AN OPEN-LABEL, ACUTE CLINICAL TRIAL TO ASSESS THE LEVEL OF KETONE PRODUCTION FOLLOWING CONSUMPTION OF AVELA™ (R)-1,3-BUTANEDIOL IN AN ADULT POPULATION

Principal Investigator	*Lowder, James Norman, M.D.* *James Lowder Consulting LLC* *132 Barn Owl Ct* *Gore, VA 22637*
Funding Sponsor	*Genomatica Inc.* *4757 Nexus Center Dr.* *San Diego, CA* *9212, U.S.*
Study Product	*Avela™ (R)-1,3-Butanediol*
Protocol Number	*CHSI-2101-02*
IRB Number	*22-GMAT-101.*

Initial version 22 March 2022
Amended 11 May 2022

Table of Contents

LIST OF FIGURES AND TABLES

List of Abbreviations

AcAc	acetoacetate
AE	adverse event
ATP	Adenosine triphosphate
BHB	β-hydroxybutyrate
BMI	body mass index
EC	Ethics Committee
GI	gastrointestinal
GRAS	Generally Recognized as Safe
HIPPA	Health Insurance Portability and Accountability Act of 1996
IRB	Institutional Review Board
mVAS	modified visual analogue scale
n	number
PHI	protected health information
PI	Principal Investigator
SAE	serious adverse event
SSS	Stanford Sleepiness Scale
U.S.	United States
VO_2	Peak oxygen uptake

Study Summary

Title	An Open-Label, Acute Clinical Trial to Assess the Level of Ketone Production Following Consumption of Avela™ *(R)*-1,3-Butanediol in an Adult Population
Short Title	Effects of *(R)*-1,3-butanediol on ketone production
IRB Number	
Protocol Number	CHSI-2101-02
Methodology	Open-label, Acute (single day) study
Study Duration	1 day for screening, 1 day for clinical study
Study Center(s)	Single-center

Objectives	Primary:
	• To evaluate the level of production of BHB following the consumption of 3 servings of Avela™, with consumption of each serving separated by 30 minutes and each serving providing 11.5 g of *(R)*-1,3-butanediol [total intake of 34.5 g of (R)-1,3-butanediol].
	Secondary:
	• To evaluate the acute effects of 3 servings of Avela™, with consumption of each serving separated by 30 minutes and each serving providing 11.5 g of *(R)*-1,3-butanediol [*i.e.,* total intake of 34.5 g *(R)*-1,3-butanediol] on:
	  ○ GI tolerance
	  ○ Sleepiness/alertness

Number of Subjects	N = 30 subjects (males and females)

Main Inclusion and Exclusion Criteria	Inclusion Criteria: • Males and females, 18 to 65 years of age • BMI 18 to <35.0 kg/m^2, inclusive • **<u>Weight ≥ 110 lbs</u>** • Subject is judged to be in good health on the basis of medical history • Subject is willing to fast for 12 hours prior to study start • Subject is willing to avoid alcohol and intense physical activity the day prior to and on the day of testing • Subject is capable of giving informed consent and complying with all study procedures/requirements. Exclusion Criteria: • Previous GI disorders (*e.g.,* inflammatory bowel disease, irritable bowel syndrome, history of surgery for weight loss, or gastroparesis) • Gastroenteritis in the 2 weeks preceding the study • Diabetes • Weight <110 lbs • History of alcohol or drug abuse • Previous diagnosis of neurological disorders, depression, or mental illness with psychosis • Unexplained alarm features (*i.e.,* unintentional weight loss >10% body weight in the last 3 months, fevers, or blood in stools) • Use of an antibiotic or any medication impacting gut transit during the 2 weeks before the study • Constipation or diarrhea (defined as, on average, less than 3 stools per week or more than 3 stools per day, respectively) • Allergy to tree nuts • Women who are pregnant or breastfeeding • Persons with medical conditions affecting the pancreas, liver, thyroid, or gall bladder.
Study Product, Dose and Route of Administration	Avela™, providing 11.5 g of *(R)*-1,3-butanediol per serving, 3 servings during the test day, with consumption of each separated by 30 minutes, for a total intake of 34.5 g *(R)*-1,3-butanediol.
Statistical Methodology	Statistical analysis plan to be completed by data management team.
Data and Safety Monitoring Plan	The PI will be responsible for data quality management and for overseeing clinical trial participant safety.
BMI = body mass index; GI = gastrointestinal; N = number; PI = Principal Investigator; IRB = Institutional Review Board.	

Background and Study Rationale

1 Introduction

This document is a protocol for a pilot clinical research study.

1.1 Background

The purpose of the pilot clinical trial described herein is to determine the level of ketone production [measured as β-hydroxybutyrate (BHB)] following consumption of *(R)*-1,3-butanediol in a beverage and to record gastrointestinal (GI) symptomology, as well as any effects on alertness/sleepiness.

There are two distinct stereoisomers of 1,3-butanediol: the R form and the S form. The R stereoisomer of 1,3-butanediol is naturally occurring, has been well studied, and appears to be the configuration that can be converted to physiological ketone bodies most proficiently (Desrochers *et al.,* 1992). Both the R or S stereoisomers of 1,3-butanediol undergo interconversion *via* the enzyme BHB dehydrogenase, producing the metabolites BHB and acetoacetate (AcAc). In the liver and blood, BHB is converted to other ketones, such as AcAc and acetone; this is similar to the endogenous pathway of AcAc formation in the liver from fatty acids (Shivva *et al*., 2016; Poff *et al*., 2019). Circulating ketones enter other tissues including the brain, kidney, skeletal muscle, and heart, and act as energy sources generating adenosine triphosphate (ATP) *via* the citric acid cycle. Excess circulating ketones that are not taken up into the tissues are eliminated in the form of acetone *via* exhalation through the lungs, or BHB and AcAc *via* the kidneys (Shivva *et al*., 2016; Poff *et al*., 2019). Under normal physiological conditions, blood levels of ketones are <3 mg/100 mL and urinary levels of ketones are ≤125 mg/24-hour; however, during periods of limited glucose availability, humans increase ketone production to approximately 150 g of ketone bodies per day (Reichard *et al*., 1974). During fasting conditions, blood BHB levels as high as 5 mM (42 to 52 mg/100 mL) have been observed (Cahill, 1970).

For over a century, the classic ketogenic diet has been used to induce ketosis; the ketogenic diet is a low-carbohydrate, high fat diet or a low-calorie diet (Avgerinos *et al.,* 2020). More recently, ketogenic diets have proved popular among individuals seeking to achieve weight loss (*e.g.*, the “Atkins diet”); these diets differ markedly from the classic ketogenic diet in that protein consumption is generally not restricted. With excessive protein consumption, amino acids undergo conversion to glucose, and as such, diets such as the Atkins diet that do not restrict protein intakes are far less ketogenic than the traditional “classic” ketogenic diet.

This study aims to evaluate the level of BHB production when Avela™ *(R)*-1,3-butanediol is consumed at a level of 34.5 g within a single test day.

