of the study. Thus, in order to achieve a total of 25 pairs (50 individuals) completing the study, we anticipate screening and enrolling approximately 78, but up to 100 individuals over 3 years. Arm 2: We propose to study a total of 50 patients both male and female consisting of patients with a history of mild TBI (n=25) and healthy matched controls (n=25), ages 18-70. As the mTBI subjects will be selected for study based on their history and symptom complex, we expect that screening failures will be low. Arm

3: We propose to study a total of 25 patients both male and female consisting of patients with a history of COVID-19 ages 18 – 70. As PASC subjects will be selected for the study based on their history and symptom complex, we expect that screening failures will be low.

ARM 1:

1. mTBI Inclusion criteria Male or female with a diagnosis of mild TBI.
2. At least 6-month post-injury.
3. Ages 18 to 70 years.
4. Lives in same household as paired control that meets inclusion/exclusion criteria.
5. Participant is willing and able to give informed consent for participation in the study.

mTBI Exclusion criteria

1. Unable to walk unassisted.
2. Significant heart, liver, kidney, blood or respiratory disease.
3. History of chest pain or coronary heart disease.
4. Uncontrolled Diabetes mellitus.
5. Any history of a recently (12 months) diagnosed cancer other than a skin cancer (excluding melanoma).
6. Current alcohol or drug abuse.
7. Premorbid history of psychiatric disorder.
8. Premorbid history of head trauma.
9. Pregnancy or become pregnant during the trial.
10. Subjects who are deficient in cortisol or thyroid at screening will be excluded until hormone abnormalities have been corrected.
11. Subjects with chronic pain who are being managed with narcotics will be excluded as the effects of central nervous system depressants may interfere with study test results.
12. Subjects with a history of inflammatory bowel disease, Celiac disease or active diverticular disease.
13. Subjects with MRI incompatible devices.
14. Subjects with severe claustrophobia.
15. Other medical condition or medication administration deemed exclusionary by the study investigators.

Household Control Inclusion criteria

1. Ages 18 and up.
2. Lives in same household as paired mTBI subject that meets inclusion/exclusion criteria.
3. Participant is willing and able to give informed consent for participation in the study.

Household Control Exclusion criteria

1. Significant heart, liver, kidney, blood or respiratory disease.
2. Uncontrolled Diabetes mellitus.
3. Any history of a recently (12 months) diagnosed cancer other than a skin cancer (excluding melanoma).
4. Current alcohol or drug abuse.
5. Premorbid history of psychiatric disorder.
6. Premorbid history of head trauma.

7. Pregnancy or become pregnant during the trial.
8. Subjects who are deficient in thyroid at screening will be excluded until thyroid hormone is replaced.
9. Subjects with a history of inflammatory bowel disease, Celiac disease or active diverticular disease.
10. Other medical condition or medication administration deemed exclusionary by the study investigators.

ARM 2:

mTBI Inclusion criteria

1. Male or female with a diagnosis of mild TBI.
2. At least 6-month post-injury.
3. Ages 18 to 70 years.
4. Participant is willing and able to give informed consent for participation in the study.

mTBI Exclusion criteria

1. Unable to walk unassisted.
2. Significant heart, liver, kidney, blood or respiratory disease.
3. History of chest pain or coronary heart disease.
4. Uncontrolled Diabetes mellitus.
5. Any history of a recently (12 months) diagnosed cancer other than a skin cancer (excluding melanoma).
6. Current alcohol or drug abuse.
7. Premorbid history of psychiatric disorder.
8. Premorbid history of head trauma.
9. Pregnancy or become pregnant during the trial.
10. Subjects who are deficient in cortisol or thyroid at screening will be excluded until hormone abnormalities have been corrected.
11. Subjects with chronic pain who are being managed with narcotics will be excluded as the effects of central nervous system depressants may interfere with study test results.
12. Subjects with a history of inflammatory bowel disease, Celiac disease or active diverticular disease.
13. Other medical condition or medication administration deemed exclusionary by the study investigators.

Healthy Control Inclusion criteria

1. Ages 18-70 years.
2. Participant is willing and able to give informed consent for participation in the study.

Healthy Control Exclusion criteria

1. Significant heart, liver, kidney, blood or respiratory disease.
2. Uncontrolled Diabetes mellitus.
3. Any history of a recently (12 months) diagnosed cancer other than a skin cancer (excluding melanoma).
4. Current alcohol or drug abuse.
5. Premorbid history of psychiatric disorder.
6. Premorbid history of head trauma.
7. Pregnancy or become pregnant during the trial.

