Official Title:	**Randomized, Single-blinded, Controlled, Parallel-arm Study to Evaluate the Effect of Fast Bar™ on Physiological Fasting Condition**
Short Title:	**"Fast Bar"**
Protocol Number:	**[XXXXXX]**
Principal Investigator(s):	**William C. Hsu, MD**
Sponsor:	**L-Nutra, Inc.**
Funded by:	**L-Nutra, Inc.**
Version Number:	**v.1.0**
Date:	**June 19, 2020**

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form will be obtained before any participant is enrolled. Any amendment to the protocol will obtain review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

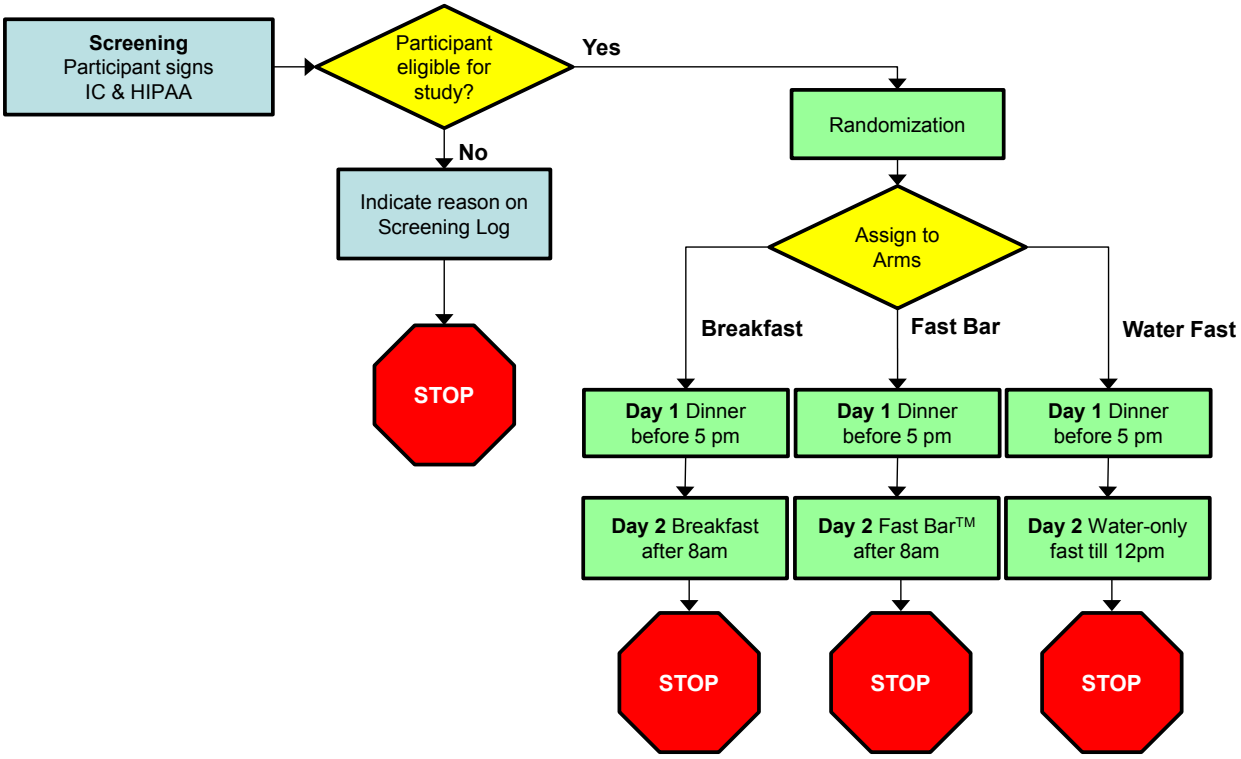
1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Randomized, Single-blinded, Controlled, Parallel-arm Study to Evaluate the Effect of Fast Bar™ on Physiological Fasting Condition
Study Description:	This study evaluates how Fast Bar™, a specially formulated energy bar, affects the physiological condition in participants after an overnight fasting. Participants will fast for 19 hours (Fast Group), consume a breakfast Bar (Breakfast Group) or a Fast Bar™ (Fast Bar Group) after an approximately 15-hour overnight fasting. Participants will be assessed for physiological parameters associated with fasting.
Objectives:	**Primary Objective:** The objective of this study is to evaluate the metabolic effects of consuming a novel food product (Fast Bar™), as compared to consuming a bar that resembles a typical breakfast, after an overnight fasting. **Secondary Objectives:** To assess the effect of a Fast Bar™ on facilitating fasting by modulating subjective variables.
Endpoints:	**Primary Endpoint:** Physiological condition associated with fasting: • Blood ketone (BHB) level • Blood glucose level **Secondary Endpoint:** Questionnaires: • Food diary • Food evaluation • Self-evaluation
Study Population:	A total of 108, adult male and female, generally healthy subjects.
Phase:	N/A

