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Acute Nutritional Ketosis and Its Implications for Plasma Glucose and Glucoregulatory Peptides in Adults with Prediabetes: A Crossover Placebo-Controlled Randomized Trial

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ABSTRACT

Background: The potential of a ketone monoester (β-hydroxybutyrate; KEβHB) supplement to rapidly mimic a state of nutritional ketosis offers a new therapeutic possibility for diabetes prevention and management. While KEβHB supplementation has a glucose-lowering effect in adults with obesity, its impact on glucose control in other insulin-resistant states is unknown.

Objectives: The primary objective was to investigate the effect of KEβHB-supplemented drink on plasma glucose in adults with prediabetes. The secondary objective was to determine its impact on plasma glucoregulatory peptides.

Methods: This randomized controlled trial [called CETUS (Cross-over randomizEd Trial of β-hydroxybUtyrate in prediabeteS)] included 18 adults [67% men, mean age = 55 y, mean BMI (kg/m^2) = 28.4] with prediabetes (glycated hemoglobin between 5.7% and 6.4% and/or fasting plasma glucose between 100 and 125 mg/dL). Participants were randomly assigned to receive KEβHB-supplemented and placebo drinks in a crossover sequence (washout period of 7–10 d between the drinks). Blood samples were collected from 0 to 150 min, at intervals of 30 min. Paired-samples *t* tests were used to investigate the change in the outcome variables [β-hydroxybutyrate (βHB), glucose, and glucoregulatory peptides] after both drinks. Repeated measures analyses were conducted to determine the change in concentrations of the prespecified outcomes over time.

Results: Blood βHB concentrations increased to 3.5 mmol/L within 30 minutes after KEβHB supplementation. Plasma glucose AUC was significantly lower after KEβHB supplementation than after the placebo [mean difference (95% CI): −59 (−85.3, −32.3) mmol/L × min]. Compared with the placebo, KEβHB supplementation led to significantly greater AUCs for plasma insulin [0.237 (0.044, 0.429) nmol/L × min], C-peptide [0.259 (0.114, 0.403) nmol/L × min], and glucose-dependent insulinotropic peptide [0.243 (0.085, 0.401) nmol/L × min], with no significant differences in the AUCs for amylin, glucagon, and glucagon-like peptide 1.

Conclusions: Ingestion of the KEβHB-supplemented drink acutely increased the blood βHB concentrations and lowered the plasma glucose concentrations in adults with prediabetes. Further research is needed to investigate the dynamics of repeated ingestions of a KEβHB supplement by individuals with prediabetes, with a view to preventing new-onset diabetes. This trial was registered at www.clinicaltrials.gov as NCT03889210. *J Nutr* 2021;151:921–929.

Keywords: prediabetes, ketosis, β-hydroxybutyrate, insulin, C-peptide, glucagon, amylin, glucagon-like peptide 1, glucose-dependent insulinotropic peptide, randomized controlled trial

Introduction

Ketosis, a metabolic state characterized by elevated circulating levels of ketones, is a normal physiological response to starvation or energy deficit. Ketones [β-hydroxybutyrate (βHB), acetoacetate, and acetone] are an efficient oxidizable source of energy as these conserve glucose/gluconeogenic substrates and regulate the preferential oxidization of other fuel substrates (e.g., fatty acids) (1). Ketones are synthesized mostly in the liver from fatty acids (mobilized from the adipose tissue).

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Subsequently, they are transported to metabolically active tissues (e.g., brain, skeletal muscle, heart), where they are metabolized to generate energy (i.e., ATP) (2). Classically, ketones were thought to be toxic byproducts of impaired carbohydrate metabolism due to the mid-19th century discovery of ketones in the urine of patients in a diabetic coma (3). Almost half a century later, a broader role of ketones in maintaining metabolic homeostasis, lipid metabolism, and regulating gene expression was established (4). However, the metabolic potential of ketones outside of energy crisis or diabetic ketoacidosis is poorly understood.

A growing body of evidence supports a possible therapeutic benefit of mild to moderate nutritional ketosis (ketones range: 0.5–7 mmol/L) (5) in aiding the management of hyperglycemia in type 2 diabetes (6) and improving weight loss (7). Acute exogenous delivery of ketones using a drink formulation containing a monoester of ketone βHB (KEβHB) provides a safe strategy for mimicking the biochemical appearance of nutritional ketosis (8, 9). Further, this state of "achieved" nutritional ketosis has a glucose-lowering effect in healthy adults (10, 11). The metabolic effect of this KEβHB-supplemented drink has primarily been examined in relation to exercise performance and when ingested along with a standard meal or oral glucose tolerance test (OGTT) (8, 10–12). Recently, a study that used OGTT demonstrated the glucose-lowering effect of the KEβHB-supplemented drink in obese individuals (13). In that study, ingestion of the KEβHB supplement was followed by an 11% reduction in glucose concentrations. While that study points to the beneficial effects of the KEβHB supplement in regulating glucose and improving metabolic homeostasis in adults with obesity, its effect in other insulin-resistant states has not been fully examined. Critical to evaluating the metabolic actions of the KEβHB supplement is to investigate its impact on glucose control in adults with prediabetes (as they are at a high risk for new-onset diabetes).

The primary aim was to investigate the effect of a single KEβHB-supplemented drink (without the intake of any other nutritional stimulants) on plasma glucose in adults with prediabetes. Given the paucity of data on the impact of KEβHB supplementation on hormonal regulation in insulin-resistant states, the secondary aim was to investigate the effect of the KEβHB-supplemented drink on glucoregulatory peptides [insulin, C-peptide, amylin, glucagon, glucose-dependent insulinotropic peptide (GIP), and glucagon-like peptide 1 (GLP-1)].

