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The Effects of Exogenous Beta-Hydroxybutyrate Supplementation on Metrics of Safety and Health

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Abstract: The ketogenic diet is a high-fat, very low-carbohydrate, moderate-protein diet that will induce a state of ketosis. Ketosis is a metabolic state characterized by elevated ketone body production in response to the absence of carbohydrates. Some drawbacks of the ketogenic diet are that it can be difficult to adhere to due to its restrictive nature, and it can also cause some undesirable side effects like gastrointestinal distress and increases in apoB-lipoproteins. In order to maximize the benefit of ketosis and to minimize side effects, supplementing with exogenous beta-hydroxybutyrate may induce a state of temporary ketosis without undesirable side effects. In the present study, 22 healthy male and female adults consumed 12.75 grams of beta-hydroxybutyrate salts or maltodextrin placebo twice daily for 90 days. Comprehensive blood safety analysis, body composition, bone densitometry, psychological and immune surveys, and blood pressure were administered at baseline, 30, 60, and 90 days. There were no significant differences in any measures collected, indicating that exogenous beta-hydroxybutyrate had no detrimental impact on fasting blood values such as electrolyte levels, glucose, hemoglobin A1c, complete blood count, body composition, bone density, psychological well-being, immune status, or blood pressure. We conclude that supplementing with exogenous beta-hydroxybutyrate is safe and well-tolerated by healthy adults.

Keywords: Beta-Hydroxybutyrate, Ketosis, Safety, Exogenous Ketones

1. Introduction

The ketogenic diet is categorized as a high-fat, very-low carbohydrate, and moderate protein dietary strategy that is meant to mimic a fasted state by restricting carbohydrate intake. Research has commonly defined the intakes of a ketogenic diet as less than 50 grams of carbohydrates per day, or 5 to 10 percent of total caloric contribution coming from carbohydrates, with fat contributing up to 90 percent of total caloric intake [1, 2]. The goal of the ketogenic diet is to induce ketosis – a metabolic state characterized by increased ketone body production in response to the absence of carbohydrates. In order to reach a state of nutritional ketosis, blood ketone concentration should be between 0.5 millimolar (mM) and 3.0 mM [3]. This rise in endogenous ketones is dependent on macronutrient availability of glucose and fatty acids, and the hormonal signaling of glucagon, insulin, and cortisol.

There are many benefits to human health for being in a state of ketosis from consuming a ketogenic diet. Weight loss occurs due to the reliance on fatty acid storage, and from the mitochondria regaining their metabolic flexibility (countering insulin resistance) [4, 5]. Young et al. demonstrated that as ketone levels rose, fat loss rose as well [6]. In addition to weight loss, research has shown that the ketogenic diet does not have a negative impact on hunger hormones despite a decline in total caloric intake [7]. To achieve an appetite suppressive effect, ketones concentrations only need to reach mild ketosis (greater than 0.5 mM) [8, 10]. Research has also demonstrated that the application of the ketogenic diet can have therapeutic benefits on diseases that impact metabolism [9]; reduce the incidence of seizures in children with epilepsy [11], improve outcomes of certain neurogenerative diseases like Parkinson's Disease [12], may help control glycolytic phenotype of various cancers by limiting glucose availability

[13], and lower glucose and hemoglobin A1c concentrations in individuals with type 2 diabetes [14, 15]. Lastly, elevated blood ketones could improve endurance performance and further optimize substrate metabolism by providing an alternative source for oxidative phosphorylation [16, 17].

In addition to being carbohydrate restrictive, adherence to the ketogenic diet can be difficult due to some undesirable side effects like gastrointestinal discomfort [18] and increases in apoB-lipoproteins [19]. Therefore, temporary and rapid rises in blood ketone concentrations with no dietary changes may be of potential interest and benefit [20]; hence, the relevance of exogenous ketones [21-23]. The safety of ketone esters has been previously explored, however, there is a void in the literature on the safety of ketone salts, which is what this study investigated. One previous study on the safety of ketone salts demonstrated that two servings of 7 grams of beta-hydroxybutyrate (BHB) combined with erythritol, L-Taurine, and L-Leucine was safe as demonstrated by no changes in complete blood count (CBC) or biomarkers of a comprehensive metabolic panel over 6 weeks [24]. Moreover, markers of cardiovascular health, such as blood pressure, improved while heart rate remained unchanged. The purpose of this study is to extend this research to 90 days with a dosage of 12.75 grams twice per day with additional metrics of safety and health.

