CLINICAL PROTOCOL COVER PAGE

Protocol Title:	An Open Label Study to Investigate the efficacy of Nic's Keto Diet on cardiovascular health in healthy adults with mildly elevated LDL levels
NCT Number:	NCT04195594
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Study Design:	Open Label
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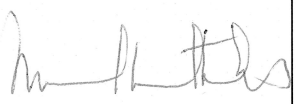
PROTOCOL SIGNATURE SHEET

The sponsor and the investigator agree to conduct the study in compliance with the clinical study protocol (and amendments), International Conference on Harmonization (ICH) guidelines for current Good Clinical Practice (cGCP) and applicable regulatory requirements.

Name	Signature	Date
Sponsor: Dr. Nicolas Tzenios +447583902071 nicolas@trccolleges.com TRCGEN+, Nic's Keto and Organic 3 Walham Yard London SW6 1JA United Kingdom		07 October 2019
Contract Research Organization: Malkanthi Evans, PhD Chief Scientific Officer KGK Science Inc. 255 Queens Ave., Suite 1680 London, ON N6A 5R8 Canada		Oct 08, 2019

1 PROTOCOL SYNOPSIS

Population: Healthy males and females with mildly elevated LDL levels

Total number of participants: 14

1.1 Inclusion Criteria

1. Provided voluntary, written, informed consent to participate in study
2. Males and females between 30 and 55 years of age
3. BMI between 20.0 to 29.9 kg/m^2, inclusive
4. Female participant is not of child-bearing potential, defined as females who have undergone a sterilization procedure (e.g. hysterectomy, bilateral oophorectomy, bilateral tubal ligation, total endometrial ablation)
   Or,
   Females of child-bearing potential must have a negative baseline urine pregnancy test and agree to use a medically approved method of birth control for the duration of the study. Acceptable methods of birth control include:
   - Non hormonal contraceptives
   - Double-barrier method
   - Intrauterine devices
   - Non-heterosexual lifestyle or agrees to use contraception if planning on changing to heterosexual partner(s)
   - Vasectomy of partner at least 6 months prior to screening
5. Subjects with mid-range (2.5–4.1mmol/L or 100-159 mg/dL) elevated LDL-C levels
6. Agrees to maintain current level of physical activity throughout the study
7. Willingness to complete all questionnaires, records, and diaries and assessments associated with the study and to complete all clinic visits.
8. Healthy as determined by medical history, laboratory results, and as assessed by Qualified Investigator (QI)

1.2 Exclusion Criteria

1. Women who are pregnant, breast feeding, or planning to become pregnant during the course of the trial
2. Women who are menopausal or post-menopausal
3. Currently following a diet (i.e. Ketogenic Diet, low-carbohydrate diet)
4. Subjects with high TSH level (>4.5mU/L) or Self reported pre-existing thyroid condition. Treatment on a stable dose of medication for at least 3 months will be considered by the QI
5. Participants with type I or II Diabetes Mellitus
6. Cancer, except skin cancers completely excised with no chemotherapy or radiation with a follow up that is negative. Volunteers with cancer in full remission for more than five years after diagnosis are acceptable.

7. Participants with previous or current pathology of the pancreas
8. Current or history of Gastroesophageal reflux disease (GERD) or any significant disease of the gastrointestinal tract
9. Self reported hypertension or on hypertensives.
10. Significant cardiovascular event in the past 6 months as assessed by the QI.
11. Major surgery in the past 3 months or individuals who have planned surgery during the course of the trial. Participants with minor surgery will be considered on a case-by-case basis by the QI
12. Gastric bypass surgery
13. Individuals with an autoimmune disease or are immune-compromised
14. Self reported HIV-, Hepatitis B- and/or C-positive diagnosis
15. History of or current diagnosis with kidney and/or liver diseases as assessed by the QI on a case-by-case basis, with the exception of history of kidney stones symptom free for 6 months
16. Self reported medical or neuropsychological condition and/or cognitive impairment that, in the QI's opinion, could interfere with study participation
17. Self reported blood/bleeding disorders as per QI
18. Current use of prescribed medications listed in Section 7.3.1
19. Current use of over-the-counter medications, supplements, foods, and/or drinks listed in Section 7.3.2
20. Alcohol or drug abuse within the last 12 months
21. High alcohol intake (>2 standard drinks per day or >10 standard drinks per week)
22. Clinically significant abnormal laboratory results at screening as assessed by the QI
23. Blood donation 30 days prior to screening, during the study, or a planned donation within 30-days of the last study visit
24. Participation in a clinical research trial within 30 days of study initiation
25. Individuals who are unable to give informed consent
26. Any other active or unstable medical condition, that, in the opinion of the QI, may adversely affect the participant's ability to complete the study or its measures or may pose significant risk to the participant

1.3 Schedule of Assessments

Procedures	Visit 1 Screening	Visit 2 Day 0 (Baseline)	Visit 3–7 Day 28–112 Week 4–16	Visit 8 Day 140 Week 20
Informed consent	X			
Review inclusion/exclusion criteria	X	X		
Review medical history	X			
Review concomitant therapies	X	X	X	X
Vitals: Height*, weight, heart rate, blood pressure **Height will only be measured at visit 1*	X	X	X	X
Urine pregnancy test	X	X		X
Physical examination		X		
Blood collection for analysis of: CBC, electrolytes (Na^+, K^+, Cl^-, P), HbA1c, fasting glucose, eGFR, creatinine, AST, ALT, total bilirubin, and TSH **TSH will only be measured at visit 1*	X			X
DEXA scan** ***DEXA will be measured at Visits, 2, 5 and 8 only*		X	X	X
ECG** ***ECG will be measured at Visits, 2, 5 and 8 only*		X	X	X
Blood Markers: Fasting glucose, HbA1c, C-reactive protein, lipid panel, Free Tri-iodothyronine (T3), ESR		X		X
Lipid Panel	X		X	
Nutritional Counselling[#] and dispense Nic's Keto Diet e-book *[#]As required, remote Nutritional Counselling will be completed on 3-day food records weekly throughout study period*		X		
Daily glucose monitors, and keto monitors Dispensed		X	X	
Daily glucose monitors, and keto monitors Returned			X	X
Food Records Dispensed	X	X	X	
Food Records Returned		X	X	X
Subject Diary Dispensed		X	X	
Subject Diary Returned			X	X
Compliance Calculated			X	X
Adverse Events	X	X	X	X

2 LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BP	Blood Pressure
BMI	Body Mass Index
CBC	Complete Blood Count
CEA	Carcinoembryonic antigen
Cl	Chloride
CPT	Carnitine Palmitoyl Transferase
CRO	Contract Research Organization
DEXA	Dual Energy X-ray Absorption
DOB	Date of Birth
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
e.g.	*"for example"*
ESR	Erythrocyte Sedimentation Rate
et al	*"and others"*
GCP	Good Clinical Practice
GERD	Gastroesophageal Reflux Disease
HbA1C	Hemoglobin A1C
HDL	High-Density Lipoprotein
HIV	Human Immunodeficiency Virus
HR	Heart Rate
lbs.	Pounds
ICF	Informed Consent Form
ICH	International Conference of Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intent-to-Treat
kg	Kilogram
LCAD	Long-Chain Acyl Dehydrogenase Deficiency
LDL	Low-Density Lipoprotein
LDL-C	Low-Density Lipoprotein Cholesterol
LOCF	Last Observation Carried Forward
MCAD	Medium-Chain Acyl Dehydrogenase Deficiency
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCT	Medium Chain Triglyceride
MCV	Mean Corpuscular Volume
mg/dL	Milligrams per Deciliter
mmol/L	Millimoles per Liter
MMRM	Mixed Model for Repeated Measures
Na	Sodium
OTC	Over-the-Counter
PP	Per Protocol

PSA	Prostate Specific Antigen
QI	Qualified Investigator
RBC	Red Blood Cells
RDW	Red Blood Cell Distribution Width
SAE	Serious Adverse Event
SCAD	Short-Chain Acyl Dehydrogenase Deficiency
SOP	Standard Operating Procedure
SST	Serum Separating Tube
TPD	Therapeutics Products Directorate
TSH	Thyroid Stimulating Hormone
UAT	User Acceptance Testing
ULN	Upper Limit of Normal
US	United States
WBC	White Blood Cell

3 TABLE OF CONTENTS

4 INTRODUCTION

North American diets are heavy in carbohydrates which constitute approximately 55% of the macronutrients in an average person's diet. Overconsumption of refined carbohydrates may have harmful effects. For instance, high intake of sugar may lead to a 44% increased prevalence of metabolic syndrome and obesity and a 26% increase in developing diabetes mellitus (1).

