Form CT
UTHSA Clinical Trial Description

This form is not mandatory. Other documents are acceptable if equivalent information is provided.

UTHSCSA Tracking Number *(internal use only)*	20220493H	**1. Original Version Date**	6/4/2022
		1.1. Revision Date(s) *add rows as needed*	8/11/2022

Title: Mechanisms to Reduce Mental and Physical Fatigue Following Exercise Training in Older Adults

2. Background

Briefly discuss the important literature relevant to the trial and that provides background for the trial. Include the importance of the trial and any relevant treatment issues or controversies.

Background and Significance

Aging Associated Fatigue: The prevalence of self-reported mental and physical fatigue among older adults is at least 25% in primary care settings (Hickie et al., 1996). Mental fatigue refers to a decrease in cognitive resources, while physical fatigue refers to the inability of muscles to maintain optimal performance. Studies suggest that mental fatigue may be present before the onset of disability and morbidity (Avlund et al., 2003) and has been reported by older adults to be a main reason for activity restriction and poor compliance with exercise interventions (Cooper et al., 2001). Though prior studies show that both components are associated with low-grade inflammation, more research is needed to pinpoint specific biological pathways to connect chronic inflammation to fatigue-associated health declines.

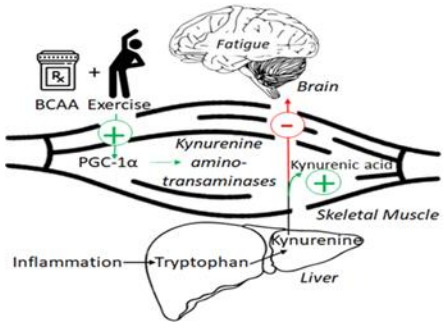
Mechanisms Leading to Fatigue in Older Adults: One pathway that may play an integral role in fatigue progression is that of the essential amino acid, tryptophan. Normally, 90-95% of tryptophan is metabolized along the kynurenine pathway in the liver. Inflammation upregulates kynurenine pathway enzymes outside the liver, causing intracellular and circulating kynurenine or oxidative kynurenine metabolite concentrations to rise. The accumulation of kynurenine and its oxidative metabolites are strongly associated with physical fatigue including weaker grip strength and slower walking speed (Westbrook et al., 2020). Further, they can readily cross into the central nervous system (**CNS**) and underlie the behavioral and cognitive impairments caused by inflammation (Allison et al., 2014).

Tryptophan Metabolism as a Mechanism Influencing Fatigue During Exercise: Evidence in animal models suggests that reductions in physical fatigue often observed with exercise training are mediated by skeletal muscle peroxisome proliferator-activated receptor γ co-activator 1α (**PGC-1α**), inducing a shift of kynurenine to kynurenic acid ((Agudelo et al., 2014; Agudelo at al., 2019). This is catalyzed by kynurenine aminotransferase (**KAT**) enzymes, which prevents oxidative metabolism of kynurenine and also its entry into the CNS, which may have an influence on mental fatigue. We have shown that reductions in skeletal muscle inflammation are associated with reductions in mental and physical fatigue following exercise training (Serra et al., 2018); however, a recent study in older adults also found that exercise training significantly increased skeletal muscle PGC-1α gene expression and KAT isoforms nearly two-fold (Allison et al., 2019). These data suggest that the tryptophan metabolism pathway may play an integral role in regulating mental and physical fatigue with exercise.

Though we have shown that exercise training can reduce the incidence of moderate-to-severe mental and physical fatigue by 30-60%, we also find that 82% of subjects continued to report mild fatigue after the intervention (Serra et al., 2018).

Further, our preliminary data suggest that when participating in exercise training, older adults tend to reduce their free-living activity by ~20% and report acute fatigue follow exercise as a contributing factor. Therefore, identifying methods to prevent acute fatigue during exercise and maximize the fatigue-reducing abilities of chronic exercise training in older adults is needed.

Figure 1. Conceptual Model: The addition of BCAA to exercise activates PGC-1α to convert kynurenine to kynurenic acid (a metabolite unable to cross the blood-brain barrier), thereby preventing mental and physical fatigue. *Adapted from Agudelo et al.* (6).