2. Methods

2.1. Subject Criteria

Twenty-two healthy male and female subjects aged 18 to 50 years old enrolled for study participation. Exclusion criteria included: hypertension, obesity (body mass index [BMI] >30 kg/m^2), smoking or using smokeless tobacco, taking any prescription medication, or having any underlying health conditions (metabolic, heart disease, diabetes, kidney disease). This study was approved by an external institutional review board (Integ Review IRB, Austin, TX, USA) and all procedures were in agreement with institutional guidelines and the Declaration of Helsinki. Prior to engagement in any study procedures, subjects provided written informed content.

Table 1. *Descriptive Subject Characteristics.*

	BHB	PLA
Sample Size	(n=11)	(n=11)
Age (years)	44.45 ± 7.30	45.55 ± 9.05
Height (cm)	166.49 ± 9.80	169.03 ± 11.15
Weight (kg)	72.79 ± 14.67	78.81 ± 18.07
Body Mass Index (kg/m^2)	26.32 ± 5.13	27.27 ± 3.66

2.2. Study Design

The study design was a randomized, double-blinded, placebo-controlled trial. Subjects were stratified into quartiles based on BMI and subjects from each quartile were randomly assigned to conditions using a random number generator (random. org). The conditions were sent to the primary investigator in white packages labeled "A" or "B". These were administered as 12.75-gram servings of a R-Beta Hydroxybutyrate (BHB) salt blend (KetoNAT™; Science Backed Solutions, LLC; Melissa, TX, USA) or a similarly flavored iso-energetic, iso-volumetric maltodextrin placebo twice daily for 90 days for a total of 25.5-grams of the respective condition, daily. The BHB salt blend treatment was 59% sodium BHB, 27% magnesium BHB, and 14% calcium BHB that was enriched in R-BHB or L-BHB (95% D-BHB and a remaining 5% L-BHB). Subjects underwent baseline testing (PRE) which included: blood draw for safety measures (complete blood count, comprehensive metabolic panel, automated differential, and hemoglobin A1c), resting blood pressure and heart rate, psychological mood assessment (Profile of Mood States; [POMS]), immune status questionnaire, body composition and bone densitometry. Following PRE testing, subjects were given a 30-day supply of either condition "A" or condition "B". Subjects were instructed to consume one serving in the morning and one serving in the afternoon with at least three hours of separation between servings. Subjects were also asked to track their caloric intake 3 days every week for the duration of the study. Lastly, subjects submitted VAS (visual analog scales) to report subjective measures of satiety, hunger, and psychological feelings (well-being, mental clarity, etc.). Testing was repeated for all study procedures in an identical manner to PRE at 30 days, 60 days, and 90 days following the original PRE testing date, with the exception of the DXA which was only performed at PRE and 90 days. Study procedures are further described below.

2.3. Bone Densitometry and Body-Composition Analysis

Bone densitometry and body composition was determined by a whole-body scan on a dual-energy x-ray absorptiometry device (Horizon A DXA System, Hologic Inc, Marlborough, MA, USA). Fat-free mass, fat mass, body fat percentage, bone mineral content, and bone density was determined for the total body with the subject lying in a supine position with knees and elbows extended. Subjects were instructed not to move for the entire duration of the scan (approximately 5 minutes). Results from each scan were uploaded and accessed on computer that was directly linked to the DXA device. Calibration of the DXA device was done against a phantom provided by the manufacturing company prior to testing.

