Acute oral intake of beta-hydroxybutyrate in a pilot study transiently increased its capillary levels in healthy volunteers

Abstract

The popularity of ketogenic diets has led to an increased interest in alternative approaches to inducing and sustaining ketosis. However, published information on the impact of exogenous ketone products in human subjects is lacking. This study aimed to characterize the circulating β-hydroxybutyrate (βHB) response in healthy men and women (n=10) following acute consumption of βHB salts. In a randomized, cross-over design, participants consumed placebo control or a combination of sodium and calcium βHB salts providing either 11.7g (full dose) or 5.85g (half dose) of βHB, with a wash-out period between intakes. Blood levels of βHB and glucose were measured and vital signs and adverse events were monitored over the following 4 hours. Consumption of 11.7g βHB led to a significant increase in circulating βHB levels above 1 mmol/L, within 15 minutes compared with the placebo. Intake of the 5.85g of βHB led to increases in βHB levels between the full dose and the placebo. The rise in βHB was comparable to that seen in physiological ketogenic situations such as when following a ketogenic diet or periods of fasting, and did not approach the range seen with pathological conditions such as diabetic ketoacidosis. Blood glucose or blood pressure was not adversely impacted during the treatment period. Pulse was seen to modestly but significantly decrease with consumption of the full dose βHB. In conclusion, consumption of 11.7g βHB can lead to transient increases in capillary βHB in line with thresholds seen in nutritional ketosis conditions.

Keywords: ketogenic diet, nutritional ketosis, β-hydroxybutyrate, exogenous ketone, cross-over trial

Introduction

A ketogenic diet refers to a dietary approach that promotes nutritional ketosis by restricting carbohydrates (usually to less than 50g per day) and increasing the intake of fat with adequate consumption of protein. With a shortage of available glucose, fat from dietary source or stored adipose deposits is metabolized into ketone bodies [acetoacetate, β-hydroxybutyrate (βHB) and acetone] that can be utilized by the cells for energy.\(^1\) Systematic reviews and meta-analyses have reported the effectiveness of ketogenic diets in treating intractable epilepsy in adults and children,\(^2,3\) preventing an increase in appetite on reduced calorie diets,\(^4\) and achieving long-term bodyweight reduction.\(^5\) Emerging studies have shown that ketogenic diets may help diabetes management and improve exercise performance.\(^6\) However, compliance with a ketogenic diet can be difficult, as many have perceived the diet to be rigid or experienced adverse effects such as gastrointestinal disturbances.\(^7\)\(^8\) It has also been reported anecdotally as well as in the literature\(^9\) that, when following ketogenic diets or very-low-calorie diets (VLCD), there is a short lag time in the increase in circulating ketone bodies in conditions of reduced glucose intake, during which individuals report symptoms described as ‘keto flu,’ including light headedness, irritability, fatigue and hunger. Hence, there has been increased interest in utilizing additional methods to enhance compliance and to facilitate the induction and maintenance of ketosis.

There has been a surge of commercially available products supplying exogenous ketones such as βHB salts. However, the oral response to such βHB salt formulations has been characterized in very few human studies. This report was intended as a proof-of-concept study to determine the circulating βHB concentration in response to acute, oral βHB supplementation. A lower dose was also investigated alongside a no-active placebo control.

Methods and materials

Study subjects

Participants were healthy adults aged 21-65y/o with normal body weight, with fasting glucose <5.55mmol/L (<100mg/dL) and fasting ketones <1mmol/L at screening. Key exclusion criteria included: currently following a ketogenic diet (<50g carbohydrate per day) or on a weight loss program; use of medications or nutritional supplements which may influence study results; known allergy or hypersensitivity to study products; significant abnormalities in medical history or physical examination; current diagnosis of serious medical conditions; history of drug or alcohol abuse. Study procedures were in accordance with the Declaration of Helsinki and were approved by the Quorum Independent Review Board (Seattle, WA). Written informed consent was obtained from all participants before enrollment.