Potential of Branched-Chain Amino Acid Supplementation to Reduce Mental and Physical Fatigue When Combined Exercise: Exhaustive exercise results in the branched-chain amino acids (**BCAA**: leucine, isoleucine and valine) being taken up by the muscle, leading to reductions in the plasma concentration. When consumed as a nutritional supplement, BCAAs may improve circulating concentration of physical fatigue substances (lactate, ammonia and 5-hydroxytryptamine), energy metabolites (glucose and free fatty acids) and muscle soreness substances (lactate dehydrogenase

and creatine kinase) (Kim et al., 2013), leading to improved physical performance. In the past two decades, numerous studies have shown the advantageous effects of BCAAs on acute and chronic exercise performance, including improvements in time to exhaustion and strength (Kephart et al., 2016; AbuMoh'd et al., 2020).

Further, studies in animal models suggest that BCAAs decreases the transport of tryptophan and its metabolites into the CNS because BCAAs and tryptophan compete for the same carrier system. We have recently shown that depletion of plasma BCAAs following dialysis is highly associated with mental fatigue (Debnath et al., 2020). In animal models, BCAAs attenuate changes in skeletal muscle PGC-1α mRNA caused by acute exercise by ~50% (Samuelsson et al., 2016). Though currently unexplored, these data suggest that when combined with exercise, BCAA supplementation may have the ability to modify mental and physical fatigue by modulating the tryptophan pathway (**Figure 1**).

3. Objectives and Endpoints *All data points collected in the study should support an objective or have a regulatory purpose. Complete the table – add rows as needed.*

3.1. Objective(s) *Clearly and concisely define the primary and secondary outcomes.*	**3.2. Endpoint** *Clearly define the endpoints. (endpoints are the basis for concluding that the objective has been met).*	**3.3. Justification for Endpoint** *Briefly explain why the endpoint(s) were chosen.*
This study will address two significant objectives that are critical to aging adults. First, we will examine whether the addition of BCAA to acute and chronic exercise has the ability to reduce mental and physical fatigue. The identification of BCAA added to exercise as superior to exercise alone could have clinical implications for older adults to improve cardiometabolic and functional risk. By reducing fatigue, older adults may be able to engage in longer periods of physical activity, thereby reducing weight gain common to older adults and improving mental and physical quality of life. This study also will address an innovative mechanistic question. This study will begin to fill a knowledge gap by determining whether changes in tryptophan metabolism occur following exercise with and without BCAA supplementation and relate to reductions in mental and physical fatigue as this may identify potential targets for future (lifestyle and pharmacologic) investigation.	Depending on which group a subject is randomized to: 1) Completion of 8 weeks of EX+BCAA 2) Completion of 8 weeks of EX+PLA (placebo) We hypothesize that 8 weeks of EX+BCAA will 1) reduce mental and physical fatigue and 2) increase PGC-1α, KATs, and kynurenic acid and decrease oxidative kynurenines compared to EX+PLA.	Justification for endpoint comes from the data generated in the past two decades that have shown the advantageous effects of branched-chain amino acids (BCAAs) on exercise performance. Further, studies in animal models suggest that BCAAs decrease the transport of tryptophan and its metabolites into the CNS because BCAAs and tryptophan compete for the same carrier system. Thus, combining BCAA with exercise may synergize to divert metabolism away from formation of neurotoxic tryptophan metabolites with known deleterious effects on mental and physical fatigue.

4. Rationale
Briefly state the reason for conducting the clinical trial.

The rationale of this study comes from the data generated in the past two decades that have shown the advantageous effects of branched-chain amino acids (BCAAs) on exercise performance. Further, studies in animal models suggest that BCAAs decrease the transport of tryptophan and its metabolites into the CNS because BCAAs and tryptophan compete for the same carrier system.