Methods

Study design and ethics

The study, called CETUS (Cross-over randomizEd Trial of β-hydroxybUtyrate in prediabeteS), was a crossover randomized controlled trial (RCT) of individuals with prediabetes. The study protocol, participant information sheet, and participant consent form were approved by the Health and Disability Ethics Committee, New Zealand (18/NTB/161). The study was conducted in compliance with the ethical standards of our institution on human subjects and the standards set by the Helsinki Declaration. The study was prospectively registered in the US clinical trials registry (clinicaltrials.gov) as NCT03889210.

The study included individuals (aged ≥18 y) with prediabetes determined based on the American Diabetes Association criteria: glycated hemoglobin (HbA1c) concentration between 5.7% and 6.4% (39–47 mmol/mol) and/or fasting plasma glucose (FPG) between 100 and 125 mg/dL (5.6–6.9 mmol/L) (14). Individuals were excluded if they were ever diagnosed with diabetes, were on insulin or other antidiabetic medications, followed a ketogenic diet or consumed other ketone supplements (e.g., ketone salts), participated in competitive sports or performed intensive physical training activity on a regular basis, had malignancy, had poor venous access for blood sampling, or had a cognitive disability. All participants provided informed consent.

Study protocol

Participants were randomly assigned to receive a KEβHB supplement–containing drink [commercially available FDA approved monoester of the ketone β-hydroxybutyrate (R-3-hydroxybutryl-1,3-hydroxybutyrate; ΔG®; TΔS Ltd)] or a placebo drink (consisting of water, flavored stevia, malic acid, and arrowroot) in a crossover sequence. Participants and the study personnel collecting the blood samples were blinded to the drink allocation. An online random-number generator (https://www.sealedenvelope.com) was used to produce a list of visit order combinations in blocks of four. The primary outcome was plasma glucose response to the KEβHB-supplemented drink compared with the placebo drink. The secondary outcome was plasma glucoregulatory peptides response (insulin, C-peptide, amylin, glucagon, GIP, and GLP-1) to the KEβHB-supplemented drink compared with the placebo drink.

Clinic visits protocol.

Participants were asked to refrain from alcohol and exercise for at least 24 h before each clinic visit. They were asked to keep a food log for the 24 h prior to their first clinic visit to facilitate the consumption of similar meals before each clinic visit.

All participants visited the clinic after an overnight fast (>8 h). At the first visit, anthropometric measurements and blood pressure readings were taken. A venous catheter with stopcock apparatus (BD Nexiva™; Becton, Dickinson and Company) was inserted in the antecubital vein to permit serial blood sample collection. Blood samples were collected in the fasting state. Immediately after the fasting blood collection, participants consumed a single drink containing the KEβHB supplement [total volume of 100 mL containing 395 mg/kg lean body weight (15)] of the KEβHB supplement and flavored stevia (SweetLeaf Sweetener®; Wisdom Natural Brands) and water [energy content ≤123 kcal (515kJ)]. In the alternative condition, participants consumed a single drink containing the placebo [total volume of 100 mL constituting water, flavored stevia (SweetLeaf Sweetener®), malic acid (BioTrace®), and arrowroot (McKenzie's®); energy content not measured]. Blood samples were collected at 30, 60, 90, 120, and 150 min following ingestion of the drinks. The catheter was kept patent using a saline flush after each blood sample collection. At the end of the first visit, participants were given a copy of their 24-h food log. They were reminded to consume similar meals 1 d before their second visit and refrain from exercise and alcohol. Participants visited the clinic in a fasted state (>8 h) after 7–10 d following their first visit. After the participants confirmed abstinence from alcohol and exercise, as well as compliance with the dietary guidelines, the protocol (as described above) was followed with the alternative drink (according to the randomized crossover design) (**Supplemental Figure 1**).

Laboratory measurements.

Blood samples were collected in EDTA and lithium heparin tubes (BD Vacutainer®; Becton, Dickinson and Company), centrifuged at 4000

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Abbreviations used: AUC, area under the curve; FPG, fasting plasma glucose; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1; HbA1c, glycated hemoglobin; KEβHB, ketone monoester (β-hydroxybutyrate); OGTT, oral-glucose-tolerance test; RCT, randomized controlled trial; βHB, β-hydroxybutyrate.

x *g* for 5 min at 4°C; the serum was separated into aliquots and stored at −80°C for further analyses. β-hydroxybutyrate was measured immediately on whole blood using a handheld monitor and Optium blood β-ketone test strip (Abbott Laboratories) (16). Plasma glucose and blood HbA1c were analyzed at the tertiary medical laboratory at Auckland City Hospital, New Zealand. Plasma glucose was measured using enzymatic colorimetric assay (F. Hoffman-La Roche Ltd), whereas blood HbA1c was measured using a boronate affinity chromatography assay (Trinity Biotech). The latter was certified by the National Glycohemoglobin Standardization Program and standardized to the Diabetes Control and Complications Trial reference assay. Plasma insulin, C-peptide, amylin, glucagon, GIP, and GLP-1 were measured using MILLIPLEX® MAP Human metabolic hormone magnetic bead panel based on Luminex xMAP® (Luminex Corporation). Protease (Sigma/Merck LGaA) and dipeptidyl peptidase IV inhibitor (Merck KGaA) were added to each sample. Results were quantified based on fluorescent reporter signals recorded by the Luminex xPONENT® software (Milliplex Analyst 5.1). The interassay and intra-assay CVs for the glucoregulatory peptides were <5% and <10%, respectively.

