Title:	**HIT HEADS Trial: Head Injury Treatment with Healthy and Advanced Dietary Supplements: A randomized, placebo-controlled, double-blinded, therapeutic exploratory clinical trial of branched chain amino acids (BCAA's) in the treatment of concussion**
Short Title	BCAA's in concussion
Drug or Device Name(s):	Branched chain amino acids
FDA IND	117570
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ABBREVIATIONS AND DEFINITIONS OF TERMS

BCAA's	Branched chain amino acids
MRI	Magnetic resonance imaging
g	Grams
g/d	Grams per day
mg/d	Milligrams per day
CCAT	Computerized cognitive assessment tool
TBI	Traumatic brain injury
LFPL	Lateral fluid percussion injury
HIPAA	Health Insurance Portability and Accountability Act
IRB	Institutional Review Board
IDS	Investigational Drug Service
SAE	Serious adverse event
AE	Adverse event
CRCU	Clinical research computing unit
CHOP	Children's Hospital of Philadelphia
HUP	Hospital of the University of Pennsylvania
CNS	Central nervous system
GABA	Gamma-aminobutyric acid
DRS	Disability rating scale
CRF	Case report form
BMI	Body mass index
PHI	Personal health information
DMS	Data management system
PI	Principal investigator
CDP	Computerized dynamic posturography

PROTOCOL SYNOPSIS

Study Title	HIT HEADS Trial: Head Injury Treatment with Healthy and Advanced Dietary Supplements: A randomized, placebo-controlled, double-blinded, therapeutic exploratory clinical trial of branched chain amino acids (BCAA's) in the treatment of concussion
Funder	Dana Foundation
Clinical Phase	Phase II
Study Rationale	Annually, between 100,000 to 140,000 children present to the emergency department for concussion in the United States.[1] The Centers for Disease Control now estimates that 1.6 - 3.8 million sports related concussions occur each year in the United States. A large proportion of these patients have enduring cognitive and neurobehavioral problems. Concussion is a heterogeneous insult to the brain that precipitates a complex pathophysiological process that can result in a cascade of deleterious side effects. At present, there are no proven therapies to mitigate or prevent the neurocognitive and neurobehavioral consequences of concussions. The limbic hippocampus, a brain structure crucial for learning and memory, is often damaged in concussion. In preclinical studies in our laboratory, analysis of ipsilateral hippocampi isolated from mice after traumatic brain injury (TBI) demonstrated that only the concentrations of the three BCAA's (valine, isoleucine, and leucine) were significantly altered (reduced) after injury. When these brain-injured animals received dietary supplementation with BCAA's, the concentrations of these amino acids were restored in the injured hippocampus and the injured animals demonstrated significant cognitive improvement to levels comparable to those obtained in non-injured control animals. In light of these results and the increasing awareness and morbidity associated with concussion, we are proposing a pilot therapeutic exploratory clinical trial to determine the effects of BCAA's in reducing the neurocognitive side effects of concussion injury.
Study Objective(s)	**Primary** • To determine whether, compared to placebo treatment, administration of BCAA's, at one or more doses, after a concussion improves processing speed recovery at one or more time-periods post concussion. **Secondary** • Determine whether administration of BCAA's improves neurocognitive recovery compared to placebo for the cognitive domains of attention, learning, and working memory.

	• Evaluate whether BCAA supplementation improves clinical symptom resolution. • Determine whether BCAA supplementation reduces the time to return to normal activities, specifically school or work, and participation in sports. • Assess dose-related compliance of subjects with daily self-administration of BCAA's and with completion of study endpoints. • Assess feasibility of enrolling potential subjects within 72 hours of concussion injury. • Assess safety of BCAA supplementation in concussed subjects.
Test Article(s)	The three BCAA's—valine, isoleucine, and leucine—will be combined together in a 1:1:1 ratio and administered twice daily by mouth.
Study Design	This study is a randomized, placebo-controlled, double-blinded therapeutic exploratory clinical trial to evaluate the effects of BCAA's on the recovery from the neurocognitive side effects of concussion injury.
Subject Population key criteria for Inclusion and Exclusion:	**Inclusion Criteria** 1. Males and females, ages 11 - 34 years, of any race. 2. Weight at least 40kg. 3. Subjects who had a concussion, as diagnosed by a qualified physician or advance practice provider, within 72 hours prior to enrollment. 4. Ability to have daily email and internet access. 5. Post-menarchal females must have a negative urine pregnancy test and must use an acceptable method of contraception. 6. Subjects must, in the opinion of the referring physician, have the capacity to provide informed consent. 7. Informed consent by the subject, or for subjects <18 years old both informed consent by a parent/guardian and child assent. **Exclusion Criteria** 1. Witnessed seizure at the time of injury or penetrating head injury. 2. Prior concussion or TBI within 90 days. 3. Concussion or TBI severe enough to require admission to an intensive care unit for observation or intervention, specifically for the brain injury. 4. Previous history of TBI or concussion requiring admission to the hospital, disabling stroke, epilepsy, brain tumor, neurodegenerative condition, or psychiatric disease.

	5. Subjects taking neurological or psychoactive medications as a regular daily prescription medication. 6. Known history of maple syrup urine disease or known family history of maple syrup urine disease. 7. Any investigational drug use within 30 days prior to enrollment. 8. Allergy to FD&C Red #40 (red dye 40) or Sucralose. 9. Lactating females. 10. Parents/guardians or subjects who, in the opinion of the investigators, may be non-compliant with study schedules or procedures.
Number Of Subjects	The study will be conducted through the Children's Hospital of Philadelphia. Recruitment will stop when approximately 72subjects are enrolled. It is expected that approximately 72 subjects will be enrolled to produce 50 evaluable subjects.
Study Duration	The study duration per subject will be up to 22 days, with up to 1 day screening and up to 21 days in the treatment phase. The entire study is expected to last approximately 2 years.
Study Phases **Screening** **Study Treatment**	(1) Screening: Physicians who evaluate patients with concussion will identify potential subjects. Subjects will be screened using the protocol inclusion and exclusion criteria. Informed consent and, if applicable, parental/guardian consent and child assent, will be obtained. (2) Intervention: Subjects will be randomized to receive either oral BCAA's 7.5, 15, 22.5, or 27 grams twice daily or placebo for a total of 21 days.
Efficacy Evaluations	Neurocognitive function will be assessed daily through the Axon Sports computerized cognitive battery, which measures processing speed, attention, learning, and working memory. Performance will be measured primarily through reaction time data, but task accuracy data will be analyzed as well. Clinical symptoms will be assessed through clinical symptom checklists, actigraphy (sleep), and dynamic posturography (balance testing optional).
Safety Evaluations	The research coordinator will ask subjects about the occurrence of any adverse events (AE's) during the study visits conducted on days 5-9, 12-16, 22-24. A standardized question will be asked of every subject to inquire about illnesses or accidents since the previous reporting period.
Statistical And Analytic Plan	The mean trajectory in decline and recovery in processing speed is unknown at this stage of development. Thus, the primary analysis will aim to determine whether there are differences in processing speed between the BCAA dose groups and placebo, and if so, at

	what time periods the differences are most pronounced. We will model processing speed as a function of dose and day of study and will allow differences between dose groups to vary as a function of time through a series of interaction terms. The primary analysis will consider three key time periods, Days 3-6, 7-10 and 11-14 when we expect differences in processing speed between the BCAA-treated groups and placebo to be largest. Likelihood ratio tests will be employed to specifically test whether there are differences in mean processing speed between placebo and any of the BCAA doses at the three key time periods.
Data And Safety Monitoring Plan	The investigators will be responsible for data quality management and ongoing assessments of safety. An experienced neurologist, Rebecca Ichord MD will act as the Independent Medical Monitor for this study.

TABLE 1: SCHEDULE OF STUDY PROCEDURES

Evaluation	Screening Day 0	Treatment Phase Days 0 – 21
Informed Consent/Assent Review Inclusion/Exclusion Criteria Pregnancy Test on Post-Menarchal Females	X	
Demographics Concussion History Randomization		X
Axon Sports CCAT		Daily
Clinical Symptom Checklist		Daily
Cognitive and Physical Activity Assessments		Daily
Sleep Assessment		Daily
Drug Accountability Assessment		Daily
Actigraphy		Continuous
Computerized Dynamic Posturography (Optional)		Days 0-4, 5-9, 12-16
Adherence Assessment		Days 0, 5-9, 12-16
Adverse Event Assessment		Days 5-9, 12-16, 22-24

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2 BACKGROUND INFORMATION AND RATIONALE

2.1 Introduction

The Centers for Disease Control now estimates that there are over 700 emergency department visits per 100,000 population for traumatic brain injury, and 1.6 - 3.8 million sports-related concussions occur each year in the United States. Many concussions are sports-related, with more than half of these concussions occur during football, particularly in adolescents and young adults. Annually, between 100,000 to 140,000 children present to the emergency department for concussion in the United States.[1] However, impacts to the head, or sudden acceleration-deceleration events, are also common in other contact sports like soccer, lacrosse, hockey, rugby, and basketball and in pursuits such as horseback riding, cycling, roller-blading, and boxing among others. No concussion is without consequences. In the short-term, patients' cognitive and athletic performance may suffer. In the long term, concussions, and perhaps even sub-concussive blows, are thought to cause alterations in cerebral structures and functions that can lead to impaired cognitive functioning and neurobehavioral problems. That is, there are enduring deleterious effects and a potential causal link between concussion and later-life cognitive and behavioral difficulties including a condition now known as chronic traumatic encephalopathy.

Concussion is a heterogeneous insult to the brain, induced by traumatic biomechanical forces, that precipitates a complex pathophysiological process that can result in a cascade of deleterious side effects. Currently, there are no proven therapies to mitigate or prevent the neurocognitive and neurobehavioral consequences of concussions. Preclinical studies in mice from our laboratory have demonstrated that the hippocampus, a brain structure involved in higher cognitive function often injured in concussion, has reduced levels of the three branched chain amino acids (BCAA's)—valine, isoleucine, and leucine— after traumatic brain injury (TBI).[2] *In vivo* administration of BCAA's restored the concentrations of these amino acids in the hippocampus, and these animals demonstrated cognitive recovery to levels comparable to non-injured animals. In light of these pre-clinical results and the increasing awareness of morbidity, including long-term sequela, associated with concussion, we propose a pilot phase II clinical trial to determine the effects of dietary supplementation with BCAA's to reduce the neurocognitive effects of concussion.

