Strategies to Augment Ketosis: Ketone Conferred Resiliency Against Sleep Restriction (STAK-Sleep)

The Ohio State University

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IRB Protocol

Title:	Strategies to Augment Ketosis Ketone Conferred Resiliency Against Sleep Restriction (STAK-Sleep)
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BACKGROUND

Only 1 in 3 U.S. Army Active Component Soldiers are estimated to get the target ≥7-hr of sleep on duty days, and ~14% have a sleep disorder [1]. Insufficient sleep has profound effects on human performance that include deficits in working memory, creativity, innovative thinking, strategic planning, mood disturbances, lapses in attention and vigilance, and impaired physical performance [2, 3]. In a classic dose-response sleep study performed at Walter Reed Army Institute of Research, it was demonstrated that limiting sleep to 3-hr per night for 7-days resulted in a steady deterioration on a psychomotor vigilance task across the week of sleep restriction [4]. Sleep restriction over 3-days has been shown to adversely affect marksmanship performance, including significantly longer time to make decisions, misidentifying friends versus foes, and believing performance did not change over time [5]. Short-term sleep restriction is also linked with impaired glucose metabolism and decreased whole body insulin sensitivity [6-8], and increases the risk of developing T2D [9].

Military personnel have few good options to counteract physical and cognitive detriments attributed to insufficient sleep. Warfighters increasingly turn to caffeine and sugar-containing energy drinks to combat sleep loss and fatigue, especially during deployment [10]. At best, these nutritional countermeasures provide a transient performance gain, and may trigger a 'rebound' hypoglycemia that exacerbates performance detriments that can increase the risk of obesity and related problems. Ketosis could improve tolerance to sleep restriction and sleep abnormalities through multiple mechanisms [11-15]. We have reported that a 1-yr ketogenic diet (KD) improved sleep quality and the proportion of people categorized as poor sleepers [14].

Several lines of evidence demonstrate that ketones are a preferred fuel for the brain, have neuroprotective effects, and may enhance neurocognitive function in various pathological or physiologically stressed states**.** At the low end of nutritional ketosis (0.5 mM), ~5% of whole brain energy metabolism is provided by ketones. At ketone concentrations of 1.5 mM (typical of KDs), ketones supply nearly 20%; at the higher end of nutritional ketosis 4-5 mM [achievable with ketone esters (KE)], half of the brain energy demands are met by ketones [18-20]. Importantly in situations where brain glucose metabolism is impaired, uptake and utilization of ketones remain fully intact [21], suggesting a hierarchy of importance placed on ketones as the preferred fuel for human brains. For example, when ketones are elevated in the circulation there is a remarkable protection from the adverse signs of hypoglycemia [16, 17].

RESEARCH OBJECTIVE

- Determine the degree to which short-term use of ketone esters (KEs) protects against fatigue from moderate sleep restriction and exercise.

Key outcome variables will include **5** major categories:

1) SLEEP OUTCOMES

a) The sleeping protocol will be monitored by investigators and personnel with experience in sleep medicine. Participants will be required to reduce sleep by 50% of normal, for 4 consecutive nights, to observe potential deficits in physical and cognitive performance [22].

b) Heart rate and sleep parameters (timing, duration, and quality of sleep) will be assessed by wrist-based acceleration with cloud technology (Polar Unite™, Polar USA). This monitor is less burdensome than other sleep monitoring technologies and less likely to interfere with normal sleep and activity demands of the cadets. We have used this watch in our work with athlete tracking in sport science with reliable and valid results. Each participant will be fitted with the watch for acclimatization and then establish 3-day baseline patterns for 3 nights before the sleep loss protocol.

c) Dietary standardization, including the same meals for all subjects and control of caffeine, will be implemented. Proper hydration will also be emphasized and evaluated at testing sessions via urine specific gravity.

2) EXERCISE PERFORMANCE

a) Since weight training is now a crucial part of any warfighter's physical preparation, we will have subjects perform two weight training workouts emphasizing all major muscle groups on Days 2, 3 and 4.

b) Workouts will be supervised by trained personnel. All physical activity will be performed in our laboratory weight room located in the Physical Activity and Educational Services (PAES) Building (305 Annie and John Glenn Avenue, Columbus, OH 43210).

c) On Days 1 and 5, cognitive and physical tests and questionnaires (McGill Pain Questionnaire, the Shortened Profile of Mood States, and the Pittsburgh Sleep Quality Index) will be examined around the sleep restriction/exercise challenge. Time of day, eating, and activity will be strictly standardized the day prior to all assessments.