Other measurements.

BMI (kg/m^2), waist and hip circumferences (centimeters), and blood pressure (millimeters of mercury) were measured using previously published standardized methods (17).

Alcohol consumption and tobacco smoking consumption were determined based on a standardized questionnaire (18) administered at the first clinic visit.

All participants underwent abdominal MRI at the Center of Advanced MRI (University of Auckland) with a 3.0-Tesla MAGNETOM Skyra scanner (Siemens). Visceral fat volume measurements were performed independently by 2 raters blinded to the participants' characteristics, as described elsewhere (19).

Sample size calculation

Based on the published literature (10, 11), we hypothesized that individuals with prediabetes will have a 15% decrease in plasma glucose after ingestion of the KEβHB supplement. Previously published data showed that individuals with prediabetes had an FPG (mean ± SD) of 5.87 ± 0.77 mmol/L (20). In line with the primary outcome of our study, to detect a 15% reduction in glucose from a mean value of 5.87 mmol/L, which corresponded to an effect size of 0.88, a sample size of 15 was determined (at an α-level of 0.05 and 80% statistical power) using the following equation (21):

$$\text{Power} = \text{pt}(\text{qt}(0.025,\ n-1,\ 0),\ n-1,\ -(\text{delta}/\text{sigma}) \times \text{sqrt}(n)) \quad (1)$$

where pt is the noncentral t function, qt is the inverse function of pt, delta is the difference in means and was set as 0.88 (effect size), and sigma is the SD of the difference and was set as 1.

Eighteen individuals were determined to be required for randomization to account for a possible 20% dropout or missing samples.

Statistical analyses

All statistical analyses were conducted using SPSS for Windows 25.0 (IBM Corporation). Data on baseline characteristics of study participants were presented as frequencies, means ± SDs (for normally distributed variables), or medians (IQRs) (for non–normally distributed variables). The subsequent statistical analyses were conducted in the following steps.

First, the total area under the curve (AUCs) for all variables (βHB, glucose, insulin, C-peptide, amylin, glucagon, GIP, and GLP-1) were calculated using the trapezoidal rule. Carryover effects for the crossover sequences (AB/BA sequences) over 2 visits were estimated using *t* tests (22). Given that the interaction between the clinic visits order (visit 1 and visit 2) was not statistically significant for the primary and secondary outcomes ($P > 0.05$), data from both the visits were used for further analyses.

Second, paired-samples *t* tests were conducted to compare the baseline measurements and total AUCs for the primary and secondary outcomes after the KEβHB-supplemented and placebo drinks. The variables insulin, C-peptide, amylin, glucagon, and GIP were log-transformed. Data were presented as means ± SEMs, unless indicated otherwise. Effect size measures were mean differences and their 95% CIs. Cohen's effect size (*d*) was calculated to assess the magnitude of the difference in the outcomes between the 2 study periods.

Third, repeated-measures ANOVAs with drink and time as fixed factors were conducted to determine the change in primary and secondary outcomes over time after the KEβHB-supplemented and placebo drinks and to measure the interaction between the drink(s) and time (drink × time). The Greenhouse-Gessier correction was applied when the assumption of sphericity was violated. The omega-squared (ω^2) metric (23), an alternative measure of the effect size, was calculated to measure the proportion of variance in the outcome variables after the KEβHB-supplemented drink. For all analyses, *P* values < 0.05 were deemed to be statistically significant.

Results

Characteristics of study participants

Eighteen individuals who met all of the eligibility criteria were randomly assigned in a crossover sequence (**Figure 1**). Nine received the KEβHB-supplemented drink, and 9 received the placebo drink on the first visit. Of the 18 participants, 10 participants met the prediabetes criteria (14) based on both HbA1c and FPG, 4 participants based on HbA1c only, and 4 participants based on FPG only. Other characteristics of the study participants are presented in **Table 1**.

Blood βHB profile in response to the KEβHB-supplemented and placebo drinks

Baseline concentrations of blood βHB after the KEβHB-supplemented drink were 0.18 ± 0.02 mmol/L and after the placebo drink were 0.17 ± 0.02 mmol/L ($P = 0.421$).

The total $AUC_{0–150}$ of βHB after the KEβHB-supplemented drink (560 ± 79 mmol/L × min) was significantly greater than after the placebo drink (50 ± 7 mmol/L × min), a mean difference (95% CI) of 510 (353, 665) mmol/L × min ($P < 0.001$, $d = 1.62$). Repeated-measures analysis showed a significant main effect of the drink ($P < 0.001$, $\omega^2 = 1.21$), time ($P < 0.001$), and a significant interaction effect of drink × time ($P < 0.001$) on βHB after KEβHB supplementation. Blood βHB concentrations were significantly higher at 30, 60, 90, 120, and 150 min after ingestion of the KEβHB-supplemented drink compared with the placebo drink, with the highest mean difference at 30 min (3.47 ± 0.22 mmol/L after KEβHB supplementation vs. 0.15 ± 0.01 mmol/L after the placebo; $P < 0.001$) (**Figure 2**).