2.4. Venous Blood Measures

Venous blood was extracted by venipuncture of the antecubital vein using a 21-gauge syringe and collected into a 10mL EDTA vacutainer tube (BD Vacutainer®, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) by a certified phlebotomist. Afterward, blood samples were centrifuged at 2500 rpm for 10 minutes at 4°C. Resulting serum samples were then aliquoted and stored at −80°C until further analysis. Samples were thawed once and analyzed in duplicate in the same assay for each analysis to avoid compounded inter-assay variance.

2.5. Blood Pressure and Heart Rate

Subjects rested in a supine position for 5 min in a quiet room at 228°C before the baseline hemodynamic measurements were obtained. Resting brachial blood pressure and heart rate were measured on the right arm with an automated digital

oscillometric sphygmomanometer (Omron, Model HEM 705-CP; Omron Corporation, Shimogyo-ku, Kyoto, Japan). Three readings separated by 1-min intervals were taken, and the mean was used for the analysis.

2.6. Immune Status Questionnaire (ISQ) and Profile of Mood States (POMS)

The Immune Status Questionnaire (ISQ) is a validated self-assessment of subjective values of seven different common symptoms associated with disease [25]. The ISQ was scored on a 5-point Likert scale from 0 to 4 for how often the subject has had the following symptoms in the past week; Never, Sometimes, Regularly, Often, and Almost Always. The values were summed up to equal a raw score. The raw score was then converted into a final score between zero and ten, with 0 being the poor immune status, and ten being excellent immune status [26].

The Profile of Mood States is a validated self-assessment of subjective values of forty different moods [25]. Those moods then fall into seven categories: Tension, Anger, Fatigue, Depression, Esteem - Related, Vigor, and Confusion. Subjects were asked to assess each of the forty moods, and if they are feeling that particular mood "right now". Subjects assessed the moods according to a 5-point Likert scale from 0 to 4: Not at All, A Little, Moderately, Quite a Lot, Extremely. The following formula was used to determine the overall POMS score:

(Tension + Depression + Anger + Fatigue + Confusion) – (Vigor + Esteem-Related) + 100

A lower score indicated a better mood, while a higher score indicated a poor mood [24].

2.7. Visual Analog Scales for Perceived Hunger and Perceived Mental Clarity

The perceptual measures collected for the study were perceived Hunger and perceived Mental Clarity. Hunger and Mental Clarity scales consisted of a scalar representation numbering from 0-10. On the Hunger Scale, visual descriptors of "not hungry", "adequately hungry" and "very hungry" presented at numbers 0, 5, and 10, respectively. On the Mental Clarity scale, visual descriptors of "Poor Mental Clarity", "Adequate Mental Clarity", and "Very Mentally Clear" are presented at numbers 0, 5, and 10, respectively.

2.8. Calorie and Macronutrient Reporting

Subjects were asked to record, and then report, their caloric intake three times per week using a mobile tracking application (MyFitnessPal, San Francisco, CA, USA).

2.9. Statistical Analysis

All statistical analyses were performed at the completion of the study using GraphPad Prism (Version 8, San Diego, CA, USA). Dependent variables were assessed for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). Two-way mixed model analysis of variance (ANOVA) was performed assuming group and time as fixed factors and subjects as a random factor. Whenever a significant F value was obtained, a post hoc test with a Bonferroni adjustment was used to for multiple comparisons purposes. The alpha level was set a $p \leq 0.05$. Data are reported as mean ± standard deviation.

3. Results

3.1. Complete Blood Count

There was no significant between or within group differences in Complete Blood Count values ($p > 0.05$, Table 2). Mean and standard deviation are displayed in Table 2.

