CLINICAL PROTOCOL COVER PAGE

Protocol Title:	A randomized, double-blind, comparator-controlled, cross-over study to investigate the safety and efficacy of RiaGev™ in healthy adults
Protocol Number:	19RNHB
Protocol Date:	October 7th, 2019
Version:	2
Study Design:	randomized, double-blind, comparator-controlled, cross-over study
Sponsor:	Bioenergy Life Science, Inc. 13840 Johnson St NE Ham Lake, MN 55304, USA
Sponsor Contact:	Alex Xue 763-746-3924 alex.xue@bioenergyls.com
CRO:	KGK Science Inc. Suite 1440, One London Place 255 Queens Ave London Ontario N6A 5R8 Canada 519-438-9374
Principal Investigator:	Trisha Shamp, PA-C, MPAS, PhD 1000 Westgate Drive Suite 149 Saint Paul, MN 55114 USA 651-641-2900

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: Alex Xue 763-746-3924 alex.xue@bioenergyls.com 13840 Johnson St NE Andover, MN 55304, USA		
Contract Research Organization: Malkanthi Evans, PhD Chief Scientific Officer KGK Science Inc. 255 Queens Ave., Suite 1440 London, ON N6A 5R8 Canada		

1 PROTOCOL SYNOPSIS

Population: Healthy male and females

Total number of participants: 18

1.1 Inclusion Criteria

1. Healthy male and females between the ages of 35 and 65 years of age, inclusive
2. BMI between 18.5 to 29.9 kg/m^2, inclusive
3. 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, complete endometrial ablation) or have been post-menopausal (natural or surgically) for at least 1 year prior to screening

   Or,

   Females of child-bearing potential must have a negative urine pregnancy test at screening and baseline and agree to use a medically approved method of birth control for the duration of the study. All hormonal birth control must have been in use for a minimum of three months. Acceptable methods of birth control include:
   - Hormonal contraceptives including oral contraceptives, hormone birth control patch (Ortho Evra), vaginal contraceptive ring (NuvaRing), injectable contraceptives (Depo-Provera, Lunelle), or hormone implant (Norplant System)
   - 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
4. Healthy as determined by laboratory results, medical history, physical exam, and EKG
5. Agrees to avoid supplementation with tryptophan and vitamin B3 or its derivatives (niacin, nicotinic acid, niacinamide) one week prior to randomization and during the study
6. Ability to complete maximal and submaximal exercise tests
7. Agrees to maintain current diet and activity level throughout the study
8. Agrees to comply to all study procedures
9. Has given voluntary, written, informed consent to participate in the study
10. Self-reported good sleeper at screening. Have a regular sleep cycle with a bedtime between the approximate hours of 9:00pm and 12:00am and regularly receive between 7-9 hours of sleep, and agrees to maintain this sleep schedule throughout the study

1.2 Exclusion Criteria

1. Women who are pregnant, breast feeding, or planning to become pregnant during the trial
2. Allergy or sensitivity to investigational product's ingredients or standard meal provided
3. Current or ex-smokers within the past year

4. Major surgery within the past 3 months which may impact the study outcomes to be assessed by the QI.
5. Untreated/unresolved/uncontrolled cardiovascular disease. Participants with no significant cardiovascular event in the past 1 year and on stable medication may be included after assessment by the QI on a case by case basis
6. Self reported current or pre-existing thyroid condition. Treatment on a stable dose medication for over 3 months will be reviewed on a case-by-case basis by the QI
7. Current or history of hypertension.
8. Type I or Type II diabetes
9. 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
10. Self reported of any autoimmune disease or immune-compromised
11. Self reported by subjects of being HIV or Hepatitis B/C positive
12. History or currently with kidney and liver diseases assessed by QI on a case by case basis, with the exception of history of kidney stones symptom free for 1 year
13. Known medical or psychological condition that, in the qualified investigator's opinion, could interfere with study participation
14. Significant gastrointestinal disease (examples include but are not limited to Celiac disease and inflammatory bowel disease)
15. Self reported of bleeding disorders.
16. Current diagnosis of gout within past three months as per the QI's assessment
17. Clinically significant abnormal laboratory results at screening as assessed by QI
18. Current use of prescribed medications or over the counter supplements that may interfere with the IP assessed by QI (See Section 7.3)
19. Alcohol consumption of >2 standard drinks/day or >14 drinks/week
20. Alcohol or drug abuse within the past 12 months
21. Use of medical marijuana
22. Frequent use of recreational drugs within 6 months of baseline assessed as per QI
23. Planned blood donation during or within 30 days following conclusion of clinical trial
24. Participation in other clinical research trials 30 days prior to baseline
25. Participants that are cognitively impaired and/or 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 pose significant risk to the participant.

1.3 Schedule of Assessments

	Period 1					Period 2			
Procedures/assessments	Visit 1 Screen	Visit 2 Day 1 (+1 Day)	Visit 3 Day 3 (+1 Day)	Visit 4 Day 5 (+1 Day)	Visit 5 Day 8 (+1 Day)	Visit 6 Day 1 (+1 Day)	Visit 7 Day 3 (+1 Day)	Visit 8 Day 5 (+1 Day)	Visit 9 Day 8 (+1 Day)
Informed consent	X								
Review inclusion/exclusion criteria	X	X							
Review medical history	X								
Review concomitant therapies	X	X	X	X	X	X	X	X	X
Vitals: Height*, weight, heart rate, blood pressure ***Height will only be measured at Visit 1***	X	X	X	X	X	X	X	X	X
Urine pregnancy test	X	X							
Electrocardiogram (EKG)	X								
Physical examination	X	X							X
Randomization		X							
CBC, electrolytes (Na, K, Cl), HbA1c*, fasting glucose, eGFR, creatinine, AST, ALT, and total bilirubin **HbA1c will only be measured at Visit 1*	X				X	X			X
Maximum heart rate (HR_{max}) treadmill test	X								
Skin fluoroscopy for NADH analysis (optional)		X			X	X			X
Blood Collection for NAD+ analysis		X	X	X	X	X	X	X	X
Blood Collection for GSH/GSSG and ATP/AMP analyses		X			X	X			X
OGTT: glucose and insulin T= 0, 15m, 30m, 45m, 60m, 90m, 2h		X			X	X			X
Questionnaire		X	X	X	X	X	X	X	X
Treadmill Exercise protocol		X			X	X			X
Salivary Collection Kit Dispensed	X	X	X	X	X	X	X	X	

Procedures/assessments	Visit 1 Screen	Visit 2 Day 1 (+1 Day)	Visit 3 Day 3 (+1 Day)	Visit 4 Day 5 (+1 Day)	Visit 5 Day 8 (+1 Day)		Visit 6 Day 1 (+1 Day)	Visit 7 Day 3 (+1 Day)	Visit 8 Day 5 (+1 Day)	Visit 9 Day 8 (+1 Day)
Salivary Collection Kit Returned		X	X	X	X		X	X	X	X
Food Records Dispensed	X	X	X	X	X		X	X	X	
Food Records Collected		X	X	X	X		X	X	X	X
IP Dispensed		X	X	X			X	X	X	
IP Returned			X	X	X			X	X	X
Subject Diary Dispensed	X	X	X	X	X		X	X	X	
Subject Diary Returned		X	X	X	X		X	X	X	X
Standardized Meal		X			X		X			X
Compliance Calculated			X	X	X			X	X	X
Adverse Events	X	X	X	X	X		X	X	X	X

7-day washout (+3 days)

2 LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATP	Adenosine triphosphate
BMI	Body Mass Index
BP	Blood Pressure
CBC	Complete Blood Count
CRF	Case Report Form
Cl	Chloride
DNA	Deoxyribonucleic acid
DOB	Date of Birth
EDTA	Ethylenediaminetetraacetic Acid
eGFR	Glomerular Filtration Rate
etc.	*"and so forth"*
e.g.	*"for example"*
et al	*"and others"*
FDA	US Food and Drug Administration
g	Gram
GCP	Good Clinical Practice
G-6-PDH	glucose-6-phosphate dehydrogenase
GSH	Glutathione
GSSG	Glutathione disulfide
GRAS	Generally Recognized as Safe
HDL	High-density lipoprotein
HR	Heart rate
lbs.	Pounds
ICF	Informed Consent Form
ICH	International Conference of Harmonization
IP	Investigational Product
IRB	Institutional Review Board
ITT	Intent-to-Treat
K	Potassium
kg	Kilogram
LDL	Low-density lipoprotein
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
Na	Sodium
NAD+	nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NR	nicotinamide riboside
OGTT	oral glucose tolerance test
OTC	Off-the-counters
PP	Per Protocol
PPP	pentose phosphate pathway
PRB	Population Reference Bureau

QI	Qualified Investigator
RBC	Red Blood Cells
RDW	Red Blood Cell Distribution Width
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SOP	Standard operating procedure
SST	Serum Separating Tube
UAT	User Acceptance Testing
WBC	White Blood Cell
WHO	World Health Organization

3 TABLE OF CONTENTS

4 INTRODUCTION

Nicotinamide adenine dinucleotide (NAD+) is one of the essential cofactors required for the proper function of living cells, and depletion in NAD has been correlated to ageing individuals as NAD is associated with oxidative stress and energy production (1). Per the Population Reference Bureau (PRB), it is estimated that by the year 2060, the number of Americans over the age of 65 will double to over 98 million (2). As well, over the years, there has been a continuous rise in obesity within older Americans, reaching 44% for women and 36% for men in the age range of 65-74 (2). One of the most common chronic diseases that are accompanied by ageing and obesity diabetes. In 2016 the WHO reported that approximately 1.6 million deaths were attributed to diabetes. Half of these individuals had high blood glucose before the age of 70 (3). Hence it is crucial to actively control blood glucose and oxidative stress during one's midlife stage.