Plasma glucose profile in response to the KEβHB-supplemented and placebo drinks

The total $AUC_{0–150}$ of glucose after the KEβHB-supplemented drink (738 ± 19 mmol/L × min) was significantly lower than after the placebo drink (797 ± 15 mmol/L × min), a mean difference (95% CI) of −59 (−85.3, −32.3) mmol/L × min ($P < 0.001$, $d = −1.10$). The intraindividual differences in the AUCs for glucose following the KEβHB-supplemented and placebo drinks are presented in **Supplemental Figure 2**A. Repeated-measures analysis showed a significant main effect of drink ($P < 0.001$, $\omega^2 = 0.64$), time ($P < 0.001$), and a significant interaction effect of drink × time ($P < 0.001$) on glucose after KEβHB supplementation. Plasma glucose concentrations were significantly lower at 60, 90, 120, and 150 min after the KEβHB-supplemented drink compared with the placebo drink, with the highest mean difference at 60 min (4.77 ± 0.15 mmol/L

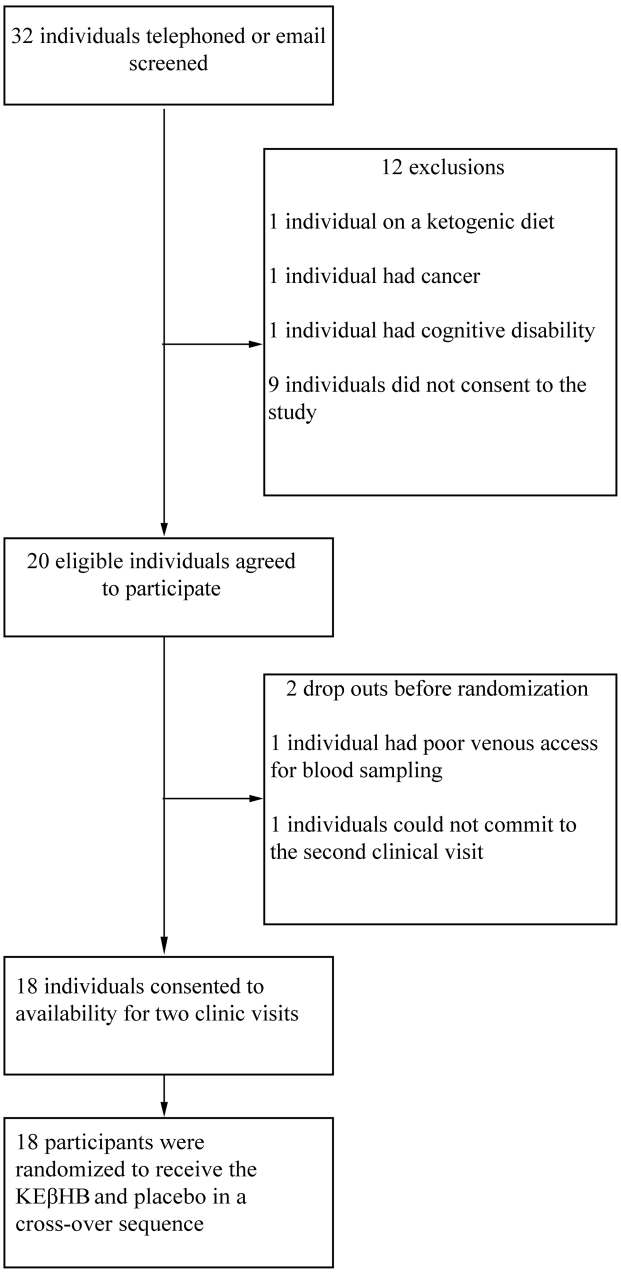

FIGURE 1 CONSORT flow diagram. CONSORT, Consolidated Standards of Reporting Trials; KEβHB, ketone monoester (β-hydroxybutyrate) supplement.

after KEβHB supplementation and 5.33 ± 0.11 mmol/L after the placebo; $P < 0.001$) (Figure 3).

Plasma glucoregulatory peptides profile in response to the KEβHB-supplemented and placebo drinks

Insulin.

The total $AUC_{0–150}$ for insulin was significantly greater after the KEβHB-supplemented drink compared with the placebo drink ($P = 0.019$) (Table 2). The intraindividual differences in the AUCs for insulin following the KEβHB-supplemented placebo drinks are presented in Supplemental Figure 2B. There was a significant main effect of the drink ($P = 0.049$, $\omega^2 = 0.26$), time ($P = 0.004$), and a significant interaction effect of drink × time ($P = 0.035$) on insulin after KEβHB supplementation. Plasma insulin concentrations were significantly higher at 30 min after the KEβHB-supplemented drink compared with the placebo drink, with the highest mean difference at 30 min (292 ± 69 pmol/L after KEβHB supplementation and 206 ± 53 pmol/L after the placebo; $P = 0.003$). Plasma insulin concentrations at 60, 90, 120, and 150 min after the KEβHB-supplemented drink were not significantly different compared with the placebo drink (Figure 4A).

TABLE 1 Baseline characteristics of study participants[1]

Characteristic	Value
Age, y	55 ± 14
Sex, n	
Men	12
Women	6
BMI, kg/m^2	28.4 ± 5.9
Waist circumference, cm	98.6 ± 15.8
Hip circumference, cm	106 ± 13
Systolic blood pressure, mm Hg	135 ± 27
Diastolic blood pressure, mm Hg	88.1 ± 12.8
Glycated hemoglobin, %	5.7 ± 0.3
Fasting plasma glucose, mg/dL	101 ± 9
Plasma insulin, nmol/L	0.13 (0.08–0.35)
Plasma C-peptide, nmol/L	0.60 (0.43–0.74)
Plasma glucagon, μg/L	0.07 (0.05–0.30)
Plasma amylin, μg/L	0.03 (0.02–0.25)
Plasma GIP, nmol/L	0.01 (0.01–0.02)
Plasma GLP-1, nmol/L	0.06 (0.05–0.09)
Visceral fat volume, m^3	0.002 ± 0.001
Alcohol use	
Alcohol consumption, g/wk	15 (4–40)
AUDIT score[2]	4 (2–11)
Smoking status	
Current smoker, n	3
Former smoker, n	7
Never smoker, n	8
Total time smoking, y	29 (7–42)

[1] Values are means ± SDs or medians (IQRs) unless otherwise indicated; $n = 18$. AUDIT, Alcohol Use Disorders Identification Test; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1.
[2] AUDIT score >6 indicated alcohol dependency.