1.2 Study Product

Avela™ *(R)*-1,3-butanediol will be consumed in the form of 3 servings of a beverage, with consumption of each serving separated by 30 minutes and each serving providing 11.5 g of *(R)*-1,3-butanediol [for a total intake of 34.5 g *(R)*-1,3-butanediol during the test day].

1.3 Generally Recognized as Safe (GRAS) for Use

Avela™ is manufactured by microbial fermentation using a strain of *Escherichia coli* that has been genetically engineered to produce *(R)*-1,3-butanediol. The isolation of *(R)*-1,3-butanediol from the production organism and fermentation media is accomplished using a series of purification and filtration steps that yield a highly purified product [≥99.7% *(R)*-1,3-butanediol]. Avela™ *(R)*-1,3-butanediol is intended to induce ketosis in the general adult population. Avela™ *(R)*-1,3-butanediol is GRAS for use in beverages, bars, and gels at a level of up to 11.5 g/serving and for 1 to 3 servings per day, yielding total intakes ranging from 11.5 to 34.5 g *(R)*-1,3-butanediol/day (equivalent to 0.16 to 0.49 g/kg body weight/day). In the pilot clinical trial described herein, study participants will consume a beverage 3 times during the test day, with consumption of each serving separated by 30 minutes and each serving providing 11.5 g of *(R)*-1,3-butanediol, for a total *(R)*-1,3-butanediol intake of 34.5 g on the test day; thus,

use of *(R)*-1,3-butanediol in the clinical trial described herein is within the parameters of the GRAS uses and use levels.

1.4 Clinical Data

There were 5 clinical studies (Kies *et al.,* 1973; Tobin *et al.,* 1975 (Study 1 and 2); Scott *et al.,* 2019; Shaw *et al.,* 2019) in which the efficacy and/or safety/tolerability of (*R, S*)-1,3-butanediol were assessed.

Kies *et al*. (1973) conducted a 4-arm crossover study in 12 healthy human volunteers [young adults, 18 to 23 years of age; number (n)=6/sex] to evaluate the protein nutriture provided by nitrogen±urea when consumed with isocaloric quantities of either 1,3 butanediol (chirality not reported but assumed to be R,S) or starch. Following a nitrogen depletion phase, the participants each partook in 4 arms of the trial. Wheat flour bread was formulated as follows for each of the 4 treatment arms: 1) Starch control supplemented with a variety of essential nutrients and minerals; 2) 5% w/w 1,3-butanediol (15 g, 90 kcal/subject/day, substituted isocalorically for starch); 3) Starch plus urea (increased nitrogen content by 4 g/subject/day); 4) 5% w/w 1,3-butanediol plus urea (increased nitrogen content by 4 g/subject/day). Participants completed each 7-day treatment arm in an order that ensured 14 consecutive days of 1,3-butanediol consumption and 14 consecutive days without, *e.g.*, 2-4-1-3 or 3-1-4-2. Samples of excrement were taken at each excretion during all arms, 7-day composites were made for each study arm, and venous blood samples were collected at study initiation at the end of each study arm, and 2 weeks after study conclusion. 1,3-butanediol, with or without urea, did not significantly impact serum protein, blood cell counts (*i.e.,* white blood cells, monocytes, eosinophils, lymphocytes, neutrophils), hematocrit, or hemoglobin levels in participants compared to treatment with starch alone. No significant difference in the blood lipid or plasma ion content was reported following 1,3-butanediol consumption compared to baseline values. The authors reported a significantly increased nitrogen balance in participants when consuming 1,3-butanediol alone compared to the starch control ($p<0.05$), accompanied by a significant decrease in urinary nitrogen excretion ($p<0.05$). After consuming 1,3-butanediol alone (arm 2), the authors also reported a significant decrease in blood glucose when compared to all other treatment arms ($p<0.05$) (Kies *et al.*, 1973). 1,3-butanediol was found to be a nontoxic metabolite providing calories for human nutrition and did not affect safety parameters, assessed using bloodwork, any differently than starch consumption.

Tobin *et al*. (1975) conducted 2 studies; one to assess the effects of 1,3-butanediol (chirality not reported) on hematological measures in a fasting state and a second to assess the effects of prior consumption of 1,3-butanediol on serum insulin, growth hormone, glucose and lipids during glucose tolerance tests. The first study was a 2-arm crossover study that included a 5-day adjustment period (dietary nitrogen was supplemented with wheat protein) and 2 study arms of 5 days each, for a total of 15 days. Following the adjustment period, study subjects (n=27 adult females) consumed for 5 days: 1) a diet containing 40 g of 1,3-butanediol (chirality not reported), or 2) a control diet containing 40 g of sucrose. Fasting blood samples were taken before and after each study arm for the analysis of hematological metrics (*e.g.*, glucose, insulin, growth hormone, cholesterol, and triglycerides). Blood glucose concentrations in the adjustment period were slightly higher than those reported in the 1,3-butanediol or control arms of the study, although not statistically different. There were no other changes measured in the blood samples between the 2 study arms, but the authors did report a "*significant*" decrease (no statistics provided) in cholesterol during the treatment phases (both 1,3-butanediol and control) as compared to the adjustment phase, and the opposite was observed for blood triglycerides. The authors concluded that there was "*no evidence of toxic reactions to the ingestion of* [1,3-butanediol] … *detected*" when administered at 40 g/day (Tobin *et al*., 1975).

The second study by Tobin *et al*. (1975) was a 2-arm crossover study that evaluated the impact of 1,3-butanediol (chirality not reported) on glucose-tolerance. In a 12-day study, 10 healthy, young-adult subjects (male & female university students) were fed a controlled diet composed of an amino acid supplemented gelatin (6 g/nitrogen/day) and starch bread as primary energy sources. Caloric intake for each subject was determined and 10% of their intake was isocalorically substituted for 1,3-butanediol or sucrose (control). Subjects ingested either the 1,3-butanediol diet or sucrose diet for 5 days, and glucose tolerance tests were conducted on Day 6 following a 10 to 14 hour fast. Study participants then

consumed the alternate diet for 5 days (Days 7 to 11), and glucose tolerance tests were conducted on Day 12 following a 10 to 14 hour fast. There were no significant changes observed in any of the metrics tested compared to that of the control group including glucose, insulin, lactate, pyruvate, or triglycerides. Based on these findings, the authors suggest that 1,3-butanediol can be supplemented in the diet up to 10% without risk of adverse effects (Tobin *et al*., 1975).