The investigating product RiaGev™ is the first and only commercially available product that contains Bioenergy Ribose® and vitamin B3 (4). It increases NAD+ in the body efficiently to promote healthy mitochondria, active immunity and cholesterol reduction. As a result, D-ribose is essential for healthy ageing (4).

Bioenergy Ribose® is a 5-carbon carbohydrate ($C_5H_{10}O_5$) called D-ribose designated as a Generally Recognized as Safe (GRAS) substance by the US Food and Drug Administration (FDA) (5). It is produced via the pentose phosphate pathway (PPP), which is fundamental for adenosine triphosphate (ATP) production (6). The PPP is a rate-limiting step that makes use of a short supply enzyme called glucose-6-phosphate dehydrogenase (G-6-PDH) (6). Supplementation of D-ribose can bypass the PPP and directly contribute to ATP production (6). In addition, to its function for ATP production D-ribose is a critical element of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and acetyl coenzyme A (6). Provided there is a reduction in ATP production; ageing is frequently due to a decline in mitochondria function. Hence, cell function and integrity are compromised, leading to chronic cardiovascular conditions and fatigue (6). With active D-ribose supplementation, improvements have been noted in several pathological conditions such as chronic fatigue syndrome, fibromyalgia, and myocardial dysfunction (6). Furthermore, D-ribose demonstrated improvements in athletic performances by recovering ATP levels and repairing cellular damage (6).

Vitamin B3 is an essential water-soluble vitamin known as either niacin, nicotinic acid, or nicotinamide. It is found in foods such as chicken, beef, fish, nuts, legumes, and grains (7). Also, vitamin B3 can be obtained from conversions of tryptophan in the body. Therefore, foods with tryptophan such as milk, eggs, meat and fish are another great source of vitamin B3 (7,8). Once vitamin B3 is consumed, it is converted into two different active forms called NAD+ or nicotinamide adenine dinucleotide phosphate (NADP) (7,8). NAD+ and NADP are essential for various metabolic redox processes with oxidized or reduced substrates. Cellular functions like genome integrity, gene expression, and cellular communication are carried out by NAD+ required enzymes (7). These required enzymes are also crucial for the production of ATP via energy transfer from carbohydrates, fats, and proteins (7). NADP is involved in fewer reactions than NAD+ such as cholesterol and fatty acid synthesis along with antioxidation (7). Lack of NAD+ has been

associated with a variety of ageing related conditions such as metabolic syndrome, cardiovascular health, and cancer (1).

This current randomized, double-blind, comparator-controlled, cross over study will investigate the efficacy and safety of RiaGev™ via evaluation of NAD+, glucose and insulin, in healthy adults of ages 36-65.

5 STUDY OBJECTIVES

The objective of this study is to evaluate the safety and efficacy of RiaGev™ in healthy adults

Primary outcome:

The change in whole blood NAD+ levels from baseline to day 8 when supplemented with RiaGev™ or comparator.

Secondary outcomes:

1. The change in serum glucose and insulin as assessed by an Oral Glucose Tolerance Test (OGTT) at t=0, 15m, 30, 45m, 60m, 90m and 2h after a 7-day supplementation with either RiaGev™ or comparator.
2. The change in serum Glutathione/Glutathione disulfide (GSH/GSSG) ratio after a 7-day supplementation with either RiaGev™ or comparator
3. The change in serum adenosine triphosphate/ adenosine monophosphate (ATP/AMP) ratio after a 7-day supplementation with either RiaGev™ or comparator
4. The change in salivary cortisol after a 7-day supplementation with either RiaGev™ or comparator
5. The change in Checklist Individual Strength (CIS) Questionnaire outcome after a 7-day supplementation with either RiaGev™ or comparator.

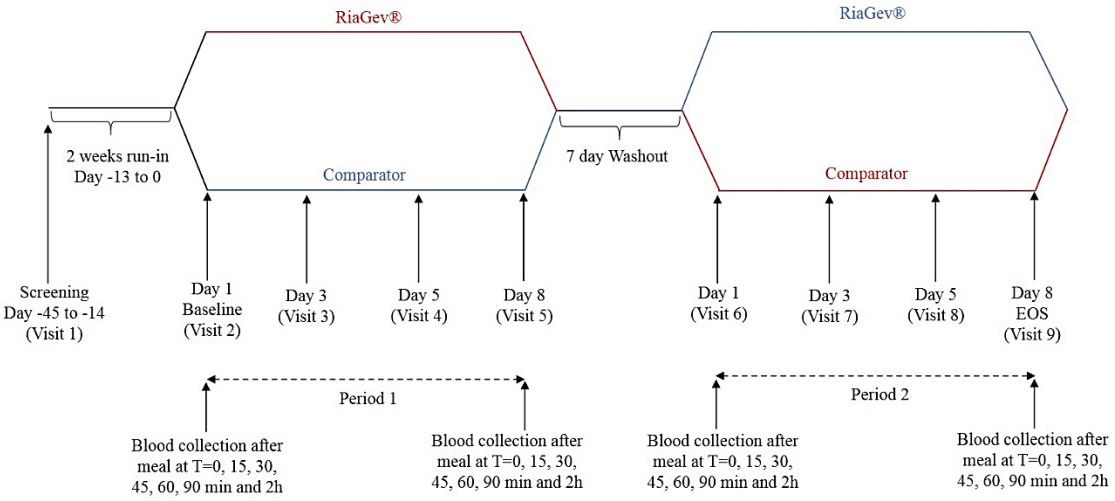
Safety outcomes:

1. Incidence of pre-emergent and post-emergent adverse events when supplemented vital signs (blood pressure (BP) and heart rate (HR)) when supplemented
2. clinical chemistry (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, creatinine, electrolytes (Na, K, Cl), fasting glucose and estimated glomerular filtration rate (eGFR))
3. 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)

(Optional) Exploratory Analysis:

1. The change in NADH before and after exercise, measured with skin fluorescence, after a 7-day supplementation with either RiaGev™ or comparator.

6 STUDY DESIGN



This will be a randomized, double-blind, comparator-controlled, crossover, safety and efficacy study conducted at Prism Clinical Research Clinic, Saint Paul, MN, USA, a KGK Science Inc. partner.

The planned sample size for this study is 18, crossing over once is equivalent to 36 participants. To evaluate the outcomes, study assessments will be conducted as per the schedule of assessments in section 1.3

Study Arm	**Number of Participants**
RiaGev™ →Comparator	N = 9
Comparator→ RiaGev™	N = 9
Total	N = 18

7 SELECTION OF STUDY POPULATION

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. Healthy male and females between the ages of 35 and 65 years of age, inclusive
2. BMI between 18.5 to 29.9 kg/m^2, inclusive
3. 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, complete endometrial ablation) or have been post-menopausal (natural or surgically) for at least 1 year prior to screening

   Or,

   Females of child-bearing potential must have a negative urine pregnancy test at screening and baseline and agree to use a medically approved method of birth control for the duration of the study. All hormonal birth control must have been in use for a minimum of three months. Acceptable methods of birth control include:
   - Hormonal contraceptives including oral contraceptives, hormone birth control patch (Ortho Evra), vaginal contraceptive ring (NuvaRing), injectable contraceptives (Depo-Provera, Lunelle), or hormone implant (Norplant System)
   - 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
4. Healthy as determined by laboratory results, medical history, physical exam and EKG
5. Agrees to avoid supplementation with tryptophan and vitamin B3 or its derivatives (niacin, nicotinic acid, niacinamide) one week prior to randomization and during the study
6. Ability to complete maximal and submaximal exercise tests
7. Agrees to maintain current diet and activity level throughout the study
8. Agrees to comply to all study procedures
9. Has given voluntary, written, informed consent to participate in the study
10. Self-reported good sleeper at screening. Have a regular sleep cycle with a bedtime between the approximate hours of 9:00pm and 12:00am and regularly receive between 7-9 hours of sleep, and agrees to maintain this sleep schedule throughout the study

7.2 Exclusion Criteria

1. Women who are pregnant, breast feeding, or planning to become pregnant during the trial
2. Allergy or sensitivity to investigational product's ingredients or standard meal provided
3. Current or ex-smokers within the past year
4. Major surgery within the past 3 months which may impact the study outcomes to be assessed by the QI.