3) NEUROPSYCHOLOGICAL ASSESSMENTS

a) The first cognitive assessment will be done via *Automated Neuropsychological Assessment Metrics* (**ANAM**) (Department of Defense, Rockville Pike, MD). This cognitive battery is a library of computer-based tests of domains including attention, concentration, reaction time, memory, processing speed, decision-

making, and executive function. The ANAM core testing battery will be used, which assess of the following areas: participant information, sleepiness scale, symptoms checklist, mood scale, simple reaction time, code substitution- learning, procedural reaction time, mathematical processing, matching to sample, code substitution-delayed, simple reaction time (repeated), and go no go.

b) The second cognitive assessment will be delivered via *Cambridge Neuropsychological Test Automated Battery* (**CANTAB**). This iPad delivery assessment will include an Information Sampling Task to test impulsivity and decision making, response time (in msec), which provides a level of sensitivity not available with traditional paper and pencil tasks, processing speed, executive functioning, and spatial memory. We have also incorporated tasks which assess the ability to rapidly identify expressed facial emotions, which may index one's ability to accurately respond to overtly threatening or non-threatening stimuli.

c) *Gradual-onset Continuous Performance Task* (**GRAD-CPT**) measures endogenous attentional control by using smooth (rather than abrupt) transitions between visual images. Scene stimuli are visually presented and smoothly transition from one scene to another. Subjects press a button when one type of scene is presented (e.g., city scene) and withhold the button press when another type of scene (e.g., mountain scene) is presented. It is a Go/No-Go test and assesses multiple metrics of executive function, including sustained attention, mind wandering, and response inhibition. The task is robust and has been used in both civilian and military samples and in disparate testing environments (lab vs. internet-based assessment).

d) *Face-Name Task*. Data in rodents suggests that KEs may impact tasks sensitive to hippocampal function. We will implement a face-name relational memory task, known to activate the hippocampus (along with prefrontal and parietal cortices) in humans using face-name pairs presented for 3-sec with an interval of 750-msec. Participants will judge which name was previously presented with the face. After a 15-min delay, participants will complete an associative face-name recognition task to assess episodic memory performance.

4) MILITARY-RELEVANT PERFORMANCE

a) Ohio State University is one of the few centers in the world equipped with a Virtual Training (VirTra) small arms indoor training simulator. Our VirTra V-100 system (VirTra Corporate HQ, Tempe, AZ) is used by both military and law enforcement agencies to provide training. The V-100 and associated compatible weapons are equipped with recoil kits for unsurpassed live-fire simulation, at distances up to 2,000-m, authoring capabilities for the creation of customized scenarios on 4 individual firing lanes. Multiple metrics of marksmanship (total number of shots fired/silhouette target presentation, percentage of targets successfully hit/min, the radial distance of a shot from the center on target, shot group tightness, and time from target presentation to trigger pull) will be collected during each visit.

b) Response time will be assessed with the Quick Board (Memphis, TN, USA) as previously described [23]:
   I. For the upper body reaction test, participants stand on the footpad at a distance where the wrists are parallel to the sides of the iPad when arms were extended forward. Participants were instructed to use only the index fingers and to touch the sensors on the left side of the screen with their left finger and on the right of the screen with their right finger. The middle sensor could be touched with either index finger. The test consists of 3 sets of 40 touches with a 0.05 second delay between correctly touching a lit sensor and subsequent stimulus.
   II. For the lower body reaction test, participants began by standing on the footpad with feet carefully aligned on either side of the middle sensor (operationally defined as the base) and the head looking forward at the iPad. Participants touch the corresponding sensor of the footpad that lit up on the iPad and then return to base before touching the next illuminated sensor. Sensors on the left side were touched with the left foot, sensors on the right side were touched with the right foot, and the sensor in the middle could be touched by either foot. Participants were instructed to keep the head up and eyes focused on the iPad, rather than looking up and down between the iPad and feet. The test consisted of 3 sets of 40 touches with a 0.05 second delay between correctly touching a lit sensor and subsequent stimulus.

c) Whole body power will be assessed with a repetitive jump test that we developed [24]. Power (peak, average, curve functions) will be assessed using an AMTI force plate with Accupower 2.0 software (Advanced Mechanical Technology Inc, Watertown, MA).

