Strategies to Augment Ketosis Variations of Ketone Metabolism

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Protocol

Strategies to Augment Ketosis: Variations in Metabolism (STAK-VKM)

Title: Variations of Ketone Metabolism
Short title: STAK-VKM

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ABBREVIATIONS

AcAc	Acetoacetate
BHB	Beta hydroxybutyrate
KE	Ketone ester
BDO	(R)-1,3 Butanediol
BTQ	Beverage tolerability questionnaire
LBM	Lean body mass
LC-MS	Liquid chromatography – mass spectrometry
GRAS	Generally recognized as safe
AUC	Area under the curve
C_{max}	Maximal concentration
C_{min}	Minimal concentration
T_{max}	Time of maximal concentration
T_{min}	Time of minimal concentration
mL	milliliters
mg	Milligrams
h	Hour
ANOVA	Analysis of variance
AE	Adverse event
USG	Urine Specific Gravity
yr	Year

STUDY SYNOPSIS

Study Title	Variations of Ketone Metabolism
Short Title	STAK-VKM
Study Design	Open label, single-arm, observational study testing blood ketone responses to C8 Ketone diester consumption across subjects of varying ages and metabolic statuses.
Study Participants	• Men and Women, ages 20-70 years old
Planned Sample Size	An evaluable sample of 336 participants/group is required; to allow for 20% attrition a final sample size of 400 is planned.
Study Product	1. C8 Ketone Diester
Serving Sizes	360 mg/kg of lean body mass
Planned Study Period	October 2022 – October 2025
OUTCOMES	
Primary	Difference in total plasma ketone appearance (AUC) across the metabolic ranges.
Secondary	• Differences in other blood ketone kinetic parameters (peak concentration, time of peak concentration, AUC R-BHB, capillary ketone concentrations). • Differences in ketone clearance rates via urine sample. • Differences in blood metabolites (glucose). • Differences in blood hormones (insulin). • Differences in tolerability and subjective satiety.

SUMMARY AND BACKGROUND

This project is focused on nutrition-based STRATEGIES TO AUGMENT KETOSIS (STAK), which enable people to increase blood ketone concentrations and to achieve 'nutritional ketosis' which may have beneficial effects on resilience and health. Ketone esters (KEs) represent one promising STAK, these compounds consist of ketone-promoting components joined by ester bonds. The ketone-promoting components can be ketone bodies such as beta hydroxybutyrate (BHB) or acetoacetate (AcAc), ketogenic precursors that metabolize to ketones via the classical beta-oxidation ketogenesis pathway (i.e., medium chain fatty acids) or ketone precursors that metabolize to BHB via a non-classical pathway (i.e., (R) -1,3 butanediol, BDO). One KE compound is a diester of hexanoic acid (a ketogenic medium chain fatty acid) and (R)-1,3 butanediol (C6 Di-ester). Recent studies of KEs suggest that they may have many acute functional effects, such as modulation of glucose, fatty acid and amino acid metabolism[5,6], heart rate4, acid base homeostasis[7,8,11,12] and urine output[7,8], as well as multiple other effects demonstrated in preclinical research, including modulation of oxidative stress and inflammation[13,14], that are yet to be investigated in clinical studies. As the use of KE products becomes more widespread, there is a critical need to expand our knowledge of how their metabolism varies between individuals to optimize dosing strategies for service members. Our goal is to determine the importance of age and metabolic healthy on blood ketone and glucose concentrations.

KE Metabolism Varies Between Individuals. This outcome of this study will help us to understand how the phenotype of the individual modulates the KE metabolic effect. Most studies of KE have been in homogenous populations, usually young, male athletes. However, two striking experiments using identical, body weight adjusted KE doses in healthy and obese individuals found that BHB area under the curve (AUC) and removal was reduced by obesity and poor metabolic health. Similarly, ketone infusion experiments found that diabetes, obesity, and insulin resistance alter BHB metabolism. It is important to determine how obesity affects KE 'sensitivity' (i.e., breakdown and oxidation) because the increasing prevalence of obesity as a function of age. Age may be another important source of variation in ketone metabolism. The genes that control the ketone system are regulated by a cascade of transcription factors and hormones including PPARα and FGF21, which are themselves known to be affected by aging and dietary status, and the cellular protein sensor target of rapamycin (TOR). Aberrant