Table 2. *Complete Blood Count Results.*

	PRE	30 DAY	60 DAY	90 DAYS	p Value
WBC (K/uL)					
BHB	5.13 ± 0.96	5.07 ± 0.95	5.38 ± 1.07	6.62 ± 2.49	0.1001
PLA	5.91 ± 1.03	5.91 ± 1.05	5.94 ± 1.03	6.19 ± 1.06	
RBC (M/uL)					
BHB	4.66 ± 0.41	4.61 ± 0.33	4.67 ± 0.34	4.70 ± 0.39	0.1084
PLA	4.36 ± 0.25	4.44 ± 0.34	4.37 ± 0.38	4.31 ± 0.40	
Hemoglobin (g/dL)					
BHB	14.43 ± 1.06	14.28 ± 0.85	14.37 ± 0.89	14.45 ± 1.01	0.1203
PLA	13.40 ± 1.25	13.64 ± 1.22	13.31 ± 1.29	13.15 ± 1.46	
Hematocrit (%)					
BHB	47.64 ± 3.07	46.82 ± 2.47	47.17 ± 2.76	46.92 ± 3.24	0.1233
PLA	44.44 ± 3.05	45.05 ± 3.23	45.05 ± 3.23	44.05 ± 3.45	
MCV (fl)					
BHB	102.36 ± 3.41	101.55 ± 3.53	101.00 ± 3.66	100.00 ± 3.29	0.8246
PLA	101.91 ± 6.69	101.64 ± 6.09	101.09 ± 6.27	100 ± 7.17	
MCH (pg)					
BHB	31.01 ± 1.01	31.00 ± 1.39	30.85 ± 1.11	30.85 ± 1.07	0.9716
PLA	30.73 ± 2.60	30.85 ± 2.76	30.60 ± 2.53	30.59 ± 2.81	
MCHC (g/dL)					
BHB	30.29 ± 0.46	30.50 ± 0.66	30.52 ± 0.79	30.85 ± 0.71	0.9812
PLA	30.10 ± 0.95	30.26 ± 1.08	30.24 ± 0.90	30.55 ± 1.06	
RDW (%)					

	PRE	30 DAY	60 DAY	90 DAYS	p Value
BHB	13.15 ± 0.59	13.14 ± 0.57	12.94 ± 0.43	12.96 ± 0.47	0.4562
PLA	12.73 ±3.26	13.30 ± 1.06	13.30 ± 1.04	13.50 ± 0.42	
Platelets (k/uL)					
BHB	218.64±5.86	230.73±70.03	232.27±65.23	236.73±79.15	0.4181
PLA	218.09±34.31	217.5±33.87	217.64±40.66	222.00±45.73	

Data reported in mean and standard deviation. P-value is from group by time interaction effect.

3.2. Automated Differential

There was no significant between or within group differences in any values of Automated Differential Cell Count ($p > 0.05$, Table 3). Mean and standard deviation are displayed in Table 3.