A typical ketogenic diet consists of high-fats, moderate protein, and an extremely low amount of carbohydrates. Along with a major reduction in carbohydrates, dietary protein is decreased. People who choose a ketogenic diet instead use an alternative fuel source for the brain with the release of ketone substrates from the body (2). During periods of low blood sugar, the body begins to break down stored fat into ketone bodies (ketosis). The typical dietary ratio of fats to carbohydrates and proteins in a ketogenic diet ranges from 3:1 to 4:1 (3). With ketogenic diets substantial weight loss can be observed in as little as 2 weeks after initiation with strong preservation of lean body mass (1).

The therapeutic benefits that come with ketogenic diets are well documented. For example, low-carbohydrate ketogenic diets have been shown to significantly increase weight loss (4). Studies have shown that ketogenic diets can result in a reduction of mean body weight by 6.6% and serum triglyceride decrease by 42% (5). In comparison to low-fat diets those on ketogenic diets lose substantially more fat mass than fat-free mass, and have greater reductions in serum triglyceride levels (4). Adherence to a ketogenic diet may help to maintain a heathy weight and BMI over the long term (6). Also noted by Dashti was a significant reduction in blood glucose levels following a 24 week period of ketogenic diet treatment (6). A randomized, double-blind, placebo controlled trial has even demonstrated that ketogenic diets are excellent at preserving good nutritional status (7). More recently ketogenic diets have shown therapeutic benefits in maintaining skin health, decreasing serum triglyceride levels, and increasing high-density lipoprotein (4, 8). For example, in type 2 diabetes mellitus patients, ketogenic diets have been shown to improve glycemia control in the absence of type 2 diabetes' medication (5).

Low-density lipoprotein (LDL) is a combination of fat (lipid) and protein. The lipids are bound to the proteins, which allows them to move freely through the blood (9). LDL is often thought of as "bad" cholesterol being attributed to negative effects on cardiovascular health (10). Numerous studies, however, have indicated there is no association between LDL and negative cardiovascular health risks (11-13). In fact, a study conducted on 304 women determined there to be no association between LDL-C and coronary calcification in women over the age of 55 (14). The hypothesis that high levels of LDL reduce cardiovascular health may be founded on selective exclusion of data and misleading statistics (11). For example, a meta-analysis published in 2007, 61 studies examined the association between LDL and cardiovascular health risk. The results of the analysis, however, did not include twelve different studies that showed no association or an inverse association with LDL and decreased cardiovascular health. Part of the issue is there are often overlooked factors, such as mental stress that can raise cholesterol levels and cause hypertension or platelet aggregation (15).

There are various ketogenic diets that differ in their composition of protein, fat, and carbohydrate. The main ones include a classical ketogenic diet, a medium chain triglyceride diet (MCT), a low glycemic index diet, and a modified Atkins diet. These different compositions result in different benefits based on the ratio of macronutrients and range of foods that are consumed. For example, the classical ketogenic diet is comprised of consistent foods, which results in little variation in ketones. Whereas a diet that is higher in protein like MCT allows for a greater volume to mix different kinds of fats (16).

The present study will investigate the effects of Nic's Ketogenic diet, comprised of 70% fat, 5% carbohydrate, and 25% protein on middle-aged males and females with mid-range elevated LDL. With the increasing evidence that LDL lacks in causation of decreased cardiovascular health risk further studies are warranted to examine the true relation of LDL and cardiovascular health risk. For example, a ketogenic diet study examined changes in LDL (17). Results from this study indicated that a ketogenic diet causes a shift of small dense LDL to large buoyant LDL, which may reduce negative risks associated with cardiovascular health. In this study the efficacy of Nic's Ketogenic diet in increasing LDL without cardiovascular health risk will be examined. The main outcome of the study is to investigate whether in periods of higher LDL there will be positive impacts on cardiovascular health and inflammation.

5 STUDY OBJECTIVES

The objective of this study is to investigate the efficacy of Nic's Ketogenic Diet on cardiovascular health and inflammation in healthy adults with mildly elevated LDL-C.

Primary outcomes:

The change in inflammation, as assessed by the change in erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) following Nic's Keto Diet for 140 days. The main outcome of the study is to investigate whether in periods of higher LDL there will be positive impacts on cardiovascular health and inflammation.

Secondary outcomes:

Changes to the following parameters from baseline to day 140 at monthly intervals following Nic's Keto Diet:

1. Weight
2. Blood pressure (BP)
3. Lipid panel

Changes to the following parameters from baseline to days 70 and 140 following Nic's Keto Diet:

1. Body composition (total body fat (%), android fat (%), gynoid fat (%), android/gynoid fat ratio, and muscle mass (%)) as assessed by DEXA Scan

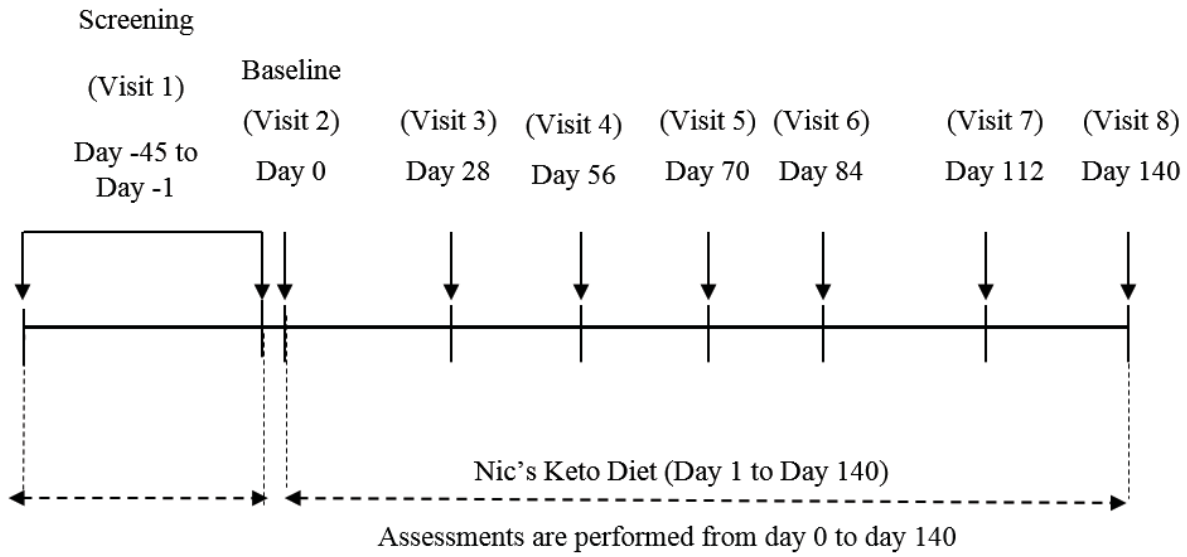
Changes to the following parameters from baseline to day 140 following Nic's Keto Diet:

1. HbA1c
2. Fasting glucose
3. Free Tri-iodothyronine (T3)

Safety outcomes:

1. Incidence of pre-emergent and post-emergent adverse events
2. Electrocardiogram (ECG) after 70 and 140 days of following Nic's Keto Diet
3. Vital signs: heart rate (HR)
4. Anthropometrics: BMI calculation
5. Clinical chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, creatinine, electrolytes (Na^+, K^+, Cl^-, P), HbA1c, fasting glucose, and estimated glomerular filtration rate (eGFR))
6. Hematology: white blood cell (WBC) count with differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), red blood cell (RBC) count, hemoglobin, hematocrit, platelet count, RBC indices (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), and mean platelet volume (MPV)

6 STUDY DESIGN



7 SELECTION OF STUDY POPULATION

This study will enroll 14 middle-aged, healthy males and females with mildly elevated LDL. Each participant must fulfill the inclusion criteria and not meet any of the exclusion criteria as described in Sections 7.1 and 7.2, respectively.

7.1 Inclusion Criteria

1. Provided voluntary, written, informed consent to participate in study
2. Males and females between 30 and 55 years of age
3. BMI between 20.0 to 29.9 kg/m^2, inclusive
4. Female participant is not of child-bearing potential, defined as females who have undergone a sterilization procedure (e.g. hysterectomy, bilateral oophorectomy, bilateral tubal ligation, total endometrial ablation)
   Or,
   Females of child-bearing potential must have a negative baseline urine pregnancy test and agree to use a medically approved method of birth control for the duration of the study. Acceptable methods of birth control include:
   - Non hormonal contraceptives
   - Double-barrier method
   - Intrauterine devices

- Non-heterosexual lifestyle or agrees to use contraception if planning on changing to heterosexual partner(s)
- Vasectomy of partner at least 6 months prior to screening

5. Subjects with mid-range (2.5–4.1mmol/L or 100-159 mg/dL) elevated LDL-C levels
6. Agrees to maintain current level of physical activity throughout the study
7. Willingness to complete all questionnaires, records, and diaries and assessments associated with the study and to complete all clinic visits.
8. Healthy as determined by medical history, laboratory results, and as assessed by Qualified Investigator (QI)

7.2 Exclusion Criteria

1. Women who are pregnant, breast feeding, or planning to become pregnant during the course of the trial
2. Women who are menopausal or post-menopausal
3. Currently following a diet (i.e. Ketogenic Diet, low-carbohydrate diet)
4. Subjects with high TSH level (>4.5mU/L) or Self reported pre-existing thyroid condition. Treatment on a stable dose of medication for at least 3 months will be considered by the QI
5. Participants with type I or II Diabetes Mellitus
6. Cancer, except skin cancers completely excised with no chemotherapy or radiation with a follow up that is negative. Volunteers with cancer in full remission for more than five years after diagnosis are acceptable.
7. Participants with previous or current pathology of the pancreas
8. Current or history of Gastroesophageal reflux disease (GERD) or any significant disease of the gastrointestinal tract
9. Self reported hypertension or on hypertensives.
10. Significant cardiovascular event in the past 6 months as assessed by the QI.
11. Major surgery in the past 3 months or individuals who have planned surgery during the course of the trial. Participants with minor surgery will be considered on a case-by-case basis by the QI
12. Gastric bypass surgery
13. Individuals with an autoimmune disease or are immune-compromised
14. Self reported HIV-, Hepatitis B- and/or C-positive diagnosis
15. History of or current diagnosis with kidney and/or liver diseases as assessed by the QI on a case-by-case basis, with the exception of history of kidney stones symptom free for 6 months
16. Self reported medical or neuropsychological condition and/or cognitive impairment that, in the QI's opinion, could interfere with study participation
17. Self reported blood/bleeding disorders as per QI
18. Current use of prescribed medications listed in Section 7.3.1
19. Current use of over-the-counter medications, supplements, foods, and/or drinks listed in Section 7.3.2
20. Alcohol or drug abuse within the last 12 months
21. High alcohol intake (>2 standard drinks per day or >10 standard drinks per week)
22. Clinically significant abnormal laboratory results at screening as assessed by the QI
23. Blood donation 30 days prior to screening, during the study, or a planned donation within 30-days of the last study visit
24. Participation in a clinical research trial within 30 days of study initiation

25. Individuals who are unable to give informed consent
26. Any other active or unstable medical condition, that, in the opinion of the QI, may adversely affect the participant's ability to complete the study or its measures or may pose significant risk to the participant

7.3 Concomitant Medications

Participants who are taking any prescribed medications that are considered not to affect the study outcomes must agree to maintain their current dosing regimen during the study unless otherwise recommended by their family physician.

7.3.1 Prescribed Medications

Participants on the following concurrent prescribed medications and/or treatments will be excluded during enrollment unless they have been taken off these therapies by their family physician. In the latter event, the frequency of use and/or dosage may be considered by the QI on a case by case basis prior to recommending an appropriate washout or their enrollment in the study.

1. Corticosteroids (pills, nasal sprays, injections)
2. Hormonal Medication (contraceptives)
3. Opioid pain medications
4. Beta blockers and Blood pressure medications (clonidine, propranolol)
5. Statins
6. Selective serotonin reuptake inhibitors (SSRIs)

7.3.2 Over-the-counter Medications, Supplements and Foods/Drinks

Participants who are currently consuming the following over-the-counter (OTC) Medications, supplement and food/drinks will not be allowed to participate unless willing to undergo a 14-day washout period prior to their baseline visit and agree not to take the supplements during the study. Other OTCs supplement and food/drinks will require a case-by-case assessment by the QI based on dose and/or frequency of use to determine adequate washout.

1. NSAIDs, with the exception of ibuprofen
2. Calcium supplements
3. Protein supplements
4. Orlistat
5. Caffeine supplements
6. Carbohydrate boosting Supplements
7. Exogeneous ketone supplements
8. Supplements known to affect weight and/or fat loss or lipid metabolism, including but not limited to:
    a. Omega 3 and 6 supplements
    b. Hemp seed oil supplement
    c. Flaxseed oil supplement

7.4 Early Withdrawal

Personal reasons

As stated in the Informed Consent Form, a participant may withdraw from the study for any reason at any time.

Removal by Qualified Investigator

Participant discontinuation should be considered at the discretion of the qualified investigator. The circumstances of any discontinuation must be documented in detail in the participant file and final report. If possible, the evaluations planned for the end of treatment will be carried out at the time when the participant is withdrawn from the study. A participant leaving the study prematurely will NOT be replaced by another. It is understood by all concerned that an excessive rate of withdrawals can render the study un-interpretable, thus unnecessary withdrawal of participants should be avoided.

Criteria for removal of participants from the study includes:

Clinical reasons

A participant may be withdrawn from the study if, in the opinion of the qualified investigator, it is not in the participant's best interest to continue. Any participant who experiences a serious adverse event (SAE) may be withdrawn from the trial at the discretion of the qualified investigator. A participant will also be withdrawn due to adverse events causing clinically significant illness or the need for prohibited medication(s) during the trial. Any female participant who becomes pregnant during the course of the trial will be withdrawn.