hyperactivation of TOR with aging may reduce ketogenesis, while we observed that a long-term ketogenic diet specifically up-regulated PPARα activity. Our preliminary work revealed substantial changes across mouse lifespan in the expression of ketone-related genes in the liver such as *Hmgcs2* (rate limiting for ketone production) and *Bdh1* (rate limiting for BHB oxidation) between young, middle-aged, and old mice, with a nadir of gene expression in middle age before increasing again late in life (**Fig 1A & 1B)**. We also found substantial age differences in response to matched doses of oral KE in mice (**Fig 1C)** and in rats. These data may have important implications for treating people of different ages and for translating KE technologies into the Department of VA. Therefore, we plan to study individual responses to KE ingestion across the lifespan, against the background of varying metabolic health

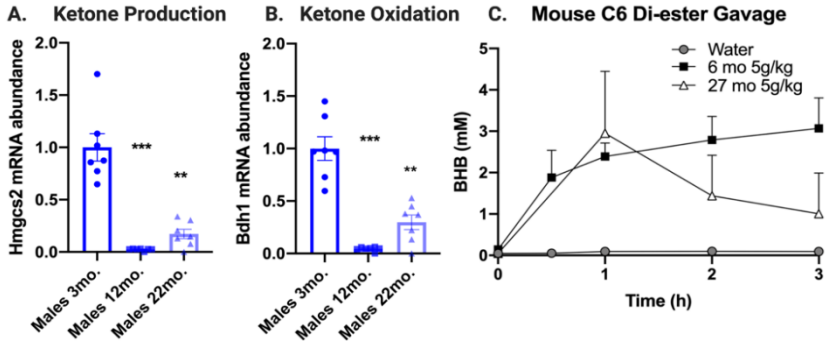

Figure 1 *Change in mRNA expression for ketone oxidation enzyme (Bdh1) and production (Hmgcs2) between young, middle aged and old male mice. Blood BHB kinetics with a matched oral KE dose in young and old male mice.*

IMPACT

KEs could represent a practical STAK method that does not require any change in diet. However, there is a critical need to elucidate how KE metabolism varies between individuals to inform KE use in the field. This high impact project will contribute important knowledge that aims to develop next-generation KE molecules and formulations that are designed to meet the needs of operators at different points in their career. Our long-term goal is to create evidence-based guidelines for deployment of KE compounds based on individual characteristics.

RESEARCH STRATEGY

This is an open label, one arm study to characterize the effects of age and metabolic health on ketone responsiveness. We will enroll a large group representing a range of ages and metabolic health status. N of 300 will be recruited and tested at the Physical Activity and Education services facility at the Ohio State University, Columbus, OH, and an N of 100 will be recruited and tested at the Buck Institute in Novato, California. Data/sample collection will include blood (capillary and whole venous blood) and study product tolerability.

OUTCOMES

Primary

- Difference in total plasma ketone appearance (AUC) across various age and metabolic ranges

Secondary

- Differences in other blood ketone kinetic parameters (peak concentration, time of peak concentration, AUC of different ketone bodies, capillary ketone concentrations).
- Differences in blood metabolites (glucose, free fatty acids).
- Differences in subjective tolerability and subjective satiety measured by questionnaires
- Differences in blood hormones (insulin).

PARTICIPANTS

This study aims to recruit equal numbers of male and female participants between the ages of 20 to 70yr. Thus, we will recruit a balanced sample of men and women with a range of ages (equal representation in each of the 5 included decades; 20-29yr, 30-39yr, 40-49yr, 50-59yr, 60-70yr) and metabolic health via HbA1c clinical ranges (normal: <5.7, prediabetes: 5.7-6.4, diabetes: >6.4). Each participant must meet all the inclusion criteria and none of the exclusion criteria at screening in order to participate.