8. Subjects who are deficient in thyroid at screening will be excluded until thyroid hormone is replaced.
9. Subjects with a history of inflammatory bowel disease, Celiac disease or active diverticular disease.
10. Other medical condition or medication administration deemed exclusionary by the study investigators.

ARM 3 (PASC):

Inclusion criteria

1. Male or female with a history of COVID with diagnosis confirmed by PCR test.
2. Minimum of 6 months since diagnosis of COVID by PCR test.
3. Ages 18 to 70 years.
4. Score of 3 or higher on any question 1-3 of the Brief Fatigue Inventory (BFI) questionnaire.
5. Participant is willing and able to give informed consent for participation in the study.

Exclusion criteria

1. Current COVID infection.
2. Unable to walk unassisted.
3. Significant heart, liver, kidney, blood or respiratory disease as determined by Principal Investigator.
4. Uncontrolled diabetes mellitus.
5. Any history of a recently (12 months) diagnosed cancer other than a skin cancer (excluding melanoma).
6. Current alcohol or drug abuse.
7. History of psychosis.
8. Pregnancy or become pregnant during the trial.
9. Subjects who are being managed with narcotics will be excluded as the effects of central nervous system depressants may interfere with study test results.
10. Other medical condition or medication administration deemed exclusionary by the study investigators.

Sources of materials. We will obtain blood, urine, fecal, questionnaire and imaging data from the mTBI and PASC subjects and blood, urine, fecal and questionnaire data from the control subjects. All data/samples will be collected solely for the purpose of experimentation, except for the safety check visit for mTBI and PASC patients at month 1. The blood will be used for screening and to measure metabolites, amino acids and hormones. Fecal samples will be used for determination of the GI microbiome. Questionnaire data will be used to assess GI health and quality of life.

Recruitment Methods and Consenting Process (Arm 1)

a. Potential mTBI subjects may be patients of the investigators.

b. mTBI subjects will be recruited through ads and flyers around the community as well as in Dr. Urban's Internal Medicine Endocrinology clinic. The study will also be listed on the UTMB clinical research website, researchmatch.org and clinicaltrials.gov. Potential subjects may be

previous research subjects of the investigator. Interested mTBI subjects in clinic will be directed to call the study team for more information. Interested mTBI subjects calling the study team in response to a physician referral, ad, flyer, researchmatch.gov or CT.gov will be given detailed information regarding the study. They will be informed that to participate in this study they must have an eligible partner in their household willing to participate with them. The study team will email (postal mail, if needed) a study information packet that will include a Fast Fact sheets for both groups explaining the screening process (including all risks of the overnight fast that is required for the screening process) and the study in bulleted points and the consent forms for both mTBI and control groups (see attached Fast Fact sheet). Potential mTBI subjects can read the study information packet with their families and decide if they would like to enroll. If interested, the potential mTBI subject will contact the study team for a phone pre-screening to go over eligibility requirements (pre-screening) for the study (see attached pre-screening sheet). If potential mTBI subject passes the pre-screening, the household control subject will have the detailed study explained to them and if they are interested, will be asked to complete a phone pre-screening. If still interested, the potential mTBI subject and the potential household control subject will be asked to come to the ITS-CRC at a time that is mutually convenient for both the subjects and the study team for consenting and medical screening, including fasting labs.

c. It is anticipated that mTBI subjects and their household control will want to be together for the consenting process. In this circumstance, the study team will go over in detail the consent forms for both mTBI group and household control group with both subjects in a private room and then they will be given time alone together to discuss whether they would like to enroll. Each participant will be given the opportunity to speak to the study team alone, if they choose to.

d. To minimize the possibility of coercion or undue influence all subjects will be given a copy of the approved consent forms and encouraged to discuss with their families prior to meeting the coordinator for the consenting process. The study team will be make it very clear to the potential mTBI participants that to meet inclusion criteria for this study, they must have an eligible household partner. If either one of the pair withdraws consent or is terminated, the other will be terminated by the study team.

e. We are requesting an alteration of the consenting process so that we can obtain written consent and perform the medical screening in the same visit. The medical screening requires an overnight fast, which the IRB deems as research. Subjects will be provided a copy of the approved consent form for their review prior to their consenting/screening visit. The risk involved is an overnight fast. An overnight fast for medical tests is routine for medical care and would not require consent outside the scope of research. When subject arrives at CRC for the screening visit, the study team will begin with the consenting process as described above. We are only requesting the waiver of written consent for fasting to occur during the consenting visit. Written consent would be obtained prior to any study related procedures.