Table 3. *Automated Differential Cell Count*

	PRE	30 DAY	60 DAY	90 DAYS	p Value
Lymphocytes (%)					
BHB	35.77 ± 8.38	35.34 ± 7.83	35.68 ± 8.83	30.99 ± 10.14	0.2397
PLA	35.86 ± 7.02	38.42 ± 7.16	37.58 ± 6.68	37.25 ± 6.28	
Monocytes (%)					
BHB	8.17 ± 5.42	6.01 ± 2.38	5.81 ± 2.45	6.70 ± 3.96	0.4749
PLA	6.35 ± 1.79	6.21 ± 2.10	5.90 ± 1.81	5.72 ± 1.73	
Eosionophil (%)					
BHB	2.02 ± 1.28	2.26 ± 1.32	2.35 ± 1.34	2.17 ± 1.35	0.6470
PLA	2.01 ± 1.21	2.02 ± 1.34	1.92 ± 1.16	2.05 ± 1.02	
Basophil (%)					
BHB	1.34 ± 0.27	1.22 ± 0.40	1.09 ± 0.32	1.05 ± 0.23	0.4171
PLA	1.15 ± 0.43	1.14 ± 0.46	1.03 ± 0.35	1.13 ± 0.74	
Granulocytes (%)					
BHB	54.76 ± 8.55	55.16 ± 8.82	54.15 ± 9.98	59.03 ± 12.18	0.4594
PLA	54.63 ± 8.75	52.25 ± 6.94	53.38 ± 7.07	53.98 ± 7.24	
Lymphocytes (k/uL)					
BHB	2.03 ± 0.62	1.76 ± 0.31	1.93 ± 0.37	1.86 ± 0.40	0.2579
PLA	2.13 ± 0.53	2.27 ± 0.58	2.23 ± 0.46	2.29 ± 0.53	
Monocytes (k/uL)					
BHB	0.33 ± 0.15	0.28 ± 0.11	0.30 ± 0.10	0.41 ± 0.25	0.0812
PLA	0.38 ± 0.08	0.35 ± 0.10	0.35 ± 0.12	0.34 ± 0.10	
Eosionophil (k/uL)					
BHB	0.09 ± 0.05	0.12 ± 0.06	0.12 ± 0.06	0.14 ± 0.08	0.1565
PLA	0.13 ± 0.05	0.13 ± 0.06	0.12 ± 0.08	0.13 ± 0.06	
Basos (k/uL)					
BHB	0.09 ± 0.03	0.07 ± 0.05	0.06 ± 0.05	0.08 ± 0.04	0.4746
PLA	0.07 ± 0.05	0.08 ± 0.04	0.07 ± 0.05	0.08 ± 0.06	
Granulocytes (k/uL)					
BHB	2.60 ± 0.99	2.85 ± 0.89	2.98 ± 0.94	4.15 ± 2.62	0.1234
PLA	3.26 ± 0.84	3.11 ± 0.77	3.21 ± 0.87	3.35 ± 0.81	

Data reported in mean and standard deviation. P-value is from group by time interaction effect.

3.3. Comprehensive Metabolic Panel

There was no significant between or within group differences in any values of the comprehensive metabolic panel ($p > 0.05$, Table 4). Mean and standard deviation are displayed in Table 4.

Table 4. *Comprehensive Metabolic Panel*

	PRE	30 DAY	60 DAY	90 DAY	p Value
Total Protein (g/dL)					
BHB	6.65 ± 0.35	6.48 ± 0.28	6.51 ± 0.38	6.48 ± 0.43	0.5227
PLA	6.58 ± 0.41	6.56 ± 0.44	6.47 ± 0.36	6.42 ± 0.38	
Albumin (g/dL)					
BHB	4.49 ± 0.22	4.28 ± 0.22	4.28 ± 0.22	4.44 ± 0.28	0.4284