Lastly, in the 2 clinical studies conducted by Scott *et al.* (2019) and Shaw *et al.* (2019), the effects of 1,3-butanediol on exercise performance were assessed; in the study by Scott *et al*. (2019), chirality is unknown and in the study by Shaw *et al*. (2019), (*R, S*)-1,3-butanediol was used, but the proportional distribution of the stereoisomers is unknown. Both studies were randomized, placebo-controlled, and crossover in design, with a 1-day intervention period. A washout period of 7 to 10 days was included in the study by Shaw *et al*. (2019), whereas the duration of the washout period was not reported in the study by Scott *et al*. (2019). One study was double-blind (Scott *et al*., 2019), whereas the other study was single-blind (Shaw *et al*., 2019). In both studies, only male subjects were included, and mean body mass index (BMI) and peak oxygen uptake (VO_2 peak) at baseline were similar across the 2 studies. Scott *et al*. (2019) described the eleven subjects as healthy runners and did not provide further details on their athletic status, while Shaw *et al*. (2019) described the 9 subjects as trained cyclists, amongst whom the average amount of time spent training was 12.3 ± 2.3 hours/week and the mean maximal power output was 389.3 ± 50.4 W. In the study by Scott *et al*. (2019), subjects first completed a 60-minute submaximal run, followed by a 5-km run time trial. In the study by Shaw *et al*. (2019), subjects first completed an 85-minute steady-state cycling period, followed by a cycling time trial to achieve 7 kJ/kg (which was reported to be approximately 25 to 35 minutes of cycling). In both studies, 1,3-butanediol was consumed as part of a beverage. In the study by Scott *et al*. (2019), the dose of 1,3-butanediol was 0.5 g/kg body weight and provided *via* approximately 650 mL of the test drink, which was consumed in 3 divided doses (twice before and once after the pre-time trial exercise). In the study by Shaw *et al*. (2019), the dose of 1,3-butanediol was 0.35 g/kg body weight and provided *via* approximately 278 mL of the test drink, which was consumed in 2 divided doses (once before and once during the pre-time trial exercise). Across the 2 studies, outcome measures related to exercise performance, GI tolerability, and hydration status, as well as blood biomarkers (*i.e.*, BHB, glucose, and lactate), were assessed.

Across both studies, there were no significant differences between the 1,3-butanediol and placebo phases on the time to complete the time trial, and the magnitudes of the effect sizes observed were interpreted as “unclear”. Similarly, no significant differences between phases were observed for power output (Shaw *et al*., 2019) and pacing (Scott *et al*., 2019) during the time trial; the magnitudes of the effects were interpreted as “unclear” or “very likely trivial” (for pacing for the first 3-km of the 5-km time trial). Thus, the consumption of 1,3-butanediol compared to placebo did not result in statistically significant or clinically significant improvements in time trial performance. Additionally, the consumption of 1,3-butanediol compared to placebo significantly increased blood BHB levels throughout exercise. However, the consumption of 1,3-butanediol compared to placebo did not result in statistically or clinically significant, sustained improvements in supportive outcomes of exercise performance. Furthermore, the evidence to support an effect of 1,3-butanediol on the sparing of glucose reserves is unclear. It should be noted that, in the study by Scott *et al.* (2019), BHB was elevated following administration of 1,3-butanediol but was not sufficient to induce moderate or high levels of ketosis.

In the study by Shaw *et al.* (2019), there was no difference in the change of body mass (corrected for fluid intake) between trials. Within the group consuming butanediol, two participants experienced low levels of nausea, euphoria, dizziness. Five participants reported low to moderate levels of belching and burping, and one participant reported severe abdominal pain. All participants reported that they disliked the taste of butanediol, which resulted in retching in four participants. In the study by Scott *et al.* (2019), no adverse GI effects were reported.

It should be noted that, in the study by Shaw *et al.* (2019), a pilot test was conducted prior to the primary study to determine a testing dose of 1,3-butanediol in a beverage that was well tolerated and would provide optimal BHB blood concentration. In the pilot test, single bolus doses of 0.5 g/kg and 0.7 g/kg of 1,3-butanediol resulted in nausea, euphoria, and dizziness. To resolve these issues, the authors determined a split dose of 0.35 g/kg (providing about 24.5 g/dose for a 70-kg individual) at the initiation of

the cycling trial and another at the midway point, which resulted in 0.7 g/kg 1,3-butanediol (49 g/trial for a 70-kg individual), elicited maximal BHB concentration and minimal side effects.

1.5 Conclusions

Avela™ *(R)*-1,3-butanediol is GRAS for use in beverages, bars, and gels at a level of up to 11.5 g/serving and for 1 to 3 servings per day, yielding total intakes ranging from 11.5 to 34.5 g *(R)*-1,3-butanediol/day (equivalent to 0.16 to 0.49 g/kg body weight/day). In the clinical trial described herein, study participants will consume a beverage 3 times during the test day, with each serving providing 11.5 g of *(R)*-1,3-butanediol, for a total *(R)*-1,3-butanediol intake of 34.5 g on the test day; thus, use of *(R)*-1,3-butanediol in the clinical trial described herein is within the parameters of the GRAS uses and use levels.
Studies in humans demonstrated no significant adverse effects when 1,3-butanediol provided up to 10% of energy intake for 5 days (Tobin *et al*., 1975). More recent studies reported that 3 doses up to 35 g/day (Scott *et al*., 2019) and split-dosing up to 49 g/day (Shaw *et al.*, 2019) of (*R, S*)-1,3-butanediol (racemic mixture) were well-tolerated by healthy athletes. Consumption of a single bolus dose of 35 or 49 g "*tended to result in nausea, euphoria, and dizziness*" (Shaw *et al.*, 2019), suggesting that a dosing regimen involving 2 or 3 doses may mitigate the potential GI effects that were observed with consumption of 1 single bolus dose of (*R, S*)-1,3-butanediol.

1.6 Dose Rationale and Risk/Benefits

The study dose was chosen based on the level of *(R)*-1,3-butanediol that was determined to be GRAS for use. Thus, during the test day, study participants will consume 3 servings of Avela™; each serving will provide 11.5 g of *(R)*-1,3-butanediol. Effects on ketone (BHB) production, GI tolerance, and sleepiness/alertness will be assessed.

2 Study Objectives

2.1 Primary Objective

To evaluate the level of production of BHB following the consumption of 3 servings of Avela™, with consumption of each serving separated by 30 minutes and each serving providing 11.5 g of (R)-1,3-butanediol [total intake of 34.5 g of (R)-1,3-butanediol].

2.2 Secondary Objectives (if applicable)

To evaluate the acute effects of 3 servings of Avela™, with consumption of each serving separated by 30 minutes and each serving providing 11.5 g of (R)-1,3-butanediol [i.e., total intake of 34.5 g (R)-1,3-butanediol] on:

- GI tolerance
- Sleepiness/alertness

3 Study Design

3.1 General Design

The clinical trial described herein is an open-label, acute study involving a total of 30 healthy male and female adults (ages 18 to 65 years; BMI of 18 to <35.0 kg/m^2, inclusive).

Prior to the clinical study, subjects will be screened according to the study inclusion and exclusion criteria, using an online survey (**Appendix A**). Subjects meeting all the inclusion criteria and none of the exclusion criteria will be invited to participate in the study (Table 3.1-1). These subjects will be sent study equipment and materials, including 1 consent form. Subjects will be asked to read the consent form and e-mail either the Research Associate or the Principal Investigator with any specific questions. If there are no questions, or once the questions have been resolved, those that wish to participate will be asked to sign the consent form and send a picture of the signed page to the Research Associate of the study.

Therefore, the subject will maintain a copy of the signed consent form, as per the U.S. Food and Drug Administration's regulations.