5. Untreated/unresolved/uncontrolled cardiovascular disease. Participants with no significant cardiovascular event in the past 1 year and on stable medication may be included after assessment by the QI on a case by case basis
6. Self reported current or pre-existing thyroid condition. Treatment on a stable dose medication for over 3 months will be reviewed on a case-by-case basis by the QI
7. Current or history of hypertension.
8. Type I or Type II diabetes
9. 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
10. Self reported of any autoimmune disease or immune-compromised
11. Self reported by subjects of being HIV or Hepatitis B/C positive
12. History or currently with kidney and liver diseases assessed by QI on a case by case basis, with the exception of history of kidney stones symptom free for 1 year
13. Known medical or psychological condition that, in the qualified investigator's opinion, could interfere with study participation
14. Significant gastrointestinal disease (examples include but are not limited to Celiac disease and inflammatory bowel disease)
15. Self reported of bleeding disorders.
16. Current diagnosis of gout within past three months as per the QI's assessment
17. Clinically significant abnormal laboratory results at screening as assessed by QI
18. Current use of prescribed medications or over the counter supplements that may interfere with the IP assessed by QI (See Section 7.3)
19. Alcohol consumption of >2 standard drinks/day or >14 drinks/week
20. Alcohol or drug abuse within the past 12 months
21. Use of medical marijuana
22. Frequent use of recreational drugs within 6 months of baseline assessed as per QI
23. Planned blood donation during or within 30 days following conclusion of clinical trial
24. Participation in other clinical research trials 30 days prior to baseline
25. Participants that are cognitively impaired and/or 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 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. Warfarin (anticoagulant)
2. Clonidine and other antihypertensive drugs
3. Selective estrogen receptor modulator
    - Tamoxifen
    - Raloxifene
    - Toremifene
4. Antidepressants like Selective serotonin re-uptake inhibitors (SSRI)
    - Citalopram
    - Fluoxetine
    - Fluvoxamine
    - Paroxetine
    - Sertraline
5. Oral corticosteroids (immunosuppressant)
6. Isoniazid
7. Pyrazinamide

7.3.2 Over-the-counter Medications, Supplements

Participants who are currently consuming the following supplements will not be allowed to participate unless willing to undergo the specified washout period prior to their baseline visit and agree not to take the supplements during the study. Other supplements will require a case-by-case assessment by the QI based on dose and/or frequency of use to determine adequate washout.

1. Vitamin B3 derivatives supplements (7-day washout)
2. Tryptophan supplements (7-day washout)
3. Chromium supplements (7-day washout)

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 PRODUCT

8.1 Manufacturing and Storage

The investigational product will be provided to Prism Clinical by the Sponsor. The investigational product will be carefully stored at the study site in a lockable, limited access area, accessible only to study team personnel in compliance with pertinent regulations. Only authorized persons will have access to the investigational product. In an ambient condition the product will be stored at room temperature and will not be exposed to direct sunlight or heat. The product and placebo will be provided in single use sachet, both blinded in label. The investigational products will be kept in a locked investigational product storage room at Prism Clinical on receipt. An accountability log will be kept for the investigational products.

All unused investigational product will be returned to the study sponsor by Prism Clinical (at the sponsor's expense) or destroyed on receipt of written confirmation from the sponsor at study closeout (within one month of last participant visit).

Manufactured by:

Bioenergy Life Science, Inc.

8.2 Labelling and Coding

The investigational product will be labeled according to the requirements of ICH-GCP guidelines and applicable local regulatory guidelines. Investigational product will be randomized and coded by an unblinded person at KGK Science Inc. who is not involved in data collection or analysis.

8.3 Investigational Product (RiaGev™)

Dietary Ingredient	Quantity (2 g/sachet, in 3 capsules)
Bioenergy Ribose® (D-ribose)	1280mg
Vitamin B3 (Nicotinamide)	240mg

Non-medical ingredients: 480mg Palm oil

8.4 Comparator

Ingredients: 480mg Palm oil and 1280mg Dextrose, packaged in 3 capsules in a sachet

8.5 Directions

Participants will be instructed to take two doses of the IP daily, once in the morning and once in the evening. Each dose will be 3 capsules, one dose will be administered immediately before breakfast and the other dose will be administered immediately before dinner. In both study periods, on day 1 only the evening dose will be administered and on day 8 only morning dose will be administered. The Clinic staff will instruct participants to save all unused and open packages and return them to Prism Clinical for a

determination of compliance. If a dose is missed participants are instructed to consume the missed dose anytime on the same day, except at bedtime. Participants will be advised not to exceed two servings daily.

8.6 Randomization

A randomization schedule will be created and provided to the Investigator indicating the order of randomization. Each participant will be assigned a randomization code according to the order of the randomization list generated using www.randomization.com. Enrolled participants will be randomized to the different treatment arms at day 1 in a double-blind manner.

8.7 Unblinding and Allocation Concealment

Unblinding should not occur except in the case of emergency situations. In the event that a serious adverse event occurs, for which the identity of the investigational product administered is necessary to manage the participant's condition, the treatment received by the participant will be unblinded and the investigational product identified. Concealment of the allocation of treatment will be employed through the use of opaque sealed envelopes, each labeled with a randomization number. Each envelope will contain information regarding the treatment associated with each randomization number. These envelopes will be readily available for the investigator to open in the event that it becomes necessary to know which product a participant is taking for the sake of the participant health care. The sponsor must be notified of any unblinding within 24 hours. Details of participants who are unblinded during the study will be included in the Final Report.

9 STUDY ASSESSMENTS

** 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 randomization at baseline.*

9.1 Visit 1 – Screening (Day -45 to Day -14) *

Participants will show up at the clinic in a fasted state (8h) for visit 1. 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 provided with the option of taking 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. Each volunteer will be sequentially assigned a screening number to be entered in the screening and enrollment log.

Screening assessments include:

1. Review medical history, concomitant therapies and current health status
2. Assess inclusion and exclusion criteria
3. Urine pregnancy test for female potential participants that are of child-bearing potential
4. Seated resting blood pressure and heart rate measurements
5. Weight and height measurements (BMI calculation)
6. Physical exam
7. EKG
8. Collect fasted blood samples for the analysis of Complete Blood Count (CBC), HbA1c, electrolytes (Na, K, Cl), fasting glucose, eGFR, creatinine, AST, ALT, and total bilirubin
9. Complete maximum heart rate treadmill test (Section 9.12.6)
10. Dispense 3-Day Food Record and sleep diary
11. Dispense salivary collection kit and instruct participants on use (Section 9.12.5)

The next visit will be scheduled for potentially eligible participants for baseline (Day 1).

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

1. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food/drinks as per Section 7.3.2
2. Complete and return the Food record for the three days prior to baseline visit for review
3. Maintain current level of physical activity and maintain and record sleep schedule in diary
4. Participants will be advised follow the dietary guidelines (see appendix 15.3)
5. Participants will collect baseline salivary cortisol the morning of their Baseline Visit (Section 9.12.5)

9.2 Run-in period – 2 weeks (Day -13 to Day 0)

Participants will undergo a 14 days run-in period.

Participants are reminded to follow the dietary guidelines 15.3, avoid the consumption of the medications and supplements as per 7.3.1 and 7.3.2, collect saliva the morning of the next in-clinic visit, maintain current level of physical activity and maintain and record sleep schedule.

9.3 Visit 2 - Baseline (period 1- Day 1+1 Day)

Eligible participants will return to the clinic fasted (8h) with completed saliva kit, sleep diary and food diary for baseline assessments.

