a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

Faculty of Health and Social Development
School of Health and Exercise Sciences
3333 University Way
Kelowna, BC V1V 1W5

Title: The effect of acute exogenous oral ketone supplementation on blood glucose levels in type 2 diabetes

Research Question: Does exogenous ketone supplementation impact glucose levels in people with type 2 diabetes?

Objectives

a) To determine whether acute ketone supplementation impacts plasma glucose levels compared to a placebo.
b) To observe blood ketone levels after ingestion of an acute dose of exogenous ketones.
c) To compare changes in insulin concentrations and C-peptide metabolism after acute ketone supplementation versus a placebo.
d) To determine whether acute ketone supplementation alters non-esterified fatty acids (NEFA) and inflammatory cytokines
e) To determine influence of acute ketone supplementation in cerebral blood flow (CBF), brain-derived neurotrophic factor (BDNF), and cognitive function
f) To evaluate influence of acute ketone supplements in histone acetylation and Beta-hydroxybutyrate(BHB)-ylation in peripheral blood mononuclear cells (PBMC) /leukocytes.
g) To determine whether acute ketone supplementation influences appetite and satiety levels and causes any gastrointestinal (GI) distress

Hypothesis:

a) Exogenous ketone supplementation will lower blood glucose compared to a placebo
b) When compared to placebo, exogenous ketone supplementation will lower NEFA concentration and suppress inflammation by decreasing inflammatory markers
c) Exogenous ketone supplementation will increase neurovascular coupling, improve cognitive function, and increase BDNF compared to placebo
d) Compared to placebo, exogenous ketone supplements will increase histone acetylation and in peripheral blood mononuclear cells (PBMC) /leukocytes
e) Participants will report greater satiety over the observation period following ketone supplementation compared to placebo

Background Information

Approximately one in four Canadians have type 2 diabetes (T2D) or prediabetes, conditions which are threatening to bankrupt healthcare systems in Canada and globally [1]. Existing measures can work well in controlled environments, but drugs are costly, require prescriptions and have side-effects, while diet-induced weight

Faculty of Health and Social Development
School of Health and Exercise Sciences
3333 University Way
Kelowna, BC V1V 1W5

loss and regular exercise require precise management and have adherence issues.
Previously, increases in blood ketones have been shown to reduce blood glucose but therapeutic application was limited due to the need for ketone infusion or severe dietary restrictions. The recent discovery and commercialization of exogenous oral ketone supplements represents a nutritional breakthrough that now allows someone to raise blood ketone levels by consuming a nutritional supplement in liquid form. In three previous UBC CREB approved experiments (H16-01846), we have shown that acute ingestion of the oral ketone monoester ((R)-3-hydroxybutyl (R)-3-hydroxybutyrate) immediately lowers glucose and improves glucose tolerance in healthy humans and individuals at risk for T2D [2] . The logical next step in this research is to examine the impact of oral β-OHB supplements on blood glucose levels in T2D. With CIHR Bridge Grant funding the overall aim of this project is to test the glucose-lowering effects of oral ketone monoester supplement in humans with T2D.

In addition, β-hydroxybutyrate appears to protect cells from inflammation and oxidative stress triggers. The direct effect of β-hydroxybutyrate on inflammation has been tested in recent cell culture and animal-based studies. The results show that elevating β-OHB can not only influence innate immune cell signalling, but can attenuate pro-inflammatory activity, which has been shown to play a major role in the perpetuation of inflammation associated with various diseases, including T2D [3]. Whether oral ketone supplements can similarly suppress inflammation in humans with T2D has not been tested.

Ketones also provide an alternative fuel for the brain and have been shown in animal studies to enhance cognitive function [4]. The mechanism(s) appear related to enhanced metabolism of ketones by the brain [5], increased blood flow to the brain [6], or elevated levels of BDNF [7]. No studies have tested whether exogenous ketones can improve brain blood flow, cognitive function or BDNF in humans.

Therefore, nutritional ketosis induced by supplementing with exogenous oral ketones is an intriguing therapeutic strategy, particularly for individuals with T2D who have elevated glucose, increased inflammation, and altered brain function. Further investigation is necessary to explore the potential metabolic effects of ketone supplementation in people with T2D. Given our past findings and expertise in this area, we are poised to conduct this urgently needed research now.