	PRE	30 DAY	60 DAY	90 DAY	p Value
PLA	4.40 ± 0.20	4.29 ± 0.25	4.2 ± 0.20	4.29 ± 0.29	
Globulin (g/dL)					
BHB	2.15 ± 0.05	2.2 ± 0.18	2.25 ± 0.26	2.05 ± 0.42	0.8957
PLA	2.18 ± 0.27	2.27 ± 0.23	2.27 ± 0.21	2.13 ± 0.26	
ALB: GLOB (U/L)					
BHB	2.10 ± 0.24	1.97 ± 0.17	1.97 ± 0.17	1.91 ± 0.23	0.4320
PLA	2.05 ± 0.23	1.89 ± 0.16	1.88 ± 0.17	2.05 ± 0.30	
Bilirubin (mg/dL)					
BHB	0.66 ± 0.21	0.63 ± 0.11	0.65 ± 0.22	0.62 ± 0.17	0.8334
PLA	0.57 ± 0.21	0.55 ± 0.14	0.63 ± 0.21	0.54 ± 0.17	
Alkaline Phosphate (U/L)					
BHB	52.73 ± 11.42	50.09 ± 13.48	49.91 ± 13.23	50.09 ± 11.69	0.4123
PLA	52.64 ± 14.70	51.82 ± 15.09	52.45 ± 12.23	54.09 ± 12.93	
AST (U/L)					
BHB	23.27 ± 6.23	27.73 ± 19.89	24.82 ± 9.59	23.55 ± 10.47	0.3370
PLA	28.27 ± 16.87	23.64 ± 8.61	24.45 ± 7.53	22.64 ± 7.70	
ALT (U/L)					
BHB	23.09 ± 9.60	26.36 ± 12.92	26.09 ± 16.16	25.64 ± 17.47	0.2445
PLA	29.00 ± 27.07	23.27 ± 9.71	25.73 ± 15.11	24.82 ± 15.68	
BUN (mg/dL)					
BHB	17.00 ± 4.27	14.73 ± 3.17	15.36 ± 4.11	16.09 ± 3.96	0.1065
PLA	15.91 ± 3.75	17.64 ± 5.41	17.00 ± 6.23	17.36 ± 6.34	
Creatinine (mg/dL)					
BHB	0.86 ± 0.22	0.90 ± 0.23	0.92 ± 0.22	0.89 ± 0.23	0.3388
PLA	0.85 ± 0.13	0.91 ± 0.13	0.89 ± 0.16	1.58 ± 2.17	
BUN/Creatinine (mg/dL)					
BHB	24.23 ± 4.84	21.30 ± 0.14	21.40 ± NA	21.40 ± N/A	0.0881
PLA	22.48 ± 0.86	24.73 ± 2.11	25.23 ± 1.27	23.30 ± N/A	
eGFR (mL/min)					
BHB	88.73 ± 15.41	84.55 ± 15.71	81.36 ± 11.93	84.82 ± 12.96	0.2965
PLA	86.64 ± 9.01	80.73 ± 10.07	82.36 ± 11.53	79.00 ± 9.34	
Sodium (mg/dL)					
BHB	141.45 ± 2.21	140.91 ± 1.64	140.18 ± 1.54	139.64 ± 2.06	0.6043
PLA	141.18 ± 1.83	140.45 ± 1.21	140.00 ± 1.73	140.18 ± 2.09	
Potassium (mg/dL)					
BHB	4.35 ± 0.35	4.27 ± 0.35	4.13 ± 0.23	4.26 ± 0.24	0.4547
PLA	4.35 ± 0.34	4.19 ± 0.18	4.08 ± 0.16	4.07 ± 0.18	
Chloride (mg/dL)					
BHB	103.55 ± 2.34	102 ± 1.95	102.91 ± 1.81	102.91 ± 2.26	0.9183
PLA	105.36 ± 2.46	103.82 ± 1.47	104.18 ± 1.78	104.55 ± 2.46	
Carbon Dioxide (mL/min)					
BHB	27.55 ± 2.25	29.36 ± 1.57	28.91 ± 1.58	29.36 ± 2.42	0.1070
PLA	26.55 ± 2.07	26.64 ± 2.11	27.18 ± 1.99	27.00 ± 2.10	
Calcium (mg/dL)					
BHB	9.23 ± 0.31	8.97 ± 0.35	9.21 ± 0.35	9.19 ± 0.28	0.1681
PLA	9.14 ± 0.34	8.95 ± 0.32	9.05 ± 0.36	8.92 ± 0.37	
Glucose (mg/dL)					
BHB	73.45 ± 8.00	87.64 ± 4.43	87.27 ± 4.98	93.36 ± 8.88	0.7419
PLA	76.36 ± 12.47	89.73 ± 10.25	92.09 ± 9.02	93.36 ± 10.53	
Hemoglobin A1c					
BHB	5.25 ± 0.27	5.10 ± 0.30	5.20 ± 0.31	5.38 ± 0.26	0.3925
PLA	5.32 ± 0.25	5.25 ± 0.29	5.38 ± 0.30	5.48 ± 0.30	

Data reported in mean and standard deviation. P-value is from group by time interaction effect. (ALB:GLOB = Albumin:Globulin Ratio, AST =aspartate aminotransferase, ALT = alanine transaminase, BUN = blood urea nitrogen, eGFR = estimated glomerular filtration rate.)

3.4. Blood Pressure and Heart Rate

There was no significant between or within group differences in resting blood pressure or heart rate ($p > 0.05$, Table 5). Mean and standard deviation are displayed in Table 5.