5. Study Design: Double blind Randomized Controlled Trial

5.1. Number of Groups/Arms	2 arms, 15 participants per arm 30 screened and enrolled (n=24 completers)	**Group name(s)**	exercise plus placebo (**EX+PLA**) vs. exercise plus BCAA (**EX+BCAA**)

5.2. Overall Design
Select all applicable

☒	**Randomization**			☐	**Cluster Randomized**						
☐	**Group-Sequential**			☐	**Adaptive Design**						
☐	**Parallel Design**			☐	**Placebo-Controlled**						
☐	**Superiority**			☐	**Equivalence**			☐	**Non-inferiority**		
Device	☐	Pilot		☐	Pivotal			☐	Post-Approval		
Drug/Biologic	☐	Phase 1	☐	Phase 1/2	☒	Phase 2	☐	Phase 2/3	☐	Phase 3	☐	Phase 4
☐	**Dose escalation**	*If yes, details →*									
☐	**Dose ranging**	*If yes, details →*									
☐	**Sub-studies**	*If yes, details →*									

5.3. Other Design Details: **Experimental Test by Phase:** The experimental design of this study involves 5 phases, completed over 3-4 months. Phase 1 involves recruitment, screening, and enrollment. Phase 2 involves baseline testing. Volunteers then will be randomly assigned to exercise plus placebo (**EX+PLA**) vs. exercise plus BCAA (**EX+BCAA**) (Phase 3). Randomization will be 1:1 using a random number generator, assigning participants down the list in order of eligibility. Phase 4 consists of eight weeks of either 3x/week EX+PLA or EX+BCAA. After the intervention, subjects will undergo final research testing (Phase 5). All research testing and training will occur at the Barshop Institute. Intervention staff and participants will be blinded (double blinded) to supplement allocation. Participants will receive either a supplement or a placebo, both in powder form. Only study team member without direct participant interaction (i.e., statistician) will know supplement allocation. Refer to section 10.1 for further information on randomization and blinding.

6. Study Population

6.1. Study Population(s) Label/Name *To add more populations – select a row, copy & paste*	**6.2. Identify the criteria for <u>inclusion</u>** *The criteria that <u>every</u> potential participant must satisfy, to qualify for study entry.* All individuals in this study population must meet <u>all</u> of the inclusion criteria in order to be eligible to participate in the study	**6.3. Identify the criteria for <u>exclusion</u>** *The characteristics that make an individual ineligible for study participation.* All individuals in this study population meeting <u>any</u> of the exclusion criteria at baseline will be excluded from study participation.
30 screen and enrolled (n=24 completers); 15/group; Community-dwelling older adults, age 60-80 years of age, with subjective reporting of fatigue (reporting ≥3 on a 1-10 fatigue scale)	1) fatigue (participants reporting ≥3 on a 1-10 scale) 2) 60-80 years of age 3) lack of menses for at least one year for women 4) BMI 20-50 kg/m^2 5) untrained with regard to structured exercise training (is not currently training more than 2x/week)	1) taking an anticoagulant medication that is unable to be discontinued before biopsies 2) allergic to lidocaine 3) neurologic, musculoskeletal, or other condition that limits subject's ability to complete study physical assessments or training 4) hepatic (LFTs >2.5xWNL), renal (eGFR<45), , and uncontrolled psychiatric disease 5) cognitive impairment (MoCA<21)

		6) consuming uncommon supplements (multivitamin, calcium, vitamin D, etc are okay) 7) uncontrolled depression (CESD≥16) 8) any disease or condition considered to be exclusionary based on the clinical opinion and discretion of the PI

6.4. **Will screen failures be allowed to <u>re-screen</u> at a later date?**	☐	**No**	☒	**Yes** *If yes, describe criteria below ↓*
If participants are able to address criteria for exclusion (e.g., stop taking uncommon dietary supplements), they can be considered for rescreening at a later date.				

7. Study Intervention(s) being tested or evaluated

This can include prevention, diagnostic or therapeutic interventions (e.g., drug or device) or educational, health services or basic science interventions (e.g., educational program, health care delivery model, or examining basic physiology)

Experimental Test by Phase: The experimental design of this study involves 5 phases, completed over approximately 3-4 months. *Phase 1* involves recruitment, screening, and enrollment. *Phase 2* involves baseline testing. Participants then will be randomly assigned to exercise plus placebo (**EX+PLA**) vs. exercise plus BCAA (**EX+BCAA**) (*Phase 3*). *Phase 4* consists of eight weeks of either 3x/week EX+PLA or EX+BCAA. After the intervention, participants will undergo final research testing (*Phase 5*). All research testing and training will occur at the Barshop Institute.