Recruitment Methods and Consenting Process (Arm 2)

a. Potential mTBI subjects may be patients of the investigators.

b. mTBI subjects will be recruited through ads and flyers around the community as well as in Dr. Urban's Internal Medicine Endocrinology clinic. The study will also be listed on the UTMB

clinical research website, researchmatch.org and clinicaltrials.gov. Potential subjects may be previous research subjects of the investigator. Interested mTBI subjects in clinic will be directed to call the study team for more information. Interested mTBI subjects calling the study team in response to a physician referral, ad, flyer, researchmatch.gov or CT.gov will be given detailed information regarding the study. The study team will email (postal mail, if needed) a study information packet that will include a Fast Fact sheets explaining the screening process (including all risks of the overnight fast that is required for the screening process) and the study in bulleted points and the consent form (see attached Fast Fact sheet). Potential mTBI subjects can read the study information packet with their families and decide if they would like to enroll. If interested, the potential mTBI subject will contact the study team for a phone pre-screening to go over eligibility requirements (pre-screening) for the study (see attached pre-screening sheet). If potential mTBI subject passes the pre-screening and if still interested, the potential mTBI subject will be asked to come to the ITS-CRC at a time that is mutually convenient for both the subject and the study team for consenting and medical screening, including fasting labs.

Healthy controls will be recruited using the UTMB clinical research website, researchmatch.org and clinicaltrials.gov. Potential subjects may be previous research subjects of the investigator. Interested healthy subjects contacting the study team in response to a physician referral, ad, flyer, researchmatch.gov or CT.gov will be given detailed information regarding the study. The study team will email (postal mail, if needed) a study information packet that will include a Fast Fact sheets explaining the screening process (including all risks of the overnight fast that is required for the screening process) and the study in bulleted points and the consent form (see attached Fast Fact sheet). Potential healthy control subjects can read the study information packet with their families and decide if they would like to enroll. If interested, the potential healthy control subject will contact the study team for a phone pre-screening to go over eligibility requirements (pre-screening) for the study (see attached pre-screening sheet). If potential healthy control subject passes the pre-screening and if still interested, the potential healthy control subject will be asked to come to the ITS-CRC at a time that is mutually convenient for both the subject and the study team for consenting and medical screening, including fasting labs.

c. All subject will have privacy at each of their visits to the ITS-CRC. All subjects will be given a private room for consenting, screening as well as each study visit.

d. To minimize the possibility of coercion or undue influence all subjects will be given a copy of the approved consent forms and encouraged to discuss with their families prior to meeting the coordinator for the consenting process.

e. We are requesting an alteration of the consenting process so that we can obtain written consent and perform the medical screening in the same visit. The medical screening requires an overnight fast, which the IRB deems as research. Subjects will be provided a copy of the approved consent form for their review prior to their consenting/screening visit. The risk involved is an overnight fast. An overnight fast for medical tests is routine for medical care and would not require consent outside the scope of research. When subject arrives at CRC for the screening visit, the study team will begin with the consenting process as described above. We are only requesting the waiver of written consent for fasting to occur during the consenting visit. Written consent would be obtained prior to any study related procedures.

Recruitment Methods and Consenting Process (Arm 3)

a. Potential PASC subjects may be patients of the investigators.

b. PASC subjects will be recruited through ads and flyers around the community as well as flyers in Dr. Urban's Internal Medicine Endocrinology clinic, the UTMB post-COVID clinic and surrounding post-COVID clinics. The study will also be listed on the UTMB clinical research website, researchmatch.org and clinicaltrials.gov. Potential subjects may be previous research subjects of the investigator. Interested subjects in clinic will be directed to call the study team for more information. Interested subjects calling the study team in response to a physician referral, ad, flyer, researchmatch.gov or CT.gov will be given detailed information regarding the study. The study team will email (postal mail, if needed) a study information packet that will include a Fast Fact sheets explaining the screening process (including all risks of the overnight fast that is required for the screening process) and the study in bulleted points and the consent form (see attached Fast Fact sheet). Potential subjects can read the study information packet with their families and decide if they would like to enroll. If interested, the potential subject will contact the study team for a phone pre-screening to go over eligibility requirements (pre-screening) for the study (see attached pre-screening sheet). If potential subject passes the pre-screening and if still interested, the potential subject will be asked to come to the ITS-CRC at a time that is mutually convenient for both the subject and the study team for consenting and medical screening, including fasting labs.