Inclusion criteria:

- Ages 20 – 70 years
- Participant is willing and able to comply with all study procedures including the following prior to Test Day: fasting (>10 h; water only), no alcohol (>24 h), no exercise (>24 h), no acute illness and controlled feeding before the Test Day, maintain diet, exercise, medication, and supplement habits throughout the study.
- Participant has no health conditions that would prevent completion of the study requirements as judged by the Investigator based on health history.
- Participant understands the study procedures and signs forms providing informed consent to participate in the study and authorizes the release of relevant protected health information to the Investigator.

Exclusion criteria:

- Participant follows a low-carbohydrate diet (<30% energy from carbohydrate) or have used exogenous ketone supplements within 4-months of study participation.
- Participant has a Primary Care Physician diagnosed history or presence of uncontrolled and/or clinically important hypertension (blood pressure >150/95 mmHg), pulmonary, cardiac, hepatic, renal, endocrine (including type 1 and 2 diabetes), hematologic, immunologic, neurologic (e.g., Alzheimer's or Parkinson's diseases), psychiatric (including unstable depression and/or anxiety disorders) or biliary disorders.

- Participant has a known allergy, intolerance, or sensitivity to any of the ingredients in the study beverages, including soy and milk protein, wheat, shellfish, fin fish, eggs, tree nuts or peanuts (production facility handles nuts).
- Participant has unstable use of a medication or supplement that the Investigator considers may affect the outcomes of the trial.
- Consumption of alcohol more than 3 drinks per day or more than 18 drinks per week.
- Consumption of tobacco.
- Consumption of cannabis.
- Participant is currently in another research study or has been in the 14 days before screening.
- Participant has had a blood draw or donation in the last 8 weeks.
- Participant has a clinically important gastrointestinal (GI) condition that would potentially interfere with the evaluation of the study beverage [e.g., inflammatory bowel disease, irritable bowel syndrome, chronic constipation, severe constipation (in the opinion of the Investigator), history of frequent diarrhea, history of surgery for weight loss, gastroparesis, systemic disease that might affect gut motility according to the Investigator, medication managed reflux and/or clinically important lactose intolerance].
- Participant has a condition the Investigator believes would interfere with his ability to provide informed consent, comply with the study protocol, which might confound the interpretation of the study results, or put the participant at undue risk.

Sample Size. Our primary outcome goal is to elucidate the effects of age and metabolic health status on plasma total ketone AUC. Using a prior human data from metabolically healthy and unhealthy groups, a 10% difference of BHB AUC (based on the 0.89 ± 0.26 mM x hr values for young healthy people) corresponds to an effect size of 0.36 SD. Using this effect size with a type II error of 0.05, a sample size of 336 is necessary to detect the difference at 90% power. A total of 400 participants will be recruited to account for 20% drop off rate.

Recruitment:
Recruitment will occur through well-established methods at OSU, which include word of mouth, ResearchMatch database, social media engagement and flyers.

METHODS

Study overview:

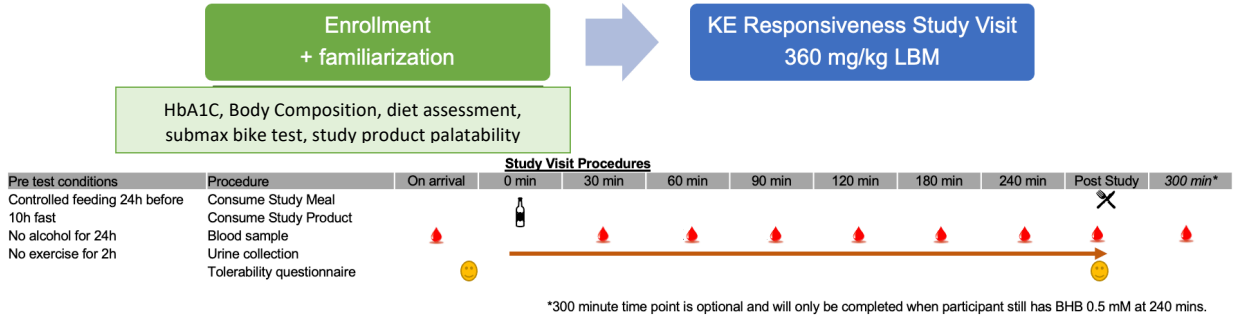

Figure 2 | Study schematic, showing timeline for a study day.