2.2 Name and Description of Investigational Product or Intervention

The three BCAA's—valine, isoleucine, and leucine—will be combined together in a 1:1:1 ratio. Four different dose levels will be evaluated: originally,15 g/d, 30 g/d, 45 g/d, and 60 g/d were planned, however during the original course of the study precipitation issues were noted at the highest ratio due to leucine precipitating at a lower concentration than expected due to the presence of the other solutes. After further testing, it was determined that the product remained in solution at a concentration of 27g/591ml bottle and therefore, the highest dose level was altered to give four dose levels of 15g/d, 30g/d, 45g/d, and 54g/d. Sodium gluconate, sucralose, and fruit punch concentrate are added to improve palatability.

2.3 Findings from Non-Clinical and Clinical Studies

2.3.1 Non-Clinical Studies

Akiva Cohen, a Co-Investigator on this proposal, has directed the preclinical animal studies leading to this clinical trial. The central hypothesis of this work is that the restoration of the delicate balance of excitation and inhibition in central nervous system (CNS) synaptic circuits after TBI may improve cognitive functioning. To examine potential changes in the excitatory (glutamate) and inhibitory (gamma-aminobutyric acid or GABA) neurotransmitters after TBI, Dr. Cohen and colleagues used high pressure liquid chromatography (HPLC) to quantify these amino acid levels in the hippocampal formation of mice (adult male C57Bl/J6) after experimental TBI using lateral fluid percussion injury (LFPI).[1] Results indicated that LPFI does not alter the concentrations of glutamate and GABA, but instead reduces the concentrations of the BCAA's, valine, isoleucine, and leucine, by 50.8, 21.1, and 52.3%, respectively in the ipsilateral hippocampus. No other amino acid was affected and BCAA levels in the LFPI contralateral hippocampus were not significantly different from those measured in sham injured mice.

After quantifying these changes in BCAA levels after brain injury, further research investigated the feasibility of using dietary treatment to restore the amino acid profile in the hippocampal formation. Therefore, both LFPI and sham injured mice received water that was either untreated (control) or contained a cocktail of all three BCAA's (100 mM of each leucine, isoleucine and valine). After consuming the treatment for 5 days, the ipsilateral hippocampus demonstrated BCAA levels similar to those from sham animals. Furthermore, neither injury nor BCAA treatment after injury appeared to significantly alter the remaining 14 tested amino acids, raise BCAA levels in contralateral hippocampus or hippocampi from sham animals, or alter the blood amino acid profile.

LFPI mice demonstrated a significant 39.0% reduction in cognitive performance (as tested by the conditioned fear response task) when compared to uninjured mice. When the LFPI animals were assessed after BCAA treatment, their cognitive performance returned to values seen in sham-injured animals. Additionally, BCAA treatment restored excitability in injured hippocampi to levels similar to those seen in sham animals. Furthermore, there was a perfect (100%) correlation between BCAA-induced cognitive improvement and restoration of ipsilateral hippocampal excitability. To our knowledge, this is the first study to identify an efficacious dietary supplement that generates a measurable change in hippocampal excitability in injured brains and, in so doing, improves cognitive function.

2.3.2 Clinical Studies

2.3.2.1 Clinical Studies in Adults

Dietary BCAA administration has been studied extensively in healthy people and in a variety of disease states over many decades.[3] BCAA therapy has been studied most extensively as it relates to exercise physiology, hepatic pathology, and various neurological and psychiatric disorders. Patients have been treated with BCAA's for variable lengths of time including for more than 2 years. In addition, many athletes and body-builders consume BCAA's as nutritional supplements.

A limited number of studies have examined BCAA's in humans after TBI. Plasma levels of BCAA's and their circulating metabolites are decreased within 24 hours after injury in both mild and severe TBI relative to healthy volunteers.[4] One study, evaluating plasma amino acid concentrations in patients approximately 2 months after TBI, found significantly reduced levels of BCAA's, among other amino acids, relative to age-matched, non-injured controls.[5] A follow-up study on a subsequent cohort of TBI patients found that plasma concentrations of amino acids were still reduced 120 days after injury, mainly driven by lower valine levels.[6] Work by the same group demonstrated that intravenous BCAA administered to patients in the rehabilitation stage after severe TBI improved Disability Rating Scale (DRS) scores when compared to placebo.[7] Additionally, they showed that BCAA's may improve recovery from a posttraumatic vegetative or minimally conscious state.[8]

2.3.2.2 Clinical Studies in Children

The lower age limit for enrollment in the proposed study is 11 years. Several clinical studies have been performed with BCAA's in children. BCAA's were given to healthy children [9] and children with cholestatic liver disease [10] to determine daily BCAA requirements. BCAA's have been administered safely to children with phenylketonuria in an attempt to inhibit entry of phenylalanine into the brain and reduce its toxic effects on the CNS.[11-13] Beneficial effects also were seen when children with end-stage liver disease awaiting transplantation were fed with a BCAA-enriched formula compared with a standard formula.[14,15] Symptoms of tardive dyskinesia were reduced in children and adolescents after treatment with BCAA's and epileptic children treated with up to 20 g/d of BCAA in conjunction with a ketogenic diet showed reduction in seizure frequency and improvements in behavior and cognitive functioning.[16,17] In all studies of children and adolescents treated with BCAA's, the supplements were well tolerated and without side effects.

2.4 Selection of Drugs and Dosages

Work from our laboratory has demonstrated that administering the equivalent of 60 g/d of BCAA's (in a 1:1:1 ratio of leucine, isoleucine, and valine) mitigates injury-induced anterograde cognitive impairments in our mouse model. Higher doses did not offer any additional benefit the animals. We originally used this dose as the maximal dose in our study, noting that it is within the ranges described in previous clinical studies.[3] For example, oral doses of 60 g/d are effective in reducing mania symptoms.[18] Benefit has also been seen in tardive dyskinesia (~15 g/d),[19] spinocerebellar degeneration (6 g/d),[20] anorexia in cancer patients (14 g/d),[21] and hepatic encephalopathy (median amount of BCAA's over 11 randomized trials was 28 g/d; range 11 to 57 g/d).[22] Aquilani and colleagues demonstrated that intravenous administration of 20 g of BCAA's over 5 hours in TBI patients was associated with improved cognitive outcomes.[7] Our preclinical work and this prior clinical work suggest that orally administering 30 g/d of BCAA's twice daily will be safe and well tolerated. However, during the original course of the study precipitation issues were noted at the highest ratio due to leucine precipitating at a lower concentration than expected due to the presence of the other solutes. After further testing, it was determined that the product remained in solution at a concentration of 27g/591ml bottle and therefore, the highest dose level was altered to 54 g/d.

Overall, BCAA's are well tolerated and associated with minimal to no side effects. Several review papers have examined BCAA administration and summarized the adverse events (AE's). [3,22] Many studies do not comment specifically on side effects, while others contain statements like "none of the subjects experienced side effects." A few report mild gastrointestinal side effects such as abdominal distention, diarrhea, and constipation. One study of patients with liver cirrhosis, a group different from our proposed study population, found these symptoms in 12% of their BCAA treatment group.[23] No serious or life threatening side effects have been reported. These studies generally indicate that humans can consume amounts of BCAA's that are comparable to those proposed here, without AE's. Indeed, many body-builders and athletes consume large amounts of BCAA's as nutritional supplements in various formulations. There is a theoretical concern that because BCAA's can alter the levels of other neurotransmitters, like dopamine and serotonin, depression or impairment of some cognitive functions may occur in susceptible individuals. These symptoms, however, have never been reported clinically.

It should be noted that the daily intake of BCAA's in a 70 kg person consuming the recommended dietary allowance for protein (0.8 g/kg/d) would be 8.4 –11.2 g [40 kg person would be 4.8 - 6.4g]. In athletes, for whom a common recommendation is at least 1.2 g protein/kg/d, the daily BCAA intake would be 12.6 – 16.8 g [40 kg person would be 7.2 – 9.6g]. The recommended dietary allowance of protein in adolescents is slightly higher than adults (1.0 g/kg/d for 11-14 year olds and 0.8-0.9 for 15-19 year olds). Therefore a 40 kg younger adolescent consuming the recommended dietary allowance for a non-athlete would be 6.0 – 8.0g and athlete would be 9.0 – 12.0g. While some studies in patients with liver cirrhosis [24] have used doses >100 g/d without reported side effects, the highest dose used in studies of neurological disease has been 60 g/d. Additionally, a single dose finding study has been done in 12 healthy volunteers and showed in a randomized, double blind, crossover design that single oral doses of 10 g, 30 g and 60 g of BCAA's were safe and well tolerated.[25] At 60 g/d, this dose represents a 2 to 4 fold increase in the daily BCAA intake for athletes (assuming a body weight of 70 kg). Given that this dose is safe and well tolerated in humans, the no observable adverse effect level (NOAEL) of BCAA intake may be considerably higher, although this has not been formally studied to our knowledge. A toxicity study in rats over 13 weeks showed that rats did not exhibit any standard measures of potential toxicity at doses greater than 100 g/d.[26]

2.5 Relevant Literature and Data

2.5.1 Computerized Cognitive Testing

Neuropsychological testing provides a valuable mechanism to detect both obvious and subtle impairments in cognitive functioning after concussion. Several computerized testing batteries are commercially available to evaluate patients after concussion. The Axon Sports Computerized Cognitive Assessment Tool (CCAT) is optimal for use in a context where frequent repeated measurements of cognitive performance are required. The CCAT consists of four computerized cognitive tests, derived from theoretical and developmentally sensitive principles, allowing this tool to be sensitive to any cause of cognitive performance change in individuals over time. The tasks themselves measure processing speed (via a simple reaction time task), attention (via a choice reaction time task), learning (via a one card learning task),

and working memory (via a one-back task). These tests have been widely validated in both healthy and concussed individuals. Normative scores exist for men and women greater than 18 years, as well as, children and adolescents aged 10-18. Studies have shown that task performance is similar between 16-18 year olds and adults.