5) BLOOD BIOMARKERS

a) Continuous quantification of interstitial fluid glucose and beta-hydroxybutyrate (BHB) will be monitored using sensor-based devices developed and manufactured by Abbott Biowearables. The systems consist of three components: 1) a sensor that incorporates a subcutaneously implanted electrochemical glucose/ketone sensor and associated on-body electronics. 2) a disposable, sensor application device, which is used to adhere the sensor to the skin of the user and to insert the sensor tail just below the surface of the skin. And 3) a handheld device (reader or phone app) which activates the sensor by near-field communication and continuously obtains glucose and BHB readings at one-minute intervals from the sensor via Bluetooth short-range wireless communication. A separate sensor will be used for glucose and BHB monitoring. Sensor values will be blinded to the participant and research personnel such that the reader (or phone) only displays whether or not the matching sensor is functional or not. Glucose and BHB sensor data is therefore not accessible for medical or non-medical decision-making. Readers (or Phones), sensors, and Patch Delivery Units are uniquely identifiable through individual serial numbers. Readers (or Phones) require periodic charging

with a power chord (at least weekly). Sensors do not require charging. The wear period for a sensor is 14-days and thus we will have participants apply new sensors during the second cross-over period after the 14-day washout period. Sensors will be placed on the non-dominant arm (mid-triceps region) during each experimental phase.

b) Fasting venous blood will be collected at baseline and on Day 1 and Day 5 of the sleep restriction protocol to measure metabolites and hormones in serum and plasma. Participants will be exposed to a maximum of 4 blood draws during the entire study.

METHODS

Experimental Approach.

We will conduct a double-blind, placebo-controlled, two-period cross-over study (**Figure 1**). The order of treatment will be balanced (i.e., half start with KE and half start with Placebo). Participants (n = 60) will be familiarized with all the physical performance tests to minimize learning effects and undergo baseline assessments. Subjects will then be randomized to either the KE or Placebo condition. On the morning of Day 1, prior to administration of KE or Placebo, subjects will undergo Pre-Testing inclusive of a blood draw, physical, and cognitive performance. For that night, and the next 4 nights, all subjects will restrict their sleep by 50%. On the morning of Day 5, the same testing battery performed on Day 1 will be replicated. KE or Placebo ingestion will occur on Days 2, 3, and 4 (2 doses/day), and include 1 dose on Day 5. After a 2-wk washout period, during which time participants return to their normal sleep and exercise habits, they will replicate the exact same 5-day protocol except they will receive the opposite treatment.

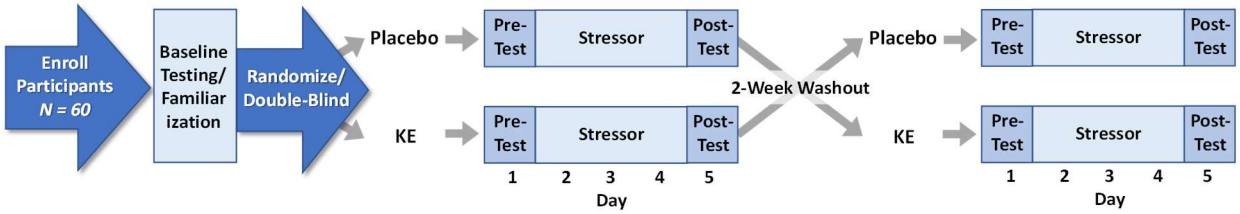

Figure 1 | Experimental Design

Rationale for Design.

We expect to show that short-term sleep restriction combined with physical exertion is associated with disturbed glucose metabolism as well as impaired cognitive function and physical performance. Based on emerging animal and human evidence indicating that ketosis is associated with enhanced cognition and improved physiological tolerance and performance in response to physical exertion [25, 26], our central **hypothesis** is that KE ingestion will lessen the detrimental effects of sleep restriction on cognitive and physical performance. This will be the first work to specifically explore how ketosis impacts cognition in the context of sleep restriction and performance tasks.

Participants.