C-peptide.

The total $AUC_{0–150}$ for C-peptide was significantly greater after the KEβHB-supplemented drink compared with the placebo drink ($P = 0.002$) (Table 2). The intraindividual differences in the AUCs for C-peptide following the KEβHB-supplemented and placebo drinks are presented in Supplemental Figure 2C. There was a significant main effect of the drink ($P = 0.001$, $\omega^2 = 0.92$), time ($P < 0.001$), and a significant interaction effect of drink × time ($P = 0.001$) on C-peptide after KEβHB supplementation. Plasma C-peptide concentrations were significantly higher at 30, 60, and 90 min after the KEβHB-supplemented drink compared with the placebo drink, with the highest mean difference at 30 min (0.88 ± 0.13 nmol/L after KEβHB supplementation and 0.50 ± 0.06 nmol/L after the placebo; $P < 0.001$). Plasma C-peptide concentrations were not significantly different at 120 and 150 min after the KEβHB-supplemented drink compared with the placebo drink (Figure 4B).

Amylin.

The total $AUC_{0–150}$ for amylin did not differ significantly after the KEβHB-supplemented drink compared with the placebo

FIGURE 2 Blood β-hydroxybutyrate concentrations in adults with prediabetes after consuming KEβHB-supplemented and placebo drinks. Values are means (95% CIs; indicated by error bars) at that time, $n = 18$. *Different from placebo at that time, $P < 0.05$ (determined using paired-samples t test). KEβHB, ketone monoester (β-hydroxybutyrate) supplement.

drink ($P = 0.562$) (Table 2). The intraindividual differences in the AUCs for amylin following the KEβHB-supplemented and placebo drinks are presented in Supplemental Figure 2D. The main effects of the drink ($P = 0.647$, $\omega^2 < 0.01$), time ($P = 0.378$), and interaction effect of drink × time ($P = 0.432$) on amylin were not statistically significant after KEβHB supplementation (Figure 4C).

Glucagon.

The total $AUC_{0–150}$ for glucagon did not differ significantly after the KEβHB-supplemented drink compared with the placebo drink ($P = 0.833$) (Table 2). The intraindividual differences in the AUCs for glucagon following the KEβHB-supplemented and placebo drinks are presented in Supplemental Figure 2E. The main effects of the drink ($P = 0.846$, $\omega^2 < 0.01$), time ($P = 0.463$), and interaction effect of drink × time ($P = 0.332$) on glucagon were not statistically significant after KEβHB supplementation (Figure 4D).

GIP.

The total $AUC_{0–150}$ for GIP was significantly greater after the KEβHB-supplemented drink compared with the placebo drink ($P = 0.005$) (Table 2). The intraindividual differences in the AUCs for GIP following the KEβHB-supplemented and placebo drinks are presented in Supplemental Figure 2F. There was a significant main effect of the drink ($P = 0.004$, $\omega^2 = 0.54$),

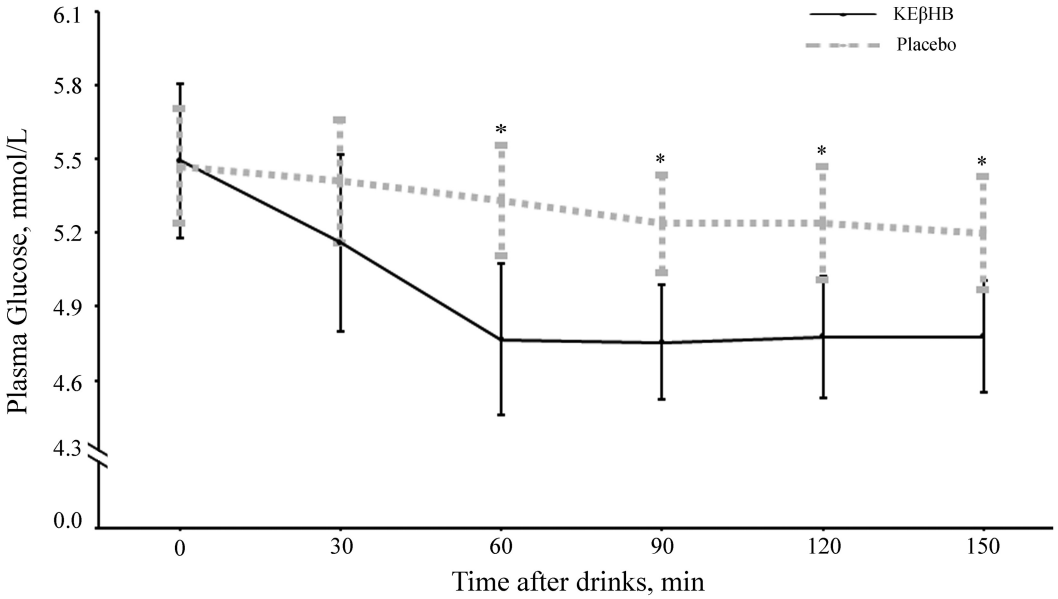

FIGURE 3 Plasma glucose concentrations in adults with prediabetes after consuming KEβHB-supplemented and placebo drinks. Values are means (95% CIs; indicated by error bars) at that time, $n = 18$. *Different from placebo at that time, $P < 0.05$ (determined using paired-samples t test). KEβHB, ketone monoester (β-hydroxybutyrate) supplement.