Participant Recruitment, Screening, and Enrollment (Phase 1):

Recruitment: We will recruit participants from the Clinics of UT Health San Antonio and the Clinical Research Core of the San Antonio Older Americans Independence Center. Additionally, recruitment methods may include posting study fliers in medical offices or senior centers, community engagement activities, and/or newspaper or web-based advertisements including social media. A UT Health website to present the project to the public will be published online and could be referenced by other institutional websites. We will recruit men and women of all races and ethnic backgrounds. The study will be published on clinicaltrials.gov. Telephone pre-screening prior to the clinic screening visit may be employed to ensure potential candidates will meet inclusion and exclusion criteria for enrollment. Those who qualify preliminarily will be scheduled for a full in-clinic evaluation and will be provided an advance copy of the consent form. All these strategies will be considered, made available and implemented/adapted according to the local policies and regulations.

Screening: Eligible volunteers will have the study explained and provide informed consent according to the guidelines of the UT Health San Antonio IRB. Participants will be asked to supply the name and contact information of their primary care provider during the consent/screening phase. An in-clinic evaluation consisting of medical history and physical exam (including EKG) will be conducted by a research clinician. Information on demographics and family medical history will be recorded. A fasting blood profile for lipids, liver, renal and hematological function will be drawn. We will draw approximately 30 ml of blood (about 2 tablespoons) during screening (Phase 1). Although we do not expect a problem with the collection of blood samples, should a problem arise, such as clotting or insufficient sample for analysis, we may draw additional blood, not exceeding 50 ml (10 teaspoons) throughout any study visit. Height and weight will be measured using a standard physician's scale. Seated blood pressures will be measured. Questionnaires to screen for depression and cognitive impairment will be administered to determine eligibility.

Enrollment: Participants will be enrolled if they provide informed consent and pass all required screening assessments.

Baseline Research Testing (Phase 2): Data for primary and secondary outcomes will be collected using standardized protocols and trained staff. All baseline testing will be conducted at the Barshop Institute over approximately 3-4 visits during approximately 2-4 week period. These tests are described in detail below.

Randomization (Phase 3): Assigned (1:1 randomization) to either EX+PLA or EX+BCAA. After randomization, vital signs and blood will be drawn before (following a 12 hour fast) and acutely (3-5 min) after completing a 400 m walk test. After the fasting blood draw, participants will receive (blinded) either ~7-10 g of BCAAs (100 mg/kg) or maltodextrin (depending upon group allocation) 30 min prior to beginning this test. Heart rate and ratings of perceived exertion will be collected throughout the 400 m walk test. Participants will be instructed to document their food intake on Food Record form during the 2 days before the 400 m walk test and to repeat this diet before the post intervention test.

Exercise + Placebo or Exercise + BCAA (Phase 4):

Exercise: The 3x/wk for eight weeks exercise protocol is designed to provide a high-volume, moderate-intensity whole body training stimulus for all participants. The sessions will occur at the Barshop Institute and be led by an exercise physiologist.

Aerobic exercise training will start conservatively with a goal of 30 minutes total duration at 40-50% maximal heart rate reserve (HRR) determined according to the formula of Karvonen: Training HR = % (HRmax - HRrest) + HRrest. HRmax is defined as peak heart rate based during a peak Cardiopulmonary Exercise Test (described above). Treadmill exercise is advanced weekly, as tolerated to an of intensity of 75-85% HRR for 30 minutes. Additionally, participants will perform resistance exercise consisting of 15 repetitions for two sets and to exhaustion on the third set for five major muscle groups: chest press, knee extension, leg curl, row, and bicep curl. Resistance is gradually increased to account for strength gains when participants are able to complete 20 repetitions on the third set.