c. All subject will have privacy at each of their visits to the ITS-CRC. All subjects will be given a private room for consenting, screening as well as each study visit.

d. To minimize the possibility of coercion or undue influence all subjects will be given a copy of the approved consent forms and encouraged to discuss with their families prior to meeting the coordinator for the consenting process.

e. We are requesting an alteration of the consenting process so that we can obtain written consent and perform the medical screening in the same visit. The medical screening requires an overnight fast, which the IRB deems as research. Subjects will be provided a copy of the approved consent form for their review prior to their consenting/screening visit. The risk involved is an overnight fast. An overnight fast for medical tests is routine for medical care and would not require consent outside the scope of research. When subject arrives at CRC for the screening visit, the study team will begin with the consenting process as described above. We are only requesting the waiver of written consent for fasting to occur during the consenting visit. Written consent would be obtained prior to any study related procedures.

Subject reimbursement for time and travel.

- mTBI and subjects will receive $50 for screening; $150 each month 0 and month 6 visits, $25 each fecal collection in month 1-5, and $25 each month (6 months) for rhGH intervention. If they complete the entire protocol, they will be reimbursed $625 for time and travel.

- PASC subjects will receive $50 for screening; $150 each month 0 and month 6 visits, $50 for the month 12 visit, $25 each fecal collection in month 1-5 and month 9, and $25 each month (9 months) for rhGH intervention. If they complete the entire protocol, they will be reimbursed $775 for time and travel.

- Control subjects will receive $25 for screening; $50 each month 0 and month 6 visits, $25 each fecal collection in month 1-5. If they complete the entire protocol, they will be reimbursed $250 for time and travel.

Potential Risks

The potential risks for mTBI and PASC subjects enrolled in this project will be the following:

Vital Signs. There are no known risks of vital signs being performed.

Phlebotomy. All patients will undergo blood draws for the medical screening tests and periodically throughout the study. The risks of phlebotomy are pain, infection, bruising and introduction of blood borne pathogens. Aseptic technique will be used to minimize these risks.

Blood draws. Repeated blood draws may expose to the risk of anemia. mTBI subjects will have approximately 120 ml of blood taken in the course of the entire study. PASC subjects will have approximately 175 ml of blood taken in the course of the entire study.

Fasting. The risks of fasting include fatigue, headache, hypoglycemia and dehydration.

Glucagon Stimulation Testing. The known side effects of the glucagon stimulation test are headache and nausea and possible vomiting.

Electrocardiogram (EKG). This is a non-invasive, painless test. There is a risk of irritation or a rash on the skin from the electrodes.

DEXA scan. The measurement of lean and fat mass by DEXA involves a brief exposure (~15 min) to a low dose of radiation (~ 0.05 mrem/whole body scan), calculated to be approximately equal to a 2 h airplane flight per scan. Otherwise, there are no known risks of measuring body composition.

Indirect calorimetry**.** There are no known risks involved with these measurements.

Magnetic Resonance Imaging (MRI) (Arm 1 only). As MRI is a procedure that does not involve radiation and is non-invasive, the main risks are accidental injury resulting from the presence of metal objects on one's clothes or person (e.g. jewelry, piercings, implants, pacemakers, some types of tattoos, IUDs). In addition, some individuals may experience claustrophobia due to the confined space within the scanner.

Cognitive Testing. There are no known risks involved with this testing.

Fecal Sampling. There is no known risk of this procedure.

Nasal Swab. Risks include momentary pain and a chance for minor bleeding from the nose similar to the risks associated with nasopharyngeal SARS-CoV-2 testing.

Performance testing and 6-minute walk. The exercise tests may cause muscle soreness, cramps, dizziness, and possibly irregular heartbeats. The risks of the 6-minute walk include falling, elevated heart rate and elevated blood pressure. All subjects will be accompanied by a trained staff member.

Questionnaires. There is no known risk to completing the questionnaires listed in this study.