Baseline (Day 1) assessments include:

1. Return completed salivary cortisol collection kit
2. Review concomitant therapies
3. Assess inclusion and exclusion criteria
4. Urine pregnancy test for female potential participants that are of child-bearing potential
5. Seated resting blood pressure and heart rate measurements
6. Review AEs
7. Weight measurements (BMI calculation)
8. Physical exam
9. Randomization of eligible participants
10. Collect Food Record and sleep diary for review
11. Collect blood sample at T=0 (pre-meal) for fasting serum glucose, insulin
12. Standardized meal provided (see Appendix 15.2)
13. Collect post-meal blood sample for serum glucose and insulin analysis at:
    a. T= 15min
    b. T= 30min
    c. T= 45min
    d. T= 60min
    e. T= 90min
    f. T= 2hrs
14. Collect blood sample at T=2 hours (post-meal) for NAD+
15. (Optional) Measure NADH with skin fluorescence
16. Complete treadmill exercise protocol (Section 9.12.7)
17. (Optional) Measure NADH with skin fluorescence
18. Collect blood sample for GSH/GSSG and ATP/AMP analysis
19. Administer CIS questionnaire
20. Dispense investigational product and instruct participants on use. Participants will be instructed to take their first dose of the investigational product in evening of Day 1.
21. Dispense saliva collection kit
22. Dispense 7-Day Food Record and subject sleep and treatment diary

The next visit will be scheduled for visit 3 (period 1- Day 3)

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

1. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food/drinks as per Section 7.3.2
2. Maintain current level of physical activity and sleep schedule

3. Complete and return the food record, sleep and treatment diary
4. Participants will be advised to follow the dietary guidelines (see appendix 15.3)
5. Collect saliva in the morning 15 minutes after waking and prior to consumption of food
6. Return to clinic one hour after consuming investigational product/placebo and breakfast on the next visit day

9.4 Visit 3 - (period 1- Day 3+1 Day)

Participants will return to the clinic one hour after consuming investigational product/placebo for Visit 3 assessments with unused investigational product, completed Subject sleep and treatment diary, saliva kit and food records.

Visit 3 assessments include:

1. Review concomitant therapies
2. Seated resting blood pressure and heart rate measurements
3. Weight measurements (BMI calculation)
4. Return unused investigational product in the original packaging and remnants and calculate compliance by counting the returned unused investigational product
5. Return saliva collection kit
6. Review AEs
7. Collect food record and subject sleep and treatment diary for review
8. Collect blood sample for NAD+ analysis
9. Administer CIS questionnaire
10. Re-dispense 7-Day Food Record
11. Dispense Subject sleep and treatment Diary
12. Dispense new saliva collection kit
13. Dispense new investigational product and instruct participants on use

The next visit will be scheduled for visit 4 (period 1- Day 5)

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

1. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food/drinks as per Section 7.3.2
2. Maintain current level of physical activity and sleep schedule
3. Complete and return the food record and sleep and treatment diary
4. Participants will be advised to follow the dietary guideline (see Appendix 15.3)
5. Collect saliva in the morning 15 minutes after waking and prior to consumption of food
6. Return to clinic one hour after consuming investigational product/placebo and breakfast on the next visit day

9.5 Visit 4 – (period 1- Day 5+1 Day)

Participants will return to the clinic one hour after consuming investigational product/placebo for Visit 4 assessments with unused investigational product, completed Subject sleep and treatment diary, saliva kit and food records.

Visit 4 assessments include:

1. Review concomitant therapies
2. Seated resting blood pressure and heart rate measurements
3. Weight measurements (BMI calculation)
4. Return unused investigational product in the original packaging and remnants and calculate compliance by counting the returned unused investigational product
5. Return saliva collection kit
6. Review AEs
7. Collect food record and subject sleep and treatment diary for review
8. Collect blood sample for NAD+ analysis
9. Administer CIS questionnaire
10. Re-dispense 7-Day Food Record
11. Dispense Subject sleep and treatment Diary
12. Dispense saliva collection kit
13. Dispense new investigational product and instruct participants on use

The next visit will be scheduled for visit 5 (period 1- Day 8)

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

1. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food/drinks as per Section 7.3.2
2. Maintain current level of physical activity and sleep schedule
3. Complete and return the food record and sleep and treatment diary
4. Participants will be advised to follow the dietary guideline (see appendix 15.3)
5. Collect saliva in the morning 15 minutes after waking and prior to consumption of food
6. Return to clinic in a fasting state (8h), without taking IP on the next visit day

9.6 Visit 5 - (period 1- Day 8+1 Day)

Participants will return to the clinic fasted (8h) for Visit 5 assessments with unused investigational product, completed Subject sleep and treatment diary, saliva kit, and food records.

Visit 5 (Day 6) assessments include:

1. Review concomitant therapies
2. Seated resting blood pressure and heart rate measurements
3. Weight measurements (BMI calculation)
4. Return unused investigational product in the original packaging and remnants and calculate compliance by counting the returned unused investigational product
5. Return saliva collection kit
6. Review AEs
7. Collect Food Record and Subject sleep and treatment diary for review
8. Collect blood sample at T=0 (pre-meal) for fasting serum glucose, insulin, CBC, electrolytes (Na, K, Cl), eGFR, creatinine, AST, ALT, total bilirubin
9. Administer the investigational product
10. Standardized meal provided (see Appendix 15.2)
11. Collect post-meal blood sample for serum glucose and insulin analysis at:
    a. T= 15min

   b. T= 30min
   c. T= 45min
   d. T= 60min
   e. T= 90min
   f. T= 2 hours
12. Collect blood sample at T=2 hours (post-meal) for NAD+
13. (Optional) Measure NADH with skin fluorescence
14. Complete treadmill exercise protocol (Section 9.12.7)
15. (Optional) Measure NADH with skin fluorescence
16. Collect blood sample for GSH/GSSG and ATP/AMP
17. Administer CIS questionnaire
18. Dispense 7-Day Food Record and Subject sleep and treatment Diary
19. Dispense saliva collection kit

The next visit will be scheduled for visit 6 (period 2- Day 1)

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

1. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food/drinks as per Section 7.3.2
2. Maintain current level of physical activity and sleep schedule
3. Complete the food record for three days before next study visit and return the food record and sleep and treatment diary
4. Participants will be advised to follow the dietary guideline (see appendix 15.3)
5. Participants will collect baseline salivary cortisol the morning of their Baseline Visit (Section 9.12.5)

9.7 Washout period (Day 9 to Day 15+3 Days)

Participants will undergo a 7-day washout prior to visit 6.

Participants are reminded to follow the dietary guidelines 15.3, avoid the consumption of the medications and supplements as per 7.3.1 and 7.3.2, collect saliva the morning of the next in-clinic visit, and maintain current level of physical activity and maintain and record sleep schedule.

9.8 Visit 6 – Baseline (period 2- Day 1+1 Day)

Participants will return to the clinic fasted (8h) for Visit 6 assessments with completed Subject sleep and treatment diary, saliva kit and food records.

Visit 6 (Day 1) assessments include:

1. Review concomitant therapies
2. Seated resting blood pressure and heart rate measurements
3. Weight measurements (BMI calculation)
4. Collect Food Record and Subject sleep and treatment diary for review

5. Return saliva collection kit
6. Review AEs
7. Collect blood sample at T=0 (pre-meal) for fasting serum glucose, insulin, CBC, electrolytes (Na, K, Cl), eGFR, creatinine, AST, ALT, total bilirubin
8. Standardized meal provided (see Appendix 15.2)
9. Collect post-meal blood sample for serum glucose and insulin analysis at:
    a. T= 15min
    b. T= 30min
    c. T= 45min
    d. T= 60min
    e. T= 90min
    f. T= 2hrs
10. Collect blood sample at T=2 hours (post-meal) for NAD+
11. (Optional) Measure NADH with skin fluorescence
12. Complete treadmill exercise protocol (Section 9.12.7)
13. (Optional) Measure NADH with skin fluorescence
14. Collect blood sample for GSH/GSSG and ATP/AMP analysis
15. Administer CIS questionnaire
16. Dispense 7-Day Food Record and subject sleep and treatment diary
17. Dispense saliva collection kit
18. Dispense new investigational product and instruct participants on use

The next visit will be scheduled for visit 7 (period 2- Day 3)

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

1. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food/drinks as per Section 7.3.2
2. Maintain current level of physical activity and sleep schedule
3. Collect saliva in the morning 15 minutes after waking and prior to consumption of food
4. Complete and return the food record and sleep and treatment diary
5. Participants will be advised to follow the dietary guideline (see appendix 15.3)
6. Return to clinic one hour after consuming investigational product/placebo on the next visit day

9.9 Visit 7 - (period 2- Day 3+1 Day)

Participants will return to the clinic for Visit 7 assessments one hour after consuming investigational product/placebo with unused investigational product, completed Subject sleep and treatment diary, saliva kit and food records.