Protocol violation

Any participant found to have entered this study in violation of the protocol will be discontinued from the study at the discretion of the qualified investigator. This will include any participant found to have been inappropriately enrolled (did not meet eligibility criteria). Participant non-compliance includes failure to show up for study visits, failure to take the investigational product as directed, or refusal to undergo study visit procedures. Participants who are found to be taking prohibited medications or supplements without the knowledge of the qualified investigator will also be withdrawn. Any major protocol deviations (i.e. those that increase the risk to participants and/or compromise the integrity of the study or its results) will result in participant discontinuation.

8 INVESTIGATIONAL DIET

8.1 Investigational Diet (Nic's Keto Diet)

Dietary Requirements	Quantity (Qty)
Calories from fat	70%
Protein	25%
Carbohydrates	5% (less than 20g of carbohydrates per day)

8.2 Directions

Participants will be instructed to follow Nic's keto diet plan for 140 days. Clinic staff will instruct participants to record results from their ketone and glucose monitor for the determination of compliance. Participants daily caloric need will be assessed for each participant as 90% of their energy requirements for weight maintenance based on their calculated base metabolic rate (BMR) and an activity factor. Participants will be advised not to exceed 20 g of carbohydrate per day (up to 5% caloric intake) and to consume the remaining 70% of calories from fat sources and 25% from protein sources. In addition to Nic's Keto Diet e-book, participants will be provided with a book summary and guidelines of foods that can be consumed and those that should not be consumed will be provided to the participant.

9 STUDY ASSESSMENTS

9.1 Visit 1 – Screening (Day -45 to Day -1)*

** At the discretion of the Qualified Investigator, any participants falling outside of the screening window (Day -45 to Day -1) due to scheduling issues will be asked to repeat eligibility/screening procedures prior to baseline visit.*

At screening, an informed consent form will be given to the potential volunteer. They will be required to read the information and will be given the opportunity to seek more information if needed, or have the option to take the consent form home to review prior to making their decision. If agreeable, the volunteer will sign the consent form and receive a duplicate of the signed copy. Once consent has been obtained, the screening visit will proceed following a prior 12-hour fast. Each volunteer will be sequentially assigned a screening number to be entered in the screening and enrollment log.

Screening assessments include:

1. Review of medical history and current health status
2. Assessment of inclusion and exclusion criteria
3. Review of concomitant prescribed and over-the-counter (OTC) medications, supplements, foods, and drinks (concomitant therapies)
4. Urine pregnancy test for female potential participants that are of child-bearing potential
5. Height and weight measurements and BMI calculation (Section 9.5.1)
6. Seated resting blood pressure and heart rate measurements (Section 9.5.2)
7. Collection of fasted blood samples for the analysis of complete blood count (CBC), electrolytes (Na+, K+, Cl-, P), HbA1c, glucose, eGFR, creatinine, AST, ALT, and total bilirubin, lipid panel and TSH level analysis
8. Dispense 3-day food records and instruct participants on completion and provide nutrition counselling

The next visit will be scheduled for potentially eligible participants for their baseline visit.

Reminders for participants prior to their next in-clinic visit:

1. Return to the clinic in a 12-hour fasted state
2. Complete 3-day food records
3. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food and drinks as per Section 7.3.2.
4. Maintain similar levels of physical activity

9.2 Visit 2 – Baseline (Day 0)

Eligible participants will return to the clinic for baseline assessments after a 12-hour fast.

Baseline (Day 0) assessments include:

1. Collection and review of food records
2. Assessment of inclusion and exclusion criteria

3. Physical exam
4. Review of concomitant therapies and adverse events
5. Urine pregnancy test for female potential participants that are of child-bearing potential
6. Weight measurements and BMI calculation (Section 9.5.1)
7. Seated resting blood pressure and heart rate measurements (Section 9.5.2)
8. Total body DEXA scan
9. Collection of fasted blood for analysis of glucose, HbA1c, C-reactive protein, lipid panel, Free tri-iodothyronine (T3), and ESR.
10. Deliver Nutrition Counselling about Nic's Keto Diet (Section 9.7.7)
11. Dispense Nic's Keto Diet e-book
12. Dispense glucose and ketone monitors and instruct participants on their use. First fasted ketone and glucose measure to be taken with monitors during baseline clinic visit.
13. Dispense or assign dates of 3-day food records
14. Dispense participant treatment diary and instruct participants on use

The next visit will be scheduled for day 28.

Reminders for participants prior to their next in-clinic visit:

1. Return to the clinic in a 12-hour fasted state
2. Complete 3- day food records weekly. Weekly records will be reviewed by nutrition team and counselling provided as required.
3. Participants will receive a 2-week follow up email from Nutrition Team (Section 9.7.7).
4. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food and drinks as per Section 7.3.2.
5. Complete subject treatment diary
6. Maintain similar physical activity and study diet
7. Monitor glucose and ketones daily

9.3 Visits 3 – 4 (Days 28, 56 ± 3 days)

Participants will return to the clinic after a 12-hour fast to complete assessments for Visits 3–4 with completed food records and subject treatment diary.

Assessments for Visits 3–4 include:

1. Collection and review of subject treatment diary
2. Collection and review of food records
3. Compliance calculation
4. Review of concomitant therapies and adverse events
5. Collection of fasted ketone and glucose and review of glucose and ketone monitors
6. Seated resting blood pressure and heart rate measurements
7. Weight measurement and BMI calculation (Section 9.5.1)
8. Collection of blood sample for lipid panel analysis (Section 9.5.2)
9. Dispense or assign dates of 3-day food records
10. Re-dispense subject treatment diary

The next visit will be scheduled for day 70

Reminders for participants prior to their next in-clinic visit:

1. Return to the clinic in a 12-hour fasted state
2. Complete 3-day food records weekly. Weekly records will be reviewed by nutrition team and counselling provided as required.
3. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food and drinks as per Section 7.3.2.
4. Complete subject treatment diary
5. Maintain similar physical activity and study diet
6. Monitor glucose and ketones daily

9.4 Visit 5 (Day 70 ± 3 days)

Participants will return to the clinic after a 12-hour fast to complete assessments for Visit 5 with completed food records and subject treatment diary.

Assessments for Visits 5 include:

1. Collection and review of subject treatment diary
2. Collection and review of food records
3. Compliance calculation
4. Review of concomitant therapies and adverse events
5. Collection of fasted ketone and glucose and review of glucose and ketone monitors
6. Seated resting blood pressure and heart rate measurements
7. Weight measurement and BMI calculation (Section 9.5.1)
8. Total body DEXA scan and ECG
9. Collection of blood sample for lipid panel analysis (Section 9.5.2)
10. Dispense or assign dates of 3-day food records
11. Re-dispense subject treatment diary

The next visit will be scheduled for day 84

Reminders for participants prior to their next in-clinic visit:

1. Return to the clinic in a 12-hour fasted state
2. Complete 3-day food records weekly. Weekly records will be reviewed by nutrition team and counselling provided as required.
3. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food and drinks as per Section 7.3.2.
4. Complete subject treatment diary
5. Maintain similar physical activity and study diet
6. Monitor glucose and ketones daily

9.5 Visits 6-7 (Day 84, 112 ± 3 days)

Participants will return to the clinic after a 12-hour fast to complete assessments for Visits 6-7 with completed food records and subject treatment diary.