Prior research has demonstrated the sensitivity of the CCAT tests to neurological impairment, including mild TBI and concussion.[27-31] The reliability and metric properties of the tests have been described and normative data are published.[27,32-40,52] Numerous publications have demonstrated that the Axon Sports CCAT has minimal practice effects (making it ideal for repeat testing as in this proposal), and good criterion, construct, test-retest, and ecological validity.[33,34] The CCAT tasks have been shown to be sensitive to concussion-related cognitive decline and symptomatic athletes have greater impairments as measured by the CCAT than asymptomatic athletes.[30,41-43]

2.5.2 Concussion Clinical Symptom Scales

In addition to neuropsychological testing, self-reported symptoms are commonly cited as a means to identify concussive symptoms and a practical method to monitor recovery from concussion. Piland and colleagues have demonstrated excellent factorial and construct validity of a 9-item self-reported symptom instrument in both high school and college athletes.[44] The items are: headache, nausea, balance problems, sleeping more than usual, drowsiness, fatigue, feeling "slowed down", feeling "in a fog", and difficulty concentrating. This 9-item Head Injury Scale is a summative 7-point Likert-type scale instrument designed to measure the overall duration (length of symptom experienced over a 24-hour period) of concussion-related symptoms. Piland also demonstrated factorial validity when athletes were queried on how severe each of the 9 symptoms has felt during the previous 24-hour period (i.e. symptom severity scale).

2.5.3 Sleep Disturbances Post-Concussion

Sleep disturbances are reported in up to 72% of patients with mild, moderate, and severe TBI up to three years post-injury. Those TBI patients with sleep disturbances have longer inpatient hospital stays, a higher cost of rehabilitation, and a greater rate of functional disability. Current clinical practice guidelines advocate aggressive management of sleep hygiene after TBI to improve neurological symptoms and cognitive outcomes; however, the evidence behind this is lacking, and the neural mechanisms underlying sleep disturbances after TBI are unknown. Moreover, our current therapeutic options for sleep disturbances are less effective in patients with TBI-related sleep problems than with other sleep disorders. Preliminary data from our laboratory suggest that after LFPI, mice show significant sleep disturbances for up to 30 days post-injury compared to sham surgery controls, including more time sleeping during the active phase and increased sleep fragmentation (defined by an increased number of sleep-wake transitions and shortened average length of sleep and wake bouts). BCAA supplementation seems to attenuate some of these sleep disturbances after TBI.

2.5.4 Balance and Vestibular Impairments Post-Concussion

Balance or postural control is necessary in activities of daily living and sport. Balance requires the CNS to process and integrate afferent information from the visual, somatosensory (proprioceptive), and vestibular systems to then perform coordinated musculoskeletal responses. Abnormal static and dynamic balance is well described in survivors of TBI and altered postural dynamics is frequently found immediately after a concussive injury. Balance deficits usually resolve within 3 to 10 days after injury, however, they may be identified even years after injury in up to a third of patients and self-reported balance difficulty often correlates with persistent post-concussion symptoms. Instrumented testing devices are considered the gold standard of balance testing since they attempt to objectively measure balance. The computerized dynamic posturography (CDP) test as an objective means to assess balance by examining the response pattern to simultaneous, multimodal sensory stimulation.[45]

2.5.5 Branched Chain Amino Acids

Branched chain amino acids are essential amino acids, meaning they cannot be synthesized *de novo* and therefore must be acquired through the diet. They are involved in numerous functions in the human brain including protein synthesis and energy production. A significant fraction of ingested BCAA's is not metabolized by the liver and passes directly into the systemic circulation, causing plasma concentrations to rise appreciably. BCAA's are important for protein metabolism throughout the body. They are transported into the brain by high affinity, low capacity transporters, located at the blood–brain barrier on capillary endothelial cells. Leucine enters the brain faster than any other amino acid, but valine and isoleucine also readily cross the blood-brain barrier.

Once in the brain, BCAA's are key amino acids involved in *de novo* glutamate (the major excitatory neurotransmitter in the CNS) synthesis, as approximately 50% of brain glutamate contains BCAA-derived nitrogen. Furthermore, *de novo* glutamate synthesis contributes approximately 40% of the releasable synaptic glutamate. It is believed that astrocytes remove released glutamate from the synaptic cleft, and amidate it to form glutamine for return to the neuron. This process, known as the glutamate:glutamine cycle, requires *de novo* glutamate synthesis to maintain sufficient presynaptic releasable neurotransmitter pools. If this process continued unabated, it would lead to a net transfer of nitrogen from astrocytes to neurons. Nature prevents this by shuttling BCAA's in the opposite direction (i.e., from the neuron to the astrocyte). Due to the intrinsic role for BCAA's in glutamate and subsequent GABA (the major inhibitory neurotransmitter in the mammalian CNS) synthesis, and the interrelationship between the glutamate:glutamine cycle and the presumed BCAA-dependent astroglial:neuronal nitrogen shuttle, changes in these three specific amino acids likely contribute to post-traumatic alterations in both hippocampal excitability and cognitive performance.

2.6 Compliance Statement

This study will be conducted in full accordance with all applicable Children's Hospital of Philadelphia (CHOP) Research Policies and Procedures and all applicable federal and state laws and regulations. The investigators will perform the study in accordance with this

protocol, will obtain consent and assent, and will report unanticipated problems involving risks to subjects or others in accordance with CHOP's Institutional Review Board (IRB) Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

3 STUDY OBJECTIVES

The purpose of this study is to determine whether dietary administration of BCAA's shows evidence of improving the recovery of neurocognitive symptoms after concussion injury, and to determine which of several doses of dietary BCAA's shows the highest promise of efficacy.

3.1 Primary Objective

The primary objective of this study is to determine whether, compared to placebo treatment, administration of BCAA's, at one or more doses, after a concussion improves processing speed recovery at one or more time-periods post concussion. In the event that differences are observed, we will determine whether differences in processing speed between BCAA-treated and placebo-treated groups reflect differences in the maximum level of impairment and/or increases in the subsequent speed of improvement.

3.2 Secondary Objectives

The secondary objectives are to:

- Determine whether administration of BCAA's improves neurocognitive recovery compared to placebo for the cognitive domains of attention, learning, and working memory, and if so determine whether the results reflect differences in maximum levels of impairment or increases in subsequent speed of improvement.
- Evaluate whether BCAA supplementation improves clinical symptom resolution.
- Determine whether BCAA supplementation reduces the time to return to normal activities, specifically school or work, and participation in sports.
- Assess dose-related compliance of subjects with daily self-administration of BCAA's and with completion of study endpoints.
- Assess feasibility of enrolling potential subjects within 72 hours of concussion injury.
- Assess safety of BCAA supplementation in concussed patients.

4 INVESTIGATIONAL PLAN

4.1 General Schema of Study Design

The proposed study is a therapeutic exploratory clinical trial that will evaluate the effects of BCAA's on the magnitude and recovery of neurocognitive deficits following concussion injury.

4.1.1 Screening Phase

Physicians and advanced practice providers who have experience in diagnosing concussion, will identify potential subjects. The investigators and co-investigators will inform the groups of physicians who will be referring patients to the study (e.g. CHOP and Penn Sports Medicine, CHOP and HUP Emergency Medicine) about the study procedures and their role in referring patients via email and presentations at local conferences. For these groups of physicians, the diagnosis of concussion is informed by the commonly published guidelines for the diagnosis of concussion. The most widely used is the Consensus Statement on Concussion in Sport: The 5th International Conference on Concussion in Sport, Berlin, October 2016.[46] Others societies and professional organizations including the American Academy of Neurology, American Academy of Pediatrics, and the American Medical Society for Sports Medicine have publications which contain similar diagnostic guidelines. A potential subject is a patient who has had a concussion, as diagnosed by a physician, within the prior 72 hours, is between the ages of 11 and 34, and, in the opinion of the physician, has capacity to consent to participate in the study. Verification of the diagnosis of concussion will be made by obtaining written documentation from the referring physician or advanced practice provider. This documentation may come in several forms including a copy of a clinic note, a copy of an ED or hospital note, summary statement or discharge document, or an email sent directly from the physician confirming the diagnosis. This documentation will be reviewed by a physician from the study staff to confirm that the patient was diagnosed by a qualified physician or advanced practice provider to have a concussion. The source document and documentation of the PI's review and confirmation of the diagnosis will be maintained in the subject's file.

Potentially eligible subjects will be referred to the study coordinator and subsequently screened by a study staff member using the protocol inclusion and exclusion criteria during an in-person interview. Post-menarchal females will have a urine pregnancy test performed, or documented as negative within 72 hours prior to enrollment. Informed consent and, if applicable, parental/guardian consent and child assent, will be obtained prior to any study related procedures being performed. If enrolled, subjects will be randomized to receive one of four different dose levels of BCAA's or placebo.

4.1.2 Study Treatment Phase

Subjects enrolled in the trial will be randomized to receive either oral BCAA's at doses of 7.5, 15, 22.5, or 27 g BID or an oral placebo BID for a total of 21 days. Subjects will complete five on-line computerized assessments daily:

1. Axon Sports Computerized Cognitive Assessment Tool
2. Clinical symptom checklists (Appendix 1)

3. Cognitive and physical activity assessments (Appendix 2)
4. Sleep assessment (Appendix 3)
5. Drug accountability assessment (Appendix 4)

Subjects will wear an actigraphy monitor for the duration of the trial to assess sleep patterns. Computerized Dynamic Posturography may be conducted at 3 time points (days 0-4, 5-9, and 12-16) during the study treatment phase.