Visit 7 assessments include:

1. Review concomitant therapies
2. Seated resting blood pressure and heart rate measurements
3. Weight measurements (BMI calculation)
4. Return unused investigational product in the original packaging and remnants and calculate compliance by counting the returned unused investigational product
5. Return saliva collection kit

6. Review AEs
7. Collect food record and subject sleep and treatment diary for review
8. Collect blood sample for NAD+ analysis
9. Administer CIS questionnaire
10. Re-dispense 7-Day Food Record
11. Dispense Subject sleep and treatment diary
12. Dispense saliva collection kit
13. Dispense new investigational product and instruct participants on use

The next visit will be scheduled for visit 8 (period 2- Day 5)

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

1. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food/drinks as per Section 7.3.2
2. Maintain current level of physical activity and sleep schedule
3. Collect saliva in the morning 15 minutes after waking and prior to consumption of food
4. Complete and return the food record and sleep and treatment diary
5. Participants will be advised to follow the dietary guideline (see appendix 15.3)
6. Return to clinic one hour after consuming investigational product/placebo on the next visit day

9.10 Visit 8 - (period 2- Day 5+1 Day)

Participants will return to the clinic for Visit 8 assessments one hour after consuming investigational product/placebo with unused investigational product, completed Subject sleep and treatment diary, saliva kit, and food records.

Visit 8 assessments include:

1. Review concomitant therapies
2. Seated resting blood pressure and heart rate measurements
3. Weight measurements (BMI calculation)
4. Return unused investigational product in the original packaging and remnants and calculate compliance by counting the returned unused investigational product
5. Return saliva collection kit
6. Review AEs
7. Collect food record and subject sleep and treatment diary for review
8. Collect blood sample for NAD+ analysis
9. Administer CIS questionnaire
10. Re-dispense 7-Day Food Record
11. Dispense Subject sleep and treatment diary
12. Dispense saliva kit
13. Dispense new investigational product and instruct participants on use

The next visit will be scheduled for visit 9 (period 2- Day 8)

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

1. Abide by instructions for medications as per Section 7.3.1 and OTC medications, supplements, and food/drinks as per Section 7.3.2

2. Maintain current level of physical activity and sleep schedule
3. Collect saliva in the morning 15 minutes after waking and prior to consumption of food
4. Complete and return the food record and sleep and treatment diary
5. Participants will be advised to follow the dietary guideline (see appendix 15.3)
6. Return to clinic in a fasting state (8h), without taking IP on the next visit day

9.11 Visit 9 – End of the Study (period 2- Day 8+1 Day)

Participants will return to the clinic fasted (8h) for Visit 9 assessments with unused investigational product, completed Subject sleep and treatment diary, saliva kit, and food records.

Visit 9 assessments include:

1. Review concomitant therapies
2. Seated resting blood pressure and heart rate measurements
3. Weight measurements (BMI calculation)
4. Return unused investigational product in the original packaging and remnants and calculate compliance by counting the returned unused investigational product
5. Physical Exam
6. Collect Food Record and Subject sleep and treatment diary for review
7. Return saliva collection kit
8. Review AEs
9. Collect blood sample at T=0 (pre-meal) for fasting serum glucose, insulin, Complete Blood Count (CBC), electrolytes (Na, K, Cl), eGFR, creatinine, AST, ALT, total bilirubin
10. Administer the investigational product
11. Standardized meal provided (see appendix 15.2)
12. Collect post-meal blood sample for serum glucose and insulin analysis at:
    a. T= 15min
    b. T= 30min
    c. T= 45min
    d. T= 60min
    e. T= 90min
    f. T= 2hrs
13. Collect blood sample at T=2 hours (post-meal) for NAD+
14. (Optional) Measure NADH with skin fluorescence
15. Complete treadmill exercise protocol (Section 9.12.7)
16. (Optional) Measure NADH with skin fluorescence
17. Collect blood sample for GSH/GSSG and ATP/AMP Analyses
18. Administer CIS questionnaire

9.12 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.

9.12.1 Height and Weight

Weight measurements will be performed with shoes removed and bladder empty. Participants will be weighed on the same scale 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.

9.12.2 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 five 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. As 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.

9.12.3 Blood Sample Collection

Blood samples will be collected during visit 2, visit 5, visit 6, and visit 9 as per the procedures for outlined below.

Participants will be placed in a comfortably seated position with their arm fully extended and supported by a pillow. Aa tourniquet will be applied to the desired arm 3-4 inches above the elbow or 4-5 inches above the wrist to facilitate locating a vein. Once chosen, the site will be cleansed with 70% isopropyl alcohol, allowed to dry, and the IV catheter will be inserted piercing the skin and into the vein to the desired depth. With the catheter in place, the positive displacement connector will be attached to the blood collection hub, a patch will be placed over the entire system, and the access device will be connected to the positive displacement connector. The vacutainer tube will then be allowed to fill as per the Order of Draw and Fill Level Chart. When required, the filled tube will be disengaged, and another tube engaged. After disengaging, each tube will be inverted gently a number of times as per the Order of Draw and Fill Level Chart. This collection process will be repeated for the remaining blood collection time points until the number of blood draws had been satisfied. The IV catheter will be kept patent with a saline. To remove the IV catheter, the patch will be peeled back, and the tape will be peeled back to ensure the system is not removed from the connector. A cotton ball will be placed at the puncture site, and the system removed in a swift motion. Pressure will be applied to the site for a minimum of 30 seconds.

Once the clotting has occurred, a bandage/cotton ball and tape will be applied over the puncture site. Alternatively, if blood drawn via the catheter is unsuccessful, direct venipuncture will be conducted.

Samples will be collected as per the following procedures for venipuncture at visit 1, visit 3, visit 4, visit 7 and visit 8.

Participants will be placed in a comfortably seated position with their arm fully extended and supported by a pillow. All appropriate tubes, needle, alcohol and cotton balls will be retrieved. A tourniquet will be applied to the desired arm 3-4 inches above the elbow or 4-5 inches above the wrist and the participant will be requested to open and close their fist a few times to facilitate locating a vein. The vein will be gently pressed to determine its direction, approximate size and depth. The tourniquet will be removed, and the chosen site will be cleansed with 70% isopropyl alcohol and cotton ball or alcohol pad. The site will be allowed to dry, and the tourniquet will be reapplied, and the participant will be required to make a tight fist. The vacutainer system (needle placed in the holder) will be held and the vein will be anchored. The needle will be inserted into the skin and vein at 30o angle. The vacutainer tube will then be allowed to fill as per the *Order of Draw* and *Fill Level Chart*. When required, the filled tube will be disengaged, and another tube engaged. After disengaging, each tube will be inverted gently a number of times as per the *Order of Draw* and *Fill Level Chart*. The tourniquet will be removed when the last collection tube is half full. A cotton ball will be placed over the site, and the needle will be removed in a swift motion. Pressure will be applied to the site for a minimum of 30 seconds. Once the clotting has occurred, a bandage/cotton ball and tape will be applied over the puncture site

9.12.4 Checklist Individual Strength Questionnaire (CIS) Questionnaire

The Checklist Individual Strength Questionnaire is a validated questionnaire for healthy working population (Appendix 15.5). This is a 20 items questionnaire scored on a 7-point scale measuring subjective fatigue experience, reduced concentration, reduced motivation and reduced physical activity level. The Questionnaire will be administered to participants on visit 2 to visit 8.

9.12.5 Saliva Sample Collection

Participants will collect salivary cortisol samples using the Salivette collection device. Saliva sample will be collected within 15 minutes of waking and prior to eating on the mornings of all visits, except screening (Visit 1). To ensure proper collection, participants will be provided instruction by Prism clinical site.

9.12.6 Maximum Heart Rate Treadmill Test

Participants will complete a graded treadmill test following a ramped Bruce protocol (9,10) at their screening visit (Appendix 15.4). Participants will continue to advance stages until volitional exhaustion by the participant. Throughout the test, participants heart rate will be monitored and recorded with a chest strap heart rate monitor. Successful competition of the test will be achieving 85% or greater of age predicted maximum heart rate (220-age) (9).

9.12.7 Treadmill Exercise

Participants will determine their max heart rate (HR) in the clinic at visit 1 (screening, Day 0) via graded treadmill test with a chest strap heart rate monitor (Section 9.12.6). Eligible participants will return and exercise on the treadmill at visit 2 (period 1, Day 1), visit 5 (period 1, Day 8), visit 6 (period 2, Day 1), and visit 9 (period 2, Day 8). On the day of the exercise, participants will be instructed to warm up on the treadmill first for five minutes at a leisurely walking pace. When the participants are ready, speed will be increased to 60% of participant's HRmax with 5% grade incline and they will be instructed to walk on the treadmill for 30 minutes or until exhaustion (11).

9.12.8 Skin Fluorescence (Optional)

The AngioExpert device may be optionally employed to evaluate the 460 nm fluorescence of the skin in response to activation by the 340nm UV light. The wavelength of 340 nm emitted in the FMSF method is only specific to NADH, thus no other substance in the skin can be excited. The AngioExpert will be used to continuously measure the 460 nm fluorescence from the most superficial skin cells in the forearm at rest, then during controlled ischemia triggered by total occlusion of the brachial artery by the brachial blood pressure cuff, and, finally, during reperfusion after deflation of the blood pressure cuff (12).