Recombinant Human Growth Hormone (rhGH). The potential risks of taking growth hormone include weight gain, joint pain, and increased blood glucose. In addition, Carpal Tunnel Syndrome, a condition characterized by numbness or pain in the wrists and fingers, has been associated with growth hormone therapy. These effects are modifiable with dose adjustment and are unlikely due to the relatively brief period of time that patients will be receiving growth hormone during the course of the study. An IGF-1 lab will be drawn at the 1-month time point. If the IGF-1 value is over 400 ng/ml, the dose of GH will not be increased.

Confidentiality. Divulgation of personal sensitive data is a general risk of clinical investigations involving human subjects.

The potential risks for control subjects enrolled in this project will be the following:

Vital Signs. There are no known risks of vital signs being performed.

Phlebotomy. All patients will undergo blood draws for the medical screening tests and periodically throughout the study. The risks of phlebotomy are pain, infection, bruising and introduction of blood borne pathogens. Aseptic technique will be used to minimize these risks.

Blood draws. Repeated blood draws may expose to the risk of anemia. Household controls will have approximately 100 ml of blood taken in the course of the entire study.

Fasting. The risks of fasting include fatigue, headache, hypoglycemia and dehydration.

Fecal Sampling. All subjects will collect fecal samples at monthly intervals throughout the study. There is no known risk of this procedure.

Questionnaires. There is no known risk to completing the questionnaires listed in this study.

Confidentiality. Divulgation of personal sensitive data is a general risk of clinical investigations involving human subjects.

B. Adequacy of Protection Against Risks

Recruitment and informed consent. Subjects will be recruited through Houston/Galveston area using flyers and other advertisements. Subjects may also be patients or previous research subjects of the investigators. The study will be listed on the researchmatch.org and clinicaltrial.gov registries. The details of the experimental procedures, including all the risks and benefits, will be explained by a study coordinator at the initial time of screening. At this time, they will be asked to

read and sign the consent form, approved by the IRB at UTMB. A copy of the signed consent form will be given to the subject.

Protections against risks. All key personnel have completed and passed the certification exam of the NIH-mandated course on protection of human subjects. The screening tests will allow us to exclude *a priori* subjects with potentially higher risk of developing complications. Randall Urban, MD will oversee the medical aspects of the experiments. Protections against risks for all patients will be the following:

Vital Signs. There are no known risks of this procedure.

Phlebotomy. The use of aseptic technique will be used to minimize the risk of infection, but will not eliminate it completely. Some patients may experience a sudden drop in blood pressure in response to the placement of catheters. Symptoms may include lightheadedness, nausea and possibly vomiting. There are no long-term effects associated with this response. In our experience, symptoms occur in 3.3% of our healthy subjects. All patients experiencing this response will be examined by a physician and given the option of discontinuing the study. If the physician, PI, study investigators, and patient find no reason to stop the study, the patient will be allowed to continue.

Blood draws. No more than ~40 ml of blood will be drawn at any visit. This is less than one tenth of a typical blood donation, and in adult individuals with normal blood count does not expose to the risk of anemia. TBI subjects will have less than 120 ml of blood drawn over the 6 month period of the study. PASC subjects will have less than 175 ml of blood drawn over the 12 month period of the study. Should we estimate blood losses from other reasons (complications of any procedure) to exceed 500 ml for the entire study, the subjects will be counseled about avoiding blood donations or participating in other studies involving blood draws until a normal hemoglobin value is restored.

Fasting. All subjects will be made aware of the risks of overnight fasting. They will be advised to drink water the night before and the morning of their visits. All subjects will be fed a meal during or after the completion of their study visits.

Glucagon Stimulation Test. Subjects will be made aware of the risks of the test.

Electrocardiogram. Subjects will be made aware of the risks of the test.

DEXA scan. The amount of radiation absorbed with each scan is very low (similar to the dose received during a 2-hour flight). Subjects will be made aware of this exposure level prior to the scans.

Indirect calorimetry**.** There are no risks involved with these measurements.

Magnetic Resonance Imaging. Subjects will be screened for the presence of implanted medical devices, embedded metal or other material for which MRI is contraindicated according to standard UTMB safety guidelines. To protect against loud noises, participants will wear foam earplugs and / or headphones to reduce the loud noises made by the scanner. To reduce claustrophobia, subjects will be able to talk to investigators throughout the study via intercom, and will be able let us know right away if they want to stop the study and get out of the scanner.

Fecal Sampling. There are no known risks of this procedure.

Nasal Swab. Care will be taken to minimize pain and/or bleeding.

Performance testing and 6-minute walk. Risk of muscle soreness or cramps can be reduced or avoided with an adequate warm-up. The subjects will be accompanied by a qualified staff member at all times.