4.2 Allocation to Treatment Groups and Blinding

The study biostatistician, Dr. Putt, will generate a randomization schedule for implementation in the randomization module of the study database system. The randomization scheme will be based on an equal allocation of each of the possible doses or placebo, and stratified based on sex. The randomization schedule is uploaded into the data management system (DMS) and provided to the Investigational Drug Service (IDS). The IDS will use the randomization schedule to prepare kits of BCAA doses or placebo for distribution. Upon confirmation of eligibility, the DMS will provide study personnel with a number that is then given to the IDS and corresponds to the kit number to be distributed. All study team members will be blinded to treatment assignment. The Database Administrator will have access to the link between the kit number and the treatment arm and will provide this information to the Investigational Drug Service (IDS) so that kits are prepared according to this scheme. BCAA and placebo treatment kits will appear identical.

4.3 Study Duration, Enrollment and Number of Sites

4.3.1 Duration of Study Participation

The study duration per subject will be up to 22 days, with up to 1 day screening and up to 21 days in the treatment phase.

4.3.2 Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted through the Children's Hospital of Philadelphia and the Hospital of the University of Pennsylvania. Recruitment will stop when 50 subjects who are evaluable for efficacy are enrolled. A subject who is evaluable for efficacy is one who completes the study up until at least day 10 of the study. It is expected that approximately 72 subjects will be enrolled to produce 50 such subjects. All subjects who enroll and receive study medication will be evaluable for safety.

4.4 Study Population

4.4.1 Inclusion Criteria

1) Males and females, ages 11 - 34 years, of any race.
2) Weight at least 40kg.
3) Subjects who had a concussion, as diagnosed by a qualified physician or advanced practice provider, within the 72 hours prior to enrollment.
4) Ability to have daily email and internet access.

5) Post-menarchal females must have a negative urine pregnancy test and must use an acceptable method of contraception including abstinence, a barrier method (diaphragm or condom), Depo-Provera, or an oral contraceptive for the duration of the study.
6) Subjects must, in the opinion of the referring physician, have the capacity to provide informed consent, defined by: 1) the ability to communicate; 2) the ability to assess their understanding of the treatment; 3) the ability to understand the risks and benefits of the treatment; and 4) the ability to understand the risks and benefits of not embarking on the treatment.
7) Informed consent by the subject, or for subjects <18 years old both informed consent by a parent/guardian and child assent.

4.4.2 Exclusion Criteria

1) Witnessed seizure at the time of injury or penetrating head injury.
2) Prior concussion or TBI within 90 days.
3) Concussion or TBI severe enough to require admission to an intensive care unit for observation or intervention, specifically for the traumatic brain injury.
4) Previous history of TBI or concussion requiring admission to the hospital, disabling stroke, epilepsy, brain tumor, neurodegenerative condition, or psychiatric disease.
5) Subjects taking neurological or psychoactive medications as a regular daily prescription medication (e.g. stimulants, antidepressants, anticonvulsants, benzodiazepines, etc.).
6) Known history of maple syrup urine disease or known family history of maple syrup urine disease.
7) Any investigational drug use within 30 days prior to enrollment.
8) Allergy to FD&C Red #40 (red dye 40) or Sucralose.
9) Lactating females.
10) Parents/guardians or subjects who, in the opinion of the investigators, may be non-compliant with study schedules or procedures.

Subjects that do not meet all of the enrollment criteria may not be enrolled. Any violations of these criteria must be reported in accordance with IRB Policies and Procedures.

5 STUDY PROCEDURES

5.1 Screening Visit

- Informed consent
- Review of inclusion and exclusion criteria
- Urine pregnancy test (for post-menarchal females only)

5.2 Study Treatment Phase

At the start of the treatment phase at Visit 1, the study coordinator or study staff member will obtain demographic information, a concussion history and measure height and weight. The study drug will be dispensed in 42 bottles at the time of enrollment. The assessments during the study treatment phase will be web-based and completed daily through the study website:

1. Axon Sports Computerized Cognitive Assessment Tool
2. Clinical symptom checklists (Appendix 1)
3. Cognitive and physical activity assessments (Appendix 2)
4. Sleep assessment (Appendix 3)
5. Drug accountability assessment (Appendix 4)

Subjects will wear an actigraphy monitor for the duration of the trial to assess sleep patterns. Subjects will have the option to complete computerized dynamic posturography (CDP) measured on visits 1, 2, and 3, which correspond to study, days 0-4, 5-9 and 12-16. If enrollment occurs on a Saturday or Sunday, CDP can be conducted the following Monday or Tuesday. The actigraph monitor will be downloaded during the CDP visits, and after being returned at the conclusion of the study (Visit 4, Days 22-24). Subjects will be screened for drug accountability via a urine assay on visits 2 and 3, which correspond to study days 5-9 and 12-16.

We recognize that the data collection we are requesting of subjects may be onerous, and return visits for study procedures are a significant burden for subjects. Early subject recruitment has demonstrated issues with consistent compliance with study procedures. Therefore, we will institute graduated rewards for continued participation during the study. Using a token economics approach, study subjects will be rewarded by text- or email-based reinforcement for each data entry completed, including visual representations of compliance to serve as reminders/motivators for sustained compliance.[52-55] These will graphically and/or by text show how close they are to meeting the 3-day-in-a-row and 7-day-in-a-row endpoints, which will have associated monetary incentives (see subject payment section).

Visit	Purpose	Main Procedures	Duration
Visit 1, Day 0	Screening	Informed consent Review inclusion/exclusion criteria Urine pregnancy test (post-menarchal women only)	15 minutes
Visit 1, Day 0	Orientation Start Study Drug	Orientation to study & study website Demographics & concussion history Baseline compliance urine test	2 hours

		Computerized balance testing (optional)* Distribute actigraphy sleep monitor Distribute study drug	
Days 0-21	Daily assessments via study website	Computerized cognitive testing Clinical symptom checklists Cognitive & physical activity assessments Sleep assessment	15 minutes
Visit 2, Day 5-9	Routine Visit	Computerized balance testing (Optional) Compliance urine test	20 minutes
Visit 3, Day 12-16	Routine Visit	Computerized balance testing (Optional) Compliance urine test	20 minutes
Visit 4, Day 22-24	End of Study	Return used and unused drug bottles Return actigraphy monitor	10 minutes

* - If enrollment occurs on a Saturday or Sunday, balance testing can be conducted the following Monday or Tuesday.

5.3 Unscheduled Visits

The study coordinator and investigators will be available to address any concerns or questions that may arise during the study. Any medically related questions will be referred to their primary treating physician.

5.4 Prior and Concomitant Medications

All medications used within 7 days prior to the screening visit and through the end of the study will be recorded. The dates of administration and reason for use will be included. Participation in the study will not preclude subjects from receiving symptomatic management for concussion related symptoms (e.g. non-steroidal anti-inflammatory drugs for headaches) or other disorders. However subjects will be discontinued from the study if they use neurological or psychoactive medications (e.g. stimulants, antidepressants, anticonvulsants, benzodiazepines, etc.) during the study. While subjects will be prohibited from using dietary supplements during the study, there will be no dietary restrictions.

5.5 Rescue Medication Administration

Subjects who experience side effects from the study medication, to a level that the subject requests medication to manage these symptoms, will be discontinued from the study.

5.6 Subject Completion/Withdrawal

Subjects may withdraw from the study at any time without prejudice to their care. They may be discontinued from the study at the discretion of the investigators for safety concerns related to any adverse event. Any subject who sustains a second concussion during the study period will be withdrawn. Additionally, any female subject who becomes pregnant while enrolled in the study will be withdrawn. Date of termination will be documented for each subject.

5.7 Early Termination Study Visit

The study coordinator will contact subjects who terminate early and request that they return all of the study drug bottles (used and unused).

6 STUDY EVALUATIONS AND MEASUREMENTS

6.1 Screening and Monitoring Evaluations and Measurements

6.1.1 Demographics

- Demographic characteristics will be obtained via interview: date of birth, gender, ethnicity, racial background, native language, handedness, and highest educational level attained.

6.1.2 Concussion History

- Concussion history will be obtained via interview: number of times diagnosed with a concussion and date of last concussion.
- History of current concussion obtained from the subject and family members, if necessary, via interview including: mechanism of injury, for sports-related concussions: sport and position and specific mechanism (head-to-head, head-to-ground, hit-to-body, etc), day and time of the injury, was the subject helmeted, presence and duration of loss of consciousness. The subject may bring a clinic note from their referring physician and refer to it for answers to these questions if they are unsure or sustained amnesia from their concussion. For CHOP patients, data may be collected directly from the medical record of their CHOP visits if subjects/parents are unable to provide.

6.1.3 Height and weight

Height and weight will be measured, or taken from the most recent visit documentation if visit was within 1 week of enrollment.

6.1.4 Laboratory Evaluations

6.1.4.1 Pregnancy Testing

A urine pregnancy test will be performed through the Translational Core Laboratories for all post-menarchal female subjects. Results will be available to the study coordinator and investigators in less than 5 minutes. If the study team is unable to access Translational Core Laboratories and/or it is not possible to obtain pregnancy test results within 5 minutes, the pregnancy test will be done by the enrolling study team member. The enrolling study team member must have documented training to do the pregnancy test. If a pregnancy test was performed by a medical facility within 72 hours prior to enrollment, and documentation can be provided or found in the CHOP medical record, then a second pregnancy test will not be required. Pregnant subjects will be excluded from the trial. Should a subject become pregnant while enrolled in the study, they will be withdrawn.

6.2 Efficacy Evaluations

6.2.1 Axon Sports Computerized Cognitive Assessment Tool (CCAT)

The Axon Sports CCAT will be completed daily over an 8-10 minute period using any computer with internet access. The CCAT consists of four computerized cognitive tests,

derived from theoretical and developmentally sensitive principles and aims to be sensitive to any cause of cognitive performance change in individuals over time. The tasks themselves measure processing speed (via a simple reaction time task), attention (via a choice reaction time task), learning (via a one card learning task), and working memory (via a one-back task). All of the four tasks are based on a 'playing-card' paradigm. Within each of the playing-card tasks, participants are shown a picture of a playing card and asked to answer a question about the card. Different tests are created by changing the question. For example, in the attention test the question 'Is the card red?' elicits a simple binary choice and is equivalent to a choice reaction time test. The question 'Is the card exactly the same as the previous card?' is used in the working memory paradigm to produce a 1-back version of the commonly used n-back working memory paradigm. In all tasks, responses are indicated by pressing the 'YES' or 'NO' buttons on a computer keyboard. Subjects will be reminded to choose an environment with minimal distractions for completing the cognitive battery. Subjects will complete a single short practice session, as previous research has demonstrated that a single practice test is sufficient to eliminate the majority of practice effects.