We expect to enroll **60** men and women. This project will leverage our ongoing relationship with the OSU Reserve Officers Training Corps (ROTC), who have been eager to participate in research on ketosis and performance, including recent work that examined extended KDs in combination with exercise training. The current ROTC Cadre leadership and cadets are enthusiastic to participate in the proposed experiments. We anticipate several participants to be recruited from Army, Navy and Air Force OSU ROTC, but we will not limit our recruitment efforts to ROTC. All participants must be men or women between the ages of 18-40 yr and have a body mass index (BMI) < 35 kg/m^2. Eligible participants will be healthy and familiarized with all aspects of the research prior to volunteering.

Participants will be excluded if they have any of the following:

- <18 or >40 years of age
- >35 body mass index (BMI).
- Diagnosed sleeping disorders (i.e., sleep apnea, insomnia).
- Gastrointestinal disorders or food allergies that would interfere with consuming the study supplements.
- Drink alcohol in excess of 3 drinks/day or 14 drinks/week
- Have any conditions or contraindications to blood draws.
- Have been diagnosed with diabetes, liver, kidney, or other metabolic or endocrine dysfunction, or use diabetic medications other than metformin
- Currently consume a low carbohydrate or ketogenic diet or have done so in the last 3 months
- Have experienced weight loss of >10% of your body weight within the last 6 months
- Are pregnant, lactating, or planning on becoming pregnant during the study
- Have any major psychiatric disorders (e.g., schizophrenia, bipolar disorder)

Participants will be recruited through posted flyers, e-mails, word of mouth, and by using ResearchMatch through the OSU CCTS. Print and email advertisements will instruct interested individuals to call the study center (Volek Lab, Kinesiology Program) for additional information about the study. Participants may either respond to the email address to set up a phone call or may call during the phone-in hours. One of the key personnel involved in the project will describe the study and determine preliminary qualifications by conducting a scripted phone interview. Participant answers to qualifying criteria questions will be recorded to assess whether or not the person calling meets the initial qualifying criteria. If it is determined that the interested participant does meet the initial qualifying criteria, an appointment will be made for the interested participant to visit the study center for a screening meeting.

Supplementation.
The ketone ester (KE) supplement we plan to use the C6 Diester (Metabolic Switch), which delivers rapid (<30-min) and sustained (~5-hr) ketosis in a predictable and dose-dependent manner. We have used this particular KE in previous studies at OSU (e.g., protocol number 2020H0005 and 2021HO425) and it is well tolerated and consistently elevates ketones into the desired range. Metabolic Switch and Placebo will be provided in a flavor and volume matched single serving nondescript plastic container in the morning prior to testing on Days 1 and 5, and then again in the evening during the sleep restriction period. The product will be consumed twice per day: once in the morning and once in the evening. Product will be distributed in a blinded manner by people not involved with testing. For more details regarding the supplement refer to *Supplementation* section below.

C6 Diester and finished product (Metabolic Switch) is manufactured under good manufacturing practice conditions for BHB Therapeutics Ltd (and its parent company, Juvenescence Ltd). BHB Therapeutics conducted a program of safety and efficacy testing

which was reviewed by an independent Expert Panel who determined that the ingredient meets the FDA GRAS definition. The findings used in this GRAS determination were peer-reviewed and published [27-30]. BHB Therapeutics currently offers Metabolic Switch for commercial sale as an ingredient in a 2.7 fl oz, chocolate flavored specialty beverage. The beverage contains 25 g of C6 Diester emulsified in a matrix of water, whey protein concentrate, modified gum acacia, natural and artificial flavors and cocoa powder. It contains 210 kcal, 0.5 g fat, 2 g carbohydrate, and 2 g protein. Manufacture of C6 Diester and the Metabolic Switch beverage involves stringent quality control processes that comply with all industry standards. Metabolic Switch has been commercialized via e-commerce in the USA since April 2021 with no serious adverse events reported. A 28-day study of healthy adults ingesting 25 g of Metabolic Switch daily found no safety or tolerability concerns [27]. We recently characterized the kinetics of ingestion of 12.5 g and 25 g servings (~150 and ~300 mg/kg body mass) of Metabolic Switch in the specialty beverage matrix and found that BHB increased ~1.7 mM above baseline and was maintained above 0.5 mM for at least 3-hr [31]. BHB Therapeutics has developed a taste and calorie matched placebo beverage which has been used in previous studies [27].

Study Procedures

Screening.