On the day of the clinical study, subjects will follow instructions for study methodology (**Appendix B**). Subjects, who will have fasted overnight for 12 hours, will self-assess and record GI symptomology [using the modified visual analogue scale (mVAS) GI symptoms tool], alertness [using the Stanford Sleepiness Scale (SSS)], and blood BHB level (using the Keto Mojo BHB monitor), at time 0 minutes, as baseline measures, using an online study portal (**Appendix C**). Subjects will then consume 3 servings of Avela™, each providing 11.5 g of *(R)*-1,3-butanediol, with the first serving consumed at 0 minutes, the second serving at 30 minutes, and the third serving at 60 minutes. After the collection of baseline measures (at 0 minutes), GI symptomology (assessed using the mVAS GI symptoms tool), alertness (assessed using the SSS), and blood BHB levels (using the Keto Mojo BHB monitor) will be assessed, again, at 30, 60, 90, 120, 180, 240, and 300 minutes. At each time point, it is important that the order of collection be maintained [*i.e.,* 1. GI symptomology; 2. Alertness/sleepiness; 3. BHB level; and 4. Avela™ (when Avela™ is to be consumed; *i.e.,* at 0, 30, and 60 minutes)]. After the assessments at 240 minutes are complete, subjects will consume 1 ALOHA Organic Plant Based Protein Bar (Caramel Sea Salt). During the study, subjects will be asked to record their blood BHB levels and questionnaire results using the online portal (**Appendix C**). The results will be recorded electronically, using a de-identified survey on Survey Monkey, designed to protect subject anonymity [*i.e.,* the survey will not be affiliated with a name or any personal information; rather, each participant with have a unique identification number, which will be used to access the survey and upload the results of the study).

Table 3.1-1 Study Groups Included in Clinical Study

Number of Subjects	Investigational Product; Dose
30 subjects (males and females)	*(R)*-1,3-butanediol; 3 doses of 11.5 g/dose

3.2 Study Endpoints

3.2.1 Primary Study Endpoint

- The measure of blood ketones levels (*i.e.,* blood BHB levels) at 0 minutes (baseline) and at 30, 60, 90, 120, 180, 240 and 300 minutes of study.

3.2.2 Secondary Study Endpoints

Using questionnaires, the following secondary outcomes will be determined:

- GI symptomology; and
- Sleepiness/alertness.

3.3 Primary Safety Endpoints

- Tolerability: unsolicited adverse events (AEs)

4 Study Population and Duration of Participation

4.1 Duration of Study Participation

The duration of subject participation is 2 days; one day for screening and one day for Avela™ *(R)*-1,3-butanediol consumption.

4.2 Total Number of Subjects and Sites

The proposed clinical study is being conducted by Genomatica. Thirty Genomatica employees (males and females) are being recruited for the study. There is no sample size calculation, as there is no control group and the study is a pilot study, with the main objective of understanding ketone (BHB) levels following consumption of (*R*)-1,3-butanediol. 30 participants are to be included, due to the inherent

variability in the GI symptomology questionnaires. Of note, in other studies of food ingredients (*e.g.,* inulin[1], sorbitol[2], erythritol + fructose[3]) where GI tolerance was an outcome of interest, sample sizes ranged from 26 to 37 participants

Genomatica employees who express interest in participating in the study and who meet the eligibility criteria for study inclusion will complete the study remotely, within their own homes, using equipment provided by Genomatica (*i.e.,* lancets, sharps container, alcohol swabs, Keto Mojo BHB monitor and ketone test strips (and glucose test strips, not to be used in the study), bandages, pregnancy test [if applicable], measuring tape, three 4 oz. (118 mL) bottles of sealed water premixed with Avela™ [each providing 11.5 g of *(R)*-1,3-butanediol], 1 shot glass with the Avela™ logo, an ALOHA Organic Plant Based Protein Bar (Caramel Sea Salt), 1 duffel bag, 1 consent form, and study instructions. All study-related data will be entered and transmitted by each study participant using an electronic platform, using surveys that will protect the identity of each participant.

4.3 Inclusion Criteria

- Males and females, 18 to 65 years of age
- BMI 18 to <35.0 kg/m^2, inclusive
- Weight is ≥ 110 lbs
- Subject is judged to be in good health on the basis of medical history
- Subject is willing to fast overnight, prior to study start
- Subject is willing to avoid alcohol and intense physical activity the day prior to and on the day of testing
- Subject is capable of giving informed consent and complying with all study procedures/ requirements.

4.4 Exclusion Criteria

- Previous GI disorders (*e.g.,* inflammatory bowel disease, irritable bowel syndrome, history of surgery for weight loss, or gastroparesis
- Gastroenteritis in the 2 weeks preceding the study
- Weight <110 lbs
- Diabetes
- History of alcohol or drug abuse
- Previous diagnosis of neurological disorders, depression, or mental illness with psychosis
- Unexplained alarm features (*i.e.,* unintentional weight loss >10% body weight in the last 3 months, fevers, or blood in stools)
- Use of an antibiotic or any medication impacting gut transit during the 2 weeks before the study
- Constipation or diarrhea (defined as, on average, less than 3 stools per week or more than 3 stools per day, respectively)
- Allergy to tree nuts
- Women who are pregnant or breastfeeding;
- Persons with medical conditions affecting the pancreas, liver, thyroid, or gall bladder.

4.5 Subject Recruitment

All subjects will be employees of Genomatica Inc., located in San Diego, California. Participation in the study is not a requirement of employment, and measures are being taken to ensure that Genomatica employees do not feel coerced to partake in the study. An employee's decision about research participation will not affect (favorably or unfavorably) performance evaluations, career advancement, or other employment-related decisions made by peers or supervisors.

[1] Example study (Bonnema *et al.,* 2010) available at: https://doi.org/10.1016/j.jada.2010.03.025
[2] Example study (Fernández-Bañares *et al.,* 2005) available at: https://pubmed.ncbi.nlm.nih.gov/16410032/
[3] Example study (Kim *et al.,* 2011) available at: https://pubmed.ncbi.nlm.nih.gov/22118754/

4.6 Early Withdrawal of Subjects

4.6.1 When and How to Withdraw Subjects

Subjects may withdraw consent or be removed from the study by the Principal Investigator (PI). In the event that a subject is withdrawn from the study, the reason for the withdrawal will be documented, with the primary reason selected from the following standard categories:

- Withdrawal of consent: the subject requests to withdraw from further participation in the study in the absence of a medical need to withdraw as determined by the PI.
- Lost to follow-up: for unknown reasons, the subject did not upload their study results on to the electronic platform.
- Poor compliance: the subject consumes only 1 or 2 of the 3 servings of the study product; is found to meet an exclusionary criterion; and/or is not complying with other study obligations.
- AE: in the judgment of the PI and in the best interests of the subject, events that require discontinuation of study product (includes all categories of study product relatedness: None, Remote, Possible, Probable, or Highly Probable).
- Death: death of the subject.
- Other: causes of premature termination from the study other than the above, such as theft or loss of study products, termination of study by Sponsor, *etc*.

4.6.2 Data Collection and Follow-up for Withdrawn Subjects

Should a subject request to withdraw or be withdrawn from the study by the PI, the subject's reason for withdrawal will be documented.