BCAA or Placebo: Participants will be randomized 1:1 to eight weeks of BCAA or maltodextrin (PLA) supplementation. Participants will be instructed to consume either ~7-10 g of BCAAs (100 mg/kg body weight) (2:1:1 leucine:isoleucine:valine) or PLA powder. In the Barshop Institute pharmacy, intervention blinded study staff wearing hair net, gloves and a facemask will weigh the required participant dose via nutrition food scale and place the daily dose in disposable condiment containers and labeled with dates for daily intake. Powders will be distributed in a double-blind fashion, identical in color, and dissolved in water or a non-calorie drink of their choice (i.e., Crystal Light). On non-exercise days, participants will be instructed to take the supplement between 9 - 10 am each morning and to log each intake in a provided spreadsheet. Powders to be consumed on non-exercise days will be pre-packaged containers and provided to participants on a weekly basis. On exercise days, the supplement will be consumed immediately after finishing exercise training. Supplement containers also will be returned every two weeks to track compliance. Assessment of side effects will occur at every exercise session.

Post Intervention Testing (Phase 5): Participants will repeat tests outlined in Phase 2 and the submaximal exercise test outlined in Phase 3.

Early Withdrawal of Subjects: If subjects are withdrawn prematurely from the study, appropriately designated research staff will make efforts to collect data throughout the protocol defined follow-up period for that subject. If a subject withdraws consent to participate in the study, attempts will be made to obtain permission to record data up to the protocol-described end of subject follow-up period. Investigator and designated research staff make it a high priority to obtain data on all subjects lost to follow up. Lost to follow up will be defined as a subject missing 3 or more consecutive visits without reasonable explanation, not answering or responding to 3 follow up phone calls to subject or emergency contacts, or returned receipt of 1 certified letter.

8. Protocol-Directed procedures, items, services or tests

List all procedures directed by the study plan - including items or services provided as part of routine or conventional care and those needed to diagnosis or treat research related complications.

Important Note – The protocol directed procedures listed must match those in the Schedule of Activities (attachment)

8.1. Drugs *(trade and generic, dosage, route of administration)*

8.2. Devices

8.3. Biologics

Participants will be randomized 1:1 to eight weeks of BCAA or maltodextrin (PLA) supplementation. Participants will be instructed to consume either ~7-10 g of BCAAs (100 mg/kg body weight) (2:1:1 leucine:isoleucine:valine) or PLA powder.

8.4. Laboratory Tests

a) Laboratory Test: We will collect plasma, serum, and whole blood for assessment of heart disease risk factors, complete blood count (CBC), comprehensive metabolic panel with liver function tests, lipid profile (e.g., triglycerides, lipoproteins, free fatty acids), glucose, insulin, systemic and cellular inflammation biomarkers, oxidative stress, antioxidant capacity, obesity-related factors (e.g., leptin and adiponectin), and amino acid metabolism.

b) Skeletal Muscle Biopsies and Analysis: Vastus lateralis muscle biopsies will be obtained with a Bergstrom needle with local anesthesia by a credentialed clinician following a 12 hour fast. Muscle tissue will quantitate local inflammation and tryptophan metabolism markers. The samples will be placed in OCT for fiber typing or immediately placed in clamps cooled in liquid nitrogen. The de-identified frozen samples will be stored at -80°C in the Barshop Institute. Participants will be instructed to refrain from vigorous physical activity and to document their food intake during the 2 days before the biopsy and to repeat this diet before the post intervention biopsy. This procedure will not be performed if IV access cannot be

obtained, if the patient is anticoagulated for any reason and primary care physician does not feel it is safe for anticoagulant to be ceased for 1 week prior to the biopsy, if the patient does not tolerate a prior biopsy, or for any other concern as determined by the PI.

8.5. Imaging Procedures

Body composition: Whole body DEXA is performed to measure lean and fat body mass and to examine the effect of the interventions on body composition at baseline and after the intervention.