Participants that meet the initial qualifying criteria will visit the study center for a screening meeting. The participant and a member of the research team will meet in a private office to discuss the informed consent form. The informed consent form will be provided to the participant for their review, the study will be described in full detail and any questions the interested participant has will be encouraged and responded to. If they choose to participate in the study, they will be asked to sign the consent form providing written consent. The participant will be informed that even though they signed the consent form, their participation in the study is dependent on the results of their questionnaires to determine if they meet the study criteria. If the participant provides consent, they will be provided with a few questionnaires including medical history, physical activity history, a menstrual history for women, a food frequency questionnaire, and a gastrointestinal health status questionnaire. All collected samples will be coded to maintain participant anonymity. We will also measure height, weight. Participants will receive a call from one of the study personnel within days of the screening visit to inform them if they are eligible or not and will provide the participants with their results from the screening visit. If the participant is eligible for the study and is still interested in participating, then he/she will be scheduled to return to the study center for baseline testing.

Testing Sessions.

A schedule of the testing battery for the 5-day sleep restriction protocol is provided in **Figure 2**. The familiarization meeting will describe all the protocols in greater detail to accustom participants to their study duties and minimize training learning effects, followed by 5, consecutive, in-person testing sessions. Days 1 and 5 comprise primarily of blood assessment, cognitive testing, virtual simulator shooting, and power/reaction time test. Days 2, 3, and 4.

Table 1 | Experimental Checklist.

	Blood Draw	HR Monitoring	CGM/CKM	Cognitive Tests	Surveys	VirTra	Power/RT
Familiarization		x	x	x	x	x	x
Day 1	x	x	x	x	x	x	x
Day 2		x	x				
Day 3		x	x				
Day 4		x	x				
Day 5	x	x	x	x	x	x	x

HR, heart rate; CGM/CKM, continuous glucose/ketone monitoring; VirTra, Virtual Training; RT, reaction time.

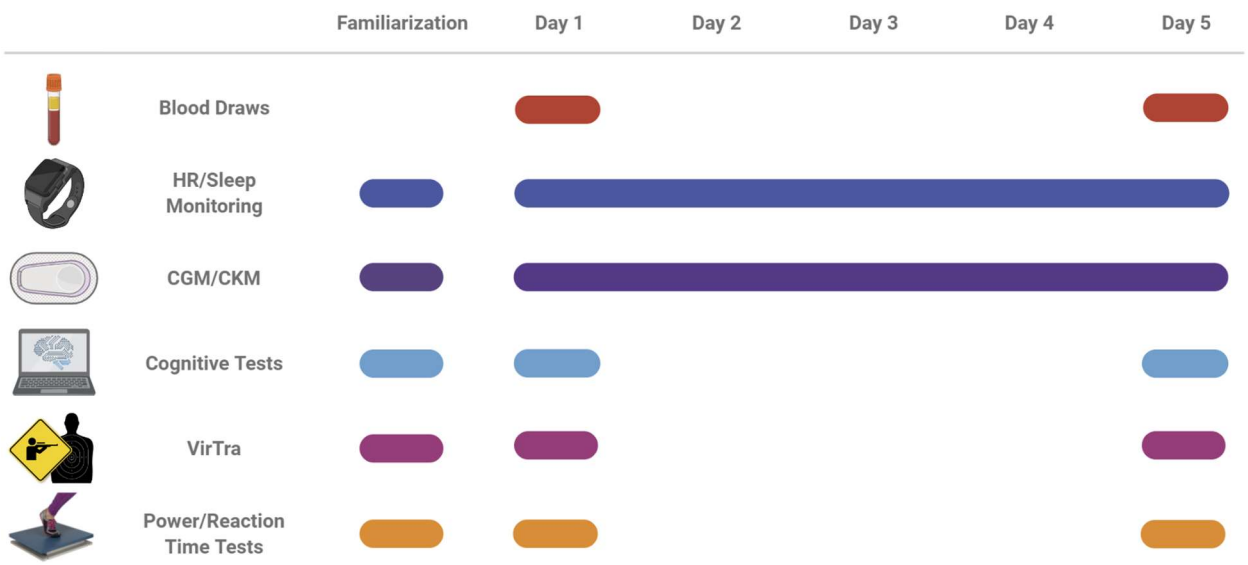

Figure 2 | Experimental Timeline During Sleep Deprivation Protocol.