Faculty of Health and Social Development
School of Health and Exercise Sciences
3333 University Way
Kelowna, BC V1V 1W5

Research Method

Experimental design

A randomized placebo-controlled, cross-over pilot study in which 20 adults with type 2 diabetes will participate in two experimental trials. All participants will provide written informed consent during an initial familiarization visit.

Following screening, eligible participants (N=20) will participate in two experimental trials of β-OHB supplementation versus placebo with order of treatment balanced, separated by ~7 days. Pre-menopausal women will be tested in the follicular phase (days 3-9) to minimize any potential changes in insulin sensitivity across the menstrual cycle [8]. Participants will be asked to refrain from structured exercise and alcohol, and limit physical activity to their activities of daily living on the day before each trial. A standardized diet with ~50% energy from carbohydrate, ~30% fat, ~20% protein (energy based on Harris-Benedict equation with an activity factor of 1.4) will be oriented on the day before each trial. Participants will then fast overnight (8-10h) and refrain from taking any medications 24 hours before the morning of the trial. Upon arrival, an indwelling intravenous catheter will be inserted and a baseline fasting blood sample will be obtained. The oral β-OHB supplement or placebo will be consumed in liquid form (~20-25 mL). Both drinks are masked with a proprietary calorie-free strawberry flavour developed by H.V.M.N®, USA, which results in the same taste profile for each. The β-OHB supplement will provide 0.3 g/kg β-OHB in the form of the ketone monoester (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate (**Δ**G®, TDeltaS, Oxford, UK). We used this dose in our supporting pilot study and published work shows it can consistently raise blood β-OHB levels to ~2-3 mmol/L within 15-30 minute[9] [2] [10]. Post consumption of the β-OHB or placebo blood samples will be obtained at 30, 60, 90,120, 150 and 180 minutes.

The primary outcomes will be total plasma glucose as represented by the 3-hour area under the curve. Secondary outcomes include plasma insulin and C-peptide levels, plasma free fatty acids (FFA), inflammatory cytokines, cerebral blood flow (CBF), cognitive function, BDNF, signalling molecules, self reported hunger and fullness by a Visual Analog Scale (VAS) and GI symptom questionnaire scores – all measured across 180 minutes post-supplementation.

Metabolite and hormone analyses

Blood samples will be collected into EDTA tubes and processed using established procedures in our lab [11] [12] [13] [14]. Blood β-OHB levels will be measured using the Abbot Freestyle Precision Neo® ketone meter. Plasma will be collected by centrifugation and will be stored at -80°C for batch analyses of glucose, insulin, FFA and cytokines as we have described [11] [12]. C-peptide will be measured by ELISA (Millipore #EZHCP-20K) in order to further characterize the impact of oral β-OHB on insulin secretion. Peripheral blood mononuclear cells will be isolated to measure histone acetylation and inflammatory signalling. Histones acetylation will be quantified with flow cytometry using the Histone H3 Acetylation Assay Kit

Faculty of Health and Social Development
School of Health and Exercise Sciences
3333 University Way
Kelowna, BC V1V 1W5

(Millipore/Sigma Aldrich, CA). Inflammatory pathways and signalling will be assessed by standard western blotting techniques.

The study will involve 2 visits to the laboratory:
Visit 1: Eligibility and Testing 1
Visit 2: Testing 2