Study Procedures

Study Day	Screening	Test Day
Visit to Study Center	X	X
Informed consent	X	
Medical intake questionnaire, diet assessment, anthropometrics[1]	X	
HbA1c Measurement[2]	X	
Submaximal Bike test[3]	X	
Compliance assessment[4]		X
Hydration Status and Urine Collection[5]		X
Consume study product[6]		X
IV Cannulation and whole blood collection[7]		X
Tolerability assessment[8]		X

1. Medical intake questionnaire and diet assessment questionnaire are attached in **Appendix** . Anthropometric data will include: Hip and Waist circumference, BMI, height and weight.
2. HbA1c measurement will be taken via fingerstick in order to stratify participants.
3. A submaximal 6 minute bike test will be conducted to predict V02 Max (Astrand Test)
4. Prior to starting each test day, participants will be asked to confirm that they meet pre-test criteria, including: fasted >10h, no alcohol for 24h, no exercise for 24h and consumed the provided pre-test food.
5. Prior to consumption of the Study Product, participants will be asked to completely void their bladder. And hydration status will be determined via urine specific gravity (USG) reporting <1.025. Participants will then void all urine into a provide container for the duration of the study visit.
6. Study Product will be consumed within +/- 60 minutes of the time established on the first test day. Participants will be provided with a choice of non-caffeinated, non-caloric beverage to remove the bitter taste of the Study Product.
7. IV cannula will be inserted at the start of each Test Day, and removed at the end of each test Day, Blood samples will be collected according to the schedule in Figure 2. Cannula will be flushed with a small volume of saline after each sample to maintain patency
8. Participants will complete a Beverage Tolerability Questionnaire prior to Study Product consumption and at the end of the Test Day (**Appendix**)

Screening Visit: 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 anthropometric measures and diet and medical questionnaire answers to determine if they meet the study criteria.

If the participant provides consent, they will be provided with questionnaires including Automated Self-administered 24-hour Dietary Assessment Tool (ASA24®),and medical history. All collected samples and data will be coded to maintain participant anonymity. We will give the participants a small volume of Study Product to screen for tolerance of the bitter tasting Study Product. We will also measure height, weight and body composition using bioelectrical impedance. Then we will use an A1CNow+, Hba1c Blood monitor kit to assess Hba1c score. We will use both body composition and Hba1c score to stratify participants to ensure various metabolic statuses are represented in the study population. The participant will then complete a 6 minutes Astrand Bike Test to determine the predicted $VO2_{Max.}$ This test does not require fasting and uses only submaximal effort upon the participant to determine cardiometabolic status by an average heart rate at 6 minutes of pedaling at a moderate intensity. If the participant is eligible for the study and is still interested in participating then they will be scheduled to return to the study center for the testing visit.

Testing Day:

Participants will report to the study center in the morning of the Test Day. Compliance with pre-test instructions (fasted > 10h, no alcohol >24h, no exercise >24h, consumed pre-test food) will be confirmed by the Investigator. Participants will complete a baseline Beverage Tolerability Questionnaire (BTQ). Participants will be asked to completely void their bladder and a sample will be analyzed for hydration status. Participants with samples reading greater $\geq$ 1.025 USG will be asked to drink 160z of water and retest again in 30 minutes. Participants will be provided with a container to void all urine during the study visit. This will be aliquoted for urine R-BHB analysis to assess the clearance of ketones in the body. A trained member of the study team will insert an IV cannula into a vein in the antecubital fossa to allow for repeated blood sampling. The cannula will be flushed with a small volume of saline after each sample withdrawal to maintain patency. At the same time as all whole blood samples, we will also collect capillary blood samples from a finger for real-time analysis of blood BHB and glucose concentration, using lancing device, commercially available test strips and a handheld monitor (KetoMojo, CA, USA).