Table 5. *Blood Pressure and Cardiovascular Results.*

	PRE	30 DAY	60 DAY	90 DAYS	p Value
Systolic BP (mmHg)					
BHB	113.00±10.25	112.18±9.66	109.00±11.25	109.09 ± 9.27	0.6480
PLA	115.91±11.38	117.45±10.35	116.64±10.60	115.00 ± 8.56	
Diastolic BP (mmHg)					
BHB	68.27 ± 10.68	64.36 ± 7.12	63.55 ± 10.00	65.45 ± 7.61	0.5610
PLA	70.82 ± 10.25	68.55 ± 11.85	70.55 ± 11.10	68.36 ± 8.54	
Heart Rate (bpm)					
BHB	62.55 ± 10.68	64.55 ± 7.03	65.00 ± 10.00	63.82 ± 8.15	0.4459
PLA	61.64 ± 8.32	64.73 ± 8.68	61.64 ± 8.41	60.91 ± 8.60	

Data reported in mean and standard deviation. P-value is from group by time interaction effect. (BP = blood pressure).

3.5. Profile of Mood States (POMS) & Immune Status Questionnaire (ISQ)

There was no significant between or within group differences for responses to the POMS questionnaire or the ISQ ($p > 0.05$, Table 6). Mean and standard deviation are displayed in Table 6.

Table 6. *Survey Results*

	PRE	30 DAYS	60 DAYS	90 DAYS	p Value
Total POMS Score (a.u.)					
BHB	82.55 ± 17.06	77.36 ± 13.43	79.55 ± 12.13	86.09 ± 9.97	0.7240
PLA	73.64 ± 9.14	73.91 ± 11.22	74.45 ± 10.82	78.09 ± 7.83	
ISQ Total Score (a.u.)					
BHB	9.00 ± 1.10	8.73 ± 1.74	9.00 ± 1.48	8.73 ± 1.42	0.2195
PLA	9.27 ± 0.47	9.45 ± 0.52	9.36 ± 0.67	9.64 ± 0.50	

Data reported in mean and standard deviation. P-value is from group by time interaction effect.

3.6. Body Composition & Bone Densitometry

There was no significant between or within group differences in any body composition or bone densitometry values ($p > 0.05$, Table 7). Mean and standard deviation are displayed in Table 7.

Table 7. *Body Composition & Bone Densitometry Results.*

	PRE	DAY 90	p Value
Total Mass (kg)			
BHB	72.66 ± 14.24	72.80 ± 14.44	0.9727
PLA	79.53 ± 17.98	79.60 ± 18.92	
Fat Mass (kg)			
BHB	23.21 ± 9.55	23.96 ± 10.48	0.8739
PLA	23.74 ± 6.87	25.31 ± 8.26	
Fat Free Mass (kg)			
BHB	49.45 ± 10.96	48.84 ± 9.69	0.5330
PLA	55.79 ± 14.28	54.28 ± 12.66	
Body Fat %			
BHB	31.66 ± 9.44	32.29 ± 9.63	0.3223
PLA	29.96 ± 5.94	31.45 ± 5.90	
Bone Mineral Density (g/cm^2)			
BHB	1.13 ± 0.11	1.11 ± 0.09	0.1530
PLA	1.16 ± 0.13	1.18 ± 0.11	
Bone Mineral Content (g)			
BHB	2291.19 ± 369.46	2248.45 ± 245.24	0.2638
PLA	2510.98 ± 501.73	2534.13 ± 409.74	

Data reported in mean and standard deviation. P-value is from group by time interaction effect.