6.2.2 Clinical Symptoms Checklists

A 9 item clinical symptom checklist, designed to measure the overall duration and severity of concussion-related symptoms, will be administered daily over a 1-2 minute period via the study website. This summative 7-point Likert-type scale instrument incorporates aspects of the two scales commonly used in the literature (Post-concussion Symptom Scale [included in the Sport Concussion Assessment Tool 2 (SCAT2)] and Graded Symptom Checklist) to capture the overall severity of symptoms. The checklist will be administered twice: first to address the duration and second the severity of symptoms including headache, nausea, balance problems, sleeping more than usual, drowsiness, fatigue, feeling "slowed down" or "in a fog", and difficulty concentrating.

6.2.3 Cognitive and Physical Activity Assessments

Subjects will be asked daily to indicate their level of cognitive and physical activity via the study website. The physical activities are based on the "Graduated Return to Play" protocol recommended in the Consensus Statement on Concussion in Sport. The cognitive activities are based on return to school guidelines used at CHOP.

6.2.4 Actigraphy Sleep and Assessment

Subjects will complete a daily brief questionnaire on their sleep via the study website and will be asked to wear an actigraphy monitor throughout the trial on the non-dominant wrist, with the exception of bathing or swimming. Actigraphy has been shown to provide a valid estimate of sleep patterns in children and adolescents. An actigraph is a non-invasive small, wristwatch sized activity monitor that provides an estimate of sleep patterns based on data collected by an internal accelerometer, and can record and store the data over an extended period of time. The data are collected continuously and then translated into epochs (typically 30 sec or 1 min) of activity. Using validated algorithms, epochs are then scored as sleep or wake. Subjects will be instructed to push the event button on the device each time they go to bed, get out of bed, take the watch off or put the watch back on if not permitted in

an academic or athletic event. The study coordinator will download the data from the actigraph device onto a study computer at each study visit and the conclusion of the study.

The actigraph monitor used in this study is called the ActiSleep+ Monitor and is produced by the ActiGraph Company from Pensacola, FL. This product is approved for marketing in the US and is an FDA cleared Class II medical device within the US (see attached material). This product is currently deployed in other concussion research studies at CHOP.

6.2.5 Computerized Dynamic Posturography (CDP)

Computerized dynamic posturography (CDP) will be measured over a 15 minute period at visits 1, 2, and 3 at The Balance Center at the Hospital of the University of Pennsylvania. CDP is a postural control test utilized to assess function balance ability.[45] The Sensory Organization Test of CDP evaluates a person's ability to effectively utilize the visual, somatosensory, and vestibular systems for the maintenance of balance. It also provides information regarding the patient's ability to maintain balance in the presence of inaccurate visual information that can confuse orientation. The motor control test measures, time to respond to a disruption of equilibrium. Testing is performed by standing a subject on a computerized support surface to measure their ability to sustain upright stance while the support surface and/or visual surround move in response to their own postural movements. Six sensory conditions are performed to assess ability to use visual, proprioceptive, and vestibular information in isolation and in concert for the preservation of balance. Medium and large forward translations for each leg will be recorded for the motor control test.

CDP will be performed on an EquiTest® system (manufactured by NeuroCom®, a division of Natus®) which is an FDA approved product. CDP will be conducted by a trained audiologist A report will be issued to the investigators who will subsequently input the results into the study database.

Balance testing is now available at CHOP, as of fall 2017, and if equivalence can be determined between the results gathered at HUP and CHOP and access can be gained to this equipment/lab then CHOP will also be added as a potential site for balance testing of subjects.

6.3 Safety Evaluation

Subject safety will be monitored by tracking AE's. See Section 8.1 for details.

7 STATISTICAL CONSIDERATIONS

7.1 Primary Endpoints

We hypothesize that neurocognitive function in concussed subjects will initially worsen and then improve in a non-linear fashion over the course of the study, and that, compared to the placebo group, one or more of the BCAA dose level groups will show a reduced nadir in cognitive performance and/or a more rapid recovery. Currently, the time course of the worsening and improvement of neurocognitive function after concussion is not well understood. Thus, part of our study will aim to identify times when maximal differences between groups may be observed. The primary analysis in this exploratory study will consider three key time periods, Days 3-6, 7-10 and 11-14 when we expect differences in processing speed between the BCAA-treated groups and placebo to be largest.

7.2 Secondary Endpoints

- Repeated measurements of neurocognitive functioning (CCAT test of attention, learning, and working memory) sampled over a period of 21 days.
- Clinical symptoms.
- Time to return to school/work, and sports participation.
- Tolerability of BCAA's based on AE's.
- Compliance.
- Safety and incidence of AE's and serious adverse events (SAE's).

7.3 Statistical Methods

7.3.1 Baseline Data

The characteristics of the dose and placebo groups will be described using standard statistics (e.g. means, medians, standard deviations, or proportions). For all analyses, high dose group will continue to be evaluated together, despite slight change in concentration. Subgroup analysis will be done to evaluate any differences in those subjects who received the full 60 g/d dosing before this dose was decreased, versus the current 54 g/d dosing. Graphical procedures will be used to assess distributional assumptions and particularly the assumption of normality of outcome.

7.3.2 Primary Efficacy Analysis

The primary intent to treat analysis will include all subjects who enroll and contribute at least 10 days of data to the study. The mean trajectory in decline and recovery in processing speed is unknown. Thus the primary analysis will aim to determine whether there are differences in processing speed between the BCAA dose groups and placebo, and if so, at what time periods the differences are most pronounced. The primary analysis will focus on results for the first 10 days of the study when we anticipate that the majority of subjects will be highly compliant. We will model processing speed as a function of dose and day of study and will allow differences between dose groups to vary as a function of time through a series of interaction terms. To ensure that the number of interaction terms does not become too large, we will consider groups of two to four time points, looking specifically at whether

there are differences between any of the dose groups and placebo at Days 1-2, 3-6, 7-10, 11-14, 15-18, 19-21. The primary analysis will consider three key time periods, Days 3-6, 7-10 and 11-14 when we expect differences in processing speed between the BCAA-treated groups and placebo to be largest. Since processing speed differs with age, the model will be adjusted for age to account for any imbalances in age between dose groups. A mixed effects model will be used to account for correlations between repeated measurements on individual subjects. Likelihood ratio tests will be employed to specifically test whether there are differences in mean processing speed between placebo and any of the BCAA doses at the three key time periods. These three global tests will be conducted at a family-wise error rate of 0.05, using a Bonferroni correction to account for the three individual global tests. If statistical significance is achieved for the global test, we will then compare individual dose levels to placebo within time periods, again using a correction for four multiple tests per key time period.

We note that with five treatment groups and 21 time points, there are a very large number of possible hypothesis tests that can be carried out with these data. If all of these tests were carried out without adjusting for multiple comparisons, we would undoubtedly find spurious associations. On the other hand, adjusting for large numbers of multiple comparisons substantially reduces the statistical power of a study. By focusing our testing for the primary efficacy analysis on three key periods where we adjust for a limited number of tests, we balance the need for a rigorous statistical testing procedure with a need to maintain statistical power.

An analysis of the Day 1-2, 15-18 and 19-21 data will follow the same format, but will be considered secondary and will not be formally corrected for multiple testing. In addition to hypothesis testing, we will estimate and plot means and confidence intervals for each group at each time point. This analysis will guide the recommendation for dose levels and evaluation times for the primary analysis in future efficacy studies.

With concerted efforts from our study coordinators and study staff we are hopeful that we can maintain excellent compliance throughout the study. In particular we expect good compliance early in the study when patients are highly motivated to feel better and return to school, work and play. However, we allow that some patients may have difficulty with compliance due to their injury, or become non-compliant once their symptoms improve. As part of this feasibility study, we will explore patterns of missing data, in particular how they may relate to the observed response (e.g. more missing data in those subjects who show better or worse processing speed) to compliance (e.g. more missing data in subjects who regularly fail to consume their supplements) and to demographics e.g. gender, age. This information will be valuable in planning future efficacy trials, and will guide our efforts to adjust for missing data. For example if the probability of missing data is associated with outcome, then non-ignorable missing data methods will be needed, whereas missing at random assumptions will be valid if the missing data can be explained by covariate information.

In addition to the primary analysis, this feasibility study will consider several approaches to modeling a smooth function for the trajectories of the individual processing speeds. The first model will fit non-linear curves to the trajectory of processing speed for the dose groups

and then compare the mean curve for the BCAA dose groups to the placebo group, using an interaction term that allows differences between groups to vary over time. The basis for this model will be a smoothing spline. This first model assumes that the trajectories are smooth over time, albeit somewhat different between groups. We will carry out a hypothesis test of the difference between the groups using a likelihood ratio test for models with and without terms for the treatment effect and treatment by time interactions. A second model conceptualizes an initial increase in processing speed followed by a period of gradual improvement and by a transition where processing speed plateaus. This transition is of interest as we hypothesize that it represents the time when processing speed has returned to baseline. Here we anticipate a model using a quadratic with a change-point,[47,48] with separate baseline functions for the dose and placebo groups and separate change-points. Correlation between repeated measurements on the same individual over time will be modeled using random effects i.e., a mixed effects model will also be used for these analyses.

7.3.3 Analysis of Secondary Endpoints

Secondary endpoints, including clinical symptoms such as the posturography and sleep assessments, and the learning, attention and working memory components of the neurocognitive battery will be modeled using a similar approach as described above for the primary efficacy analysis (Section 7.3.2), with the goal of assessing differences between groups over time, after adjustment for age differences. We will assess the distribution of the outcome and either use a transformation to achieve approximate normality or use a generalized mixed effects model. Time to event outcomes (e.g. time to return to school or sports participation) will be modeled using a Cox proportional hazards model. We will secondarily adjust our primary models for body mass index (BMI) and compliance to obtain information on whether individuals with lower BMI, and presumably higher BCAA/kg dosing have better outcome.