Familiarization:

1. The primary goal is to fit each eligible participant with a wrist-based HR monitor and a CGM/CKM device (Abbott Freestyle Libre) within one week of the Day 1 visit. This familiarization protocol is intended to capture a week of free-living data and one week of each experimental condition.
    a. Because we expect healthy, young adults, we don't anticipate screening for familiarization blood draw values. However, each consented participant will be given the opportunity to have their blood drawn by a trained phlebotomist if they have reservations about their blood values (e.g., metabolic panel, lipid panel) but still wish to participate in the study.
2. A secondary goal is to present the participants with each of the testing duties during the study, such as surveys, computer/iPad assessments, virtual shooting scenarios, how to squat jump on a force plate, and Quick Board reaction time.
3. Lab members will conduct a presentation of proper weightlifting lifting and running form expected during training days to minimize exposure to injury.

Test Day 1 & 5:

1. Participants will arrive at the testing lab (305 Annie and John Glenn Avenue, Columbus, OH 43210) between 6:00-9:00h, hydrated and after an overnight fast (<8h).
2. *Blood Draws:* After 10 minutes of rest, a trained phlebotomist will perform an intravenous blood draw from the antecubital fossa using a 21G butterfly needle. Total blood collected will be limited to two test tubes (total volume 2 x 10mL): EDTA plasma and serum.
3. *Supplement Ingestion:* The first supplement dose (either KE or PL) will be consumed before commencing the cognitive testing battery.
4. *Cognitive Tests:* A series of computer and iPad-based cognitive tests will be administered to each participant to measure their attention, information processing, memory, function, inhibition, and social and emotional domains.
5. *Surveys:* A secondary assessment of well-being will be administered in the form of questionnaires: McGill Pain Questionnaire, the Shortened Profile of Mood States, and the Pittsburgh Sleep Quality Index.
6. *VirTra:* Participants will be tested on shooting competency using compressed gas weapons. The system is designed to record each weapon action (pistol/rifle) and simulate bullet trajectory and recoil without firing an actual projectile (i.e. bullet). Measures of reaction time, accuracy, precision, and spread will be recorded by a trained team member present in the room.
7. *Force plate*: Whole body power will be assessed with a repetitive 10-jump test that we developed. Power (peak, average, curve functions) will be assessed using an AMTI force plate with Accupower 2.0 software (Advanced Mechanical Technology Inc, Watertown, MA).
8. *Response time*: Upper body and lower body reaction time will be measured using the Quick Board (The Quick Board, Memphis, TN). The Quick Board system is comprised of an iPad (Apple Inc., Cupertino, CA) Quick Board application and a footpad with 5 sensors placed equidistant from each other, with two at the front of the footpad, two at the back of the footpad, and one in the middle of the footpad. Upper and lower body reaction time will be measured using hands and legs, respectively. Prompts will be displayed by the iPad software to instruct the participant with quick time tasks.
9. After Day 1, participants will be instructed to restrict their sleep to 50% of their habitual sleeping duration. The goal is to provide a consistent start-and-stop sleep schedule:
    a. Example 1: if a participant sleeps on average **6** hours per night, then 50% reduction will result in **3** hours per night for 4 nights in a row. A target sleeping schedule will be from **3am – 6am**.
    b. Example 2: if a participant sleeps on average **8** hours per night, then 50% reduction will result in **4** hours per night for 4 nights in a row. A target sleeping schedule will be from **3am – 7am**.
10. After testing on Day 5 (i.e. after 4 nights of consecutive sleep restriction) the participants can resume regular sleeping habits for two weeks until they return to the lab to begin their second cross-over condition.

Day 2, 3, & 4:

1. Participants will be asked to maintain normal training schedule during these days.
2. The goal is to make sure that training during and between supplement condition remains same to control for confounding effects.
    a. For example, if a participant must run and train for ROTC duties Tuesday, Wednesday, Thursday (e.g. Days 2, 3, 4) then he/she will be asked to record the training regimen in the training log and closely replicate the same exercises (intensity and duration) throughout the study.

Privacy.
The consent room (PAES A023) will be used as the private consenting location. For all the data collected over the course of the study (i.e. records, biological samples and questionnaires) each participant will receive a unique subject identifier (i.e. a code) instead of using their legal name. Each unique identifier will only be available to research personnel.