5 Study Product

5.1 Description and Treatment Regimen

An outline of the study timepoints is provided in Table 5.1-1. At Screening, subject eligibility will be determined using an online survey (**Appendix A**). Participants will be enrolled in the study in groups of 10 at a time. Participants will eat a meal the night before the study and begin the study the next morning in a fasted state (*i.e.,* overnight, 12-hr fast). The time at which the last meal was consumed and the study start time should be recorded using the online portal (**Appendix C**).

The study will be approximately 300 minutes (5 hours), from start to finish. Completion of study questionnaires, assessment of blood BHB levels, and consumption of the Avela™ beverage will be completed, as per Table 5.1-1; a detailed description of the methodology for each study timepoint is provided in section 6.0 of this clinical study protocol.

Table 5.1-1 Clinical Study Outline

Assessment	Tool Utilized	Time (min)							
		Screening (up to 7 days prior to test day)	**0**	**30**	**60**	**90**	**120**	**180**	**240**	**300**
Eligibility for study participation	On-line Survey	X	-	-	-	-	-	-	-	-
GI symptomatology	mVAS GI Symptoms Tool	-	X	X	X	X	X	X	X	X
Sleepiness/ Alertness Assessment	Stanford Sleepiness Scale	-	X	X	X	X	X	X	X	X
Blood BHB levels	Keto-Mojo Monitor	-	X	X	X	X	X	X	X	X
(R)-1,3-butanediol consumption	Avela™	-	X	X	X	-	-	-	-	-
ALOHA Bar consumption	Not applicable		-	-	-	-	-	-	X	-

BHB = beta-hydroxybutyrate; GI = gastrointestinal; min = minutes; mVAS = modified visual analogue scale.

5.2 Method for Assigning Subjects to Treatment Groups

All subjects included in the study will be assigned to receive the active investigational product [Avela™ *(R)*-1,3-butanediol] (*i.e.,* there is only one group for the study, with a before, after study design). Upon enrolment, each participant will be provided with a unique identification number.

5.3 Subject Compliance Monitoring

Compliance of subjects will be assessed by using compliance records. Participants must consume all 3 doses of product, at the specified timepoints, to meet compliance requirements.

5.4 Prior and Concomitant Therapy

Subjects may continue their use of medications and/or dietary supplements throughout the study period, so long as they do not have conditions or are taking medications are not contra-indicated (see Exclusion Criteria in Section 4.4).

5.5 Handling of Study Product

5.5.1 Dispensing of Study Product

At screening, all subjects included in the study will be provided with the following:

- 3 x 4 oz (118 mL) bottles of water premixed with Avela™ [each providing 11.5 g of (R)-1,3-butanediol];
- Lancets;
- Alcohol swabs;
- 1 sharps container;
- 1 shot glass with the Avela™ logo;
- 1 Keto Mojo BHB monitor and ketone test strip;
- Bandages;
- 1 pregnancy test [if applicable];

- 1 x measuring tape;
- 1 x ALOHA Organic Plant Based Protein Bar (Caramel Sea Salt);
- 1 duffel bag;
- 1 x study consent form; and
- 1 x study instruction sheet (**Appendix B**).

5.5.2 Return or Destruction of Study Product

The PI will maintain records of the product's delivery to the study participants and its use by each subject. These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and the unique code numbers assigned to the trial subjects. Investigators should maintain records that document adequately the doses provided to subjects and consumed by subjects so as to permit complete reconciliation of all investigational product(s). As the study product is a lawful food ingredient in the United States (U.S.), there will be no requirement for study participants to return unused study product to Genomatica. Rather, any unused study product can be disposed of by the participant by flushing down the toilet or pouring down the drain. The sharps container is provided with a prepaid outer shipping carton and water-resistant sealing tape and a shipping manifest form for tracking and certificate of destruction. Study subjects will follow the mailing instructions provided with the sharps container to return the container.

6 Study Schedule

A tabular summary of the schedule of procedures is provided in Table 5.1-1. An overall review of the order of events on the test day is provided in Table 6-1. A breakdown of the order of events at each study timepoint (*i.e.,* prior to study start and at 0, 30, 60, 90, 120, 180, 240, and 300 minutes) is provided in Sections 6.2.1 to 6.3.0.

Table 6-1 Order of Events on Study Test Day

Timepoint (min)	mVAS GI Symptoms Tool	SSS	BHB Measure	Consumption of Avela™	Consumption of ALOHA Bar
0	1	2	3	4	-
30	1	2	3	4	-
60	1	2	3	4	-
90	1	2	3	-	-
120	1	2	3	-	-
180	1	2	3	-	-
240	1	2	3	-	4
300	1	2	3	-	-

6.1 Screening – Day 1 (Investigative Staff)

- Assess participant eligibility using responses to online survey (**Appendix A**).
- If participant is eligible, obtain signature of subject on study Informed Consent Form for study participation.
- Provide participants with three 4 oz (118 mL) bottles of water premixed with Avela™ [each providing 11.5 g of (R)-1,3-butanediol]; lancets; alcohol swabs; 1 Keto Mojo BHB monitor; bandages; measuring tape; pregnancy test [if applicable]; one ALOHA Bar; 1 x study consent form; and 1 x study instruction sheet (**Appendix B**).

6.2 Study Test Day – Day 2 (Study Participant)

6.2.1 Prior to Study Start

- **Pregnancy test [if applicable]**
  - If a participant answered “no” to both of the study screening questions:
    - “If you are a female, are you post-menopausal, defined as not having had a period for 12 consecutive months or longer?”
    - “If you are a female, have you had any procedure that would result in you not being able to have children, such as “tying of tubes” or a removal of your ovaries?”
  - Then the subject is to take the pregnancy test prior to study start and proceed only if the test is negative (the result of the test will be included on the online portal).

- **Setting up the Keto-Mojo Monitor**
  - The subject will be instructed to download the MyMojoHealth app from the Apple Store or Google Play.
  - The subject will sync the app with his/her Keto-Mojo Monitor.
  - The study coordinator will send the subject a request, on his/her App, to join the study account, in order to share all BHB readings acquired during the study. Each subject’s data will be downloaded *via* the account.
- The subject will familiarize themselves with the online system for data recording.

6.2.2 0 min timepoint

- Using the online portal, the subject will:
  - Record the time their last meal was consumed.
  - Record the start time of the study.
  - Complete the mVAS GI symptoms tool.
  - Complete the SSS.

- Measure their blood BHB level using the Keto Mojo Monitor and record the value on the online portal.
- Start their on-screen timer.
- Consume one (1) bottle of premixed Avela™ liquid within a 5-minute time period.

6.2.3 30 min timepoint

- Using the online portal, the subject will:
  - Complete the mVAS GI symptoms tool.
  - Complete the SSS.
- Measure their blood BHB level using the Keto Mojo Monitor and record the value on the online portal.
- Consume one (1) bottle of premixed Avela™ liquid within a 5-minute time period.

6.2.4 60 min timepoint

- Using the online portal, the subject will:
  - Complete the mVAS GI symptoms tool.
  - Complete the SSS.
- Measure their blood BHB level using the Keto Mojo Monitor and record the value on the online portal.
- Consume one (1) bottle of premixed Avela™ liquid within a 5-minute time period.