8.6. Other Research Procedures *(e.g., other safety and efficacy assessments.)*

Study Procedures:

a) Questionnaires related to their health-related quality of life (PROMIS-57), fatigue assessment scale (FAS), Center for Epidemiologic Studies Depression Scale (CES-D), energy expenditure (Minnesota Leisure Time Physical Activity Questionnaire; MLTQ), activities of daily living, and dietary habits (short health eating index: sHEI) and sleep quality (Insomnia Severity Index: ISI). See Form M.

b) Body composition: Minimum and maximum waist and hip circumference will be measured with a tape measure. Weight will be measured on a standard physician's scale.

c) Energy Intake and Expenditure: *Energy intake* will be assessed through the Automated Self-Administered 24-Hour (ASA24®) Dietary Assessment Tool. Participants will be asked to self-administer 24-hour food recalls over a 3-5-day period and enter the information into the ASA24® web-based tool that enables multiple, automatically coded, self-administered 24-hour recalls. The latest release, ASA24-2016, also permits researchers to collect data using multi-day food records, also known as food diaries. Simultaneously, an activity monitor (Actigraph™) system will be used to monitor *total daily activity energy expenditure* (kcal/d). The Actigraph™ GT9x-BT accelerometers will be provided to participants upon meeting eligibility criteria. The monitors will be worn either on a belt around the hip or the wrist as he or she would a watch. Participants will wear the accelerometer for 5-7 days including the weekend. This measurement will be taken prior to randomization (Phase 3).

d) Physical Fatigue:
Isometric Handgrip: Hand grip strength of both limbs will be assessed using a handheld dynamometer.
Strength: We will use a standardized 1-repetition maximum (1-RM) protocol that includes 4-6 trials with rest periods of the leg extension and chest press exercises. Participants also will be asked to perform the leg extension and chest press exercises at 60% 1-RM as many times as they can before fatigue.
Chair rise: The number of times that a participant can rise from a sitting to a standing position and to sit down again as quickly as they can in 30 seconds will be recorded. The time for the participants to rise five times from a sitting to a standing position and to sit down again also will be recorded.
*Cardiopulmonary Exercise Test (**CPET**):* A resting ECG will be performed prior to test. Open circuit spirometry will be performed during a cardiac exercise stress test using a customized protocol to evaluate the cardiopulmonary response (i.e., VO2peak and ventilatory threshold) to strenuous exertion under clinical supervision and continuous vital signs and ECG monitoring according to the participant's tolerance, perceived exertion, and gait stability. These tests will be reviewed and cleared for participation by a research clinician. Participants breathe through a non-rebreather mask to collect expired air. A computer interfaced with gas analyzers continuously measures ventilation. Oxygen consumption, carbon dioxide production, and minute ventilation will be measured breath-by-breath using a metabolic cart.
Six-minute Walk Distance: We will measure the maximal distance the participants can walk during six minutes. Participants will be instructed to "cover as much distance as they can" over a flat surface demarcated by traffic cones while timed by a stopwatch.

e) Frailty assessment: Frailty will be classified using the Fried phenotype criteria: 1) Self-reported unintentional weight loss of ≥10 pounds in the past year; 2) Self-reported exhaustion (CES-D); 3) Low energy expenditure using the Minnesota Leisure Time Physical Activity Questionnaire (MLTQ) to assess physical activity (duration and frequency); 4) Weakness measured via grip strength using a handheld dynamometer in the dominant hand; 5) and slowness on 10-foot walk at usual pace. These measures have been standardized in the SALSA study and will be used in this study as described (Espinoza, 2008). At the baseline, self-reported unintentional weight loss will be used as criteria for weight loss as stated above. A frailty score will be calculated as the number (0 – 5) of frailty characteristics present. Those with ≥ 3 of these 5 characteristics are categorized as frail; those with 1 or 2 are categorized as pre-frail, and those with none are categorized as non-frail (Fried et al., 2001).