4. Discussion

In this study, we demonstrated the safety of exogenous BHB under uncontrolled conditions of daily living. The most significant finding of this study was that sustained 25.5 grams of daily exogenous ketone salt consumption for 90 days was safe for healthy adults and that it had no adverse effect on any blood health markers, hemoglobin A1c, psychological well-being, or cardiovascular markers of health. Comprehensive metabolic panel, complete blood count, and automated differential cell count remained normal and unaltered after supplementing twice daily with exogenous

BHB for 90 days. Furthermore, there were no significant changes in the POMS or ISQ, resting blood pressure, or resting heart rate. The findings in this study support, and further build upon, a previous study by Holland et al. [24] demonstrating that 6 weeks of exogenous ketone salts supplementation did not negatively impact various markers of human health and safety in adults.

There are many controversial views regarding the ketogenic diet, ketosis, and exogenous ketones. Two such views are that ketosis can increase the risk of complications in the liver [27], and the kidneys [28]. However, in human studies, it was demonstrated that the ketogenic diet may improve clinical outcomes of nonalcoholic fatty liver disease [29, 31]. In the present study, we found that markers of liver health; total protein, albumin, globulin, ALB:GLOB ratio, bilirubin, alkaline phosphate, AST, and ALT; were unaffected and no different from placebo with exogenous ketone supplementation in a healthy population over 90 days.

Depending on macronutrient distribution of caloric intake, the ketogenic diet can be considered a high protein diet (≥ 20% of caloric intake). It has been posited that diets higher in protein could lower pH and increase the acidic load on the kidneys [28]. However, the previously referenced study used a high- protein, low-carbohydrate diet, while a typical ketogenic diet consists of low to moderate protein and very-low carbohydrate [30]. With a typical ketogenic diet, Kossof et al. [32] demonstrated no increased risk of kidney stones in children. Our study demonstrated no changes in kidney function markers such as BUN, creatinine, BUN/creatinine ratio, and eGFR in a healthy population over 90 days. In addition, acid-base balance was maintained as demonstrated by blood carbon dioxide and chloride levels.

The ketogenic diet has shown to lead to calcium loss, and in some cases, can increase the risk of bone loss. Previous research has suggested that the ketogenic diet can increase calcium excretion, which can lead to bone mass loss in children and adolescents [33–35]. Our study demonstrated no blood calcium or electrolyte loss, as all electrolyte levels were unchanged over 90 days in a healthy adult population. Moreover, we found no changes in bone mineral density or bone mineral content as assessed by whole-body DXA scans at PRE and Day 90. These results suggest that BHB does not lead to bone density or bone mineral content loss.

Lastly, it has been postulated that electrolytes may alter different markers of cardiovascular health such as blood pressure and heart rate [36]. Exogenous ketone salts, like the ones used in the present study, are bound to calcium and magnesium in order to improve transport and absorption across the gut-blood barrier. Therefore, it is reasonable to investigate if altering dietary intake of electrolytes with the consumption of exogenous ketone salts may have an effect on blood pressure. However, in our present study, systolic blood pressure, diastolic blood pressure, and heart rate were unaffected in the resting state. In addition, blood concentrations of sodium, potassium, chloride, and calcium all remained unaltered.

A limitation of the present study is the method of supplementation, and the lack of exercise and dietary control. Subjects were provided the supplement every 30 days when they came into the laboratory for testing. They were asked to return any unused supplements to the laboratory to help keep subjects honest and to track adherence. Lastly, diet and exercise were not controlled. However, to keep subjects accountable, they were encouraged to track and report dietary intake via a mobile application. Dietary records demonstrated no differences in daily average of total calories consumed (BHB: 1430.71 ± 370.84 vs PLA: 1560.04 ± 394.54 kcal/day, p=0.4381). Future research may seek to directly compare the effects of exogenous ketone esters and exogenous ketone salts.

5. Conclusion

Exogenous ketone salt supplementation (BHB) can be considered safe and well tolerated. BHB showed no changes in comprehensive metabolic panel, automated differential cell count, complete blood count, hemoglobin A1c, resting blood pressure and heart rate or psychological surveys over 90 days of supplementation in a healthy population when compared to PLA. This study established the safety of long term BHB supplementation, but further investigation is needed to examine the efficacy of exogenous ketone supplementation in other areas of health, longevity, cognitive function, and other chronic conditions.

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