TABLE 2 Plasma glucoregulatory peptide AUCs in adults with prediabetes after consuming KEβHB-supplemented and placebo drinks[1]

Peptide	Placebo ($n = 18$)	KEβHB ($n = 18$)	Mean difference (95% CI)	d	P
Insulin,[2] nmol/L × min	28.0 ± 6.15	35.2 ± 8.73	0.237 (0.044, 0.429)	0.61	0.019
C-peptide,[2] nmol/L × min	74 ± 8	100 ± 15	0.259 (0.114, 0.403)	0.89	0.002
Amylin,[2] μg/L × min	16.8 ± 4.20	17.4 ± 4.50	0.054 (−0.140, 0.249)	0.15	0.562
Glucagon,[2] μg/L × min	22.9 ± 4.89	22.9 ± 4.68	0.014 (−0.127, 0.155)	0.04	0.833
GIP,[2] nmol/L × min	1.48 ± 0.15	1.96 ± 0.24	0.243 (0.085, 0.401)	0.77	0.005
GLP-1, nmol/L × min	9.64 ± 0.97	9.68 ± 0.90	0.042 (−1.00, 1.09)	0.02	0.934

[1]Values are means ± SEMs of the total AUC estimated over 150 min. Mean differences (95% CIs) were obtained from paired Student's t test; d values indicate the Cohen's effect size; P values <0.05 were deemed to be statistically significant. GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1; KEβHB, ketone monoester (β-hydroxybutyrate) supplement.
[2]Analytes were log-transformed for paired Student's t test.

time ($P < 0.001$), and a significant interaction effect of drink × time ($P = 0.009$) on GIP after KEβHB supplementation. Plasma GIP concentrations were significantly higher at 30, 60, 90, and 150 min after the KEβHB-supplemented drink compared with the placebo drink, with the highest mean difference at 30 min (17.4 ± 2.9 pmol/L after KEβHB supplementation and 10.2 ± 1.2 pmol/L after the placebo; $P = 0.002$). Plasma GIP concentrations were not significantly different at 120 min after the KEβHB-supplemented drink compared with the placebo drink (Figure 4E).

GLP-1.

The total $AUC_{0–150}$ for GLP-1 did not differ significantly after the KEβHB-supplemented drink compared with the placebo drink ($P = 0.934$) (Table 2). The intraindividual differences in AUCs for GLP-1 following the KEβHB-supplemented and placebo drinks are presented in Supplemental Figure 2G. The main effects of the drink ($P = 0.866$, $\omega^2 < 0.01$), time ($P = 0.109$), and interaction effect of drink × time ($P = 0.506$) on GLP-1 were not statistically significant after KEβHB supplementation (Figure 4F).

Discussion

This RCT was the first study to investigate the effect of KEβHB supplementation on glucose in individuals with prediabetes. This study showed that blood βHB concentrations increased from 0.20 mmol/L to 3.50 mmol/L within 30 min of consuming a KEβHB-supplemented drink, and remained above the basal value for the duration of the study (150 min). Notably, a state of ketosis achieved by the elevated concentrations of βHB had a large effect ($d = -1.10$) in lowering the plasma glucose response. Further, the AUCs for glucoregulatory peptides (such as insulin, C-peptide, and GIP) were greater after supplementation with KEβHB than after the placebo.

A low-carbohydrate, high-fat "ketogenic diet" has been successful in treating several metabolism-related disorders (5–7). An increase in ketone concentrations (i.e., ketosis) may