VISITS SUMMARY

Visit 1: Baseline screening (duration 60 minutes)
Individuals with T2D will be recruited by poster and online advertisement. We will also invite past study participants who have consented to be contacted for future research.
Individuals must be able to read and understand English in order to complete the study diet and activity logs. On the initial visit potential participants will read through the information and consent form, ask any questions, and provide written informed consent. They will received a signed copy of the document. The postdoctoral fellow research lead (Dr. B. Oliveira) will ensure that the subjects understand the details of the study and consent form. Following informed consent, eligibility criteria will be confirmed by detailed medical history questionnaire and baseline measurements.
Inclusion criteria will be: i) physician-diagnosed T2D of ≥1 year; ii) current HbA1c of 6.5-8.0%; iii) treatment with lifestyle or stable (≥3 months) oral glucose-lowering medications; iv) blood pressure of <160/99 mm Hg assessed according to guidelines; v) non-smoking; vi) no prior history of CVD or stroke; vii) not on hormone replacement therapy, corticosteroids, or anti-inflammatory medications; viii) 20–75 years old and iv) if vaccinated for COVID-19, have been vaccinated at least four weeks prior to participation in the study
Exclusion criteria will include: i) being a competitive endurance athlete; ii) taking exogenous insulin or SGLT2 inhibitors; iii) following a ketogenic diet, low-calorie diet, periodic fasting regimen, or consume ketogenic supplements; iv) being unable to travel to and from the university; v) being unable to follow the controlled diet instructions; vi) being pregnant or planning to become pregnant during the study (if female); vii) disorders of fat metabolism, chronic pancreatitis, had gastric bypass surgery and/or gallbladder disease; vii) being unable to read or communicate in English.
Based on our previous studies ~55% of males and ~70% of females with T2D who volunteer will meet these eligibility criteria so we anticipate no recruitment issues. We will allow statins and anti-hypertensive medications if on a stable dose for 3 months because the majority of T2D patients are on at least one of these medications; excluding them would leave a small pool for recruitment and limit the generalizability of our findings. Excluding exogenous insulin will avoid potential interactions and risk of hypoglycemia. SGLT-2 inhibitors (which are rarely used in BC because they are not covered by the provincial drug plan) will also be excluded

Faculty of Health and Social Development
School of Health and Exercise Sciences
3333 University Way
Kelowna, BC V1V 1W5

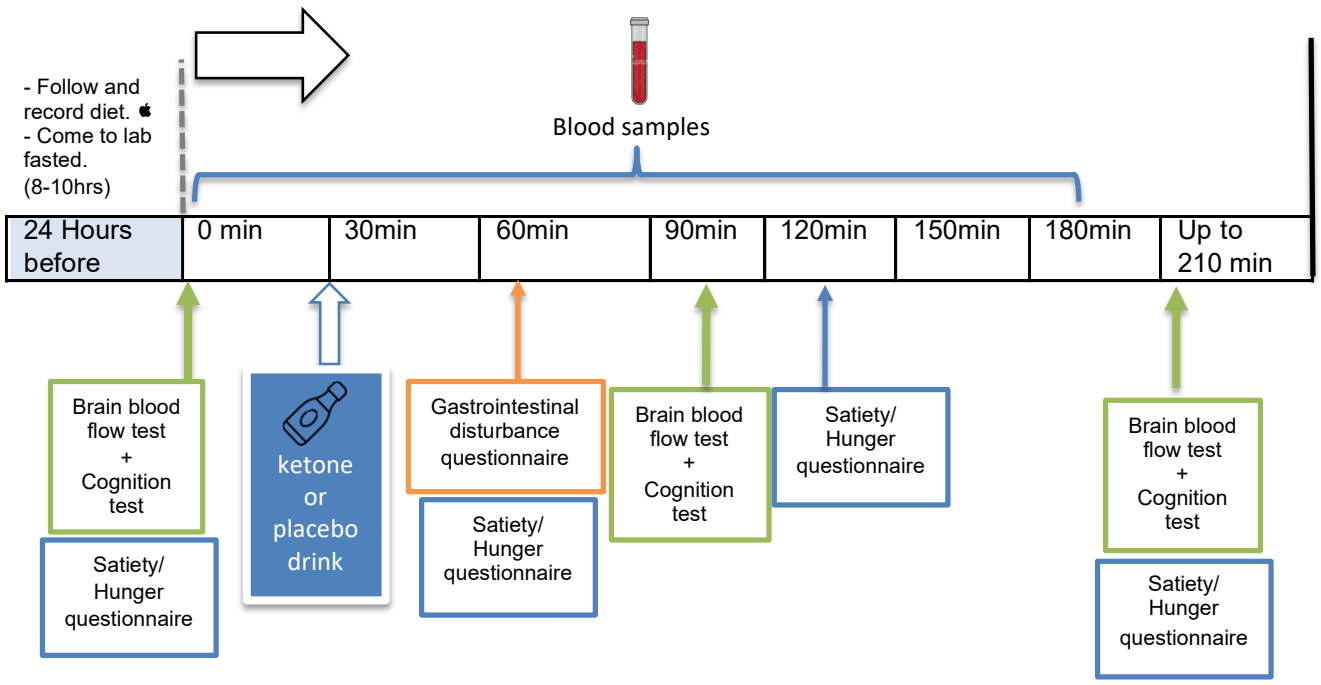
because of reports of euglycemic ketoacidosis with this drug [15]. Potential risks of hypoglycaemia with the most common diabetes medications (i.e., metformin, DPP-IV inhibitors, and GLP-1 agonists) are very low so we will be recruiting from a large pool that is representative of the T2D population.
Females will complete the menstrual cycle questionnaire in order to determine when the follicular phase is for testing purposes. Anthropometric and physiologic measurements will be collected (height, body weight, waist circumferences, blood pressure) for baseline participant characterization. The Montreal Cognitive Assessment (MoCA) will be used as a screening tool to ensure cognition levels meet the studies' requirements. Participants will be instructed about the standardized diet ~50% energy from carbohydrate, ~30% fat, ~20% protein (energy based on Harris-Benedict equation with an activity factor of 1.4) that will be oriented on the day before each trial. They will be asked to refrain from structured exercise and alcohol, and limit physical activity to daily living tasks as well as. Participants will then fast overnight (8-10 h) and refrain from taking any medications 24 hours before trial visits.