Assessments for Visits 6-7 include:

1. Collection and review of subject treatment diary
2. Collection and review of food records
3. Compliance calculation
4. Review of concomitant therapies and adverse events
5. Collection of fasted ketone and glucose and review of glucose and ketone monitors
6. Seated resting blood pressure and heart rate measurements
7. Weight measurement and BMI calculation (Section 9.5.1)
8. Collection of blood sample for lipid panel analysis (Section 9.5.2)
9. Dispense or assign dates of 3-day food records
10. Re-dispense subject treatment diary

The next visit will be scheduled for day 140

Reminders for participants prior to their next in-clinic visit:

1. Return to the clinic in a 12-hour fasted state
2. Complete 3-day food records weekly. Weekly records will be reviewed by nutrition team and counselling provided as required.
3. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food and drinks as per Section 7.3.2.
4. Complete subject treatment diary
5. Maintain similar physical activity and study diet
6. Monitor glucose and ketones daily

9.6 Visit 8 – End-of-Study (Day 140 ± 3 days)

Participants will return to the clinic after a 12-hour fast to complete end of study assessments with completed food records and subject treatment diary.

Visit 8 assessments include:

1. Collection and review of subject treatment diary
2. Collection and review of food records
3. Compliance calculation
4. Collection and review of glucose and ketone monitors
5. Collection of fasted ketone and glucose and review of glucose and ketone monitors
6. Review of concomitant therapies and adverse events
7. Weight measurements and BMI calculation (Section 9.5.1)
8. Seated resting blood pressure and heart rate measurements (Section 9.5.2)
9. Total body DEXA scan and ECG
10. Urine pregnancy test for female potential participants that are of child-bearing potential
11. Collection of fasted blood samples for the analysis of CBC, electrolytes (Na+, K+, Cl-, P), HbA1c, eGFR, creatinine, AST, ALT, total bilirubin, fasting glucose, C-reactive protein, lipid panel, free tri-iodothyronine (T3), and ESR

9.7 Clinical Assessments and Procedures

Calculations or measurements of specific parameters are required, as indicated in the schedule of assessments. Instructions for determining these parameters are provided in the following sections.

9.7.1 Height and Weight

Weight measurements will be performed with shoes removed and bladder empty on calibrated scales at all visits.

At least two separate measurements will be taken at each visit. If the two measurements are more than 0.5 kg (1.1 lbs) apart, a third measurement will be taken. The two closest values will be selected and entered in the database.

Measurement of height will be performed with the participant's shoes removed. The participant's knees will be straightened, and head held upright.

9.7.2 Blood Pressure and Heart Rate

In-office, seated, resting blood pressure assessment:

> The participant should be seated comfortably with their back supported and the upper arm bared without restrictive clothing. Feet should be flat on the floor and legs will not be crossed. The participant will rest in this position for at least 5 minutes prior to the first reading.

At screening:

> Seated blood pressure will be checked in both arms and if different the arm with the higher systolic blood pressure reading will be taken for measurements. If the systolic blood pressure is the same in both arms, the arm with the higher diastolic blood pressure will be used. If both are equal, then the left arm will be used. In the chosen arm, a second measurement will be taken at least 1 minute from the first measurement. If a difference of more than 8 mmHg exists between the two readings a third reading will be taken. An average of the two lowest readings from the chosen arm will be taken for the determination of inclusion into the study. Per the QI's opinion, a high office blood pressure should be rechecked manually after the participant is given a glass of water and is seated for 15 min. Also, participant should be queried about their usual blood pressure.
>
> The arm chosen for use at the initial visit will be documented in the study file and used in all subsequent visits.

At study visits:

> Once enrolled in the study, BP will be measured in the chosen arm. Three readings will be made, averaged and recorded in the chart.

Heart Rate (beats/min) will be measured using the reading on the automated blood pressure monitor, or manually by the clinical coordinator placing their index finger on the participant's radial artery while

observing a timer and counting the number of beats over 30 seconds and then multiplying the number by two. This is repeated for a total of three measurements.

9.7.3 Blood Sample Collection

At the screening visit, baseline visit, day 28–112 (visits 3–6), and day 140 (visit 9), an appropriately trained and qualified phlebotomist will perform the venipuncture procedure to collect the necessary blood samples. Participants will be placed in a comfortable seated position with their desired arm, at the phlebotomist's discretion, fully extended and supported with a pillow. A tourniquet will be applied 3-4 inches above the elbow with the participants opening and closing their fist a few times to allow the phlebotomist to manually determine the approximate size, depth, and location of the vein. Following the site of the venipuncture being appropriately sterilized, the phlebotomist will collect the sample using the vacutainer system according to the relevant laboratory order requirement. Once collection is complete a cotton ball will be immediately placed on the venipuncture site which will be periodically checked to ensure clotting has begun at which point a clean cotton ball will be applied and secured with *Micropore™ Surgical Tape*. All collected sample tubes will be labeled with subject identification codes, visit number, project code, DOB (Date of Birth), gender, date and time of draw.

9.7.4 Electrocardiogram

The participant electrode-skin area will be prepared for ECG by the clinic coordinator at the KGK clinic (London, ON) to ensure a good electrode contact.

The participant should be warm and relaxed in a supine position with arms flat along the sides of the body. Participants that do not fit comfortably on the exam table due to size will be instructed to cross their arms on their stomach to reduce muscle tension.

The clinic coordinator will ensure that electrodes are placed in the proper position and the electrodes have good skin contact to minimize artifact. The electrode will not be placed over bones, irritated skin, areas where there is a lot of muscle movement or incisions. The participant will be instructed to breathe calmly and not read or talk and have no metal contacts including belt, keys and coins during the ECG test.

9.7.5 Dual Energy X-Ray Absorptiometry (DEXA) Scan

The DEXA scan will be performed at the KGK clinic (London, ON) or a local facility at visits 2 (Day 0), visit 5 (Day 84), and visit 7 (Day 140) by trained technicians. The DEXA scan is a form of X-ray radiation that measures the body tissue density which will be converted and used for assessment of the study outcomes. Coordinators will remind participants 24 hours before the day of the DEXA assessment for the necessary instructions prior to the scan.

9.7.6 Food Records - Libro

Participants will be using an online food record application called Libro, by Nutritics, to record their food consumption. This will, in turn, be used to calculate their daily calories, macronutrient and micronutrient intake throughout the study. Libro is an online portal where participants will enter their food and beverage intake. KGK staff will create an online access for each participant based on their study code, void of all personal information to protect any confidential participant information. All participants will be provided

with instructions on how to use the Libro application. Participants will use this tool to track their daily food and drink intake. The food records will be reviewed by trained staff at the visits and participants will be counseled with dietary suggestions as required. In the event the access to Libro is disrupted due to unforeseen reasons, participants will be instructed to take note of the foods they consume via pen and paper, and then enter these into the database once the access is restored or be provided with a paper food record if required.

9.7.7 Nutrition Counselling

At visit 2 participants will be counselled on the specifics of the diet, including the dietary guidelines (Appendix 16.2), using the Handy Guide for Food Servings (Appendix 16.1) and Nic's Keto Diet e-book. Participants will be given a summary of Nic's Keto Diet e-book and mini e-course. Participants will also be given information on what a ketogenic diet means and what to expect on a ketogenic diet (water weight loss, keto flu, GI changes). They will be given counselling on how to use Libro (Section 9.7.6) to complete food records and to assess macronutrient intake in order to comply with the study requirements. The nutrition team will send participants a follow-up email 2 weeks after the commencement of the diet to endure they understand the requirements and answer any questions they have about the ketogenic diet. At visits 3-7 the nutrition team will counsel participants on dietary compliance, with an emphasis on the importance of dietary compliance and provide suggestions on how to comply if food records reflect noncompliance. Additionally, the nutrition team will review the dietary guidelines and answer participants questions about the ketogenic diet. The nutrition team will review participants 3-day food records weekly and provide remote (phone/e-mail) counselling on diet.

9.8 Compliance

Compliance will be determined by ketone and glucose monitors dispensed at baseline and food records. Participants will be instructed to measure their ketone and glucose levels daily before their first meal, and the corresponding values will be recorded online to the participant's e-diary.