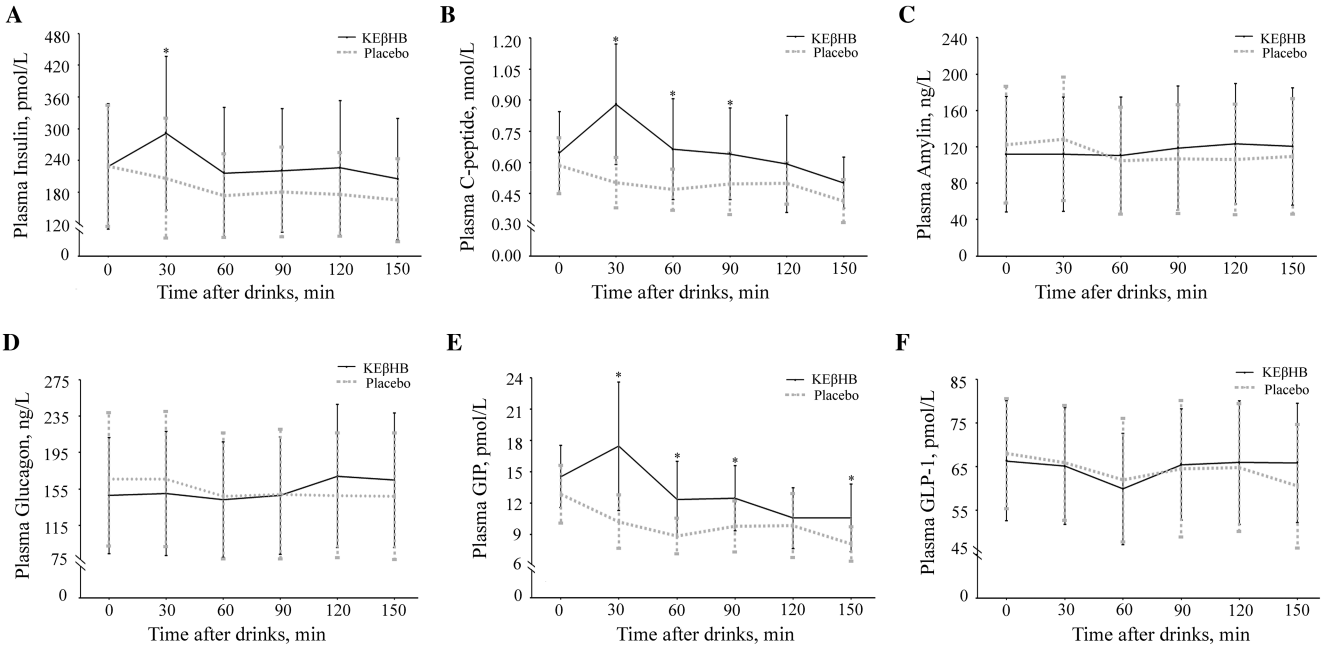

FIGURE 4 Plasma concentrations of insulin (A), C-peptide (B), amylin (C), glucagon (D), GIP (E), and GLP-1 (F) in adults with prediabetes after consuming KEβHB-supplemented and placebo drinks. Values are means (95% CIs; indicated by error bars) at that time, $n = 18$. *Different from placebo at that time, $P < 0.05$ (determined using paired-samples t test). Values for insulin, C-peptide, amylin, glucagon, and GIP were log-transformed for paired-samples t test. GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1; KEβHB, ketone monoester (β-hydroxybutyrate) supplement.

underlie the efficacy of ketogenic diets (4). However, the beneficial effects of diet-induced ketosis depend on major metabolic shifts, including depletion of the hepatic and muscle glycogen reserves and increased adipocyte lipolysis, resulting in elevated concentrations of free fatty acid substrates and endogenous hepatic ketone production (24). Achieving this state of ketosis requires strict compliance with the "ketogenic diet", which is in addition to limiting food choices and the possibility of gastrointestinal side effects (25). Further, the consumption of large amounts of dietary lipids can elicit dyslipidemia, potentially increasing cardiovascular risks (26). As an alternative, ketone supplements (e.g., KEβHB supplement), which have typically mild and infrequent gastrointestinal side effects (27), are a well-tolerated approach to achieve nutritional ketosis (ketone range: 0.5–7 mmol/L) (13, 29). Moreover, consuming KEβHB-supplemented drinks leads to improved glucose tolerance in healthy individuals (10, 11, 28). A study in 15 healthy adults showed that a single drink of the KEβHB supplement (without other nutritional stimulants) caused an acute increase in blood βHB concentrations and decreased plasma glucose concentrations (10). In that study, glucose concentrations returned to baseline values by the end of 240 min study period. In the present study, blood βHB concentrations increased within 30 min following the ingestion of the KEβHB-supplemented drink (without other nutritional stimulants) and remained elevated until the end of the study, thus mimicking a state of nutritional ketosis for at least 150 min. This state of ketosis was accompanied by a 14.5% reduction in plasma glucose, which decreased from 5.50 mmol/L (99.1 mg/dL) at baseline to 4.70 mmol/L (84.7 mg/dL) ($P < 0.01$) at 150 min (Figure 3). Although we did not determine the duration for which plasma glucose concentrations remained below the baseline values, our findings showed that, in individuals with prediabetes, a single drink of the KEβHB supplement could have a sustained glucose-lowering effect for at least 150 min. Given that repeated ingestion of the KEβHB-supplemented drink would be needed to maintain a prolonged state of ketosis, future research in prediabetes should focus on the pharmacokinetics of multiple doses of KEβHB supplement taken over an extended time.

The mechanisms underlying the glucose-lowering effect of the KEβHB supplement are not clearly understood. Evidence from animal studies suggests that the glucose-lowering effect of exogenous ketones can be attributed to its stimulatory effect on insulin secretion (29–33). However, these findings have not been consistently replicated in clinical studies (8, 10–13, 30, 34–36). For instance, some studies have shown that infusion of supraphysiological doses of βHB causes a significant decrease in glucose concentrations without a concomitant increase in insulin concentrations (30, 34, 35). In contrast, other studies have demonstrated an increase in C-peptide concentrations (but not insulin) after βHB infusion (36). Further, while the KEβHB-supplemented drink led to a two-fold increase in insulin concentrations during a postexercise hyperglycemic clamp in healthy individuals in one study (12), in another study adding the KEβHB supplement to a postexercise high-carbohydrate protein drink did not have a significant impact on insulin concentrations in healthy individuals (37). Furthermore, the KEβHB supplement, when co-administered with glucose (in an OGTT), did not appear to have a significant impact on the total AUCs for insulin and C-peptide in healthy and obese adults (11, 13). On the contrary, in the present study, KEβHB supplementation (compared with the placebo) explained 26% of the variance in plasma insulin concentrations and 90% of the variance in plasma C-peptide concentrations, leading to significantly greater AUCs for insulin and C-peptide. Although the exact mechanism underlying the insulinotropic effects of the KEβHB supplement needs to be investigated in the future, we hypothesize that an acute increase in βHB concentrations (after ingestion of the KEβHB-supplemented drink) has an incretin-like effect on the pancreatic β cells. In the presence of sub-stimulatory glucose concentrations (as seen in prediabetes) (29, 38), βHB activates the G-protein–coupled receptors expressed on β cells, leading to increased cAMP and Ca^{2+}, which results in accelerated exocytosis of insulin (39, 40). Therefore, the glucose-lowering effect of the KEβHB supplement in prediabetes may, to a certain extent, be attributable to increased insulin secretion. Whether or not it affects insulin sensitivity in prediabetes requires further research.