Experimental Trial A (duration 210 minutes)

Females will complete experimental trials in the follicular phase. Participants will report to the laboratory after an overnight fast (8-10 hours). Adherence to the 24 hour diet will be confirmed upon arrival in the laboratory, as well as information about physical activity and sleep. An indwelling intravenous catheter will be inserted into the antecubital vein for repeated blood sampling by a trained phlebotomist (Dr Jonathan Little and Dr. Barbara Oliveira). Blood (5 ml) will be drawn into EDTA tubes for measurement of plasma samples before being frozen in a -80 degree Celsius research refrigerator. There will be 7 intravenous blood draws in total for a total of ~35 ml of blood collected in each trial. The first collection will occur prior (0) to consumption of ketone or placebo drink. Another 6 blood draws will occur at 30 minute intervals (30, 60, 90, 120, 150, 180) after ingestion of the ketone or placebo drink. Thirty minutes after ingestion, participants will be instructed to complete the gastrointestinal distress questionnaire. Brain blood flow and blood pressure will be measured non-invasively and a cognitive test battery will be performed in separate intervals throughout the study. During the visit participants will answer a brief questionnaire regarding satiety and hunger. Subjects will be reminded to not perform exercise or to consume alcohol or medications 24 hours prior to the next visit.

Faculty of Health and Social Development
School of Health and Exercise Sciences
3333 University Way
Kelowna, BC V1V 1W5

Protocol timeline



Visit 2: Experimental Trial B

Subjects will return fasted to the laboratory at least 2 days after the fisrt visit. Adherence to the 24 hour diet will be confirmed upon arrival in the laboratory. Participants will be instructed to identify any deviations in diet before beginning the second trial. Protocol for the second visit will be the same as visit 1, although participants will receive the remaining condition (ketone supplementation or placebo).

Statistical Analyses

Descriptive statistics (means, SD, and frequencies) will be calculated and Q-Q plots of residuals used to test for normality and skewness. Significance will be set at $P<0.05$. Data with a time component will be analyzed using a linear mixed effects model with subjects as a random factor and condition and time as fixed factors using SAS. Main effects of condition and condition X time interactions will be probed with planned comparisons to examine differences between β-OHB and placebo at each time point. Data with skewed distributions will be log-transformed or non-linear mixed effects models will be used. All participants will be included in the intention-to-treat (ITT) analyses and missing data will not be imputed, per contemporary guidelines with use of linear mixed models [16].

Sample Size

Twenty participants will be included in this cross-over study. In our pilot study on healthy volunteers we saw a ~15% reduction in glucose area under the curve after

Faculty of Health and Social Development
School of Health and Exercise Sciences
3333 University Way
Kelowna, BC V1V 1W5

ketone supplementation versus placebo. Using means and standard deviations from our previously published study in people with T2D (N=51) [17] for fasting glucose (8.6 +/- 2.3) a 15% reduction in blood glucose corresponds to an effect size d = 0.73. Assuming 80% power with an alpha level of 0.05 and a moderate correlation between repeated measures of r=0.7 (calculated using G*Power v3.1) a total sample size of n=14 is needed to detect differences between ketone and placebo conditions. To preserve power and account for any dropouts or missing blood samples we will recruit 20 participants, aiming for equal males and females.[2] [17]

Significance

The results of this study will help determine whether supplementation with exogenous ketones can alter metabolism and subsequently improve blood glucose levels. This is a pilot study and it will provide useful data to better answer future research questions and aid in the design of longer term studies.