We will also monitor compliance including adherence to treatment and daily completion of neurocognitive testing. It is of interest to determine whether there are differences in compliance among the dosage groups versus placebo whether compliance decreases as a function of time, and whether compliance differs among groups over time. Adherence to treatment is measured in terms of number of bottles of consumed per day (0, 1, or 2) and will be modeled as an ordinal outcome. Adherence to treatment will be assessed using generalized estimating equations (GEE) models as we have repeated measures on adherence for each subject across all time points in the study and are primarily interested in differences between groups. In particular we anticipate that with regular prompting and encouragement, subjects will consistently complete their neurocognitive functioning tests up until about 10 to 14 days into the study. We anticipate that compliance may begin to decrease around the 10 to 14 day point as subjects begin to feel better and return to their normal routines. Assessment of compliance will be key information needed to design the anticipated pivotal trial of subsequent research studies.

7.3.4 Safety Analysis

All subjects enrolled into the study, who receive study medication, are evaluable for safety. The frequencies of AEs by type, body system, severity, patient BMI, and relationship to study drug will be summarized. SAEs (if any) will be described in detail. AE incidence will be summarized by dose and placebo along with the corresponding exact binomial 95% two-sided confidence intervals.

7.4 Sample Size and Power

The primary analysis of the processing speed data is broadly based, reflecting the fact that that this is a feasibility study in which we have limited knowledge regarding the temporal patterns of decline and recovery in human neurocognitive functioning following concussion. We hypothesize that BCAA-treated groups will differ from placebo at specific time periods. The power calculation is based on comparisons of each dose group to placebo at each of the key time periods. These time periods each encompass three daily measurements per subject. With 10 subjects per dose group, and three repeated measurements per subject we have 80% power to detect mean differences in processing speeds between the BCAA group and the placebo group on the order of 0.122 seconds. Putting this in the context of a mean processing speed for healthy individuals of ages 14 to 34 of 2.46 to 2.51 seconds (Axon Sports unpublished), means that detectable differences between groups are on the order of 5% of the mean of a healthy individual. The analysis is based on a standard deviation in healthy individuals of 0.08, which we inflated to 0.10 based on our experience with injured or sick subjects, where variances are generally larger than their healthy counterparts. The intra-class correlation coefficient was set to 0.5. The calculation assumes a two-sided family-wise Type I error rate of 0.05 and uses a Bonferroni correction with four contrasts for each time period. The overall sample size is thus 50 subjects. We will consider a subject who contributes data up until and including at least Day 10 to be “evaluable for efficacy.” We conservatively anticipate that no more than 72 subjects will be enrolled in order to achieve 50 evaluable subjects. We anticipate that it will take us approximately 12-18 months to recruit these subjects.

8 STUDY MEDICATION

8.1 Description

The three BCAA's, valine, isoleucine, and leucine, will be dissolved in water and combined with sodium gluconate, Tropical Punch Kool-Aid® powder, and sucralose to improve palatability.

8.1.1 Packaging

Study product will be packaged by the Investigational Drug Service (IDS) at the Hospital of the University of Pennsylvania (HUP) into 20 oz bottles containing 591 mL of blinded doses active drink (containing valine, isoleucine and leucine) or matching placebo (similar in appearance and taste but absent the three amino acids). Each bottle will be individually labeled and sealed with a tamper-evident cap, then packaged in corrugated cartons (with a carry handle) in sets of 14 (representing a one-week supply).

8.1.2 Labeling

Study product will carry labeling that clearly identifies the study, treatment code and directions, as well as the statement 'Caution: For Investigational Use Only'. Each label will identify the study day (Day 1, Day 2, etc.) and the labels will be color coded for MORNING dose (yellow) and EVENING dose (blue). A detailed label on the outside of the carton will identify the subject, date, prescriber, emergency phone number, study, federal caution statement (noted above) and detailed directions for use. A smaller removable label, will be placed on the carton and will be peeled off and pasted into the subject's study records at the time of dispense, as a clear record of which kit was issued to which subject.

8.1.3 Dosing

Four different doses of BCAA's will be used in this study, 15, 30, 45, and 54 grams per day (divided BID). Originally, 60 grams per day (divided BID) was used as the highest dose, however during the study precipitation issues were noted at the highest ratio due to leucine precipitating at a lower concentration than expected in to the presence of the other solutes. After further testing, it was determined that the product remained in solution at a concentration of 27g/591ml bottle and therefore, the highest dose level was altered to 54 grams per day. For each dose, the three BCAA's - valine, isoleucine and leucine - will be combined together in a 1:1:1 ratio and dissolved in 591 ml (20 ounces) of water (see table below). Additionally, 13 g sodium gluconate, 1.75 g unsweetened Tropical Punch Kool-Aid powder, and 9 g sucralose are added to the mixture to improve palatability. This mixture will be administered twice daily.

BCAA 27 g/bottle	BCAA 22.5 g/bottle	BCAA 15 g/bottle	BCAA 7.5 g/bottle
Valine 9 g	Valine 7.5 g	Valine 5 g	Valine 2.5 g
Leucine 9 g	Leucine 7.5 g	Leucine 5 g	Leucine 2.5 g
Isoleucine 9 g	Isoleucine 7.5 g	Isoleucine 5 g	Isoleucine 2.5 g
Sodium Gluconate 13 g	Sodium Gluconate 13 g	Sodium Gluconate 13 g	Sodium Gluconate 13 g
Unsweetened flavoring 1.75 g	Unsweetened flavoring 1.75 g	Unsweetened flavoring 1.75 g	Unsweetened flavoring 1.75 g
Sucralose 9 g	Sucralose 9 g	Sucralose 9 g	Sucralose 9 g

QS to 591 mL	QS to 591 mL	QS to 591 mL	QS to 591 mL

The solution may be chilled or at room temperature, and should be shaken prior to consumption. The bottles may not be frozen or heated. Subjects are instructed to consume exactly 2 bottles in a 24-hour period. The recommended time between the two daily doses is 6 hours. The exact time given to consume a given bottle is not prescribed, although less than 60 minutes is recommended. If less than 2 bottles are consumed in a 24-hour period, the subject is instructed not to complete the remainder of the solution the following day and proceed to the bottles scheduled for that day. A study member is available 24 hours per day, 7 days per week by email and cell phone to answer dosing and other administrative questions.

The placebo solution will contain 1.2 g sucrose octaacetate (NF grade) and 30 g of microcrystalline cellulose (NF grade) dissolved in 591 ml of water. Additionally, 13 g sodium gluconate, 1.75 g unsweetened Tropical Punch Kool-Aid powder, and 9 g sucralose are added to the solution. This will ensure that the placebo solution has similar taste, texture, consistency and appearance as the BCAA solution.

BCAA's will be supplied by Spectrum Chemical and Laboratory Products. All ingredients in both BCAA and placebo solutions are USP/NF grade (see attached material).

8.1.4 Treatment Compliance and Adherence

Each day, subjects will self-report the number of bottles of the study medication that were consumed the prior day. Subjects will return all used, partially used, and unopened medication bottles at the conclusion of the study.

The study supplement will contain 500 mg/bottle of deuterium oxide that can be detected in the urine using isotope ratio mass spectrometry. Deuterium oxide has been used to assess drug compliance in both adult and pediatric research studies.[49-51] Urine samples will be collected from all subjects for a baseline measure of deuterium oxide on Day 0 and then again on days 7 and 14 of the trial to assess adherence. These samples will be frozen and stored in the laboratory of Dr. Cohen. Isotope ratio mass spectrometry will be conducted by the CHOP Metabolomics Core facility in a batched fashion at several points throughout the study. Deuterium oxide will be supplied by Cambridge Isotope Laboratories and is of suitable quality for use in human subjects (see attached material).

To assist with treatment compliance, subjects will be sent text, email or in-app alert messages twice a day to remind subjects to consume their study drink, and to reward them for compliance if they have documented consuming their drink.

8.1.5 Drug Accountability

Adequate records of study drug receipt and disposition will be maintained by the IDS Pharmacy at HUP. Records of receipts, investigational drug orders, dispensing records, and

disposition forms will be examined during the course of the study. The IDS at HUP will prepare treatment kits in advance of enrollment for rapid distribution when a subject enrolls.

The purpose of these records is to ensure regulatory authorities and the trial sponsor, the Dana Foundation, that the investigational new drug will not be distributed to any person who is not a study subject under the terms and conditions set forth in this protocol. The study medication is to be prescribed by the investigator or designee and may not be used for any purpose other than that described in this protocol. At study completion, all study products including partially used and empty containers will be destroyed on-site by the IDS Pharmacy and complete documentation will be maintained, with copies given to the investigator/sponsor.

9 SAFETY MANAGEMENT

9.1 Clinical Adverse Events

Clinical AE's will be monitored throughout the study.

The study coordinator will ask the subject about the occurrence of any AE's during the scheduled study visits conducted on days 7, 14, and 22 and during unscheduled visits. A standardized question will be asked of every subject to inquire about illnesses or accidents since the previous reporting period. The study coordinator will collect complete information about AE's from subjects including:

- Descriptive text and key words of the event
- Assessment of the event severity (grade)
- Indication if the event is serious and type
- Relationship to study drug
- Start date and end date (if applicable)

The investigators, with input from the independent medical monitor, will evaluate all AE's to determine the relationship to the study drug. All recorded AE's will be followed until resolution or stabilization. An AE Log will be used to collect this information across study visits and prompt follow-up of previously reported events.

Additionally, at the final study visit, subjects will be asked to call the study coordinator and report any serious AE's that they experience following the last visit, and that they believe may be related to their treatment.

9.2 Adverse Event Reporting

The investigator is responsible for recording and reporting unanticipated problems related to research that occurs during and after study treatment. All on-site SAEs (CHOP or related sites) will be reported to the IRB in accordance with CHOP IRB policies. AE's that are not serious will be summarized in narrative or other format and submitted to the IRB at the time of continuing review. Since this is an FDA regulated study, Dr. Cohen (IND Sponsor) will monitor AEs and report them to the FDA.