Questionnaires. There are no known risks of this procedure.

Recombinant Human Growth Hormone Administration. The potential risks of taking growth hormone (weight gain, increased blood glucose, joint pain, and Carpal Tunnel Syndrome), will be described in the consent form. These effects are unlikely due to the relatively brief period of time that subjects will be receiving growth hormone during the course of the 6 or 9-month treatment period. An IGF-1 lab will be drawn at the 1-month time point. If the IGF-1 value is over 400ng/ml, the dose of GH will not be increased.

Confidentiality. Only the investigators, the study personnel, and the Medical Records department of the UTMB Hospital will have access to the subjects' personal information. All data containing the name of the patients will be locked in the PI office. To preserve confidentiality, immediately after enrollment each subject will be assigned a code by which each research sample will be identified for further analysis, thus avoiding identification by non-qualified individuals.

C. Potential Benefits of the Proposed Research to the Subjects and Others

The only potential benefits for the participation in this protocol are related to the knowledge of the results of the screening tests that will be released to the volunteer. No other benefits are expected for the subjects. The knowledge collected from the present studies may be of benefit to society, as these studies will help develop the scientific basis for designing treatments for improving the symptom complex of mTBI and PASC (cognition, performance and perceptual fatigue) in individuals with mTBI or PASC.

D. Importance of Knowledge to be Gained

Characterization of the GI microbiome in patients with history of mTBI and PASC in relation to healthy controls will provide further insight into the mechanisms and regulation of the gut-brain axis. Recognition that the microbiome of mTBI and PASC patients are consistently altered will aid the development of future diagnostic and/or treatment plans for mTBI and PASC patients.

E. Data Safety and Monitoring Plan

Study Title:

Brain and Gut Plasticity in mild TBI or post-acute COVID syndrome Following Growth Hormone Therapy

Type of Research Data or Events to be Monitored:

Total subject accrual, including screen fails, dropouts and terminations will be monitored as well as adverse events, protocol deviations, violations and unanticipated problems. Since the risk level associated with this study is estimated to be moderate, subjects will be monitored by the principal investigators for safety throughout the study (i.e. subjects' lab values, medical history, reactions to injections, and rhGH treatment).

Methods and Frequency of Analysis:

Plan for Monitoring and Safety Review:

a. Randall Urban, MD, the Principal Investigator is the individual responsible for monitoring the safety environment of the participants. He will work with, Dr. Dillon, or the study coordinator, to oversee safety of the participants.

b. Thorough monitoring of the recruitment, enrollment, retention, informed consent process, adverse events, study procedures, and the evaluation of primary and secondary endpoints will be carried out.

c. Potential subjects will be informed of all procedures involved in the study. If screening reveals any clinical abnormalities, subjects will be advised, by either a study team physician or nurse practitioner, to consult his/her primary physician. If the subject agrees to participate, written consent will be obtained in the presence of one of the following: the Principal Investigator, Co-Investigator, or Study Coordinator. There will always be at least one qualified member of the research team or one CRC nurse with the subject during the study protocol.

Plan for Data Management:

a. All the study data will be collected by the research team and recorded on case report forms. To preserve confidentiality, immediately after enrollment each subject will be assigned a code by which each research sample will be identified for further analysis to avoid identification by non-qualified individuals. Dr. Urban, the Principal Investigator, is the individual responsible for storage of data. Recognizable personal subject information will be stored in Dr. Urban's laboratory in a locked cabinet.

b. Data will be reviewed on a weekly basis by the PIs and the research team.

c. The conditions that would necessitate early termination of the study include non-compliance with study medication dosing.

d. The PI with the aid of the Co-Investigators and the Study Coordinator will perform aggregate analysis of data and adverse events.

Persons Responsible for Data Monitoring:

The ultimate responsibility for data and safety monitoring, and submitting reports of unanticipated problems, adverse events, protocol deviations and protocol violations rests with the Principal Investigator, Randall Urban, MD. Every effort will be undertaken to monitor and minimize the risks

to subjects. Prior to obtaining informed consent, subjects will be encouraged to thoroughly read the informed consent form and ask questions regarding the outlined procedures and risks. To ensure ongoing protection of study subjects all procedures will be performed under the supervision of Randall Urban, M.D., or a physician willing to provide backup medical coverage. All study physicians will be familiar with the study protocol.