OUTCOMES MEASUREMENTS AND METHODS

The primary outcome measure is the plasma glucose concentrations over the 3-hour period following the ingestion of the beverages. Secondary measures will include plasma insulin and C-peptide, free fatty acids, inflammatory cytokines, CBF, cognitive function and neurovascular coupling, inflammation signalling molecules, appetite/satiety levels and gastrointestinal distress questionnaire.

Anthropometric Measures

Waist circumference, height (cm) and weight (kg) will be measured using standard procedures. Body mass index (BMI) will be calculated as kg/m^2.

Physiological Measures

Resting blood pressure and heart rate will be collected for descriptive purpose. Before both measures, participants will be instructed to sit quietly and rest for 5 minutes. A finger tip heart rate monitor will track heart rate. Blood pressure will be collected using a cuff around the upper arm, a stethoscope will be placed in the cubital fossa for systolic and diastolic blood pressure.

Blood Sampling

Venous blood samples for plasma glucose, insulin, free fatty acids, C-peptide and cytokines will be obtained by a researcher trained in phlebotomy (Dr. Little and Dr.

Faculty of Health and Social Development
School of Health and Exercise Sciences
3333 University Way
Kelowna, BC V1V 1W5

Oliveira). Repeated blood samples will be obtained by intravenous catheter in an antecubital vein kept patent with sterile saline. The procedure will follow sterile conditions within our laboratory (Arts Building Rm 115B) with biosafety and blood collection accreditation as approved previously (e.g., H14-01636; H15-00205; H12-02268). Approximately 5 mL of blood will be collected into vacutainer tubes and processed at 7 timepoints for a total of 35 mL per test day. Plasma insulin will be measured on a 96-well plate using a commercially available high-sensitivity human insulin ELISA (Mercodia, Upsulla, Sweden). Plasma glucose will be measured using a hexokinase method and free fatty acids by colorimetric assay. Glucose, insulin, free fatty acids and cytokines will all be analyzed on a Chemwell 2910 clinical chemistry analyzer (Awarness Technologies, Connecticut, United States of America). C-peptide will be measured by ELISA (Millipore #EZHCP-20K). Plasma will also be analyzed through metabolomics technology by UVic-Genome BC Proteomics Centre. De-identified samples containing frozen plasma in microtubes (samples identified by an ID code only with no personal identifiers) will be sent on dry ice to UVic following UBC Biosafety guidance for transmission of biological materials. Results containing the analyte concentration are sent by secure email back to UBC PI.
Histones acetylation will be quantified with flow cytometry using the Histone H3 Acetylation Assay Kit (Millipore/Sigma Aldrich, CA). PBMCs will be isolated and inflammatory pathways and signalling assessed by standard western blotting techniques. They will also Blood β-OHB levels will be measured using the Abbot Freestyle Precision Neo® ketone meter, Abbott, England after ketone or placebo.

Cognitive Function

The Montreal Cognitive Assessment (MoCA) will be used as a screening tool ensure that all participants are 'cognitively normal'. The tests will be administered and interpreted by a trained researcher (Dr. Oliveira). In the unlikely event that a participant's performance on the MoCA is below the cognitively normal range, Dr. Walsh will encourage participants to contact their family physician to discuss their performance.
Cognitive function will be assessed using an iPad-based app (BrainBaseline, Digital Artefacts). A battery of 3 tests that collectively assess executive functions and processing speed will be used. Each test will take less than 3 minutes to complete. There is no prognostic validity to these tests (i.e., assessing cognitive impairment), rather they are intended to demonstrate within- and between-subject changes for research purposes. There are no risks associated with this measure.

