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THE UNIVERSITY OF BRITISH COLUMBIA

Faculty of Health and Social Development
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The Effect of Different Ketone Supplements on Blood β-OHB and Blood Glucose in Healthy Individuals

Research Question

How do different ketone supplement types affect blood beta-hydroxybutyrate (β-OHB) and blood glucose in healthy individuals?

Objectives

a) To determine the effect of acute ketone supplementation on blood β-OHB across different types of ketone supplements.
b) To compare changes in blood glucose following acute supplementation with different types of ketone supplements.
c) To evaluate the influence of acute ketone supplementation on blood pressure and heart rate across different types of ketone supplements.
d) To investigate the effect of acute supplementation with different ketone supplements on gastrointestinal discomfort, hunger, and fullness.
e) To explore supplement acceptability and taste across different ketone supplements.

Hypotheses

a) All ketone supplements will raise blood β-OHB.
b) All ketone supplements will lower blood glucose.

This is an exploratory/descriptive pilot study that is designed to inform future research; therefore, we do not pose specific hypotheses as to how the ketone supplements will differ with respect to their effects on the above-outlined objectives.

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Background Information

Acute increases in blood ketones (beta-hydroxybutyrate, β-OHB) have been found to temporarily reduce blood glucose [1] but therapeutic application has traditionally been limited due to the need for ketone infusion or severe dietary restrictions to achieve nutritional ketosis. With the recent development of exogenous ketone supplements that can be consumed orally [2], it is now possible to acutely raise blood β-OHB concentration without the need for other nutritional modifications or invasive procedures. In our lab, we have previously shown that acute ingestion of oral ketone supplements potently increases β-OHB and immediately lowers blood glucose [3-6]. These findings suggest exogenous ketones to be a promising therapeutic strategy aimed at lowering blood glucose and improving blood glucose control in individuals with impaired glucose metabolism, particularly individuals with prediabetes or type 2 diabetes [7].

The first ketone supplements available on the market were in the form of either ketone salts (i.e., β-OHB bound to a mineral salt) or ketone monoesters (i.e., β-OHB bound to a ketone precursor such as butanediol) [2]. Ketone salts can lead to gastrointestinal discomfort in many individuals [8] and ketone monoesters have an extremely unpleasant bitter taste. This makes adherence to a nutritional supplement regimen using ketone salts or monoesters challenging. Since then, new formulations have been developed that differ with respect to ingredients (e.g., include flavouring to mask the bitter taste and improve palatability) and chemical properties (e.g., different precursors and/or types of molecule the β-OHB is bound to). In particular, two new ketone supplements (beta-hydroxybutyric acid and 1,3-Butanediol) have recently become commercially available that promise an improved consumer experience.

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However, little is known about the pharmacokinetics of these supplements, which can be expected to differ from the original supplements based on differences in formulation. This, in turn, may affect the glucose-lowering properties of the exogenous ketone supplements. While qualities such as an improved taste and gastrointestinal tolerability are desirable in ketone supplements, it is of importance for the field of research focusing on the therapeutic value of exogenous ketones to establish the effect of these different ketone supplements on blood β-OHB and blood glucose and determine how they compare to the effects of the by now well-established ketone monoester.

The overall aim of this pilot study is therefore to test the β-OHB-raising and glucose-lowering effect of these two new types of commercially-available oral ketone supplements (beta-hydroxybutyric acid and 1,3-Butanediol) in comparison to a ketone monoester, which we have used in our previous studies on ketone supplementation.

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Research Method

Experimental design

A double-blind randomized cross-over pilot study in which *N* = 12 healthy adults will participate in three experimental trials with a different ketone supplement in each trial. Each ketone supplement has FDA GRAS (Generally Recognized As Safe) status as a food additive and is available for online purchase in Canada:

1) "KE4" (by KetoneAid)
    - Beta-hydroxybutyrate monoester
2) "Kenetik" (by DrinkKenetik)
    - Beta-hydroxybutyric acid
3) "Ketone 2.0" (by HVMN)
    - 1,3-Butanediol

All participants will provide written informed consent before start of data collection. Following screening and informed consent, eligible participants will participate in three experimental trials of β-OHB supplementation with the order of treatment randomized, balanced, and separated by at least 48 hours. 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 [9]. Participants will be asked to refrain from structured exercise and alcohol on the day before each trial. Furthermore, participants will be instructed to record their dietary intake on the day prior to their first experimental visit, and to consume the same foods and quantities on the days before the subsequent experimental visits. Participants will then be asked to fast overnight (at least 8 hours) and refrain from taking any medications on the morning of the trial.

Upon arrival, a baseline fasting blood sample will be obtained via finger prick. The oral ketone supplement will then be consumed within 5 minutes. The different

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supplement types will have an overall similar yet likely distinguishable flavour and will be provided at different volumes (based on the manufacturer's formulation) – however, participants will not be aware which flavour and volume will correspond to which ketone supplement. Furthermore, all supplements will be provided in opaque plastic bottles (labelled with "A", "B", or "C" according to a code that only the principal investigator has access to) such that neither the participant nor the research lead will be able to visually identify the supplement type. Each ketone supplement will be provided at a dose of 10 g active ingredient (i.e., 10 g of beta-hydroxybutyrate monoester, beta-hydroxybutyric acid, or 1,3-Butanediol). This is the recommended serving size for the two new ketone supplements to be tested (beta-hydroxybutyric acid and 1,3-Butanediol) and is in line with a previous pharmacokinetic study that showed a dose of ~ 10 g beta-hydroxybutyrate monoester to successfully raise β-OHB within 30 minutes [2]. Specifically, this leads to one trial each with:

1) 20 mL of "KE4" (i.e.,10 g beta-hydroxybutyrate monoester)
2) 237 mL of "Kenetik" (i.e., 10 g beta-hydroxybutyric acid)
3) 35 mL of "Ketone 2.0" (i.e., 10 g 1,3-Butanediol)

The primary outcome will be total β-OHB as represented by the area under the curve across 4 hours. Secondary outcomes include blood glucose, blood pressure, heart rate, taste and supplement acceptability, hunger and fullness, and gastrointestinal tolerability - all measured across the 240-minute post-supplementation period. Blood samples will be collected via finger prick; blood β-OHB and glucose levels will be measured using the "Keto-Mojo" handheld monitor at baseline and 15, 30, 60, 90, 120, 180, and 240 minutes after ingestion of the ketone supplement.

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Visits Summary

The study will involve a total of 3 visits to the laboratory.

Visit 1: Baseline screening & first experimental trial (duration 270 minutes)

Individuals will be recruited by word-of-mouth. Individuals must be able to read and understand English in order to understand the study instructions. On the initial visit, the potential participant will read through the information and consent form, be able to ask any questions, and provide written informed consent. The research lead (Kaja Falkenhain, PhD Candidate) will ensure that the participant understands the details of the study and consent form.

Inclusion criteria will be: i) over the age of 18; and ii) able to fast overnight. **Exclusion criteria** will include: i) being a competitive endurance athlete; ii) following a ketogenic diet, low-calorie diet, periodic fasting regimen, or regularly consuming ketogenic supplements; iii) being unable to travel to and from the university; iv) being unable to follow the diet instructions; v) being pregnant or planning to become pregnant during the study; vi) having been diagnosed with a chronic disorder of glucose or fat metabolism, including type 2 diabetes, chronic pancreatitis, or gallbladder disease; vii) being unable to read or communicate in English.

Females will complete the menstrual cycle questionnaire to confirm they are in the follicular phase. Anthropometric and physiologic measurements will be collected (height, body weight, waist circumferences, blood pressure) for baseline participant characterization. The research lead will review the dietary record with the participant and confirm the participant is fasted and has refrained from structured exercise and alcohol the day prior.

A baseline finger prick will then be performed before ingestion of the ketone supplement. Another 7 finger pricks will be performed at 15, 30, 60, 90, 120, 180,

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and 240 minutes after ingestion of the supplement. Throughout the 240-minute period, participants will be allowed to do quiet work or perform other sedentary activities (reading, watching a movie). Participants will be asked to complete the questionnaire on gastrointestinal discomfort 60 minutes after ingestion of the supplement, and the questionnaires on hunger and fullness as well as taste and supplement acceptability at the end of the 240-minute post-supplementation period.

At the end of the visit, the participant will be reminded to consume the same diet on the day before the next experimental trial, and to refrain from structured exercise and alcohol consumption in the 24 hours prior.

Visits 2 & 3: Second and third experimental trial (duration 240 minutes)

The second and third experimental trial will be identical to the first visit (except no baseline anthropometric measurements will be performed), with the only difference being the different ketone supplement provided to the participant.

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Statistical Analyses

Descriptive statistics (means, SD, and frequencies) will be calculated. Significance will be set at $P < 0.05$. Data across time will be analyzed in R using a linear mixed effects model with subjects as a random factor and condition and time as fixed factors. Assumptions will be tested using Q-Q plots of residuals. 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 [10].

Sample Size

We will recruit 12 participants to be included in this cross-over study, which has blood β-OHB (as represented by the area under the curve across the 4 hours following ingestion of the supplement) as the primary outcome. A previous pharmacokinetic study exploring the effects of a ketone monoester provided at a dose of ~10 g in healthy adults found that blood β-OHB was raised to 1.4 ± 0.6 mM within 30 minutes, and to an average of 0.5 ± 0.5 mM across the entire 4-hour post-supplementation period [2], which represents an effect size of $d > 1.0$ compared to baseline. Using the outcome data from this study ($N = 15$) and assuming 80% power with an alpha level of 0.05 and a moderate correlation between repeated measures of 0.5, a total sample size of $N = 6$ would be sufficient to detect an increase in average blood β-OHB compared to baseline (calculated using G*Power v3.1). In order to allow for a more nuanced evaluation of differences between ketone supplements and to account for any dropouts or missing blood samples, we will recruit 12 participants (aiming for equal males and females), which is in line with the sample size of previous pharmacokinetic studies of ketone supplements [2].

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Significance

The results of this study will help determine the effect of different types of ketone supplements on blood β-OHB and glucose. 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.

Outcome Measurements and Methods

The primary outcome will be total β-OHB as represented by the area under the curve across 4 hours. Secondary outcomes include blood glucose, blood pressure, heart rate, hunger and fullness, gastrointestinal discomfort, and taste and supplement acceptability.

Anthropometric Measures

Waist circumference (cm), 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 and throughout the study visits (at the same time points as blood samples are collected). Before measurements, 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.

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Blood Sampling

Blood samples will be collected via finger prick. Blood β-OHB and glucose levels will be measured using the "Keto-Mojo" handheld monitor (which we have used extensively in our previous studies) at baseline and 15, 30, 60, 90, 120, 180, and 240 minutes after ingestion of the ketone supplement.

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