9.12.9 Food Records

Participants will be asked to record their food consumption during the study. This data will be used to calculate and analyze their daily calories, macronutrient and micronutrient intake throughout the study using a software called Nutritics. The food records will be reviewed by trained staff at the visits and participants will be counseled with dietary suggestions as required. All participants will be provided with instructions on how to complete their food records.

9.13 Compliance

Compliance will be assessed by counting the returned unused study product at each visit. Compliance is calculated by determining the number of dosage units taken divided by the number of dosage units expected to have been taken multiplied by 100.

$$\frac{number\ of\ dosage\ units\ taken}{number\ of\ dosage\ units\ expected\ to\ have\ been\ taken} \times 100\%$$

In the event of discrepancy between the information in the treatment diary and the amount of study product returned, use will be based on the product returned unless an explanation for loss of product has been provided. Participants found to have a compliance of <80% or >120% will be counseled. A compliance of <70% or >130% will be considered as non-compliant and any participant demonstrating non-compliance for two consecutive visits will be withdrawn from the study.

9.14 Laboratory Analyses

Blood samples will be drawn from the participants at screening (Visit 1, Day 0), baseline (Visit 2, Day 1), V3, V4, V5, V6, V7, V8, and at the End of Study visit (V9) 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 or the product they received.

At screening (Visit 1), 8 hour fasting:

1. Two EDTA vacutainer tubes to generate whole blood for:
   a. CBC analysis
   b. Hb-A1c analysis

2. One SST vacutainer tube to generate serum for:
   a. electrolytes (Na, K, Cl), fasting glucose, eGFR, creatinine, AST, ALT, and total bilirubin analysis

At baseline (Visit 2)

1. One BD vacutainer tube to generate whole blood for:
   a. NAD+

2. Two SST vacutainer tubes to generate Pre-Prandial serum for:
   a. Fasting Glucose analysis
   b. Fasting Insulin analysis

3. One BD vacutainer tube to generate whole blood for:
   a. ATP/AMP analysis
   b. GSH/GSSG analysis

4. Twelve SST vacutainer tubes to generate serum for:

a. Fasting Glucose analysis (one per time point tube)
b. Fasting Insulin analysis (one per time point tube)

Visit 3:

1. One BD vacutainer tubes to generate whole blood for:
   a. NAD+

Visit 4:

1. One BD vacutainer tubes to generate whole blood for:
   a. NAD+

Visit 5

1. One BD vacutainer tube to generate whole blood for:
   a. NAD+

2. Two SST vacutainer tubes to generate Pre-Prandial serum for:
   a. electrolytes (Na, K, Cl), eGFR, creatinine, AST, ALT, and total bilirubin, fasting Glucose and Fasting Insulin analysis

3. One BD vacutainer tube to generate whole blood for:
   a. ATP/AMP analysis
   b. GSH/GSSG analysis

4. Twelve SST vacutainer tube to generate Post-Prandial serum for:
   a. Fasting Glucose analysis (one per time point tube)
   b. Fasting Insulin analysis (one per time point tube)

5. One EDTA vacutainer tubes to generate whole for:
   a. CBC analysis

Visit 6

1. One BD vacutainer tube to generate whole blood for:
   a. NAD+

1. Two SST vacutainer tubes to generate Pre-Prandial serum for:

   a. electrolytes (Na, K, Cl), eGFR, creatinine, AST, ALT, and total bilirubin, fasting Glucose and Fasting Insulin

2. Twelve SST vacutainer tube to generate Post-meal serum for:

   a. Fasting Glucose analysis (one per time point tube)
   b. Fasting Insulin analysis (one per time point tube)

3. One BD vacutainer tube to generate whole blood for:
   a. ATP/AMP analysis
   b. GSH/GSSG analysis

4. One EDTA vacutainer tubes to generate whole blood for:
    a. CBC analysis

Visit 7

1. One BD vacutainer tube to generate whole blood for:
    a. NAD+

Visit 8

1. One BD vacutainer tube to generate whole blood for:
    a. NAD+

At the end of the study (Visit 9)

1. One BD vacutainer tube to generate whole blood for:
    a. NAD+

2. Two SST vacutainer tube to generate Pre-Prandial serum for:
    a. electrolytes (Na, K, Cl), eGFR, creatinine, AST, ALT, and total bilirubin, fasting Glucose and Fasting Insulin analysis

3. One BD vacutainer tube to generate whole blood for:
    a. ATP/AMP analysis
    b. GSH/GSSG analysis

4. Twelve 8.5mL SST vacutainer tube to generate Post-Prandial serum for:
    a. Fasting Glucose analysis (one per time point tube)
    b. Fasting Insulin analysis (one per time point tube)

5. One EDTA vacutainer tubes to generate whole blood for:
    a. CBC analysis

The total blood volume collection for the laboratory assessments listed above will be approximately 250 mL, over the period from screening to end of study (approximately 20 days). At any study visit, blood loss per volunteer is not expected to exceed 131.5 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.

HCMC is the central laboratories that will be used in this study to measure blood parameters

University of Washington is the laboratory that will be used for NAD, GHS/GSSG and ATP/AMP analyses in this study.

Urine pregnancy test will be performed at the Prism clinical site.

9.15 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.

9.16 Protocol Amendments

If amendments to the study protocol are required after approval such changes will be captured in writing the reasons for the change documented and 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 product 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 product, whether or not it is considered related to that product. 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 product to the adverse event will be assessed by the qualified investigator as either:

Most probable:	There is a reasonable relationship between the investigational product and AEs. The event responds to withdrawal of investigational product (dechallenge) and recurs with rechallenge when clinically feasible.
Probable:	There is a reasonable relationship between the investigational product and AEs. The event responds to dechallenge.
Possible:	There is a reasonable relationship between the investigational product and AEs. Dechallenge information is lacking or unclear.
Unlikely:	There is a temporal relationship to the investigational product administration but there is no reasonable causal relationship between the investigational product and the AEs.
Not related:	There is no temporal relationship to investigational product administration or there is a reasonable causal relationship between non-investigational product, 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 product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

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 product
3. Requiring a change in concomitant therapy

This applies to any protocol and non-protocol specified laboratory result from tests performed after the first dose of the investigational product, 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 product 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 product related SAEs and unexpected adverse reactions.

11 STATISTICAL EVALUATION

11.1 Determination of Sample Size

The planned total sample size will be 18 with 9 participants randomized to each treatment schedule. This sample size will enable detection of a difference of 8.7 pMol/mg protein in NAD+ concentration between the two treatments at an overall 5% significance level and 80% power. This sample size takes into account a loss to follow up rate of 20%.

This calculation is based on a critical difference in NAD+ concentration, between NR and placebo of 6.1 pMol/mg protein and a standard deviation of 11.04 units reported in a study by Martens et al. (2018)(13).

11.2 Analysis Plan

The **Safety Population** will consist of all participants who received any amount of either product, and on whom any post-randomization safety information is available.

The **Intent-to-Treat (ITT) Population** consists of all participants who received either product and on whom any post-randomization efficacy information is available.

The **Per Protocol (PP) Population** consists of all participants who consumed at least 80% of treatment or placebo doses, 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

The primary outcome is the change in whole blood NAD+ levels from baseline to day 6 compared between RiaGev™ and comparator.

An effectiveness analysis based on the intent-to-treat population and an efficacy analysis based on the per protocol population will be performed. Safety analysis will be done for the safety population.

Variables will be tested for normality and transformed to the log scale if found to be non-normally distributed. Log-normally distributed variables will be analyzed in the logarithmic domain. Intractably non-normal outcome variables will be analyzed by appropriate non-parametric tests.

Missing values in the intent-to-treat and per protocol analyses will be imputed using the most recent previously-available value (LOCF, or "last-observation-carried-forward" imputation) or multiple imputation methods. No imputation will be performed for missing safety values.

For each continuous endpoint, a summary table will be prepared with a variety of summary statistics including mean, standard deviation, median and range values for each time point-treatment pairing. To account for any a priori differences between groups, the summary statistics of the changes from baseline will also be provided. For parameters requiring a logarithmic transformation, the summary statistics will be provided as non-transformed values. Mean values will be displayed as graphs, with a separate line for each product, and error bars indicating standard errors or standard deviations. Mean changes from baseline will be graphed similarly. Although imputed data will be used for significance testing, non-imputed data will be used for presenting summaries. Categorical variables will be summarized as counts and percentages.

Continuous efficacy endpoints will be tested for significance between groups by Analysis of Covariance (ANCOVA). The dependent variable will be the measurement at each visit, the factor will be the treatment group, and the value at baseline (Day 1) will be the covariate. A within group analysis on effectiveness/efficacy endpoints will be done using the Student's paired t-test or, in the case of intractable non-normality, the Wilcoxon sign rank test. Categorical variables will be compared using Chi-squared or Fisher's Exact tests as appropriate.