CBF Measurement

Intracranial blood flow velocity will be measured non-invasively with transcranial Doppler ultrasound (TCD) in the middle and posterior cerebral arteries simultaneously. Our group within the Centre for Heart Lung and Vascular Health (CHLVH) has conducted both validation and reproducibility studies involving TCD. The day-to-day reproducibility of middle cerebral artery velocity in adults has a

Faculty of Health and Social Development
School of Health and Exercise Sciences
3333 University Way
Kelowna, BC V1V 1W5

coefficient of variation of <4.5%. The TCD probe that will be placed lightly against the skin between the temple and ear (temporal window) and will be held in place by a specialized headband that will worn by participants for the entirety of the experiment. Blood pressure will be measured non-invasively using a finger cuff affixed to the middle finger of the participant's non-dominant hand. The combination of intracranial blood flow velocity and blood pressure will be used to calculate an index of vascular conductance (i.e., vasodilation) during the neurovascular coupling (NVC) protocols.

Assessment of Neurovascular Coupling (NVC)

Using neuronal activation (via a visual stimulus) we evoke localized changes in blood flow in the brain. We will activate the occipital lobe (while measuring velocity in the posterior and middle cerebral arteries) using checkerboard displayed on a tablet. Eight cycles will be completed, each consisting of 20 s eyes-closed followed by 40 s of activation (eyes open). An auditory prompt will provide notification of "eyes-open" and "eyes-closed" periods. The cerebral blood velocity response will be averaged across the 8 trials for each participant.

Questionnaires:

Medical History Questionnaire:
A customized Medical and Health Status Questionnaire will be used to assess inclusion/exclusion criteria and prior history of medical conditions.

Female menstrual cycle questionnaire
Female participants will be asked about the timing of their menstrual cycle in order so that all participants can be tested in the follicular phase.

Gastrointestinal Distress Questionnaire
Gastrointestinal (GI) disturbances will be measured using a 10 cm visual analogue scale (7). The questionnaire will report subjective GI symptoms after ingestion of either the ketone supplement or placebo.

Self reported hunger/fullness Visual Analog Scales (VAS)
Participants will rate each of the following 4 questions by marking vertically on a horizontal line with descriptive anchors on either side ("not all" to "extremely"): 1) How hungry do you feel; 2) How satisfied do you feel; 3) How full do you feel; 4) How much do you think you can eat. The VAS scores will be converted to a 0–100 scale, as previously described (Flint et al, 2000).

All questionnaires are included as attachments.

Protection of Human Subjects:
All participants will be given a unique study code, with all data and information gathered connected with this code. Only the PI will have access to the master list linking the codes with participant names. Participant information and data will be

Faculty of Health and Social Development
School of Health and Exercise Sciences
3333 University Way
Kelowna, BC V1V 1W5

either stored in a locked filing cabinet, or on an internal UBC network drive accessed only through a dedicated LAN internet connection on a password protected computer in the PIs laboratory with Salto passcard access only to the members of the research lab.

POTENTIAL PROBLEMS AND ALTERNATIVE STRATEGIES

Diet compliance: We will encourage accurate recording of diet by providing clear instructions and training participants on the necessary detail required. Together with the assessment of dislikes and allergies we will personalize each intervention diet and provide all food in order to enhance compliance, as the foods provided will be based on preference, except altering the macronutrient composition of meals. Participants will complete a food log to record consumption of the study diets (and any additions/subtractions) and be asked to return any food not consumed.

Blood sampling: The insertion of a needle for blood sampling (intravenous and finger pricks) is a common medical practice and involves minimal risk provided proper precautions are taken. There may be some discomfort, discoloration/bruising with the cannula/venipuncture needle insertion. But the risk of pain and infection will be minimized by using an experienced trained phlebotomist, and of course by ensuring the needle is inserted and removed under completely sterile conditions.

Protection of personal data: All participants will be given a unique study code, with all data and information gathered connected with this code. Only the PI will have access to the master list linking the codes with participant names. Participant information and data will be either stored in a locked filing cabernet, or on an internal UBC network drive accessed only through a dedicated LAN internet connection on a password protected computer in the PIs laboratory with Salto passcard access only to the members of the research lab.

Informed consent and ethics: Prior to beginning the study Informed consent will be obtained from participants and they will be informed they can withdraw at any time.