6.2.5 90 min timepoint

- Using the online portal, the subject will:
  - Complete the mVAS GI symptoms tool.
  - Complete the SSS.
- Measure their blood BHB level using the Keto Mojo Monitor and record the value on the online portal.

6.2.6 120 min timepoint

- Using the online portal, the subject will:
  - Complete the mVAS GI symptoms tool.
  - Complete the SSS.
- Measure their blood BHB level using the Keto Mojo Monitor and record the value on the online portal.

6.2.7 180 min timepoint

- Using the online portal, the subject will:
  - Complete the mVAS GI symptoms tool.
  - Complete the SSS.
- Measure their blood BHB level using the Keto Mojo Monitor and record the value on the online portal.

6.2.8 240 min timepoint

- Using the online portal, the subject will:
  - Complete the mVAS GI symptoms tool.
  - Complete the SSS.
- Measure their blood BHB level using the Keto Mojo Monitor and record the value on the online portal.
- Consume the provided ALOHA Bar within 7 minutes.

6.2.9 300 min timepoint

- Using the online portal, the subject will:

  - Complete the mVAS GI symptoms tool.
  - Complete the SSS.
- Measure their blood BHB level using the Keto Mojo Monitor and record the value on the online portal.

6.3.0 End of Study

Subjects will be instructed to connect to the Keto Mojo App to allow it to sync with the Keto-Mojo Monitor. Once the study is complete, the subject will be instructed to close all browser windows and to dispose of any unused study product (either by flushing down the toilet or pouring down the drain). The sharps container can be mailed using the provided return envelope for disposal.

7 Study Procedures

7.1 Clinical Evaluations

There are no clinical evaluations to be completed as part of this study protocol.

7.2 Laboratory Evaluations

7.2.1 Clinical Laboratory Evaluations: Blood BHB Measurement

To determine blood BHB levels, each participant will be provided with a Keto-Mojo Monitor[4] (Keto-Mojo, Amsterdam, Netherlands). The monitor allows for each subject to measure their own ketone levels (*i.e.,* BHB levels), through sampling of capillary blood. Moore *et al.* (2021) tested the Keto-Mojo meter for accuracy *versus* the Abbott Lab Precision Xtra glucose/ketone device (Abbott's ketone meter) and reported there was excellent agreement between the two meters. Abbott's ketone meter has been validated in comparison to mass spectrometry (the gold standard of ketone measurement) in animal studies (Pineda and Cardoso*,* 2015; Bach *et al.,* 2016) and in humans at ketone levels below 3 mM (Janssen *et al.,* 2010; Yu *et al.,* 2011). In their publication, Moore *et al.* (2021) indicate:

> "*For the purpose of identifying nutritional ketosis, the Keto-Mojo device (Meter 2) is a reliable and accurate portable blood ketone measurement device that is more cost-effective than another commonly used portable ketone meter, the Precision Xtra (Meter 1). The acceptable measurement error, consistent level of agreement, and high diagnostic performance of Meter 2 compared to Meter 1 makes it appropriate for use by recreational or professional athletes interested in verifying that they are in a state of [nutritional ketosis] on a daily basis.*"

Capillary blood BHB levels will be measured at baseline (0 min) and at 30, 60, 90, 120, 180, 240, and 300 minutes of the study. A total of 10 fingersticks will be taken [8 study timepoints + 2 control timepoints (maximum)]. A typical blood drop contains ~35 µl of blood[5]. It is likely that 2 to 3 drops, per fingerstick, will be required (*i.e.,* 105 µl of blood). Total blood collection during the study will be: (8+2) X 105 µl = 1,050 µl or 1.050 ml. Frequent fingerstick blood sampling is common when examining post-prandial glycaemic responses, as capillary blood is more ideal (subject to less variability) than venous blood, as per the International Organization for Standardization (ISO 26642:2010[6]). Also, as per this standard, the number of fingerstick blood sampling is 8, which is very similar to the number in our protocol. Although our interest is in post-prandial ketone (BHB) responses, the analogy is relevant with respect to the acceptability of the blood sampling schedule and frequency of blood draws.

[4] Available at: https://keto-mojo.com/video/why-keto-mojo-is-the-gold-standard-for-testing-blood-ketones/.
[5] Available at: Not Every Drop of Blood Is Identical | The Partnership for Global Health Technologies (bu.edu)
[6] Available at: https://www.iso.org/standard/43633.html

7.3 Questionnaire Assessments

7.3.1 GI Symptoms Assessment

A validated tool to measure GI tolerability of *(R)*-1,3-butanediol will be utilized. The mVAS GI symptoms tool is a self-administered questionnaire, which is modelled after the validated "gold standard" measurement tool predominantly used to test clinical gastroenterology scenarios (Bengtsson *et al.,* 2013). Gaskell *et al.* (2019) tested the reliability of the mVAS in assessing GI symptoms during exercise, with and without dietary interventions. The mVAS has been modified slightly for this clinical study protocol, as there is no exercise employed in this study. The mVAS uses a 10-point rating scale to self-report on GI symptoms; 1–4 is indicative of mild GI symptoms (*i.e.,* sensation of GI symptoms, but not substantial enough to interfere normal activities); 5–9 is indicative of severe GI symptoms (*i.e.,* GI symptoms substantial enough to interfere with normal activities), and 10 is indicative of extremely severe GI symptoms warranting cessation of all other activities. If no specific GI symptom is reported, this would be recorded as a 0. A sample of the questionnaire is available within the screen captures of the online portal (**Appendix C**). In the study by Gaskell *et al.* (2019), GI symptoms, such as regurgitation and defecation, were rated dichotomously, either as 0 (no symptom) if they did not result in a cessation of exercise or as 10 (extremely severe symptoms) if they resulted in either a temporary or complete cessation of exercise; in this clinical study, these have been modified to a full 10-point VAS scale. All subjects will complete the mVAS at baseline (0 minutes), and at 30, 60, 90, 120, 180, 240, and 300 minutes.

7.3.2 Assessment of Alertness

The SSS (Shahid *et al.*, 2012) is a subjective tool to assess how alert a subject is feeling at specific moments in time. The scale requires respondents to select a rating of 1 to 7, where a "1" indicates the subject is "feeling active, vital, alert, or wide awake" and the highest score of "7" indicates the subject is "no longer fighting sleep, sleep onset soon; having dream-like thoughts" (Hoddes *et al.*, 1973). The scale is validated, correlating r = 0.68 with performance on the Wilkinson tests. All subjects will complete the SSS at baseline (0 minutes) and at 30, 60, 90, 120, 180, 240, and 300 minutes. The SSS is available in the screen captures of the online portal (**Appendix C**).

8 Statistical Analysis Plan

Statistical analyses to be conducted will be completed by the data management team.

9 Safety and Adverse Events

9.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency [*i.e.*, not described in study-related documents such as the Institutional Review Board (IRB)-approved protocol or consent form];
- Related or possibly related to participation in the research (*i.e.*, 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 subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Serious Adverse Event
AEs are classified as serious or non-serious. A serious adverse event (**SAE)** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect

- an important medical event

Important medical events are those that may not be immediately life-threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All AEs that do not meet any of the criteria for serious should be regarded as ***non-serious AEs***.