Description of Sites/Facilities Enrolling Participants:	Participants will be recruited via email or telephone. Participants will be consented via Zoom tele-conference. The study will be carried out at participants' home.
Description of Study Intervention:	Study subjects will consume a standardized ready-to-eat meal as dinner the day before testing and asked to fast for approximately 15 hours. Study subjects will either continue to fast for 6 hours (Fast Group), consume either a breakfast (Breakfast Group) or a Fast Bar™ (Fast Bar Group).
Study Duration:	3 months
Participant Duration:	2 days

1.2 SCHEMA



1.3 SCHEDULE OF ACTIVITIES (SOA)

	Screening	Day 1	Day 2				
Day		**1**	**T0**	**T1**	**T2**	**T3**	**T4**
Hours			Baseline	+1 h	+2 h	+3 h	+4 h
Example clock times			8 am	9 am	10 am	11 am	12 pm
Procedures							
Informed consent	X						
Randomization	X (1)						
Blood draw (Fingerstick) • ketone (BHB) • glucose		X (morning, fasting)	X	X	X	X	X
Intervention							
Healthy Eating Style	X (2)						
Dinner, followed by 15 h water-only fasting		X (before 5 pm)					
Breakfast Bar			X (after T0 test)				
Fast Bar™			X (after T0 test)				
Water-only fast			X	X	X	X	X
Questionnaires/ Surveys							
Demographics	X						
Contact Information	X						
Medical history Concomitant medications	X						
24-h dietary recall	X						
Food diary		X					
Self-evaluation			X (after T0 test)			X	
Food evaluation				X			

(1) Subjects will be assigned to study arms according to the randomization scheme generated by using the web site Randomization.com (*http://www.randomization.com*).

(2) Subjects will be provided with a USDA MyPlate Plan according to his/her BMI and physical activity level (*https://www.choosemyplate.gov/resources/MyPlatePlan*) and encouraged to adopt the recommended healthy eating style for 2-3 days before starting the trial.

2 INTRODUCTION

2.1 STUDY RATIONALE

The objective of this study is to evaluate the metabolic effects of consuming a novel food product (Fast Bar™) after overnight fasting.

Interest in fasting-based programs (i.e. intermittent fasting) for improvement of health and longevity continues to grow [1-3]. While benefits of intermittent fasting have been convincingly demonstrated in rodent models, the more limited data in humans is less clear. As such, additional investigations of the

effects of fasting-based protocols in humans are needed. The short-term fasting (e.g. 12 to 48 hours in duration) utilized in many intermittent fasting programs are considered safe, but some individuals may find them subjectively difficult. As such, the question of whether the benefits of fasting can be obtained while small amounts of food are consumed is of substantial interest. An attractive strategy may be to consume small quantities of foods specifically formulated to maintain a fasted or pseudo-fasted state. If such a strategy is successful, the benefits of fasting may be maintained even though a small amount of food consumption, which will alleviate subjective feelings of hunger and promote adequate nutritional intake.

One commercially available product that is designed to be consumed during periods of intermittent fasting is the Fast Bar™ (*www.fastbar.com*), which stems out of the well-researched fasting-mimicking diet [4, 5] to assist prolonged fasting. The unique formulation of the Fast Bar™, which consists of nuts and other select ingredients, is thought to minimize deviations in metabolic biomarkers associated with a fasting state. This may allow for extension of a fasting period through reduced subjective difficulty of fasting. However, these items have yet to be evaluated for its effects during intermittent fasting through a controlled research trial. As such, the objective of this study is to evaluate the metabolic and subjective effects of consuming a novel food product (Fast Bar™) after a short period of fasting.