8.7 Attach a Schedule of Activities (SOA) Excel File [Download the Template here: *Schedule of Activities*]	*Check to indicate that the SOA Excel File is attached →*	☒

9.	**Preparation/Handling/ Storage/Accountability of Investigational Drug, Biologic, or Device**
☐	*N/A - This study does not include any investigational products (e.g. drugs, devices or biologics)*
☒	*N/A - An Investigator Brochure is attached*
☒	*N/A - A Drug/Device Manual is attached*

9.1. Acquisition and accountability

State how the study intervention and control product will be provided to the investigator. Describe plans about how and by whom the study intervention will be distributed, including participation of a drug repository or pharmacy, and plans for disposal of expired or return of unused product.

This study will utilize over-the-counter dietary supplement powders, neither of which require regulation by the FDA. Each will be purchased online and shipped to the Barshop Institute and stored in the clinical pharmacy.
The BCAA supplement we will utilize is manufactured and distributed by Naked Nutrition. 1 scoop (1Tbsp) contains 2500 mg of L-leucine, 1250 mg of L-isoleucine, and 1250 mg of L-valine and sunflower lecithin.
The maltodextrin placebo we will utilize is manufactured and distributed by nutricost. 1 scoop (15 g) contains 14 g of carbohydrate (maltodextrin).
Study staff will distribute the supplement to participants weekly and will ask for unused containers to be returned weekly. The distribution of supplement will be logged by study staff. Participants also will keep a log of supplement intake.
Expired and returned product will be discarded following pharmacy protocol.

9.2. Formulation, Appearance, Packaging, and Labeling

Describe the formulation, appearance, packaging, and labeling of the study intervention and control product, as supplied. Information in this section can usually be obtained from the IB or the package insert, or device labeling. This section should include the name of the manufacturer of the study intervention and control product.

Intervention blinded study staff wearing hair net, gloves and a facemask will weigh the required participant dose via nutrition food scale and place the daily dose in disposable polypropylene condiment containers and labeled with dates for daily intake.

9.3. Product Storage and Stability

Describe storage and stability requirements (e.g., protection from light, temperature, humidity) for the study intervention and control product. For studies in which multi-dose vials are utilized, provide additional information regarding stability and expiration time after initial use (e.g., the seal is broken).

The supplement will be stored per distributor recommendations (cool, dry place) in the Barshop clinical pharmacy.

9.4. Preparation

Describe the preparation of the study intervention and control product, including any preparation required by study staff and/or study participants. Include thawing, diluting, mixing, and reconstitution/preparation instructions in this section. For devices, include any relevant assembly or use instructions.

On exercise days, both powders will be dissolved in ~400 ml of a non-caloric drink of their choice (i.e., Crystal Light) in the clinical pharmacy by study staff. On non-exercise days, participants will be provided with the Crystal Light and provided written instruction to prepare the mixture.

10. Study Intervention Additional Details

10.1. Measures to Minimize Bias: Randomization and Blinding

This section should contain a description of randomization and blinding procedures (if applicable to the study design). It should include a description or a table that describes how study participants will be assigned to study groups, without being so specific that blinding or randomization might be compromised. Plans for the maintenance of trial randomization codes and appropriate blinding for the study should be discussed. The timing and procedures for planned and unplanned breaking of randomization codes should be included. Include a statement regarding when unblinding may occur and who may unblind. Provide the criteria for breaking the study blind or participant code. Discuss the circumstances in which the blind would be broken for an individual or for all participants (e.g., for serious adverse evets (SAEs)). Indicate to whom the intentional and unintentional breaking of the blind should be reported.

Randomization codes will be created at random and kept electronically by a study team member not directly involved with participants (i.e., statistician). We anticipate that blinding will only be broken prior to completion of the study if requested by the DSMB due to safety concerns. Unblinding prior to study completion will be reported to the IRB, study sponsor and DSMB.
Randomization code: a random number generator will be used utilizing numbers 1 and 2 where 1=placebo group 2= BCCA group

10.2. Study Intervention Compliance

Define how adherence to the protocol (e.g., administration of study intervention, use of device,) will be assessed, and verified (if applicable, e.g., plasma assays, electronic monitoring devices, daily diaries).

Compliance will be assessed weekly via intake logs and return of unused supplement.

10.3. Permitted Concomitant Therapy

This section should be consistent with the medication restrictions in the inclusion/exclusion criteria previously listed. Describe how allowed concomitant therapy might affect the outcome (e.g., drug-drug interaction, direct effects on the study endpoints).