9.3 Definition of an Adverse Event

An AE is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). An AE is any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

All AE's (including SAE's) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event.

9.4 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death,
- A life-threatening event (at risk of death at the time of the event),
- Requires inpatient hospitalization or prolongation of existing hospitalization, or
- A persistent or significant disability/incapacity

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

A distinction should be drawn between serious and severe AE's. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but would not be an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

9.4.1 Relationship of Serious Adverse Events to Study Drug or Other Intervention

The relationship of each SAE to the study intervention will be characterized using one of the following terms: definitely, probably, possibly, unlikely, or unrelated.

9.5 IRB/IEC Notification of Serious Adverse Events and Other Unanticipated Problems

The investigator will promptly notify the IRB of all on-site unanticipated, SAE's that are related to the research activity. Other unanticipated problems related to the research involving risk to subjects or others will also be reported promptly to the IRB. Written reports will be filed using the eIRB system and in accordance with the timeline below. External SAE's that are both unexpected and related to the study intervention will be reported promptly after the investigator receives the report.

Type of Unanticipated Problem	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs All other SAEs	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Brief Summary of important AEs may be reported at time of continuing review

9.5.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. The investigator is responsible for ensuring that all SAE's are followed until either resolved or stable.

9.6 Investigator Reporting of a Serious Adverse Event to Sponsor

Reporting will be consistent with regulatory and sponsor requirements.

9.7 Medical Emergencies

If a medical emergency occurs during the screening process, the subject will immediately be referred to the CHOP or HUP emergency department. If the study coordinator or investigators learns of a medical emergency while communicating with a subject who is not on-site, the subject will immediately be referred to a local hospital emergency department.

10 STUDY ADMINISTRATION

10.1 Treatment Assignment Methods

10.1.1 Randomization

The study biostatistician, Dr. Putt, will generate a randomization schedule that will be implemented in the randomization module of the study database system. The randomization scheme will be based on equal allocation of BCAA's to placebo and stratified based on sex. Upon confirmation of eligibility in the DMS, the randomization module will be programmed to generate a randomization number that corresponds to a unique kit number of BCAA or placebo treatment. This kit number will be used to distribute treatment.

10.1.2 Blinding

All primary study team members will be blinded to treatment arm. The Database Administrator will have access to the information that links the kit number to the treatment arm. He/she will provide this information to the Investigational Drug Service (IDS) so that kits are prepared according to this scheme. BCAA and placebo treatment kits will appear identical. They will be labeled with the following information:

1. Kit number
2. Study name and number
3. Bottle # X of 42.
4. Subject ID # [blank line to record this number]

10.1.3 Unblinding

Unblinding can occur if the PI instructs the IDS to reveal this information. A direct request from the PI to IDS must be documented.

10.2 Data Collection and Management

Procedures to assure confidentiality will be strictly observed. All identifiable personal health information (PHI) data will be 1) kept in confidential locked files; 2) identified by subject number only; and 3) kept separately from identifying information used for subject tracking and follow-up contact. Identifying information will be kept in separate locked files. No identifying information will be disclosed in reports, publications, or presentations.

Protection of subjects depends on the joint activities of all clinical staff as well as the database administrative team. Extensive efforts will be made to ensure that subjects' confidentiality is maintained. Each subject will be assigned a unique study identification number and is never tracked through the study by name, medical record number, or any other personal identifier. A log of the subject names, ID numbers, and pertinent registration information (e.g., home address, telephone number, and emergency contact information) is maintained in a separate, locked area at the clinical site. The database administrative staff will not have access to this information. Only the subject ID number and date of birth are given to the database administrative staff and registered in the study database. Any communication between the database administrative staff and investigators regarding subject

data will occur via the subject ID number. Any forms or documents sent to the IRB or other regulatory authorities will have all personal information removed.

Study data will be entered into a web based data management system (DMS). This DMS uses a secure connection between the client browser at the clinical site and the web server at the Penn Clinical Research Computing Unit (CRCU). Data transmitted over this connection is authenticated by the use of digital certificates and is encrypted as it travels the internet to the CRCU. Data is being gathered and stored through the CRCU because the expertise of the CRCU staff was required to construct the secure website subjects will access to complete daily assessments and to build the DMS on an Oracle platform so it can communicate in a subject-specific manner directly and securely with the Axon Sports system. The Oracle development tools integrate seamlessly with the DMS to allow a full range of programming functionality which allow us to: 1) capture data from remote locations over the internet using secure connections, 2) incorporate data quality checks at entry and after entry using simple or complex rule sets as needed, 3) assign specific privileges for users to reflect their role and needs on the project, to provide an electronic audit trail of all data changes, 4) incorporate and enforce workflow logic where appropriate, 5) integrate with external data and software systems, and 6) provide secure and convenient access to data for authorized study personnel. Using this platform, the DMS automatically transmits a subject's ID number and date of birth to the Axon Sports system. The Axon Sports system then securely sends coded data to the DMS where it is stored. At no point is PHI except date of birth (for the purposes of computing a participant's age) collected by the Axon Sports system or stored in the DMS. The Axon Sports system does not have direct access to the DMS. All information transmitted between the Axon Sports system and the DMS is coded solely by subject ID number.

The DMS will be designed to prevent unauthorized access to trial data and to prevent data loss due to equipment failure or catastrophic events. The procedures to do so encompass user account management, user privilege assignment, data loss prevention (database backup), computer systems validation, performance monitoring, and the DMS change management. User access will be controlled by assignment of confidential usernames, passwords, and role assignment. The system will meet the applicable Federal regulatory requirements and those described in the E6 Good Clinical Practice Guidelines to ensure the confidentiality of trial subjects.

CRCU data networks comprise a set of physical data networks all linked in a point-to-point configuration to form a single logical data network. The network is protected through the use of several Juniper® firewall devices. These devices perform as firewalls and logging devices for data network traffic, as Intrusion Detection to alarm information technology personnel to possible network attack scenarios, and as an email spam filter and eliminator.

Security requirements at the computer systems' level start with separation of duties with the system administrators. Administrators each are assigned specific accounts that dictate what services they have access to. All accounts are individually assigned and logged, regardless of access level. The systems are configured to vendor specification with the published operating system releases and published patches, before connected to any data network. Once configured, the systems operating system is imaged and TripWire® software is loaded

to ensure all system and operating files maintain their current configurations. Any changes to the recorded and monitored files results in an alert being sent to the responsible administrator.

All users of the database applications have unique and permanent user identifications assigned to them for use to gain access to the systems and to the appropriate databases. Passwords are aged and also restricted so good password creation and usage are enforced. Users are trained on log-in procedures and also application usage. A support helpdesk is maintained during normal business hours to ensure problems are quickly discovered and resolved for the users.

The data is stored in several disk storage subsystems that are backed up and stored off site, depending on the critical nature of the data. Standard operating procedures are in place to provide business continuity, disaster recovery, and file restoration. All hardware/system software components are covered by vendor supported maintenance contracts with a 4-hour or less turn-around on parts and assistance.

Computer system security will be enhanced for the applications through the use of the Oracle database repositories. Users of the applications will not be allowed to log into any of the project servers directly. All users of the project will only log into specific applications where access is controlled and limited.

10.3 Confidentiality

Patient confidentiality will be maintained at all times and study information will be protected against release to unauthorized people. All data and records generated during this study will be kept confidential in accordance with institutional policies and the Health Insurance Portability and Accountability Act (HIPAA) on subject privacy. The investigator and all other personnel will not use such data and records for any purpose other than conducting the study. Subjects will be assigned study numbers for entry into the protocol; this number alone will identify them. Subjects are free to refuse participation in the study and can withdraw at any time without compromising clinical care. The investigator will obtain a data use agreement between the provider (the PI) of the data and any recipient researchers (including others at CHOP) before sharing a limited dataset (PHI limited to dates and zip codes).

10.4 Regulatory and Ethical Considerations

10.4.1 Data and Safety Monitoring Plan

Rebecca Ichord MD, an experienced neurologist, will act as the independent Medical Monitor for adverse events during this study. Dr. Ichord has served in prior and ongoing clinical trials as endpoint adjudicator and was on the Clinical Event Committee for the pediatric EXCOR Berlin heart trial. She is presently a member of the endpoint adjudication committee for the Thrombolysis in Pediatric Stroke trial. The Medical Monitor will review all acute AE's regardless of how the investigator reports the relationship between the event and the study intervention. All patient deaths will be similarly reviewed. The study will also undergo monitoring by the CHOP Office of Research Compliance and Regulatory Affairs

after each subject for the next six subjects enrolled and then at least annually throughout the duration of the study.

10.4.2 Risk Assessment

Risks for this study are a minor increase above minimal. Subject safety will be monitored by AE's on a weekly basis. Expected adverse experiences during this study may include gastrointestinal symptoms such as dyspepsia either from the BCAA's themselves or the 20 ounces of fluid consumed. Prior studies have shown that these symptoms when related to BCAA's are mild and transient. An informal inquiry of athletes (both male and female) from many sports indicated that drinking 20 ounces of fluid twice daily is not burdensome and within the volume typically consumed while training or competing. Should a subject report new onset gastrointestinal symptoms while on therapy, the subject will be monitored to see if these symptoms either have an alternative explanation (e.g. development of acute gastroenteritis from an identifiable source) or spontaneously resolve in less than 24 hours. The study therapy will not be held unless these symptoms persist for more than 24 hours or are severe enough to require medical attention. In this case the subject will be removed from the study and the therapy not restarted. Taking the CCAT or CDP may temporarily exacerbate concussion symptoms, which typically resolve after a short period of rest. Neurological and psychiatric symptoms induced by changes in neurotransmitters like serotonin, dopamine, norepinephrine are theoretically possible with BCAA therapy but have never been observed clinically. Emergence of any of these symptoms will result in discontinuation of the subject's participation in the study. BCAA therapy will be discontinued for any SAE related to its use.