Participants will then consume the C8 Ketone Diester Study Product. They will be given 5 minutes to consume the Product. After C8 Ketone Diester consumption, they will remain at the study center for ~4-5h, with blood sampling occurring at regular intervals (see

Figure 2). A total of 7 whole blood samples (~8 mL each) will be collected each Test Day. Capillary blood samples will be collected at the same time as whole blood samples. At the 4th hour finger stick, participants whose capillary ketones levels have not returned to baseline will be asked to stay for a 5th hour to assess the complete metabolism of the study product. Participants will complete satiety questionnaires each hour, and will complete a BTQ at the end of the Test Day.

Participants will be asked to minimize ambulatory movement during the Test Day. Non caloric beverages (i.e., water) will be permitted *ad libitum* and intake volumes will be recorded. At the end of the Test Day, the IV cannula will be removed and a dressing will be applied to the cannula site. Participants will be given a snack to consume.

Sample Processing and Analysis

Blood Processing: Whole blood collected through the IV cannula will be processed to plasma (EDTA tubes) or serum (clot activator serum collection tubes) and then snap frozen in liquid nitrogen for storage prior to analysis (details below). We will collect capillary blood samples for real-time analysis of blood BHB and glucose concentration, using commercially available test strips and a handheld monitor (KetoMojo, CA, USA), this will also allow comparison of ketone values obtained via different methods.

Blood Sample Analytical Methods:

- *Ketones* will be determined by multiple methods to provide a robust measure of ketosis and allow cross validation. Firstly: by capillary BHB concentrations obtained from a finger stick using a handheld device (KetoMojo, USA). Secondly: plasma and urine ketones (R-BHB) will be measured using ultra-performance liquid chromatography – tandem mass spectrometry (LC-MS).
- *Plasma hormone* (insulin) will be analyzed using commercially available ELISA assay kits (Cayman Chemical, USA).
- *Capillary glucose* concentrations obtained from a finger stick using a handheld device (KetoMojo, USA).

Beverage Tolerability Questionnaire: The BTQ used in this study is similar to that used in previous tolerability studies[1516,17,18] . Ten tolerability issues are included in the BTQ: gas/flatulence, nausea, vomiting, abdominal cramping, stomach rumbling, burping, reflux (heartburn), diarrhea, headache, and dizziness. Participants are asked if the issue was present (pre- beverage - baseline) or had occurred since they took the study beverage (post-beverage – 4h) at the following intensities: none, mild (awareness of symptoms but easily tolerated), moderate (discomfort enough to interfere with but not prevent daily activity) or severe (unable to perform usual activity). These correspond to scores of 0–3, respectively for each issue, giving a maximal composite score, defined as the sum of the ten items, of 30.

Satiety Visual Analogue Scale: We will use a 3-item visual analogue scale, that assesses hunger, fullness and desire to eat by participant's marking on a line anchored at either end with 'not at all' and 'extremely.' Distance along the line is measured in mm.

Body Composition: We will use the Quantum V Segmental BIA which provides quantitative regional body composition assessments that are similar to those produced by Dual-energy X-ray Absorptiometry (DXA) scanners. This is performed by using eight hand and foot electrodes (left and right side) with the subject in a supine position and measuring 13 resistance and reactance regions on the human body. 811

STUDY PRODUCTS

Background: The exogenous ketone compound, C8 Ketone Diester, is commercially available in as the product 'Metabolic Switch' (Juvenescence Ltd, NJ, USA).

Quality Control: As only small amounts of product are needed for this study, and products are shelf stable, our aim is that all product inventory will be acquired at the start of the study to ensure the same product lots are used throughout. We plan to obtain Certificates of Analysis to confirm product exogenous ketone content.

Serving Size Rationale: Previous work has shown that lean body mass (LBM) is a major covariate in KE responsiveness[9]. Therefore, we will standardize exogenous ketone serving sizes to LBM (assessed using DXA) for all trials at 360 mg/kg LBM, which for a participant with 70 kg of lean mass corresponds to ~ 25 g. These serving sizes are representative of typical commercial serving sizes and are expected to elevate blood BHB in the range of 1.5 - 2 mM

Storage: The study products will be stored in a dry secure location at ambient temperature (15- 77°F) and are best served chilled. Study product supplies are to be used only in accordance with this protocol and under the supervision of the Investigator.