2.2 BACKGROUND

Negative energy balance induced by calorie restriction (CR) or increased energy expenditure induced by exercise (EX) improves all metabolic coronary heart disease (CHD) risk factors, including plasma LDL-cholesterol, total cholesterol/HDL ratio, HOMA-IR index, and CRP concentrations [6, 7]. However, chronic calorie restriction, whose beneficial effects have been known for decades, has not been adopted by even a small percentage of the US population, in part because compliance to chronic and extreme diets is very low.

In healthy human subjects, three monthly cycles of fasting-mimicking diet (FMD) lasting 5 days reduced body weight, trunk and total body fat, and blood pressure [5]. In the same study, a post hoc analysis demonstrated that body mass index, blood pressure, fasting glucose, triglycerides, total and low-density lipoprotein cholesterol, and C-reactive protein were more beneficially affected in those participants with the highest baseline levels for the same variables, suggesting that FMD may be particularly effective in participants with established metabolic syndrome. Moreover, FMD cycles restore insulin secretion and glucose homeostasis in both type 2 and type 1 diabetes mouse models. In mice, a 4-day fasting mimicking diet (FMD) induced a stepwise expression of Sox17 and Pdx-1, followed by Ngn3-driven generation of insulin-producing b cells, indicating that FMD promotes the reprogramming of pancreatic cells to restore insulin generation in islets from T1D patients and reverse both T1D and T2D phenotypes in rodent models [8].

The Fast Bar™ is a designed to provide energy without interfering with the protective and metabolic effects of fasting. The Fast Bar™ diets consist of 100% ingredients, which are generally regarded as safe (GRAS).

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

A 14-18 hours overnight fasting is a common practice. However, it is possible that some participants may experience fatigue, weakness, low blood sugar.

Fingerstick is a common practice for checking blood glucose. Participants could experience pain, discomfort, bruising or infection (a slight risk any time the skin is broken). Fingerstick devices should never be used for more than one person.

Although participants will be screened for food allergies, it is possible that an allergic reaction could occur upon ingestion of the study foods.

2.3.2 KNOWN POTENTIAL BENEFITS

Short term known potential benefits:

N/A

Long term known potential benefits:

N/A

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

RISKS

The study intervention (overnight fasting and study food consumption) and procedure (fingerstick) pose a minimal risk to the study subjects.

BENEFITS

Participants may not receive any direct benefit from taking part in this study.

3 OBJECTIVES AND ENDPOINTS

The research questions to be addressed are:

1. What are the metabolic effects of consuming a Fast Bar™, as compared to consuming a typical breakfast, after an overnight fast?
2. Based on the observed metabolic effects, does consumption of a Fast Bar™ allow for maintenance of the fasting state?
3. Does consumption of a Fast Bar™ facilitate fasting by modulating subjective variables?

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Physiological condition associated with fasting	• Blood ketone (ß-hydroxybutyrate, BHB) level • Blood glucose level	When hepatic glycogen stores are exhausted (after 12-24 hours of total fasting), the liver produces ketones (ketogenesis: acetoacetate (AA) and BHB to provide an energy substrate for peripheral tissues.
Secondary		
Questionnaires	• Food evaluation • Self-evaluation	The secondary objective is to assess the subjective variables

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is quantitative research with a randomized, single-blinded, controlled, parallel-ar design.

Total length of subject participation is 2 days.

The study will be carried out off-site (at participants' home).

After the explanation of the study and informed consent signature, eligible participants will be randomized to three groups.

1. Subjects in all groups will be asked to consume a standardized ready-to-eat dinner meal before 5 pm on day 1.
2. Breakfast Group: overnight fasting (approximately 15 hours), then consume a breakfast on day 2;
3. Fast Bar Group: overnight fasting (approximately 15 hours), then consume a Fast Bar™ on day 2.
4. Water-only fasting group: overnight fasting (approximately 19 hours);
5. Subject all groups will be asked not to consume any food or calorie-containing drinks other than the study foods till 2 pm on day 2.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This research will allow for scientific evaluation of the application of the Fast Bar™ during intermittent fasting. That is, this research will allow for quantification of the changes in metabolic biomarkers after consumption of a Fast Bar™, following a period of acute fasting, to determine whether deviations from the fasted state are smaller and more transient than when consuming a breakfast bar. This comparison will facilitate a better understanding of whether the Fast Bar™ is a viable strategy for extending the benefits of intermittent fasting despite the consumption of food.