Study design

This was a randomized, double-blind, placebo-controlled cross-over trial in which βHB concentrations in capillary blood were evaluated following consumption of 2 different doses of βHB salts and a placebo. Each treatment took place on a different study day separated...
by a wash-out period of at least 48 hours and not more than 1 week. Participants attended the Functional Medicine Research Center (Gig Harbor, WA) following a 10-hour overnight fast. Participants were advised on composition of evening meal prior to the study days, and were requested to avoid alcohol and physical activity the day before each study visit (Figure 1). The βHB salts or placebo were mixed with 12 oz water and consumed within 5 minutes. Blood was collected immediately prior to consumption, and 0.25, 0.5, 1, 2 and 4 hours post consumption via finger-stick for glucose and βHB assessments (Figure 1). During the 4 hours post consumption period, all subjects were fasted and were permitted to consume plain water only throughout this time. Body weight was measured at each study visit, and vital signs (pulse, blood pressure, temperature, heart rate) were measured before product consumption and at 4 hours post-consumption. AE’s and tolerance information was recorded at the end of each study arm. Circulating glucose and ketone levels were determined using the commercially available monitoring system Precision Xtra™ (Abbott Diabetes Care Inc., Alameda, CA). The area-under-the-curve (AUC) for βHB was determined using the trapezoidal rule.

Study products

The full-dose and half-dose study products provided, respectively, 11.7g/serving and 5.85g/serving of βHB from calcium and sodium βHB in addition to flavors and excipients to improve product taste tolerability. The placebo control matched flavors and excipients to the full-dose βHB product. βHB was supplied by NNB Nutrition (Frisco, TX).

Statistical analyses

For normally distributed data, differences between treatment arms were assessed by repeated measures analysis of variance (RM-ANOVA) with post-hoc testing if significance was identified. Paired t-tests were used to assess within group differences between two time-points. Non-normally distributed data was transformed via log or square root transformation, and normal distribution re-examined. If normality could not be coax, Friedman test was used to assess differences between the three treatment groups, and Dunnett’s test for multiple comparisons used for post-hoc testing if a significant between-group difference was seen.

Results

All 10 participants (8 women and 2 men; all Caucasians) recruited for this study completed the study. Their mean age (mean±SD) was 31.4±12.0 y/o and mean BMI was 23.7±1.3. Baseline fasting βHB and glucose were 0.17±0.08mmol/L and 5.09±0.45mmol/L, respectively. Complete blood count and lab parameters were all within normal range (data not shown). The full-dose product (βHB-full) rapidly increased capillary βHB concentrations during the first 60 min reaching peak levels of 1.04±1.63mmol/L at 15 min, and returned towards baseline values 2 hours following intake (Table 1). The half-dose product (βHB-half) also resulted in an increase in βHB within the same timeframe, although the overall magnitude of the increase was less pronounced, and the βHB concentrations were not significantly different from placebo.

Differences between groups assessed with Friedman test, with Dunnett’s test for multiple comparisons used if overall group differences identified (p<0.05). Between-treatment differences denoted as *a,b,c* with treatments not sharing a letter considered significantly different (p<0.05). Friedman test revealed a significant between-group difference in βHB AUC (Figure 2). Dunnett’s post-hoc tests identified a significant difference between βHB-full and placebo (p=0.001) but not between βHB-full and βHB-half (p=0.35). Mean βHB AUC for placebo was lower than βHB-half but the between-group difference did not reach statistical significance (p=0.08). No difference in blood glucose occurred in any of the treatment groups at any of the time points as assessed by RM-ANOVA (Figure 3). Paired t-tests did not identify any differences between baseline and 4 hours for βHB-full, βHB-half, or for placebo.

Figure 1 (A) Study design flow chart and (B) protocol at each clinic visit.
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Table 1 Circulating $\beta$HB concentrations (mean±SE) from baseline to 4 hours post study treatment consumption

| Product   | $\beta$HB-full | Placebo | $\beta$HB-half | P value (main effect) |
|-----------|----------------|---------|----------------|-----------------------|
|           | Pre            | Post    | Pre            | Post                 |                      |
| $\beta$HB-full | 112.6 (3.7)   | 113.7 (3.9) | 113.3 (3.7)   | 116.5 (3.6)         |                      |
| Systolic BP (mmHg) | 67.8 (3.1)   | 67.2 (3.8)   | 69.8 (2.3)    | 71.2 (4.4)          |                      |
| Diastolic BP (mmHg) | 63.6 (4.7)$^a$ | 56.8 (4.0)$^b$ | 58.8 (4.0)   | 60.0 (3.6)         | 98.0 (0.1)$^a$ |
| Pulse (bpm) | 97.7 (0.1)$^a$ | 98.0 (0.1)$^b$ | 98.1 (0.1)$^b$ | 98.1 (0.1)$^b$     | 153.7 (4.3)         |
| Temperature (°F) | 153.4 (4.3) | 152.2 (4.4) | 154.2 (4.7) | 153.8 (4.7)        |                      |

Data displayed as mean (SE). Significant within-treatment arm differences between baseline and 4-hours are denoted by $^a,b$. Time-points not sharing a letter are considered significantly different (p<0.05) as assessed by paired t-tests.
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There were no significant differences in any of the vital sign related variables at baseline between the 3 groups as assessed by RM-ANOVA (Table 2). The small but statistically significant increase in temperature (by a mean of 0.3°F) indicated potential influence of the study environment. There was a statistically significant reduction in pulse with βHB-full consumption. One individual reported mild loose stool after taking βHB-full and one reported moderate migrane after taking βHB-half although the individual had a history of migrane.