Table 1: Study product details

Study Active Ingredient	Serving Size	Form	Other product ingredients [ALLERGENS]	Example Commercial Product (Company)
C8 Ketone Di-ester	360 mg/kg	Ready to drink beverage	Water, high fat whey protein concentrate, modified gum acacia, citric	Metabolic Switch

			acid, soy lecithin, natural flavors, stevia leaf extract, pectin, sodium carboxymethyl cellulose, potassium sorbate. [DAIRY AND SOY]	(Juvenescence Ltd, NJ, USA)

DATA ANALYSIS AND STATISTICAL METHODS

Primary outcomes

- Difference in total plasma ketone appearance (AUC) between the ranges of age and metabolic status

Secondary outcomes

- Differences in other blood ketone kinetic parameters (peak concentration, time of peak concentration, AUC of different ketone bodies, capillary ketone concentrations).
- Differences in blood metabolites (glucose).
- Differences in subjective tolerability and subjective satiety measured by questionnaires
- Differences in blood hormones (insulin).

Statistical Plan: Statistical analysis of data will take place on completion of the study in collaboration with Buck Bioinformatics Core. All decisions regarding exclusion from the analysis population will be documented prior to database lock. Missing data will not be imputed, and only observed data will be included in the statistical analysis. To understand the relationship between BHB AUC and covariates such as age and metabolic health, we will first use multiple linear regression where BHB AUC is regressed on the covariates. P-values will be used to determine covariates that significantly predict BHB AUC. From the effect size of the coefficients, we can also assess which covariates have a greater impact on BHB AUC. The significance of interaction between covariates can also be investigated.

To explore the pharmacokinetics of KE metabolism, we will develop a nonlinear mixed effect model (NLME). A one-compartment model with first-order absorption and elimination, where a constant proportion of ketone is absorbed/eliminated per unit time, has previously been observed to fit the pharmacokinetics of BHB Monoester metabolism[19]. The major goal of modeling the ketone data is to illustrate the relationships between the pharmacokinetic parameters such as C_{max}, clearance(CL), volume of distribution(V_d) and the covariates. We will carry out preliminary analysis of the data after 200 participants have completed the testing visit to determine effect size and determine if additional recruitment is required. Our anticipated results from this experiment are to

show that blood ketone responses to a standard KE dose can be predicted based on age and metabolic health, and to inform individualized dosing guidelines.

STUDY MONITOTRING

Concomitant Medication/Supplements and Treatment

All concomitant medications/supplements used 1 months prior to Screening Visit and during the study will be reported to the study personnel for assessment and recorded in the participant's study documents.

Adverse Event Monitoring

An AE is defined as any untoward medical occurrence in an investigation participant following written informed consent that does not necessarily have a causal relationship with the study product. An AE can be any unfavorable or unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product. This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures (including laboratory test abnormalities).

Some side effects or GI symptoms could occur as an outcome of these dietary interventions; side effects listed in the beverage tolerability questions and reported will not be categorized as AEs but recorded as study outcomes. Side effects, outside of what is expected as a result of study product consumption, reported by participants and judged by the Investigators as medically relevant events and related to study product will be recorded as AEs.

Events should be considered AEs if they:

- Result in discontinuation from the study,
- Require treatment or any other therapeutic intervention,
- Require further diagnostic evaluation (excluding a repetition of the same procedure to confirm the abnormality),
- Are associated with clinical signs or symptoms judged by the Investigator to have a significant clinical impact.