4.3 JUSTIFICATION FOR DOSE

Fasting leads to the mobilization of hepatic glycogen stores. When the glycogen stores are exhausted (after 12-24 hours of total fasting), the liver produces ketones (ketogenesis: acetoacetate (AA) and ß-hydroxybutyrate (BHB) to provide an energy substrate for peripheral tissues. Consumption of food, depending on the composition of the food, i.e., the absolute levels and ratios of carbohydrate, protein and fat, may markedly affect the ketogenesis state. We hypothesis that the specially formulated Fast Bar™ will not significantly impact the overnight fasting-induced ketogenesis state.

4.4 END OF STUDY DEFINITION

The end of the study is defined as completion of the procedures shown in the Schedule of Activities (SoA) in the trial (Section 1.3).

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

An individual of any race and ethnicity who meets all of the following criteria will be included for participation in this study:

- Ability and willingness to provide written informed consent;
- Ability and willingness to use Zoom teleconference;
- Ability and willingness to perform the study tests and adhere to study protocol (to the best of the participant's knowledge);
- 18-65 years of age (inclusive) at screening;
- BMI 20-35 kg/m^2 (inclusive) at screening;

- In good health (as determined by medical history to evaluate acute or ongoing chronic medical diagnoses/conditions that have been present for at least 90 days).

5.2 EXCLUSION CRITERIA

An individual of any race and ethnicity who meets any of the following criteria will be excluded from participation in this study:

- Has any medical disease or condition that, in the opinion of the principal investigator (PI) or appropriate study personnel, precludes study participation* (*Including acute, subacute, intermittent or chronic medical disease or condition that would place the subject at an unacceptable risk of injury, render the subject unable to meet the requirements of the protocol, or may interfere with the evaluation of responses or the subject's successful completion of this trial);
- History of gastric bypass (based on medical history provided at screening);
- Under medications aimed at keeping blood glucose under control (based on medical history provided at screening);
- Type 1 diabetes (based on medical history provided at screening);
- Taking insulin, insulin analogs, or octreotide (based on medical history provided at screening);
- Food allergies which would make the subject unable to consume the food provided (based on medical history and information provided at screening) (participants will be asked to review the ingredient lists for the dinner meal, the breakfast and the Fast Bar™, and to state that they are not allergic to the ingredients to the best of their knowledge);
- Women who are pregnant;
- Alcohol dependency (alcohol intake greater than two drinks per day for women and three drinks per day for men) (based on medical history and information provided at screening).

5.3 LIFESTYLE CONSIDERATIONS

Participants will be asked to:

- Finish the dinner (provided) by 5 pm on day 1, followed by an approximate 15-hour water-only fast;
- Self-monitor blood glucose on day 1 (morning before breakfast) and on day 2;
- Self-monitor blood ketone (BHB) on day 1 (morning before breakfast) and on day 2.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the trial but are not subsequently randomly assigned to the study intervention or entered in the study.

A minimal set of screen failure information including demography, screen failure details, eligibility criteria will be retained to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Potential participants will be recruited via telephone and email.
Participants will be enrolled via Zoom teleconference (https://www.zoom.com).

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Participants will be asked to consume a ready-to-eat dinner before 5 pm on day 1.

Participants will be asked to refrain from consuming any food or drinks (except water) for 15 hours.

On day 2, subjects will be asked

- to continue the water-only fast for 4 more hours (Water Fast Group);
- to consume a breakfast, and then continue to water-only fast for 4 more hours (Breakfast Group);
- to consume a Fast Bar™, and then continue to water-only fast for 4 more hours (Fast Bar Group).

6.1.2 DOSING AND ADMINISTRATION

Participants will undergo 15 to 19 hours of water-only overnight fasting.

Participants will consume a breakfast or a Fast Bar™.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

This study will use food products developed by the L-Nutra company.

Study coordinator will be responsible to keep the inventory of products.

Prepared study kits that includes all necessary study materials will be mailed to participants once they are enrolled and consented.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

L-Nutra company handles the formulation, appearance, packaging and labeling of all the food products for this study.