9.9 Laboratory Analyses

Blood samples will be drawn from the participants at screening (Visit 1), baseline (Visit 2, Day 0), Visit 3, Visit 4, Visit 5, Visit 6, Visit 7, and at the End of Study visit (Visit 8, day 140) as indicated in the schedule of assessments.

Protection of subject confidentiality will extend to all data generated from the assaying of these samples. These samples will be alphanumerically coded and the persons performing the analysis will not be aware of the subject's identity.

At screening (Visit 1), 13 mL of ≥12 hours fasted whole blood will be collected in:

1. Two 4mL EDTA vacutainer tubes to generate plasma for:
   a. CBC analysis (one tube)
   b. Hb1Ac analysis (one tube)

2. One 5mL SST vacutainer tube to generate serum for:
   a. Electrolytes (Na^+, K^+, Cl^-, P), lipid profile, fasting glucose, eGFR, creatinine, AST, ALT, TSH, and total bilirubin

At baseline (Visit 2), 13 mL of ≥12 hour fasted whole blood will be collected in:

1. Two 4mL EDTA vacutainer tubes to generate plasma for:
   a. Hb1-Ac (one tube)
   b. ESR (One Tube)

2. One 5mL SST vacutainer tube to generate serum for:
   a. Fasting glucose, C-reactive protein, lipid panel, Free T3 (one tube)

At Visit 3, 5 mL of ≥ 12 hour fasted whole blood will be collected in:

1. One 5mL SST vacutainer tube to generate serum for:
   a. Lipid Panel (one tube)

At Visit 4, 5 mL of ≥ 12 hour fasted whole blood will be collected in:

1. One 5mL SST vacutainer tube to generate serum for:
   a. Lipid Panel (one tube)

At Visit 5, 5 mL of ≥ 12 hour fasted whole blood will be collected in:

1. One 5mL SST vacutainer tube to generate serum for:
   a. Lipid Panel (one tube)

At Visit 6, 5 mL of ≥ 12 hour fasted whole blood will be collected in:

1. One 5mL SST vacutainer tube to generate serum for:
   a. Lipid Panel (one tube)

At Visit 7, 5 mL of ≥ 12 hour fasted whole blood will be collected in:

1. One 5mL SST vacutainer tube to generate serum for:
   a. Lipid Panel (one tube)

At the end of the study (Visit 8), 17 mL ≥ 12 hour fasted whole blood will be collected in:

1. Three 4mL EDTA vacutainer tubes to generate plasma for:
   a. CBC analysis (one tube)
   b. Hb1Ac analysis (one tube)

c. ESR (One Tube)

2. One 5mL SST vacutainer tube to generate serum for:
   a. Electrolytes (Na^+, K^+, Cl^-, P), fasting glucose, eGFR, creatinine, AST, ALT, CRP, Lipid Panel, Free T3, and total bilirubin (one tube)

The total blood volume collection for the laboratory assessments listed above will be approximately 68 mL, over the period from screening to end of study (approximately 140 days). At any study visit, blood loss per volunteer is not expected to exceed 18 mL. Additional blood samples may be collected during the course of the study in order to perform or repeat laboratory tests outlined in the Schedule of Assessments if needed.

Dynacare Laboratory will be used in this study to measure blood parameters.

Urine pregnancy test will be performed at the KGK Science clinic site.

9.10 Termination of the Trial

In the case of premature termination of the trial, participating investigators/participants, and the Institutional Review Board must be promptly informed of the termination. In the event of early termination, as many assessments will be completed as agreed upon by participant.

9.11 Protocol Amendments

If amendments to the study protocol are required after approval such changes will be captured in writing with the reasons for the change documented, signed, and dated by the sponsor. Any such amendments may be subject to IRB review/approval prior to implementation. Exception: if it becomes necessary to alter the protocol to eliminate an immediate hazard to participants, an amendment may be implemented prior to IRB approval. In this circumstance, the Investigator must notify IRB in writing within five (5) working days of the implementation.

10 SAFETY INSTRUCTIONS AND GUIDANCE

10.1 Adverse Events and Laboratory Abnormalities

10.1.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation participant who has been administered an investigational diet and does not necessarily have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a diet, whether or not it is considered related to that diet. Pre-existing conditions that worsen during a study are to be reported as AEs.

During the study, participants should record any adverse effects in their diary. At each visit the participant will be asked, "Have you experienced any difficulties or problems since I saw you last?". Any adverse events (AEs) will be documented in the study record and will be classified according to the description, duration, intensity, frequency, and outcome. The qualified investigator will assess any AEs and decide causality.

Intensity of AEs will be graded on a three-point scale (mild, moderate, severe) and reported in detail in the study record.

Mild:	Awareness of event but easily tolerated
Moderate:	Discomfort enough to cause some interference with usual activity
Severe:	Inability to carry out usual activity

The causality relationship of investigational diet to the adverse event will be assessed by the qualified investigator as either:

Most probable:	There is a reasonable relationship between the investigational diet and AEs. The event responds to withdrawal of investigational diet (dechallenge) and recurs with rechallenge when clinically feasible.
Probable:	There is a reasonable relationship between the investigational diet and AEs. The event responds to dechallenge.
Possible:	There is a reasonable relationship between the investigational diet and AEs. Dechallenge information is lacking or unclear.
Unlikely:	There is a temporal relationship to the investigational diet administration but there is no reasonable causal relationship between the investigational diet and the AEs.
Not related:	There is no temporal relationship to investigational diet administration or there is a reasonable causal relationship between non-investigational diet, concurrent disease or circumstance and the AEs.

10.1.2 Serious Adverse Event

A serious adverse event (SAE) is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that results in any of the following outcomes:

1. Death
2. A life-threatening adverse event
3. Inpatient hospitalization or prolongation of existing hospitalization
4. A persistent or significant disability or incapacity
5. A congenital anomaly/birth defect in the offspring of a participant who received the study treatment

Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or the development of drug dependency or drug abuse.

10.1.3 Unexpected Adverse Reaction

An unexpected adverse reaction is an adverse reaction, the nature and severity of which is not consistent with the applicable diet information.

10.1.4 Laboratory Test Abnormalities

The investigator must assess the clinical significance of all abnormal laboratory values as defined by the compendium of normal values for the reference laboratory.

Any treatment emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the AEs form in the study record:

1. Accompanied by clinical symptoms
2. Leading to interruption or discontinuation of the investigational diet
3. Requiring a change in concomitant therapy

This applies to any protocol and non-protocol specified laboratory result from tests performed after the first day of the investigational diet, which falls outside the laboratory reference range and meets the clinical significance criteria for liver and kidney tests as well as for hematology and clinical chemistry, etc. (i.e. AST and/or ALT > 2 x ULN).

This does not apply to any abnormal laboratory result which falls outside the laboratory reference range but which does not meet the clinical significance criteria or those which are a result of an AE which has already been reported.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being reported as an AE in the study record.

10.2 Treatment and Follow-Up of AEs And Laboratory Abnormalities

10.2.1 Treatment and Follow-up of AEs

AEs, especially those for which the relationship to the investigational diet is suspected, should be followed up until they have returned to baseline status or stabilized.

If after follow-up, return to baseline status or stabilization cannot be established, an explanation should be recorded in the study record.

10.2.2 Treatment and Follow-up of Laboratory Abnormalities

In the event of participant-initiated withdrawal or clinically significant unexplained abnormal laboratory test values, the participant will be withdrawn from the treatment and will remain in the study and be required to attend all remaining study visits as part of a safety arm.