Adverse Event Reporting Period
The study period during which AEs must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as the evening of the day on which the clinical study takes place.

Pre-existing Condition

A pre-existing condition is one that is present at the start of the study. A pre-existing condition should be recorded as an AE if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
As the study product is a food ingredient that is GRAS in the U.S., there are no general examinations planned for this clinical study.

Post-study Adverse Event
All unresolved AEs should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the AE is otherwise explained. While adverse events related to GI intolerance are being solicited, the study participants will also be able to provide information on any other adverse events experienced at the end of the mVAS questionnaire, where space is provided for detailing “other” adverse events, including those that are not GI related.

Hospitalization, Prolonged Hospitalization or Surgery
Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as a SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE if hospitalization or prolonged hospitalization is for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

9.2 Recording of Adverse Events

Although AEs are not expected, should any non-serious or serious AEs occur, it is recommended that the participant seek immediate treatment. All AEs occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAEs that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported to the IRB immediately.

9.3 Reporting of Serious AEs and Unanticipated Problems

The Investigator must conform to the AE reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
- (see definitions, section 9.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- An assessment of the association between the event and study treatment by a qualified person, as needed

9.3.1 Investigator reporting: Notifying the IRB

Investigators are responsible for safety reporting to their local IRB. Investigators are responsible for complying with their local IRB's reporting requirements, though must submit the required reports to their IRB no later than 10 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the investigator's study file.

10 Study Administration, Data Handling and Record Keeping

10.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization.

10.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. In the case of the clinical trial proposed herein, examples of original documents and data records include: consent forms and the online study portal data (blood BHB levels and questionnaires).

10.3 Records Retention

Genomatica will retain records for this clinical trial for a period of 2 years from completion of the trial, defined as the day the final subject completes the study. The files will be stored with Human Resource/Legal/Finance files – locked with restricted access; at 4757 Nexus Centre Drive, San Diego CA 92121.

11 Study Monitoring, Auditing, and Inspecting

11.1 Study Monitoring Plans

The clinical study described herein is a pilot study intended to determine the level of ketosis (BHB) achievable with consumption of the food ingredient, *(R)*-1,3-Butanediol. The study is not being conducted in accordance with Good Clinical Practice, and therefore, there is no clinical study monitoring.

11.2 Auditing and Inspecting

The clinical study described herein is a pilot study intended to determine the level of ketosis (BHB) achievable with consumption of the food ingredient, *(R)*-1,3-Butanediol. The study is not being conducted in accordance with Good Clinical Practice, is not a regulated pharmaceutical trial, and therefore, will not be audited or inspected.

12 Ethical Considerations

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or IRB, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate.

The subjects for the study proposed herein are being recruited from the pool of employees at Genomatica. Participation in the study is not a requirement of employment, and measures are being taken to ensure that Genomatica employees do not feel coerced to partake in the study. For example, employees will not be directly solicited to enroll in research, neither orally nor through individual mailings or individual e-mails. Acceptable methods of recruitment will include flyers and other recruiting materials (approved by an IRB), displayed in the workplace where public announcements are permitted and/or mass e-mailers. The IRB will be provided with specific plans for ensuring the privacy of employees that participate in research. Also, policies will be clearly spelled out in the informed consent for issues such as injury or sickness as a result of the research. It will be made clear to employees that an employee's decision about research participation will not affect (favorably or unfavorably) performance evaluations, career advancement, or other employment-related decisions made by peers or supervisors.

13 Study Finances

13.1 Funding Source

This study will be financed by Genomatica Inc.

13.2 Subject Stipends or Payments

All participants will be provided with a Keto-Mojo ketone/glucose monitor kit (complete with ketone and glucose test strips, lancets, alcohol swabs, sharps container and bandages), a measuring tape, a shot glass with the Avela™ logo and a duffel bag, which they are permitted to keep, following the study. There will no costs incurred by subjects.

14 Publication Plan

Genomatica Inc. plans to register this study at ClinicalTrials.gov and to publish the results of the study.

15 References

Avgerinos KI, Egan JM, Mattson MP and Kapogiannis D, 2020. Medium Chain Triglycerides induce mild ketosis and may improve cognition in Alzheimer's disease. A systematic review and meta-analysis of human studies. Ageing Research Reviews, 58, 101001 [11pp]. DOI:10.1016/j.arr.2019.101001.

Bach KD, Heuwieser W, McArt JAA (2016). Technical note: Comparison of 4 electronic handheld meters for diagnosing hyperketonemia in dairy cows. J Dairy Sci 99(11):9136-9142. DOI:10.3168/jds.2016-11077.

Bengtsson, M., Persson, J., Sjölund, K., & Ohlsson, B. (2013). Further validation of the visual analogue scale for irritable bowel syndrome after use in clinical practice. Gastroenterology Nursing, 36, 188–198. PubMed ID: 23732784 doi:10.1097/SGA.0b013e3182945881

Cahill GF Jr (1970). Starvation in man. New Engl J Med 282(12):668-675. DOI:10.1056/NEJM197003192821209. Cited In: Veech et al., 2001.

Desrochers S, David F, Garneau M, Jetté M, Brunengraber H (1992). Metabolism of R-and S-1,3-butanediol in perfused livers from meal-fed and starved rats. Biochem J 285(2, Part 2):647-653. DOI:10.1042/bj2850647.

Gaskell, S, Snipe R, Costa R. (2019). Test–Retest Reliability of a Modified Visual Analog Scale Assessment Tool for Determining Incidence and Severity of Gastrointestinal Symptoms in Response to Exercise Stress. Int J Sport Nutr and Exer Metab 29:411-419.

Grove R, Prapavessis H (1992). Preliminary evidence for the reliability and validity of an abbreviated Profile of Mood States. Int J Sport Psychol 23:93-109.

Hoddes E, Zarcone V, Smythe H, Phillips R, Dement WC (1973). Quantification of sleepiness: a new approach. Psychophysiology 10(4):431-436. DOI:10.1111/j.1469-8986.1973.tb00801.x.

Janssen MJ, Hendrickx BH, Habets-van der Poel CD, van den Bergh JP, Haagen AA, Bakker JA (2010). Accuracy of the Precision® point-of-care ketone test examined by liquid chromatography tandem-mass spectrometry (LC-MS/MS) in the same fingerstick sample. Clin Chem Lab Med 48(12):1781-1784. DOI:10.1515/CCLM.2010.351.

Kies C, Tobin RB, Fox HM, Mehlman MA (1973). Utilization of 1,3-butanediol and nonspecific nitrogen in human adults. J Nutr 103(8):1155-1163. DOI:10.1093/jn/103.8.1155.

McNair D, Lorr M, Droppleman L (1971). *Manual for the Profile of Mood States*. San Diego (CA): Educational and Industrial Testing Service (EdITS). Cited In: Grove and Prapavessis, 1992.

McNair D, Lorr M, Droppleman L (1992). *Profile of Mood States Manual, revised edition*. San Diego (CA): Educational and Industrial Testing Service (EdITS). Cited In: van Wijk, 2011.