The custom ready-to-eat dinner prior to fasting will be provided by Nutrition For Longevity, a subsidiary of L-Nutra.

Dinner meal ingredients: Sockeye Salmon, Organic Red Cabbage, Organic Cooked White Rice, Organic Stir Fry Greens, Organic Broccoli, Organic Cauliflower Rice, Organic Carrots, Organic Onion, N4L Imported EVOO, N4L Teriyaki Sauce (Organic Pineapple Juice, Organic Japanese Rice Wine, Organic Ginger Root, Organic Tamari), Organic Garlic, Organic Scallions.

Contains Fish, Soy, Tree Nuts.

The breakfast bar is formulated to mimic a typical breakfast in energy contents.

Breakfast ingredients: Tapioca Syrup, Dates, Almond Butter, Almonds, Soy Protein Crisps (Soy Protein Isolate, Rice Flour, Malt Extract (may contain wheat), Micellar Casein, Whey Protein Concentrate, Pea Protein, Flaxseed, Almond Protein, Maltodextrin, Sunflower Lecithin, Sea Salt, Natural Flavor.
Contains Milk, Soy and Almonds. May contain Wheat.

Fast Bar™ ingredients: Macadamia Nuts, Pecan Pieces, Inulin, Almond Meal, Almond Butter, Honey, Coconut Flour, Flaxseed Meal, Vanilla Powder, Sea Salt, Rosemary oleoresin.

Product Contains: Tree nuts (almonds, coconut, macadamia nuts, pecans). Manufactured on equipment that also processes other tree nuts, peanuts, soy, wheat, egg, and milk.

6.2.3 PRODUCT STORAGE AND STABILITY

Food items need to be stored in a cool, dry place.

6.2.4 PREPARATION

N/A

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

After screening, participants will be block randomized (12 blocks of 9 each) in a 1:1:1 allocation to the breakfast, Fast Bar and water-only fast groups using a predetermined randomization table.

The following measures will be performed to minimize biases:

- Subjects will be randomly assigned to either one of the two arms of the study;
- Data and/or specimens will be labeled with a code that the research team can link to personal identifying information;
- The study foods will be labelled with the participants' code (by research personnel not directly interacting with the participants).

6.4 STUDY INTERVENTION COMPLIANCE

Participants will be recruited via email and/or telephone.

Potential participants will be provided with study-related documents over the email or mail.

Potential participants will be consented over a Zoom conference with the study coordinator. A signed consent form will be emailed back to the PI using Docusign.

Participants will be monitored via Zoom teleconference by the study personnel.

6.5 CONCOMITANT THERAPY

N/A

6.5.1 RESCUE MEDICINE

N/A

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

N/A

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance;
- If any clinical adverse event (AE), biomarker abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant;

In case of discontinuation/withdrawal, the data collected up to the time of discontinuation from that specific participant will still be used as part of the trial database and statistical analysis of trial outcome.

7.3 LOST TO FOLLOW-UP

N/A

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Participant outcomes will be reminded before starting fasting (day 1) and the end of fasting (day 2) via telephone calls, text message and/or email.

Questionnaires will be self-administered through HIPAA compliant E-platform CRIO.

Procedure List:

- Screening (SCR): study explanation, informed consent, medical history, and instructions.
- Day 1
  - finish dinner by 5 pm followed by water-only fasting;
- Day 2
  - Continue the water-only fast, consume a breakfast or a Fast Bar™.

Examination and tests will include:

- Fingerstick for blood glucose and ketone (BHB) before breakfast on day 1;
- Fingerstick for blood glucose and ketone (BHB) on baseline timepoint (before breakfast), and 1, 2, 3, and 4 hours after consuming the breakfast bar or the Fast Bar™;
- Questionnaires and Surveys:
  - Nurse's Health Survey 2016 [9-11]: to evaluate medical history, newly developed conditions, changes in health conditions and healthcare utilization/medications;
  - Food evaluation;
  - Self-evaluation;

8.2 SAFETY AND OTHER ASSESSMENTS

N/A

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a))

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating. Of note, the term "severe" does not necessarily equate to "serious".
- **Extreme** – life-threatening or disabling

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.

- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.3.3.3 EXPECTEDNESS

William W Hsu, MD, will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study or upon review by a study personnel.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Angie Huang, MS, RDN, will record all reportable events with start dates occurring any time after informed consent is obtained until the last day of study participation. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

Following adverse event (AE) or serious suspected adverse reaction will be reported to IRB within 10 working days after investigators becoming aware of the event.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

Following serious adverse event (SAE) or serious suspected adverse reaction will be reported to IRB within 10 working days after investigators becoming aware of the event.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Angie Huang, MS, RDN, will report eventual AE and/or SAE to the participant. Any adverse effects will be reported to participant directly in person or via phone/video conference/email.

8.3.8 EVENTS OF SPECIAL INTEREST

N/A

8.3.9 REPORTING OF PREGNANCY

N/A

8.3.10 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.3.11 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

8.3.12 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Angie Huang, MS, RDN, will report eventual UPs to the participant. Any adverse effects will be reported to participant directly in person, via phone or Zoom.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

The primary trial outcome will be the ketone (beta-hydroxybutyrate, BHB) levels 3 hours (T3) after consuming the Fast and changes in BHB area under the curve (AUC) between 0 to 4 hours after consuming the study foods on day2.

We hypothesize that the breakfast bar group will show significant lower blood BHB level compared to the Fast Bar group.

9.2 SAMPLE SIZE DETERMINATION

Sample-size estimation is based on two considerations:

1. Sample-size estimation is based on pilot studies assuming ketone (BHB) levels of 0.25 (SD: 0.19) and 0.16 (SD: 0.05) mM for the Fast Bar and the Breakfast groups, respectively. With t test (one-

tailed) of difference between two independent means, a total sample size of 62 will be necessary to achieve 80% power with an α error probability of 0.05. Sample size calculation was performed using the GPower v3.1.9.6 (Franz Faul, University Kiel, Germany; Actual power = 0.8164386; Actual α = 0.0291400).*

2. Sample-size estimation is based on Fisher's exact test (one tailed) for proportion difference between the breakfast and Fast Bar groups: food consumption will reduce blood ketone in 67% vs. 33% of the participants in the breakfast and Fast Bar groups, respectively. A total sample size of 64 will be necessary to achieve 80% power with an α error probability of 0.05. **

Based on prior experience in similar dietary intervention studies, we anticipate an approximate 10% dropout and/or data loss. 36 subjects per group will be enrolled. Sample size calculation was performed using the GPower v3.1.9.6 (Franz Faul, University Kiel, Germany; Actual power = 0.8164386; Actual α = 0.0291400). A fasting only group (32 subjects) will also be included for comparison. A total 108 subjects will be enrolled.

*** t tests –** Means: Difference between two independent means (two groups)

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	One
	Effect size d	=	0.6478342
	α err prob	=	0.05
	Power (1−β err prob)	=	0.8
	Allocation ratio N2/N1	=	1
Output:	Noncentrality parameter δ	=	2.5505258
	Critical t	=	1.6706489
	Df	=	60
	Sample size group 1	=	31
	Sample size group 2	=	31
	Total sample size	=	62
	Actual power	=	0.8096862

**** Exact –** Proportions: Inequality, two independent groups (Fisher's exact test)

Options:	Exact distribution		
Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	One
	Proportion p1	=	0.67
	Proportion p2	=	0.33
	α err prob	=	0.05
	Power (1−β err prob)	=	0.8
	Allocation ratio N2/N1	=	1
Output:	Sample size group 1	=	32
	Sample size group 2	=	32
	Total sample size	=	64
	Actual power	=	0.8164386
	Actual α	=	0.0291400

9.3 POPULATIONS FOR ANALYSES

All participants will be included in the intention-to-treat analyses of the results. Participants who are removed from study for non-compliance or for safety reasons will still have their data included in the final analysis, unless they withdraw consent to have their data included, and will not be replaced.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Continuous trial outcome variables will be evaluated for normality; normalizing transformations or nonparametric statistics will be used as needed. All group comparisons on post-randomization efficacy outcomes will include randomized treatment (Fast Bar vs breakfast). A 1-sided level of significance set at $P<0.05$ will be used for the primary outcome; secondary and exploratory outcomes will not be adjusted for multiple hypothesis testing.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary trial outcome will be the ketone (beta-hydroxybutyrate, BHB) levels 3 hours (T3) after consuming the study foods (Breakfast Bar or Fast Bar) and changes in BHB area under the curve (AUC) between 0 to 4 hours after consuming the study foods on day2.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

The correlations of the time-dependent blood BHB levels between the groups (Fast Bar vs. Fasting and Fast Bar vs. Breakfast) will be carried out using Pearson correlation coefficient or Spearman's rank correlation analyses.