Grading and Severity

The Investigator will evaluate all AEs with respect to their severity, and record the outcome and action taken on the AE study documents . AEs will be graded as:

Mild: Awareness of symptoms but easily tolerated
Moderate: Discomfort enough to interfere with but not prevent daily activity

Severe: Unable to perform usual activity

Relationship
The Investigator will also judge the likelihood that the AE was related to the study beverage and document this on the appropriate study documents as:

NOT RELATED	This category applies to those adverse experiences which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).
UNLIKELY	In general, this category can be considered applicable to those experiences that after careful medical consideration at the time they are evaluated, are judged to be, unlikely related to the study beverage.
POSSIBLY	This category applies to those adverse experiences for which, after careful medical consideration at the time they are evaluated, a connection with the study beverage administration appears possible but cannot be ruled out with certainty.
PROBABLY	This category applies to those adverse experiences that, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the study beverage.
DEFINITELY	This category applies to those adverse experiences which, the Investigator feels are incontrovertibly related to the study beverage.

Appropriate therapeutic action and follow-up measures will be performed by the Investigator in accordance with appropriate medical practice standard of care.

Serious Adverse Event Definition/Qualification
A SAE is defined as an AE that results in any of the following outcomes:

- Death (note that death is the outcome of a SAE and the cause of death should be listed as the AE),
- Life-threatening event,
- In-patient hospitalization or prolongation of existing hospitalization,
- A persistent or significant disability/incapacity,
- Congenital anomaly or birth defect,
- Any other important medical event that may not result in death, be life-threatening, or require hospitalization, may be considered a SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

In the event of a SAE, the participant may be dropped from the study if the Investigator deems it necessary.

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.

CONDUCT OF THE STUDY

1. **Ethics and Regulatory Considerations**

This study will be conducted according to Good Clinical Practice Guidelines, the Declaration of Helsinki (2004) and United State Code of Federal Regulation Title 21. Signed written informed consent for participation in the study will be obtained from all

participants before protocol-specific procedures are carried out. Participants will be informed of their right to withdraw from the study at any time. Participants will be informed that their participation in the study is completely voluntary, personal information will be both deidentified to preserve anonymity.

2. **Institutional Review Board**

The Investigator will ensure that an appropriately constituted IRB, in compliance with the requirements of 21 CFR 56, reviews and approves the clinical study. Before the study is started, the Investigator will forward copies of the protocol and consent form for this study to the IRB for review and approval. IRB approval must refer to the study by exact protocol title and number, identify the documents reviewed, and state the date of review. The IRB must be informed of all subsequent protocol amendments. No alterations, modifications to IRB-approved documents, including the protocol, protocol summary, consent form, recruitment materials and questionnaires will be allowed. The IRB must also be informed of all SAEs and of unexpected AEs as outlined in the IRB's SOPs or reporting guidelines.

3. **Informed Consent and Protected Health Information**

The study will be explained verbally as well as on the informed consent document. Each participant will be given ample opportunity to inquire about details of the study and to read and understand the consent form before signing it. It will be made clear that participants can withdraw from the study at any time.

Each participant's signed informed consent document must be kept on file by the Investigator. The participant should receive a copy of the informed consent document. A participant may not be admitted to the study unless informed consent of the participant (or his/her legally authorized representative) has been obtained.

4. **Participant Confidentiality**

The Investigator is responsible for ensuring that participants' anonymity will be maintained. For all the data collected over the course of the study for each participant (i.e., records, biological samples and questionnaires) a unique subject identifier (i.e., a code) will be assigned and used instead of the subject's name. The code for each participant which links the subject name with their identifier will only be available to research personnel. Electronic CRFs or other documents will identify participants by initials, number, or code, and not by name. The Investigator will keep a separate log showing codes, names, and addresses. Any records that contain the subject's name and identifier will either be stored in the Kinesiology file storage room in a file cabinet (locked) or protected on a computer via password protection on the individual digital file and password protection on the computer the file(s) are stored on. All other records that contain the subject identifier only will also be kept in either a file cabinet in our locked file

storage room or on a password protected computer. 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. 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

5. **Withdrawal of Participants from the Study**

Participants may be removed from the study for any of the following reasons:

- A participant requests discontinuation;
- The Investigator initiates removal for medical or compliance reasons;
- Occurrence of any AE or condition that could, in the Investigator's opinion, interfere with the evaluation of the effect of the study beverage 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. In the event that a participant is withdrawn from the study, the reason for the withdrawal will be documented in the eCRF.

References

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