10.4.3 Potential Benefits of Trial Participation

By taking BCAA supplementation subjects may accelerate the recovery of cognitive symptoms after concussion injury. Successful development of this compound may modify clinical protocols for treating concussion and other types of TBI.

10.4.4 Risk-Benefit Assessment

Although this study involves more than minimal risk (i.e. administration of a test substance), there is the potential for benefit directly or through new knowledge. We believe that the risk to benefit ratio is favorable and the risks are reasonable in relation to the potential benefit and new broadly applicable knowledge.

10.5 Recruitment Strategy

Physicians with experience in diagnosing patients with concussion, will identify potential subjects and refer them to the study coordinator for participation in the study. These providers typically see patients within a few days of their suspected concussion. Recruitment will primarily occur through CHOP Sports Medicine, the CHOP Emergency Department, University of Pennsylvania Sports Medicine, the Hospital of University of Pennsylvania (HUP) Emergency Department, and Penn Presbyterian Medical Center Emergency Department (Presby ED).

For CHOP Sports Medicine, providers will identify potential subjects and with subject's permission, the provider will contact the study coordinator with the subject's contact information. Physicians in the CHOP Emergency Department will identify potential subjects for this study. Once a patient has been diagnosed by an ED physician with a concussion, a referral will be made to the study coordinator and a HIT-HEADS Study informational sheet will be provided to the patient. The study coordinator will arrange to complete the remainder of the screening process in a designated office or clinical exam after discharge from the ED.

For Penn Sports medicine, providers will identify potential subjects and with the subject's permission, the provider will contact the study coordinator with the subject's contact information.

Providers in the HUP ED, and Presby ED may also identify potential subjects for this study. They will provide a HIT-HEADS informational sheet to a patient once they have been diagnosed with a concussion. The patient will then contact the study coordinator if they are interested in study participation. Providers from these entities will not collect or transmit patient information to the study coordinator. Once the study coordinator has made contact with a potential subject, he or she will make arrangements with the potential subject to complete the remainder of the screening process in a designated office or clinical exam room.

Subjects may self-refer to the study. Informational flyers will also be displayed and distributed in University of Pennsylvania campus, including student gathering places, locker rooms, training facilities and sports medicine clinics to inform subjects about the study. Patients greater than 10 years of age that had a sports physical in the last year will be contacted and will receive an informational flyer, a letter from the PI, and a business card in the mail. Those patients that selected to receive CHOP emails will also receive an email from the PI.

Marianne Chilutti, Department of Biomedical and Health Informatics (DBHi) will query the CHOP EPIC system for patients, ages 11 - 34 years, who, in the previous 24 hours, have been diagnosed with a concussion (ICD-9-CM codes 850, 850.0, 850.1, 850.10, 850.11, 850.5, 850.9) or made a call to the triage nurse after business hours with the complaint of a concussion . The search will be limited by those departments listed above. The report will be run daily and the results emailed to the study team. A medical record review using the EPIC database will be conducted by the study team to determine which patients are eligible for contact about the study. Specifically, the medical record will be searched for the manner and time of the concussion to determine which patients are eligible for contact. A study team member will then contact potential subjects via phone to provide information about the study.

High school administrative and athletic personnel from the school districts in the greater Philadelphia area will be given information about the study through informational fliers. Additional information about how to refer concussed players to the study will be communicated to nurses, coaches and team medical staff if the school is interested in participation. As with recruitment from other domains, potential subjects will be required to

contact the study coordinator directly and the diagnosis of concussion will be confirmed by a physician prior to enrollment.

Upon approval from high school administrative personnel, a member of the study team will attend back to school night to distribute approved study materials including flyers and business cards. Approval will be documented in the form of a letter/email of confirmation from a high school administrator.

The UNTOLD Foundation will display the approved flyer on the organization's homepage, http://www.theuntoldfoundation.org/.

The Recruitment Enhancement Core (REC) may provide assistance with recruitment plan development and may assist in identifying and contacting potential participants using the CRU, the CHOP Recruitment Registry, social media and internal communication resources. The REC also may engage community partners and facilitate outreach on behalf of the Research Institute and this CHOP research study.

10.6 Informed Consent/Assent and HIPAA Authorization

Informed consent is an ongoing process that takes place between the investigator/study staff and study participants. The PI or their designee (physician co-investigators) will obtain informed consent for this research study. The consent process will occur in a designated office or clinical exam room at CHOP or Penn. It is the responsibility of the PI to ensure that informed consent has been properly obtained. The consent will include use of the information obtained during the clinical battery before enrollment and all data obtained after enrollment. Subjects may take any additional time needed to review the consent document, however, initiating the trial may not begin more than 72 hours after the time of injury. Adult subjects age ≥18 will sign the Informed Consent after full review and verbalized understanding of the protocol. If the subject is a <18 years old, enrollment will require both parental permission and subject assent.

10.7 Payment to Subjects/Families

Subjects will receive a possible total of $100 for participation in this study. Amount received will depend on compliance with study procedures and will be offered in a graduated schema to encourage ongoing participation.[56] Payment will be placed on a reloadable CHOP-issued card and money added throughout the study. Subjects will receive $20 on the card at the time of visit 1. Each day that they input all required study data will add $1, with a bonus of $2 each time 3 days in a row are met and $5 each time 7 days in a row are met, for a possible total of $50 if all 21 days of data are entered completely. $10 will be added for each in-person research visit that they attend (possible total of $30 for 3 visits)..

10.7.1 Reimbursement for travel, parking and meals

Subjects who park at a CHOP facility that requires paid parking for any of their 4 research visits will receive parking vouchers.

11 PUBLICATION

The principal investigator and co-investigators will prepare the data for publication.

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13 APPENDICES

13.1 APPENDIX 1: Clinical Symptom Checklist

Here is a list of symptoms that people often feel when they have a concussion. For each symptom, please select the number that best describes ***how long you experienced each symptom in the past day [insert date]***.

Symptom Duration	Never experienced this symptom	Very Briefly		Sometimes Today (about half the day long)			All Day Long
1. Headache	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
2. Nausea	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
3. Balance problems	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
4. Sleeping more than usual	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
5. Drowsiness	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
6. Fatigue	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
7. Feeling "slowed down"	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
8. Feeling like "in a fog"	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
9. Difficulty concentrating	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6

Here is a list of symptoms that people often feel when they have a concussion. For each symptom, please select the number that best describes ***how severe the symptom has felt in the past day [insert date]***.

Symptom Severity	Not experiencing this symptom	Mild		Moderate		Severe	
10. Headache	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
11. Nausea	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
12. Balance problems	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
13. Sleeping more than usual	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
14. Drowsiness	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
15. Fatigue	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
16. Feeling "slowed down"	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
17. Feeling like "in a fog"	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6
18. Difficulty concentrating	❑0 0	❑1 1	❑2 2	❑3 3	❑4 4	❑5 5	❑6 6

13.2 APPENDIX 2: Physical and Cognitive Activity Assessments

Please indicate your level of cognitive activity in the past day [insert date]:

1. **Cognitive Activity:**

- ❑0 Complete cognitive rest
- ❑1 Not attending school/work, but doing light cognitive activity, (e.g. watching TV, pleasure reading or using the Internet)
- ❑2 Not attending school/work, but doing moderate cognitive activity, (e.g. homework, academic reading, school/work-related computer use)
- ❑3 Attending school/work with decreased workload (e.g. not taking exams, taking a reduced number of courses)
- ❑4 Full unrestricted participation in school/work including all exams, assignments, and homework

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Please indicate your level of physical activity in the past day [insert date]:

2. **Physical Activity:**

- ❑0 Complete physical rest
- ❑1 Light aerobic activity (e.g. walking, swimming or stationary cycling)
- ❑2 Moderate non-contact exercise (e.g. running, skating drills in hockey, running drills in soccer or football)
- ❑3 Complex activity or drills involving partial participation (e.g. passing or shooting drills in sports, partial participation in gym class or exercise classes)
- ❑4 Complex activity or drills with full participation (e.g. full contact practice, normal training activities, full participation in gym class or exercise classes)
- ❑5 Normal activity level without limitation, including normal game or scrimmage play

13.3 APPENDIX 3: Sleep Assessment

1. How many naps did you take yesterday? [insert date]?
   ❑0 None ❑2 Two ❑4 Four
   ❑1 One ❑3 Three ❑5 Five

For each nap that you took, please record the approximate start time and duration. If you did not take a nap, please continue to Question 2.

	Approximate Start Time		Duration of Nap	
a. 1st Nap	__ __ : __ __	❑1 a.m. ❑2 p.m.	__ __	❑1 minutes ❑2 hours
b. 2nd Nap	__ __ : __ __	❑1 a.m. ❑2 p.m.	__ __	❑1 minutes ❑2 hours
c. 3rd Nap	__ __ : __ __	❑1 a.m. ❑2 p.m.	__ __	❑1 minutes ❑2 hours
d. 4th Nap	__ __ : __ __	❑1 a.m. ❑2 p.m.	__ __	❑1 minutes ❑2 hours
e. 5th Nap	__ __ : __ __	❑1 a.m. ❑2 p.m.	__ __	❑1 minutes ❑2 hours

2. What time did you go to bed yesterday? __ __ : __ __ ❑2 p.m. ❑1 a.m.

3. How long did it take you to fall sleep? _____ ❑1 minutes ❑2 hours

4. How many times did you wake during the night?
   ❑0 Never
   ❑1 1-2 times
   ❑2 3-4 times
   ❑3 more than 4 times

5. What time did you get up today? __ __ : __ __ ❑1 a.m. ❑2 p.m.

6. About how many hours did you sleep last night? __ __ hours

7. Please comment on any unusual activities yesterday (insert date): (i.e. sickness, holiday, daylight savings time, travel, etc.)

13.4 APPENDIX 4: Drug accountability assessment

1. During the prior day [insert date], did you take your supplement? ❑$_1$ Yes ❑$_0$ No

   a. If **Yes**, how many complete bottles did you take?
   - ❑$_1$ Zero
   - ❑$_2$ One
   - ❑$_3$ Two