Data displayed as mean (SE). Significant within-treatment arm differences between baseline and 4-hours are denoted by a,b. Time-points not sharing a letter are considered significantly different (p<0.05) as assessed by paired t-tests.

Discussion

In this study, consumption of 11.7g of βHB led to a significant increase in capillary βHB levels during the first hour compared to control. Increases in βHB with 5.85g of βHB (half-dose) led to increases in βHB between full dose and placebo control. The magnitude of the rise in βHB was comparable to that seen in physiological ketogenic situations such as when following a ketogenic diet or periods of fasting,\(^1\) and did not approach the range seen with pathological conditions such as diabetic ketoacidosis. No severe gastrointestinal distress was observed.

When analyzing capillary specimens for βHB levels, we noticed data points from one subject (Subject 101) that might be potential outliers. For this subject, the circulating [βHB] measured 15min after the βHB-full and βHB-half intake was 5.2mmol/L and 1.3mmol/L, respectively, compared with 0.2-0.8mmol/L for the other 9 subjects. The circulating [βHB] measured 4h after the placebo intake for was 0.7mmol/L for this subject as opposed to 0.2-0.3mmol/L for the rest of the group (Supplementary Figure 1). The same statistical analysis excluding this subject revealed that the capillary βHB originally peaked at 15min after βHB-full intake was reduced to 0.58±0.23mmol/L and the between-group difference did not reach statistical significance. Instead, it peaked at 30min at 0.62±0.08mmol/L. The rest of the results including AUC data remained similar (Supplementary Table 1 & Supplementary Figure 2). Whether the cause of the unusual βHB levels seen in this subject was due to hydration status, difference in ketone body metabolism, or measurement error remains to be explored.

Supplementary Figure 1 Circulating βHB concentrations from Subject 101 vs. group average (n=10; mean + SD).

Identification of potential outliers

Subject 101’s [βHB] level measured 15 min after the βHB-full and βHB-half intake was 5.2mmol/L and 1.3mmol/L, respectively. The circulating [βHB] measured 4 h after the placebo intake for was 0.7mmol/L for this subject as opposed to 0.2-0.3mmol/L for the rest of the group (Supplementary Figure 1).

Supplementary Table 1 Circulating βHB concentrations (mmol/L) at baseline and 4 hours post study treatment consumption (n=9)

| Time     | βHB-full | Placebo | βHB-half | P value (main effect) |
|----------|----------|---------|----------|-----------------------|
| Baseline | 0.13 (0.02) | 0.17 (0.02) | 0.17 (0.02) | 0.282 |
| 15 min   | 0.58 (0.23)\(^a\) | 0.17 (0.02)\(^b\) | 0.44 (0.23)\(^ab\) | 0.136 |
| 30 min   | 0.62 (0.08)\(^a\) | 0.16 (0.02)\(^b\) | 0.46 (0.06)\(^a\) | <0.001 |
| 60 min   | 0.54 (0.04)\(^a\) | 0.19 (0.01)\(^b\) | 0.28 (0.04)\(^a\) | <0.001 |
| 120 min  | 0.29 (0.03) | 0.19 (0.02) | 0.32 (0.09) | 0.234 |
| 180 min  | 0.21 (0.01) | 0.24 (0.03) | 0.20 (0.02) | 0.369 |
| 240 min  | 0.19 (0.03) | 0.22 (0.01) | 0.26 (0.03) | 0.172 |

Differences between groups assessed with Friedman test, with Dunnett’s test for multiple comparisons used if overall group differences identified. Between-group differences denoted as a,b,c with treatments not sharing a letter considered significantly different (p<0.05).

Statistical analysis omitting βHB data from Subject 101

βHB-full resulted in a rapid increase in capillary βHB concentrations within 60 minutes, with return towards baseline values thereafter. βHB-half also resulted in an increase in βHB within the same time-frame, although the overall magnitude of the increase was less pronounced (Supplementary Table 1).

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**Conflict of interests**

All authors are employees of Metagenics, Inc.

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