Moore AR, Holland-Winkler AM, Ansley JK, Boone EDH, Schulte MKO (2021). Reliability and diagnostic performance of a new blood ketone and glucose meter in humans. J Int Soc Sports Nutr 18(1):6 [9pp]. DOI:10.1186/s12970-020-00404-2.

Pineda A, Cardoso FC (2015). Technical note: Validation of a handheld meter for measuring β-hydroxybutyrate concentrations in plasma and serum from dairy cows. J Dairy Sci 98(12):8818-8824. DOI:10.3168/jds.2015-9667.

Poff AM, Rho JM, D'Agostino DP (2019). Ketone administration for seizure disorders: history and rationale for ketone esters and metabolic alternatives. Front Neurosci 13:1041 [13pp]. DOI:10.3389/fnins.2019.01041.

Reichard GA Jr, Owen OE, Haff AC, Paul P, Bortz WM (1974). Ketone-body production and oxidation in fasting obese humans. J Clin Invest 53(2):508-515. DOI:10.1172/JCI107584.

Scott BE, Laursen PB, James LJ, Boxer B, Chandler Z, Lam E, et al. (2019). The effect of 1,3-butanediol and carbohydrate supplementation on running performance. J Sci Med Sport 22(6):702-706. DOI:10.1016/j.jsams.2018.11.027.

Shahid A, Wilkinson K, Marcu S, Shapiro CM, editors (2012). Stanford Sleepiness Scale (SSS). In: *STOP, THAT and One Hundred Other Sleep Scales*. New York (NY): Springer Science+Business Media, LLC, pp. 369-370.

Shaw DM, Merien F, Braakhuis A, Plews D, Laursen P, Dulson DK (2019). The effect of 1,3-butanediol on cycling time-trial performance. Int J Sport Nutr Exerc Metab 29(5):466-473. DOI:10.1123/ijsnem.2018-0284.

Shivva V, Cox PJ, Clarke K, Veech RL, Tucker IG, Duffull SB (2016). The population pharmacokinetics of D-β-hydroxybutyrate following administration of *(R)*-3-hydroxybutyl *(R)*-3-hydroxybutyrate. AAPS J 18(3):678-688. DOI:10.1208/s12248-016-9879-0.

Tobin RB, Mehlman MA, Kies C, Fox HM, Soeldner JS (1975). Nutritional and metabolic studies in humans with 1,3-butanediol. Fed Proc 34(12):2171-2176.

Van Wijk C (2011). Mood States are not associated with BMI in mentally healthy adults. Psychology 2(5):492-496. DOI:10.4236/psych.2011.25076.

Veech RL, Chance B, Kashiwaya Y, Lardy HA, Cahill GF Jr (2001). Ketone bodies, potential therapeutic uses. IUBMB Life 51(4):241-247. DOI:10.1080/152165401753311780.

Yu H-YE, Agus M, Kellogg MD (2011). Clinical utility of Abbott Precision Xceed Pro® ketone meter in diabetic patients. Pediatr Diabetes 12(7):649-655. DOI:10.1111/j.1399-5448.2011.00768.x

16 Appendices

Appendix A Screen Captures of Online Screening Survey
Appendix B Detailed Instructions for Participants
Appendix C Screen Captures of Online Portal

Appendix A Screen Captures of Online Screening Survey

Appendix B Detailed Instructions for Participants

B-1 Detailed instructions for participants

Day prior to study start

1. Final meal should be consumed to ensure a minimum of a **12-hour fast** prior to study start. You are permitted to drink water when you wake up; do not consume any food. Record the time at which the last meal was consumed using the online portal.
2. Place Avela™ test beverages in the fridge for consumption of chilled beverages on study date.
3. Download the Keto-Mojo App from the Apple Store or Google Play and make sure it is paired with your Keto-Mojo Monitor.
4. You will be sent a request on your App to join the study account, in order to share all BHB readings acquired during the study; please accept.
5. Test blood BHB levels
    - Use the Keto-Mojo monitor to measure your blood BHB level. The manual provided with the monitor provides step by step instructions. Alternatively, you can review these videos for assistance:
        - How to take a reading on the Keto-Mojo meter: https://keto-mojo.com/video/gkplus-meter-testing-demo/
        - How to use the lancet device: https://keto-mojo.com/video/gkplus-meter-lancet-device/

Study Start

6. Please ensure 12 hours have elapsed since the last food product was consumed.
7. Retrieve from the fridge the bottles of water containing the premixed Avela™ product.
8. On the day of study, if you are a female and you answered "no" to both of these questions during the study screening:
    i. "If you are a female, are you post-menopausal, defined as not having had a period for 12 consecutive months or longer?" and
    ii. "If you are a female, have you had any procedure that would result in you not being able to have children, such as "tying of tubes" or a removal of your ovaries?"

    Take the pregnancy test provided prior to study start. Proceed with the study only if the test is negative (the result of the test will be included on the online portal).

Baseline (0 minutes)

9. Using the online portal, complete the mVAS for GI symptoms, followed by the SSS.
10. The on-screen timer will start automatically upon reading the welcome screen and completing age, height, last meal questions.
11. Use the Keto-Mojo monitor to measure your blood BHB level and record this value on the online portal.
12. **Consume one of the Avela™ beverages within 5 minutes.**

30 minutes

13. Using the online portal, complete the mVAS for GI symptoms, followed by the SSS.
14. Use the Keto-Mojo monitor to measure your blood BHB level and record this value on the online portal.
15. **Consume one of the Avela™ beverages within 5 minutes.**

60 minutes

16. Using the online portal, complete the mVAS for GI symptoms, followed by the SSS.
17. Use the Keto-Mojo monitor to measure your blood BHB level and record this value on the online portal.
18. **Consume one of the Avela™ beverages within 5 minutes.**

90 minutes

19. Using the online portal, complete the mVAS for GI symptoms, followed by the SSS.
20. Use the Keto-Mojo monitor to measure your blood BHB level and record this value on the online portal.

120 minutes

21. Using the online portal, complete the mVAS for GI symptoms, followed by the SSS.
22. Use the Keto-Mojo monitor to measure your blood BHB level and record this value on the online portal.

180 minutes

23. Using the online portal, complete the mVAS for GI symptoms, followed by the SSS.
24. Use the Keto-Mojo monitor to measure your blood BHB level and record this value on the online portal.

240 minutes

25. Using the online portal, complete the mVAS for GI symptoms, followed by the SSS.
26. Use the Keto-Mojo monitor to measure your blood BHB level and record this value on the online portal.
27. Consume the ALOHA Bar that has been provided, within a 7-minute timeframe.

300 minutes

28. Using the online portal, complete the mVAS for GI symptoms, followed by the SSS.
29. Use the Keto-Mojo monitor to measure your blood BHB level and record this value on the online portal.

Congratulations! You have now completed the study! Please open the Keto-Mojo App to allow it to sync with the Keto-Mojo Monitor and mail back the sharps container using the prepaid outer shipping carton, when convenient.

.

Appendix C Screen Captures of Online Portal