Confidentiality*.*
Prior to signing the informed consent form all participants will be informed that their participation in the study is voluntary and that they may withdraw at any time. Participants who provide written consent are considered enrolled in the study. All records or biological data obtained after signing of the informed consent (including the screening visit, even for subjects that are not eligible for participation in the study) are treated with the same confidentiality safety measures as those subjects who qualify. Any information obtained during the prescreening for participants that were not eligible will be deleted. Hard copy data storage will be stored away in a locked file cabinet. Digital data will be protected on a computer via password. Subject names will never be used in any presentation or publication resulting from this study. The records will be maintained until the data are published and up to a maximum of ten years after the completion of the study.

Sleep Deprivation Disclaimer
According to the National Institute of Health, sleep deprivation is known to affect driving safety. Participants who undergo study-supervised sleep restriction are prone to falling asleep when stopped at a light and while driving. This situation increases the likelihood of motor vehicle accidents and traffic law violations, respectively. To avoid these risks we plan to collaborate with OSU-approved ride services (i.e., Lyft) to provide transportation for participants who commute to campus via car. 100% of the cost will be re-imbursed using university accepted PCards or check.

***Withdrawal from the Study*.**
Participants may be removed from the study for any of the following reasons:

- Discontinuation request;
- Medical or compliance reasons;
- Occurrence of any adverse event or condition that could, in the investigator's opinion, interfere with the evaluation of the effect of the study methods or put the participant at undue risk.

It is understood by all concerned that an excessive rate of withdrawals can render the study uninterpretable, therefore, unnecessary withdrawal of participants should be avoided. Should a participant decide to withdraw, all efforts will be made to complete and report observations as thoroughly as possible and documented in the eCRF.

Serious Adverse Event Reporting Instructions.

If in the opinion of the Investigator the event meets the criteria of a SAE the following procedures will be followed:

- The Investigator will notify Institutional Review Board (IRB) of the SAE within the parameters and timeframe specified under the IRB Standard Operating Procedures (SOP). An initial report followed promptly by a complete report will be forwarded to the IRB, when applicable.
- If a participant is hospitalized or hospitalization is prolonged due to the SAE, the hospital discharge summary will be obtained if possible.
- If a death occurs and an autopsy is performed, a copy of the autopsy report will be obtained if possible.
- All efforts must be undertaken to obtain follow-up information promptly.

Recording of Adverse Events.

All AEs (AE or SAE) will be recorded on the AE study documents. For participants who have an ongoing AE at their final study visit, follow-up information will be captured in the AE eCRF page which will be completed after 30 days.

Serious Adverse Event Follow-Up.

For all ongoing SAEs occurring during the study, the Investigator must submit follow-up reports regarding the participant's subsequent course. All SAEs that are ongoing at the end of the study or upon discontinuation of the participant's participation must be followed until either:

- The event resolves, or
- The event/condition has stabilized (e.g., in the case of persistent impairment), or
- The event returns to baseline, if a baseline value is available, or
- The participant dies, or
- The event can be attributed to other than the study beverage, or to other than the study conduct.

Participant Compensation.

Participants who complete all study duties will be compensated with a $300 PCard or a check. No compensation will be provided for completing the screening visit. Payments will be prorated by condition: each 5-day condition will result in $150; completion of both conditions (KE and Placebo) will result in $300. Any external costs associated with the study (i.e., transportation) will be covered by the study team.

Data analysis.

This will be the first prospective study to compare responses between a group that receives a ketogenic diet and/or ketone supplements to a group that receives the same treatment undergoing a placebo. Statistical analyses will occur in collaboration with the OSU Center for Biostatistics. For data reduction purposes, z-scores within a given cognitive domain will be averaged to generate a composite measure for that domain. Pre- vs. post-stressor differences in the composite cognitive score will be calculated and summarized for all subjects across treatment arms and time points. Differences in cognitive scores between treatment arms will be analyzed using two-way repeated-measures ANOVA. An interaction term between time and treatment will test whether there was any carry-over effect. Normality and heteroscedasticity will be checked using residual plots. Sample size calculations were based on a randomized crossover design of male athletes, which found an effect size of 0.7 in pre- vs. post-test cognitive performance between placebo and KE [32]. Our sample size of 60 subjects has 96.5% power (α=0.05) to detect this effect size (standardized mean difference) between treatments.

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