Probabilities < 0.05 will be considered statistically significant. All statistical analysis will be completed using the R Statistical Software Package Version 3.5.2 (R Core Team, 2018) for Microsoft Windows.

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

For adverse events, a descriptive analysis will be given. Adverse events will be presented in a frequency table by category and treatment. Furthermore, description, frequency, severity and causality will be reported for each adverse event.

Continuous safety parameters (e.g. hematology, clinical chemistry, heart rate and blood pressure) will be summarized using a table including mean, standard deviation, median, minimum value, and maximum value for each measurement point. The changes from baseline will also be summarized similarly.

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. Existing, modified, and newly developed eCRFs (electronic case report forms) and clearly defined instructions for their correct use will be used to gather the study data. Quality assurance will be conducted on the database prior to collecting the data (anticipating problems with data collection), and quality control activities (such as recognition, monitoring and troubleshooting) will be conducted during and after the data is collected to ensure the integrity and validity of study results. The data will be handled and stored in OpenClinica Enterprise Version 4 (a cloud-based electronic case report form compliant with the industry regulations) and MS Access database.

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

Prism Clinical Research and 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.

Prism Clinical Research and 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 venipuncture and the associated risks. 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.

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
5. The study product is being stored correctly and an accurate record of its dispensation to the study participants is being maintained (accountability)

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 OpenClinica Enterprise Version 4 (OC4) study instance designed specifically for this study. The database design starts by revising the finalized study protocol and source documents. eCRFs (electronic case report form) will be created and coded as per study protocol and source documents. The two environments for the database will be created: test instance and production instance. A UAT (User Acceptance Testing) of the database will be performed in the test environment. The database will be reviewed thoroughly to ensure that all datapoints have been completed and that the data collected is accurate and obtained as specified in the study protocol. After conducting this quality control on content, functionality and validation checks, the database will be approved be moved to the production environment. A password protected user ID's will be created which will give access to the limited and trained authorized

personnel. Only properly trained Data management staff will be granted access to perform database designing, according to SOP PC 115 GCP - Designing Database in OpenClinica Enterprise Version and SOP PC 114 GCP - Creating user in OpenClinica Enterprise Version. A study specific Data Management Plan will be generated after the finalization of the database.

The standard data validation and edit checks will be performed on the production environment of the study by designing study specific validation checks and restrictions as per study protocol. Automatic and manually generated discrepancies will be queried and managed according to the SOP PC 116 GCP - Discrepancy Management SOP in OpenClinica Enterprise Version 4. Data will be extracted and tables will be created, queried and exported during and at the end of study using PostgreSQL tool (pgadminIII 9.5) and MS 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 will 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 2 years from the date of completion of the study in accordance with ICH Harmonised Guideline for Good Clinical Practice.

REFERENCE LIST

1. Aman Y, Qiu Y, Tao J, Fang EF. Therapeutic potential of boosting NAD+ in aging and age-related diseases. Transl Med Aging. 2018 Jan;2:30–7.

2. Mark Mather, Linda A. Jacobsen, Kelvin M. Pollard. Aging Baby Boomers to Face Caregiving, Obesity, Inequality Challenges [Internet]. population reference bureau; 2016 Jan. Available from: https://www.prb.org/unitedstates-population-bulletin/

3. Diabetes [Internet]. world health organization; 2018 Oct. Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes

4. RiaGev® [Internet]. bioenergy life science Inc.; Available from: http://bioenergylifescience.com/#products

5. Compound Summary for CID 10975657 D-Ribose [Internet]. National Center for Biotechnology Information; [cited 2019 Feb 28]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/D-ribopyranose#section=Food-Additives-and-Ingredients

6. Mahoney DE, Hiebert JB, Thimmesch A, Pierce JT, Vacek JL, Clancy RL, et al. Understanding D-Ribose and Mitochondrial Function. Adv Biosci Clin Med. 2018 Feb 13;6(1):1.

7. Niacin Fact Sheet for Health Professionals [Internet]. National Institutes of Health; 2019. Available from: https://ods.od.nih.gov/factsheets/Niacin-HealthProfessional/#h9

8. Meyer-Ficca M, Kirkland JB. Niacin. Adv Nutr. 2016 May 1;7(3):556–8.

9. Will PM, Walter JD. Exercise testing: Improving performance with a ramped Bruce protocol. Am Heart J. 1999 Dec;138(6):1033–7.

10. Milani RV, Lavie CJ, Mehra MR, Ventura HO. Understanding the Basics of Cardiopulmonary Exercise Testing. Mayo Clin Proc. 2006 Dec;81(12):1603–11.

11. Dickman JR, Koenig RT, Ji LL. American Ginseng Supplementation Induces an Oxidative Stress in Postmenopausal Women. 2009;28(2):219–28.

12. Bugaj O, Zieliński J, Kusy K, Kantanista A, Wieliński D, Guzik P. The Effect of Exercise on the Skin Content of the Reduced Form of NAD and Its Response to Transient Ischemia and Reperfusion in Highly Trained Athletes. Front Physiol. 2019 May 15;10:600.

13. Martens CR, Denman BA, Mazzo MR, Armstrong ML, Reisdorph N, McQueen MB, et al. Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD+ in healthy middle-aged and older adults. Nat Commun [Internet]. 2018 Dec [cited 2018 Dec 3];9(1). Available from: http://www.nature.com/articles/s41467-018-03421-7

15 APPENDICES

15.1 Appendix I: Schedule of Assessments

	Period 1					Period 2			
Procedures/assessments	Visit 1 Screen	Visit 2 Day 1 (+1 Day)	Visit 3 Day 3 (+1 Day)	Visit 4 Day 5 (+1 Day)	Visit 5 Day 8 (+1 Day)	Visit 6 Day 1 (+1 Day)	Visit 7 Day 3 (+1 Day)	Visit 8 Day 5 (+1 Day)	Visit 9 Day 8 (+1 Day)
Informed consent	X								
Review inclusion/exclusion criteria	X	X							
Review medical history	X								
Review concomitant therapies	X	X	X	X	X	X	X	X	X
Vitals: Height*, weight, heart rate, blood pressure ***Height will only be measured at Visit 1***	X	X	X	X	X	X	X	X	X
Urine pregnancy test	X	X							
Electrocardiogram (EKG)	x								
Physical examination	X	X							X
Randomization		X							
CBC, electrolytes (Na, K, Cl), HbA1c*, fasting glucose, eGFR, creatinine, AST, ALT, and total bilirubin **HbA1c will only be measured at Visit 1*	X				X	X			X
Maximum heart rate (HR_{max}) treadmill test	X								
Skin fluoroscopy for NADH analysis (optional)		X			X	X			X
Blood Collection for NAD+ analysis		X	X	X	X	X	X	X	X
Blood Collection for GSH/GSSG and ATP/AMP analyses		X			X	X			X
OGTT: glucose and insulin T= 0, 15m, 30m, 45m, 60m, 90m, 2h		X			X	X			X
Questionnaire		X	X	X	X	X	X	X	X

Procedures/assessments	Visit 1 Screen	Visit 2 Day 1 (+1 Day)	Visit 3 Day 3 (+1 Day)	Visit 4 Day 5 (+1 Day)	Visit 5 Day 8 (+1 Day)		Visit 6 Day 1 (+1 Day)	Visit 7 Day 3 (+1 Day)	Visit 8 Day 5 (+1 Day)	Visit 9 Day 8 (+1 Day)
Treadmill Exercise protocol		X			X		X			X
Salivary Collection Kit Dispensed	X	X	X	X	X		X	X	X	
Salivary Collection Kit Returned		X	X	X	X		X	X	X	X
Food Records Dispensed	X	X	X	X	X		X	X	X	
Food Records Collected		X	X	X	X		X	X	X	X
IP Dispensed		X	X	X			X	X	X	
IP Returned			X	X	X			X	X	X
Subject Diary Dispensed	X	X	X	X	X		X	X	X	
Subject Diary Returned		X	X	X	X		X	X	X	X
Standardized Meal		X			X		X			X
Compliance Calculated			X	X	X			X	X	X
Adverse Events	X	X	X	X	X		X	X	X	X

7-day washout (+3 days)

15.2 Standardized Meal Items

A menu for the standardized meal will be provided to the participants at visits 2, 5, 6 and 9. Participants should be encouraged to consume the entire meal. All food that is not eaten is to be weighed and recorded. Participants will then be provided with an equivalent meal at their next visit.