10.3 Reporting of SAEs And Unexpected Adverse Reactions

The qualified investigator will be responsible for classification of an AE as an SAE within 24 hours of notification. Causality should be signed off by the qualified investigator prior to reporting to ethics and regulatory bodies. Notification of any serious adverse events must be made in writing to the study sponsor. The IRB will be notified of all diet related SAEs and unexpected adverse reactions. All blinded SAE's or unblinded-participant-on-active product SAE's will be reported to the Therapeutics Products Directorate (TPD) in an expedited manner.

KGK Science must notify the TPD of all blinded or unblinded-participant-on-active product serious adverse events and reactions as follows:

> If it is neither fatal or life threatening, within 15 calendar days after the day on which the sponsor becomes aware of the information; and
>
> If it is fatal or life threatening, must be reported as soon as possible, but not later than seven (7) days after the day on which the sponsor becomes aware of the information.

11 STATISTICAL EVALUATION

11.1 Determination of Sample Size

Because this is a pilot study to explore the efficacy and safety of Nic's Ketogenic Diet, sample size will not be calculated. A total of 14 participants will be enrolled.

11.2 Analysis Plan

The **Safety Population** will consist of all participants who start the diet, and on whom any safety information is available.

The **Intent-to-Treat (ITT) Population** will consist of all participants who start the diet and on whom efficacy information is available.

The **Per Protocol (PP) Population** will consist of all participants who comply with the diet , do not have any major protocol violations and complete all study visits and procedures connected with measurement of the primary variable.

11.3 Statistical Analysis Plan

All test of significance will be performed at 2-sided, alpha level = 0.05 unless otherwise specified.

All the primary and secondary endpoints will be analysed as continuous variables.

For each primary and secondary endpoint, descriptive statistics including number of subjects, arithmetic mean, standard deviation, median, minimum and maximum values will be presented for each study day and for the changes from baseline (Day 0) to each subsequent study day.

Changes in continuous endpoints from baseline will be calculated as:

$$Change\ to\ V_i = Value\ at\ V_i - Value\ at\ V_{baseline}$$

The continuous endpoints will be analysed with Mixed Model for Repeated Measures (MMRM). The model will include baseline value and visit as independent variables. P-values for the changes from baseline will be obtained from the model. No adjustment on p-values for multiple primary endpoints will be made.

Missing data for the primary and secondary endpoints will be imputed with last-observation-carried-forward (LOCF) method or multiple imputation as a sensitivity analysis for ITT Population. No imputation will be performed for PP and Safety Population.

11.3.1 Premature Discontinuation Description

For each premature discontinuation, the following parameters will be listed: participant number, dates of start and end of treatment, and the reason of premature discontinuation.

11.3.2 Safety

Adverse events (AEs) will be divided into pre-emergent and post-emergent AEs and presented separately as the number and percentage of subjects for each system organ class, preferred term, and lower level term. Furthermore, the lower level term, intensity, relationship, action taken, and outcome will be reported for each adverse event.

Continuous safety parameters (vital signs, anthropometrics, clinical chemistry, haematology) will be summarized with number of subjects, mean, standard deviation, median, minimum value, and maximum value for each measurement point.

11.4 Protocol Deviation Description

Protocol deviations will be listed in the final study report.

11.5 Protocol Amendments

Once the protocol has been approved by the IRB, any changes to the protocol must be documented in the form of an amendment. All amendments will be documented in the final study report.

12 DATA COLLECTION AND STORAGE

All data collection and record storage will be done in compliance with ICH GCP Guidelines and applicable local regulatory guidelines.

13 ETHICAL ASPECTS OF THE STUDY

This study will be conducted with the highest respect for the individual participants (i.e., participants) according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki, and the ICH Harmonised Tripartite Guideline for GCP.

13.1 IRB Approval

KGK Science Inc. will supply relevant documents for submission to an IRB for the protocol's review and approval. The following must be submitted to the IRB: this protocol, a copy of the informed consent form, and, if applicable, volunteer recruitment materials and/or advertisements and other documents required by all applicable laws and regulations. The IRB's written approval of the protocol and volunteer informed consent must be obtained before commencement of the study. The IRB approval must refer to the study by exact protocol title, number, and version date; identify versions of other documents (e.g., informed consent form) reviewed; and state the approval date.

KGK must adhere to all requirements stipulated by the IRB. This may include notification to the IRB regarding protocol amendments, updates to the informed consent form, recruitment materials intended for viewing by volunteers, local safety reporting requirements and submission of the investigator's annual/final status report to the IRB.

13.2 Volunteer Information and Informed Consent

Written consent documents will embody the elements of informed consent as described in the declaration of Helsinki and the ICH Guidelines for GCP and will be in accordance with all applicable laws and regulations. The informed consent form describes the planned and permitted uses, transfers, and disclosures of the volunteer's personal and personal health information for purposes of conducting the study. The informed consent form further explains the nature of the study, its objectives, and potential risks and benefits, as well as the date informed consent is obtained. The informed consent form will detail the requirements of the volunteer and the fact that he or she is free to withdraw at any time without giving a reason and without prejudice to his or her further medical care.

13.3 Potential Risks and Procedures to Minimize Risk

All potential risks are disclosed to study participants prior to their participation. The potential risks associated with this study include those associated with venipuncture and switching from a carbohydrate-rich diet to a ketogenic diet. Risks associated with venipuncture include pain, bruising, and infection at the site. Alcohol swabs and proper venipuncture procedure will be followed to minimize the risk of infection. The potential short-term side effects of the ketogenic diet are nausea, vomiting, headache, fatigue, dizziness, insomnia, and constipation.

14 QUALITY ASSURANCE AND QUALITY CONTROL

14.1 Auditing

All material used in clinical studies are subjected to quality control. Quality assurance audits may be performed by the sponsor or any health authority during the course of the study or after its completion.

The Investigator agrees to comply with the sponsor and regulatory requirements in terms of auditing of the study. This includes access to the source documents for source data verification.

14.2 Monitoring

An initiation meeting will be conducted by the sponsor or an approved representative (CRO). At this meeting, the protocol and logistical aspects of the study will be reviewed with the Investigator and all study staff.

Source documents will be reviewed to ensure that all items have been completed and that the data provided are accurate and obtained in the manner specified in the protocol. The participant files will be reviewed to confirm that:

1. Informed consent was obtained and documented
2. Enrolled participants fulfilled all inclusion criteria and did not meet any exclusion criteria;
3. AE/SAE reporting has been performed as applicable
4. Study visits have been conducted as per protocol and information has been recorded in the appropriate place in the source document

Incorrect, inappropriate, or illegible entries in the participant files will be returned to the Investigator or designee for correction. No data disclosing the identity of participants will leave the study center. The Investigator and any designees will maintain confidentiality of all participant records.

The Investigator will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspections and will allow direct access to source data and documents for these purposes.

14.3 Data Management

Data required for the analysis will be acquired from source documentation (including laboratory reports) and entered into Open Clinica Enterprise Version study instance designed specifically for this study. The two instances for the database would be created, test instance and production instance. A UAT (User Acceptance Testing) of the database would be performed in the test instance and then moved to the production instance. A password protected user id's will be created which would give access to the limited authorized personnel. Only properly trained Data management staff will be granted access to perform

database designing, according to SOP - Designing Database in Open Clinica Enterprise Version and SOP - Creating user in Open Clinica Enterprise Version. A study specific Data Management Plan will be generated after the finalization of the database.

The standard data validation and edit checks would be performed on the production instance of the study by designing study specific rules and restrictions as defined in the eCRF. The discrepancies will be queried and managed according to the SOP - Discrepancy Management SOP in Open Clinica Enterprise Version. Data tables will be created, queried and exported during and at the end of study using PostgreSQL tool (pgadminIII 9.5) and Microsoft Access.