Ketone supplements: Ketone supplements are indicated to be safe for consumption and have GRAS (generally recognized as safe) status by the FDA [9]. The protocol for this study was reviewed by the CIHR Nutrition and Health Panel and received Bridge Grant Funding (reviews and our responses attached). We have conducted two previous (H16-01260, H16-01846) and have one ongoing trial (H18-02930) using this supplement in similarly-designed studies. We have not

Faculty of Health and Social Development
School of Health and Exercise Sciences
3333 University Way
Kelowna, BC V1V 1W5

seen any GI distress or adverse effects of the supplement in any of these experimental trials (>200 individual consumption events with both the supplement and placebo). Participants will be under close supervision after ingestion of ketone supplements, and the researchers will confirm that the participant is not experiencing any adverse effects prior to leaving the laboratory.

References

1. Canadian Diabetes Association, A.C., *An economic tsunami: th cost of diabetes in Canada*. I2009: http://www.diabetes.ca/documents/get-involved/FINAL_Economic_Report.pdf.
2. Myette-Côté, É., et al., *Prior ingestion of exogenous ketone monoester attenuates the glycaemic response to an oral glucose tolerance test in healthy young individuals.* The Journal of physiology, 2018. **596**(8): p. 1385-1395.
3. Neudorf, H., et al., *Oral Ketone Supplementation Acutely Increases Markers of NLRP3 Inflammasome Activation in Human Monocytes.* Molecular nutrition & food research, 2019: p. 1801171.
4. Kashiwaya, Y., et al., *A ketone ester diet exhibits anxiolytic and cognition-sparing properties, and lessens amyloid and tau pathologies in a mouse model of Alzheimer's disease.* Neurobiology of aging, 2013. **34**(6): p. 1530-1539.
5. Krikorian, R., et al., *Dietary ketosis enhances memory in mild cognitive impairment.* Neurobiology of aging, 2012. **33**(2): p. 425. e19-425. e27.
6. Puchowicz, M.A., et al., *Diet-induced ketosis increases capillary density without altered blood flow in rat brain.* American Journal of Physiology-Endocrinology and Metabolism, 2007. **292**(6): p. E1607-E1615.
7. Park, C.H. and Y.-S. Kwak, *Analysis of energy restriction and physical activity on brain function: the role of ketone body and brain-derived neurotrophic factor.* Journal of exercise rehabilitation, 2017. **13**(4): p. 378.
8. Diamond, M.P., D.C. Simonson, and R.A. DeFronzo, *Menstrual cyclicity has a profound effect on glucose homeostasis.* Fertility and sterility, 1989. **52**(2): p. 204-208.
9. Clarke, K., et al., *Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects.* Regulatory Toxicology and Pharmacology, 2012. **63**(3): p. 401-408.
10. Cox, P.J., et al., *Nutritional ketosis alters fuel preference and thereby endurance performance in athletes.* Cell metabolism, 2016. **24**(2): p. 256-268.
11. Barry, J.C., et al., *Hyporesponsiveness to the anti-inflammatory action of interleukin-10 in type 2 diabetes.* Scientific reports, 2016. **6**: p. 21244.
12. Robinson, E., et al., *Short-term high-intensity interval and moderate-intensity continuous training reduce leukocyte TLR4 in inactive adults at elevated risk of type 2 diabetes.* Journal of applied physiology, 2015. **119**(5): p. 508-516.
13. Wan, Z., et al., *One-week high-fat diet leads to reduced toll-like receptor 2 expression and function in young healthy men.* Nutrition research, 2014. **34**(12): p. 1045-1051.
14. Wan, Z., et al., *Reduction of AMPK activity and altered MAPKs signalling in peripheral blood mononuclear cells in response to acute glucose ingestion following a short-term high fat diet in young healthy men.* Metabolism, 2014. **63**(9): p. 1209-1216.
15. Fralick, M., S. Schneeweiss, and E. Patorno, *Risk of diabetic ketoacidosis after initiation of an SGLT2 inhibitor.* New England Journal of Medicine, 2017. **376**(23): p. 2300-2302.
16. Chakraborty, H. and H. Gu, *A mixed model approach for intent-to-treat analysis in longitudinal clinical trials with missing values.* 2009.
17. Francois, M.E., et al., *Combined interval training and post-exercise nutrition in type 2 diabetes: a randomized control trial.* Frontiers in physiology, 2017. **8**: p. 528.

Faculty of Health and Social Development
School of Health and Exercise Sciences
3333 University Way
Kelowna, BC V1V 1W5