Standardized Food Menu

BREAKFAST (standardized, before blood collection at t=2 h)
¼ cup blueberries
1 medium banana
¾ cup plain Greek yogurt
½ tbsp honey
¼ cup granola
1 slice whole grain toast
1 tbsp almond butter
1 x Babybel original cheese portion
250 ml decaffeinated coffee
\water, *ad libitum* water will be provided

15.3 Dietary Guideline

Participant Initials: _____ Participant Number: _____

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:

- Energy drinks, Vitamin Water, and sports drinks
- Yeast spread (vegemite, marmite, etc.)
- French bread
- Condensed tomato soup and related products
- Ready-to-eat cereals
- Game meat
- Turkey

Foods to Limit (No more than 1 serving per day):

- Dairy products (milk, cheese, yogurt)
- Beans/legumes/lentils
- Soybeans/soy products
- Poultry (chicken, duck etc.)
- Red meat (beef, veal, lamb, pork)
- Organ meats
- Fish, shellfish and crustaceans
- All commercial and homemade beers, and homemade wines

Suggested Breakfast Options

- Fried eggs with white toast and breakfast potatoes/hash browns
- Fruit salad
- Yogurt with fruit (no granola)

Suggested Lunch Options

- Quinoa salad with bell peppers and corn
- Oven-baked chicken strips with roasted potatoes
- Garden salad with grilled chicken or hard-boiled egg

Suggested Dinner Options

- Vegetable stir fry with steamed rice
- Beef, chicken, or vegetable tacos with corn tortillas (not flour)
- Baked chicken or pork with broccoli and potatoes

Suggested Snack Options

- Apple with nut butter
- Fruit salad
- Celery and carrot sticks with dressing

15.4 Maximum Heart Rate Treadmill Test Protocol (9)

Table I. Ramped Bruce protocol

Duration	Stage segment	Speed (mph)	Grade (%)	Speed (m/min)	Exercise $\dot{V}o_2$	Rest $\dot{V}o_2$	Total $\dot{V}o_2$	Total METs
	1	1	0	26.67	2.7	3.5	6.2	1.8
	2	1	1	26.67	3.1	3.5	6.6	1.9
	3	1	2	26.67	3.6	3.5	7.1	2
	4	1.1	3	29.33	4.5	3.5	8	2.3
	5	1.2	4	32	5.5	3.5	9	2.6
	6	1.2	5	32	6.1	3.5	9.6	2.7
	7	1.2	5	32	6.1	3.5	9.6	2.7
	8	1.3	6	34.67	7.2	3.5	10.7	3.1
	9	1.4	7	37.33	8.4	3.5	11.9	3.4
	10	1.5	8	40	9.8	3.5	13.3	3.8
	11	1.6	9	42.67	11.2	3.5	14.7	4.2
3 min	12	1.7	10	45.33	12.7	3.5	16.2	4.6
	13	1.8	10	48	13.4	3.5	16.9	4.8
	14	1.8	10	48	13.4	3.5	16.9	4.8
	15	1.9	11	50.67	15.1	3.5	18.6	5.3
	16	1.9	11	50.67	15.1	3.5	18.6	5.3
	17	2	11	53.33	15.9	3.5	19.4	5.5
	18	2	11	53.33	15.9	3.5	19.4	5.5
	19	2.1	12	56	17.7	3.5	21.2	6.1
	20	2.2	12	58.67	18.5	3.5	22	6.3
	21	2.3	12	61.33	19.4	3.5	22.9	6.5
	22	2.4	12	64	20.2	3.5	23.7	6.8
	23	2.5	12	66.67	21.1	3.5	24.6	7
6 min	24	2.5	12	66.67	21.1	3.5	24.6	7
	25	2.5	12	66.67	21.1	3.5	24.6	7
	26	2.6	13	69.33	23.2	3.5	26.7	7.6
	27	2.6	13	69.33	23.2	3.5	26.7	7.6
	28	2.7	13	72	24	3.5	27.5	7.9
	29	2.8	13	74.67	24.9	3.5	28.4	8.1
	30	2.9	14	77.33	27.2	3.5	30.7	8.8
	31	3	14	80	28.2	3.5	31.7	9
	32	3.1	14	82.67	29.1	3.5	32.6	9.3
	33	3.2	14	85.33	30	3.5	33.5	9.6
	34	3.3	14	88	31	3.5	34.5	9.9
	35	3.4	14	90.67	31.9	3.5	35.4	10.1
9 min	36	3.4	14	90.67	31.9	3.5	35.4	10.1
	37	3.5	14	93.33	32.9	3.5	36.4	10.4
	38	3.5	14	93.33	32.9	3.5	36.4	10.4
	39	3.6	15	96	35.5	3.5	39	11.1
	40	3.6	15	96	35.5	3.5	39	11.1
	41	3.7	15	98.67	36.5	3.5	40	11.4
	42	3.8	15	101.33	37.5	3.5	41	11.7
	43	3.9	16	104	40.4	3.5	43.9	12.5
	44	4	16	106.67	41.4	3.5	44.9	12.8
	45	4	16	106.67	41.4	3.5	44.9	12.8
	46	4.1	16	109.33	42.4	3.5	45.9	13.1
	47	4.2	16	112	43.5	3.5	47	13.4
12 min	48	4.2	16	112	43.5	3.5	47	13.4
	49	4.2	16	112	43.5	3.5	47	13.4
	50	4.3	16	114.67	44.5	3.5	48	13.7
	51	4.3	17	114.67	46.6	3.5	50.1	14.3
	52	4.4	17	117.33	47.6	3.5	51.1	14.6
	53	4.4	17	117.33	47.6	3.5	51.1	14.6
	54	4.5	17	120	48.7	3.5	52.2	14.9
	55	4.6	18	122.67	52	3.5	55.5	15.9
	56	4.7	18	125.33	53.1	3.5	56.6	16.2
	57	4.8	18	128	54.3	3.5	57.8	16.5
	58	4.9	18	130.67	55.4	3.5	58.9	16.8
	59	5	18	133.33	56.5	3.5	60	17.2
15 min	60	5	18	133.33	56.5	3.5	60	17.2

15.5 Checklist Individual Strength Questionnaire

********* CIS20R *********
Checklist Individual Strength
University Hospital Nijmegen
Department of Medical Psychology

Instruction:
On the next page you find 20 statements. With these statements we wish to get an impression of how you have felt during the past two weeks. For example:

I feel relaxed

If you feel that this statement is not true at all, place a cross in the right box; like this:

I feel relaxed	**yes**, that is true	X							**no**, that is not true

If you feel that this statement is not true at all, place a cross in the right box; like this:

I feel relaxed	**yes**, that is true							X	**no**, that is not true

If you feel that this statement in not "yes, that is true", but also not "no, that is not true", place a cross in the box that is most in accordance with how you have felt.
For example, if you feel relaxed, but not very relaxed, place a cross in one of the boxes close to "yes, that is true": like this:

I feel relaxed	**yes**, that is true			X					**no**, that is not true

Do not skip any statement and place only one cross for each statement.

1. I feel tired	yes, that is true								no, that is not true
2. I feel very active	yes, that is true								no, that is not true
3. Thinking requires effort	yes, that is true								no, that is not true
4. Physically I feel exhausted	yes, that is true								no, that is not true
5. I feel like doing all kinds of nice things	yes, that is true								no, that is not true
6. I feel fit	yes, that is true								no, that is not true
7. I do quite a lot within a day	yes, that is true								no, that is not true
8. When I am doing something, I can concentrate quite well	yes, that is true								no, that is not true
9. I feel weak	yes, that is true								no, that is not true
10. I don't do much during the day	yes, that is true								no, that is not true
11. I can concentrate well	yes, that is true								no, that is not true
12. I feel rested	yes, that is true								no, that is not true
13. I have trouble concentrating	yes, that is true								no, that is not true
14. Physically I feel I am in a bad condition	yes, that is true								no, that is not true
15. I am full of plans	yes, that is true								no, that is not true
16. I get tired very quickly	yes, that is true								no, that is not true
17. I have a low output	yes, that is true								no, that is not true
18. I feel no desire to do anything	yes, that is true								no, that is not true
19. My thoughts easily wander	yes, that is true								no, that is not true
20. Physically I feel in a good shape	yes, that is true								no, that is not true

SCORING CIS20R
For the items: **2, 5, 6, 7, 8, 11, 12, 15, 20** is the scoring as follows:

	yes, that is true	1	2	3	4	5	6	7	**no**, that is not true

For the items: **1, 3, 4, 9, 10, 13, 14, 16, 17, 18, 19** is the scoring as follows:

	yes, that is true	7	6	5	4	3	2	1	**no**, that is not true

Subsequently **the four subscales** are calculated by summing the respective items

subscale 1: Subjective feeling of fatigue	items 1, 4, 6, 9, 12, 14, 16, 20
subscale 2: Concentration	items 3, 8, 11, 13, 19
subscale 3: Motivation	items 2, 5, 15, 18
subscale 4: Physical activity	items 7, 10, 17