Reporting Unanticipated Problems, Adverse Events, Protocol Deviations and Protocol Violations:

a. Dr. Urban, the Principal Investigator, is the individual responsible for monitoring and reporting the occurrence of unanticipated problems, adverse events, protocol deviations and protocol violations throughout the study. The subjects will be monitored throughout the study at screening and follow up visits.

b. Anticipated Adverse Events, as discussed above will be included in the consent form. All other anticipated adverse events will be addressed by the PI and study team. If an adverse event occurs Dr. Urban will be immediately notified and a note will be entered in to the subject's CRC medical chart using the grading scale listed below.

c. Adverse Event Grading Scale (UTMB – CRC Scale):

0 = No Adverse Event or within normal limits

1 = Mild Severity: Transient laboratory test alterations; discomforts noted but no disruption of daily activities; no therapy, or only symptomatic therapy required.

2 = Moderate Severity: Laboratory test alterations indicating injury without long-term risk; discomfort sufficient to modify normal daily activity; specific therapy required (i.e., more than symptomatic).

3 = Serious Severity: Laboratory test indicating a serious health threat or permanent injury; incapacity, inability to work, inability to perform normal daily activity; hospitalization required or prolonged; emergency treatment required; life-threatening events; death.

The attribution scale assesses the relation of the event to the study procedures. The Principal Investigator will judge whether or not an adverse event is: 1) not related; 2) possibly related; 3) probably related; 4) definitely related to the study procedures and interventions.

All unanticipated, serious, fatal and/or life-threatening adverse events will be reported to the IRB, CRC Program Director and Pfizer, Inc within 24 hours of occurrence. The PI and UTMB Institutional Review Board (IRB) are responsible for determining whether modifications to the protocol and consent form are required. If a determination is made that participants are found to be exposed to excessive risks in relation to anticipated benefits, the study will be immediately suspended. Studies will not resume until modifications are made that are deemed to result in an acceptable risk/benefit ratio by the PIs and IRB. Aggregate reports of adverse events will be prepared on an annual basis and forwarded to the IRB and CRC at annual review.

Stopping Rules:

Pairs enrolled in Arm 1:

mTBI Subjects:

1. If paired household control subject withdraws consent, mTBI subject will be terminated from the study.
2. If subject reports they have missed more than 7 consecutive days of study medication administration, they will be terminated.

Household Control Subjects:

1. If paired mTBI subject withdraws consent or is terminated, household control subject will be terminated from the study.

Subjects enrolled in Arm 2 & 3:

mTBI/PASC Subjects:

1. If subject reports they have missed more than 7 consecutive days of study medication administration, they will be terminated.

Procedures and Time Frames for Communicating Outcomes:

Monitoring Reports will be prepared and forwarded to the IRB on an annual basis.

Precautions for Maintaining Data Integrity:

Careful monitoring of the recruitment, enrollment, retention, adverse events, and study procedures will help to protect the safety of study subjects, the quality of data, and the integrity of the study. As part of the safety plan for this study, the PI will review individual study subject records to ensure that appropriate mechanisms to protect the safety of study participants are being followed, that protocol requirements are being adhered to, and that data is accurate, complete, and secure. Subject records include consent forms, case report forms, flow of data forms, laboratory specimen records, inclusion/exclusion forms, cumulative toxicities and/or adverse event logs, and medical charts.

Records Retention

The investigator shall retain the records for 15 years.

Inclusion of Women and Minorities

Women and men will be included, as GENOTROPIN is approved for both.

Minorities will be included in the proportion found in the general population (see Targeted Enrollment Table)

Targeted/Planned Enrollment Table

Study Title: Brain and Gut Plasticity in mild TBI or post-acute COVID Syndrome Following Growth Hormone Therapy

Total Planned Enrollment: Up to 125 subjects. We would like to complete n=50 TBI subjects (enrolled in arm 1 and arm 2), n=50 control subjects (enrolled in arm 1 and arm 2) and n=25 PASC subjects (arm 3).

Targeted/ Planned Enrollment: Number of Subjects			
Ethnic Category	Sex/Gender		
	Females	Males	Total
Hispanic or Latino	13	12	25
Not Hispanic or Latino	50	50	100
Ethnic Category: Total of All Subjects *	63	62	125
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	13	12	25
White	50	50	100
Racial Categories: Total of All Subjects *	63	62	125

Inclusion of Children.

Children will not be included in this study. The age range for inclusion is 18 and over.

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