Separate analyses by treatment groups will test for difference in mean levels of continuous or prevalence of categorical outcomes.

9.4.4 SAFETY ANALYSES

Incidences of adverse events and serious adverse events will be compared between treatment groups using Fisher's exact tests.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Baseline comparability between randomized groups will be evaluated for demographics, age, sex, BMI. Group differences will be evaluated with t-tests or nonparametric rank sum tests for continuous variables, and chi-square tests for categorical variables. Standardized group differences (group difference divided by standard error of difference) will also be computed for each baseline variable.

9.4.6 PLANNED INTERIM ANALYSES

An interim analysis will be carried out when 50% of the trial has been completed. Interim analysis will assess the accrual rates, basic characteristics of the study population, protocol adherence, and answer following questions:

1. Are the assumptions under which the original sample size was computed appropriate?
2. Is it likely that a relevant intervention difference will be demonstrated?

9.4.7 SUB-GROUP ANALYSES

N/A

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

N/A

9.4.9 EXPLORATORY ANALYSES

N/A

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

All institutional and Federal regulations concerning the Informed Consent form will be fulfilled.

The study will be conducted in adherence to ICH Good Clinical Practice.

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol: Informed Consent.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Safety: determination of unexpected, significant, or unacceptable risk to participants;
- Study conduct: low accrual, poor retention.

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor and IRB.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study participant's contact information will be securely stored at Clinical Research IO (CRIO) E-platform (*https://www.clinicalresearch.io*) for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at L-Nutra. Individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at L-Nutra.

- Research procedures will be conducted in participant's home.
- Data will be captured and reviewed in a private setting.
- Only authorized research study personnel will be present during research related activities.
- The collection of information about participants is limited to the amount necessary to achieve aims of the research.
- Participants will not be approached in a setting or location that may constitute an invasion of privacy or could potentially stigmatize them

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at L-Nutra. After the study is completed, the de-identified, archived data will be stored at L-Nutra for use by other researchers including those outside of the study.
This study does not store biological specimens.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Name and contact information of the Principal Investigator and the Study Coordinator.

Principal Investigator	**Co-Investigator/Study Coordinator**
William C. Hsu, MD	Angie W. Huang, MS, RDN
L-Nutra, Inc.	L-Nutra, Inc.
8000 Beverly Blvd, Los Angeles CA 90048	8000 Beverly Blvd, Los Angeles CA 90048
Tel: 617.655.4394	Tel: 323.628.3158
Email: *whsu@l-nutra.com*	Email: *awu@l-nutra.com*

10.1.6 SAFETY OVERSIGHT

N/A

10.1.7 CLINICAL MONITORING

The PI, William C Hsu, MD, to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonization Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

Angie W Huang, MS, RDN, will be overseeing the collected exams. This include biochemical readout and subjective reporting by the subject of any change in diet use and compliance as well as adverse/unexpected symptoms and events.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the trial staff at the site under the supervision of the Principle Investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into E-platform developed by Clinical Research IO (CRIO), a 21 CFR Part 11-compliant data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

At the conclusion of the study, data will be retained for study record keeping purposes for at least 3 years. These documents will be retained for a longer period, however, if required by local regulations.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP). The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

Protocol deviations will be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

10.1.12 CONFLICT OF INTEREST POLICY

L-Nutra, Inc. received patent of the food used in this study from the University of Southern California and may develop products that can be sold. If L-Nutra makes money from these products, participants will not receive any money.

10.2 ADDITIONAL CONSIDERATIONS

N/A

10.3 ABBREVIATIONS

AE	Adverse Event
BHB	ß-hydroxybutyrate
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRIO	Clinical Research IO
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practices
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
ITT	Intention-To-Treat
PI	Principal Investigator
SAE	Serious Adverse Event
SOA	Schedule of Activities
SOP	Standard Operating Procedure
UP	Unanticipated Problem

10.4 PROTOCOL AMENDMENT HISTORY

N/A

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