There are no medication restrictions except that subjects taking anticoagulant medications must be able to stop temporarily prior to biopsy procedures. Additionally, subjects may not take supplements other than standard multivitamins (calcium, vitamin D, etc.)

10.4. Rescue Medicine

List all medications, treatments, and/or procedures that may be provided during the study for "rescue therapy" and relevant instructions.

☒ *N/A, no rescue medicine*

11. Study Intervention Discontinuation

11.1. Discontinuation of Study Intervention

Describe the criteria for discontinuing the study intervention (e.g., halting rules), including any monitoring test(s) and associated clinical decision point(s). Include reasons for temporary discontinuation of the study intervention (e.g., type and quantity of adverse events), clearly stating the length of time, if applicable, and describe the data to be collected at the time of study intervention discontinuation and approaches for restarting administration of or re-challenging with study intervention.

Side effects (expected to be minimal) may include gastrointestinal distress (e.g., cramps and gas) and acne, and will be monitored at each exercise session. Any unanticipated or serious side effects will be reported immediately to the study clinical staff, IRB and DSMB. Halting the study will be at the discretion of the study clinical staff and/or DMSB. There is no risk of stopping the study supplements early or abruptly.

11.2. Continued Follow-up Discontinuation of Study Intervention

Describe efforts that will be made to continue follow-up of participants who discontinue the study intervention, but remain in the study for follow-up, especially for safety and efficacy study endpoints (if applicable). Reasonable efforts must be made to undertake protocol-specified safety follow-up procedures to capture adverse events (AE), serious adverse events (SAE), and unanticipated problems involving risks to subjects or others (UPIRSOs).

Participants who withdraw from the study will be contacted by study staff via both telephone and mail to encourage post-intervention testing.

12. Statistical Considerations

12.1. Statistical Hypotheses

State the formal and testable null and alternative hypotheses for primary and key secondary endpoints, specifying the type of comparison (e.g., superiority, equivalence or non-inferiority, dose response) and time period for which each endpoint will be analyzed.

Our central hypothesis is that eight weeks of BCAA added to exercise will increase expression of KATs, shifting kynurenine metabolism towards enhanced synthesis of kynurenic acid, thereby reducing fatigue.

12.2. Sample Size Determination

Include number of participants to recruit, screen, and enroll to have adequate power to test the key hypotheses for the study. Provide all information needed to validate your calculations and judge the feasibility of enrolling and following the necessary number of participants.

An important goal of statistical analyses is to determine effect sizes to make certain that future studies evolving from the current work are adequately powered. While data do not exist to provide more precise effect sizes for the current experiment, based upon previous studies (Millischer et al., 2017), a 0.64±0.19 µmol/L decrease in kynurenine concentration was observed following exercise training. The anticipated additive effect of BCAA supplementation is 0.25 µmol/L. Assuming a SD of 0.25 µmol/L, 12 subjects per group are necessary to achieve at least 80% power with a 5% level of significance. To allow for a 20% dropout, we will recruit a total of 30 subjects with the intent of having 24 complete.

12.3. Populations for Analyses

Clearly identify and describe the analysis datasets (e.g., which participants will be included in each).

exercise plus placebo (**EX+PLA**) vs. exercise plus BCAA (**EX+BCAA**)

12.4. Statistical Analyses

Include analysis of primary efficacy endpoints, secondary endpoints, safety analyses, and any planned interim analyses

In collaboration with RC3 (Trial Design and Integrative Informatics Core), two separate independent sample t-tests will be used for each outcome measure: one to compare change before and after acute exercise (during the 400 m walk) between the two groups and one to compare change before and after chronic (8-week) exercise training between the two groups. This will determine the significance of our primary outcomes (fatigue and tryptophan metabolism) change over time. All statistical tests will be 2-sided and unadjusted for multiple comparisons. We are eschewing the mixed model analysis of variance (ANOVA) to account for the time effect. The repeated measures ANOVA would be testing more effects than we are exploring here, and the study is not powered for this advanced analysis.