For Statistical analysis, the validated soft lock copy of the blinded study database will be sent to the Statistician to perform the analysis. The study database would be a read only file to ascertain changes in the data are not made during or after the analysis.

High safety standards for the transfer and storage of study data are guaranteed by the use of technologies such as password protection, firewalls and periodic backup to protect stored data.

All study data is archived for a period not less than 25 years from the date of completion of the study in accordance with Health Canada regulatory requirements.

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16 APPENDIX

16.1 Handy Guide to Serving Size

Handy Guide to Serving Sizes

Learn how to use your hand to estimate Canada's Food Guide serving sizes and compare them to the food portions you eat.

Vegetables and Fruit: Canada's Food Guide recommends 7 to 10 servings of Vegetables and Fruit a day depending on your age and gender. Here's what a Food Guide serving looks like.

Fresh, frozen or canned vegetables
1/2 cup (125 mL) = 1/2 fist

Leafy vegetables
1 cup (250 mL) = 1 fist

Whole fruit
1 fruit = 1 fist

Fresh, frozen or canned fruit
1/2 cup (125 mL) = 1/2 fist

Dried fruit
1/4 cup (60 mL) = Cupped hand

100% fruit juice
1/2 cup (125 mL) = 1/2 fist

Grain Products: Canada's Food Guide recommends 6 to 8 servings of Grain Products a day depending on your age and gender. Here's what a Food Guide serving looks like.

Bread
1 slice = Size of hand

Bagel
1/2 small bagel = Size of hand

Rice
1/2 cup (125 mL) = 1/2 fist

Pasta
1/2 cup (125 mL) = 1/2 fist

Cold Cereal
30g = 1 fist

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Visit **www.unlockfood.ca/handyguide** to use the interactive version of the Handy Guide to Serving Sizes and watch videos to help you manage your food portions.

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Handy Guide to Serving Sizes

Learn how to use your hand to estimate Canada's Food Guide serving sizes and compare them to the food portions you eat.

Milk and Alternatives: Canada's Food Guide recommends 2 to 3 servings of Milk and Alternatives a day depending on your age. Here's what a Food Guide serving looks like.

Milk or fortified soy beverage
1 cup (250 mL) = 1 fist

Yogurt
3/4 cup (175 mL) = 1 fist

Cheese
1½ oz (50g) = 2 thumbs

Meat and Alternatives: Canada's Food Guide recommends 2 to 3 servings of Meat and Alternatives a day depending on your age and gender. Here's what a Food Guide serving looks like.

Meat and Poultry
2½ oz (75g) = Palm of hand

Fish
2½ oz (75g) = Palm of hand

Peanut butter
2 tbsp (30 mL) = 2 thumbs

Nuts and seeds
1/4 cup (60 mL) = Cupped hand

Legumes
3/4 cup (175 mL) = 1 fist

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Visit **www.unlockfood.ca/handyguide** to use the interactive version of the Handy Guide to Serving Sizes and watch videos to help you manage your food portions.

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Handy Guide to Serving Sizes

Find out how to manage your portions of these foods:

Fats: Canada's Food Guide recommends 2 - 3 tbsp of unsaturated fat each day and limiting the total amount of fat you eat. Here's what a tbsp and tsp look like.

Margarine or butter
1 tsp (5 mL) = 1 thumb tip
1 tbsp (15 mL) = 1 thumb

Oil
1 tsp (5 mL) = 1 thumb tip
1 tbsp (15 mL) = 1 thumb

Mayonnaise
1 tsp (5 mL) = 1 thumb tip
1 tbsp (15 mL) = 1 thumb

Sweet and Salty Foods: Canada's Food Guide recommends limiting foods that are high in sugar, salt and fat. These can add a lot of calories without a lot of nutrition.

Chocolate

If you enjoy chocolate, have a few pieces once in a while. Buy a small chocolate bar instead of a large one. Avoid buying a large bag of single serving chocolates.

Baked goods

Cookies, muffins, tarts, croissants, brownies and cake slices from bakeries and coffee shops are often high in calories. Buy one as a treat only once in a while and share it with a friend.

Salty snacks

Put a handful of chips, pretzels or nachos on a plate instead of eating out of the bag. This will help you control how much you eat. Try to avoid second helpings of salty snacks.

French fries

It's easy to overdo it when eating fries. At restaurants, ask for a small portion or share one plate of fries with friends.

Beverages: Canada's Food Guide recommends limiting beverages that are high in fat and sugar. These can add a lot of calories without a lot of nutrition.

Water

Quench your thirst with water. Add extra flavour with cucumber slices, mint, berries, lemon, lime or orange wedges.

Coffee

Sweetened coffee beverages can be high in sugar and fat. Ask for a plain latte or cappuccino with skim, 1% or 2% milk.

Pop

Try to avoid drinking pop. If you occasionally drink pop, limit how much you drink.

Fruit drinks

Fruit punch, fruit drink, fruit cocktail and fruit flavoured beverages are high in sugar and are not part of Canada's Food Guide. Limit or avoid these drinks.

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16.2 Dietary Guidelines

Dietary Guidelines

Please use the following guidelines to help you make meal and snack choices between study visits that are appropriate for the study.

Foods to Avoid

- All sweeteners other than Erythritol and Stevia
- Soybeans and soy products (tofu, tempeh, natto, edamame, soy milk, etc.)
- Grains
- Beans, peas and pulses
- Protein bars, shakes, and supplements
- Starchy vegetables (potato, yam, carrot, etc.)
- Low-fat dairy products
- Alcohol except spirits
- All fruits except berries listed below

Foods/Items to Limit

- Alcohol (no more than 2 standard servings of liquor/spirits per day)
    - Wine, beer, cocktails, coolers, ciders are all **not permitted**
    - Permitted: vodka, gin, whiskey, etc.
- Broccoli, cabbage, brussels sprouts
- Fruit such as strawberries, blueberries, and raspberries

Suggested Foods:

- Vegetables: *Green leafy and cruciferous vegetables.*
    - Lettuce
    - Arugula
    - Spinach
    - Mushrooms
    - Asparagus
    - Bok choy
    - Kale
    - Collard greens

- Meats: *Fattier sources of protein are encouraged. Watch out for added sugars.*
    - **Fish**: Wild caught fish such as catfish, cod, flounder, halibut, mackerel, mahi-mahi, salmon, snapper, trout, and tuna.

  - **Shellfish:** Clams, oysters, lobster, crab, scallops, mussels, and squid.
  - **Beef:** Ground beef, steak, roasts, and stew meat.
  - **Pork**: Ground pork, pork loin, pork chops, tenderloin, and ham.
  - **Poultry**: Chicken, duck, quail, pheasant and other wild game.
  - **Organ meats:** Heart, liver, kidney, and tongue.
  - **Other Meat**s: Veal, Goat, Lamb, Turkey and all kinds of wild game.
  - **Bacon and Sausage**: Check labels and avoid anything cured in sugar or containing artificial fillers.

- Fats and Oils: *Organic and grass-fed sources are always best.*
  - Lard
  - Tallow
  - Butter
  - Macadamia/Brazil Nuts
  - Butter/Ghee
  - Mayonnaise
  - Coconut Butter
  - Cocoa Butter
  - Olive Oil
  - Coconut Oil
  - Avocado Oil
  - Macadamia Oil
  - MCT Oil

- Eggs
- Fermented foods (sauerkraut, kimchi, lacto-fermented vegetables, etc.)
- Avocado
- High fat dairy (cheese, heavy cream, butter, etc.)