For the first time, we investigated the effect of the KEβHB supplement on GIP. The KEβHB-supplemented drink had a large effect (Cohen's $d = 0.77$) on the total AUC for GIP. Plasma GIP concentrations showed a sustained elevation over time (at 30, 60, 90, and 150 min) after KEβHB supplementation compared with the placebo (Figure 4C). Glucose-dependent insulinotropic peptide is an incretin, synthesized within and released from the enteroendocrine K cells in response to nutrient stimulants (in particular, fats). In response to nutrient ingestion, GIP binds to a specific α_s-G-protein–coupled receptor and activates the intracellular second messenger adenyl cyclase (cAMP)/protein kinase A pathway to regulate glucose homeostasis (41). Based on our findings, we speculate that the KEβHB supplement potentiates GIP release, which stimulates insulin to promote a reduction in glucose concentrations. Given that a marked increase in C-peptide (a marker of insulin secretion) was observed up until 90 min only, the effect of KEβHB supplementation on plasma glucose may involve other putative mechanisms (such as the GIP–IL-6 pathway or the gut–brain signaling pathway) (42, 43). More mechanistic studies are warranted to evaluate the interaction between the gut-associated pathways and the KEβHB supplement.

The present study also examined, for the first time, the effect of the KEβHB supplement on amylin—a pancreatic hormone co-secreted from β cells in a 20:1 molar ratio of insulin to amylin (44). The KEβHB-supplemented drink did not have a significant effect on amylin concentrations compared with the placebo drink ($P = 0.562$). In addition, we also studied glucagon and GLP-1 response to the KEβHB-supplemented drink. To date, only 2 studies have investigated the effects of exogenous βHB on glucagon in humans. One study showed that the infusion of βHB did not alter glucagon concentrations in healthy adults (36). The other study showed a significant increase (albeit marginally, $P = 0.05$) in glucagon concentrations after the ingestion of the KEβHB-supplemented drink (with OGTT) by obese individuals (13). In the present study, plasma glucagon did not change significantly after the KEβHB-supplemented and placebo drinks ($P = 0.833$). As insulin and glucagon are counterregulatory hormones (45), it would be reasonable to suggest that the lack of a significant change in glucagon concentrations was due to an increase in insulin (or C-peptide) concentrations after KEβHB supplementation. To the best of our knowledge, the effect of exogenous βHB on GLP-1 has been investigated using a KEβHB-supplemented drink only (11, 13, 28). Those studies showed that consuming the KEβHB-supplemented drink (taken along with OGTT or a dextrose drink) significantly lowered plasma GLP-1 in healthy and obese individuals. In contrast, our findings did not show a significant change in plasma GLP-1 after KEβHB

supplementation compared with the placebo ($P = 0.934$). The discrepancy could be explained by the fact that we studied the effect of the KEβHB supplement in the absence of additional carbohydrates (oral glucose), which is a primary nutritional stimulus for GLP-1 secretion.

The present study has several strengths. First, we used a ketone supplement containing βHB monoester (instead of βHB salt), an efficient and safe method to achieve nutritional ketosis (14). The KEβHB supplement has only the D-isoform of βHB, which is rapidly metabolized by the gut, without altering the acid-base balance in the blood and urine, and has milder gastrointestinal effects compared with βHB salts (10, 27). Second, we used the robust American Diabetes Association diagnostic criteria to identify individuals with prediabetes. Third, we investigated the effect of KEβHB supplementation on a large panel of glucoregulatory peptides without the compounded impact of other nutrient stimulants (such as carbohydrates and proteins). This enabled us to study the "pure effect" of ketones in the absence of confounders.

The study also has limitations that need to be acknowledged. First, we did not investigate the effect of KEβHB supplementation on other glucoregulatory mechanisms such as fatty acid metabolism (46, 47), hepatic gluconeogenesis (35, 48), and altered digestion. Since our study was the first to investigate the effects of a KEβHB supplement in prediabetes, it was not powered to investigate all the mechanisms involved in βHB and glucose metabolism. Detailed phenotyping studies are warranted to understand comprehensively the complex metabolism of KEβHB supplementation in individuals with prediabetes. Second, we did not investigate other ketone bodies (acetoacetate and acetone) and their effect on glucose metabolism. Some of the βHB (from the KEβHB supplement) is possibly converted to acetoacetate in the liver, affecting the plasma glucose concentrations (49, 50). However, given that the conversion of βHB to acetoacetate and acetone is relatively small and slow (ratio of βHB to acetoacetate is 6:1) (10), we believe that the metabolic interconversions of ketones did not impact the glucose response to the KEβHB-supplemented drink. Moreover, our study was not adequately powered to investigate the kinetics of ketone body metabolism. Future studies to assess the redox homeostasis and mitochondrial bioenergetics after KEβHB supplementation and its implications on glucose homeostasis are warranted. Third, not all participants had FPG concentrations in the prediabetes range. This was because we included participants with FPG and/or HbA1c in the prediabetes range (as per the American Diabetes Association guidelines) to increase the generalizability of our findings. However, a post hoc analysis constrained to the participants with elevated FPG only did not change the results materially (data not shown). Fourth, since we investigated the effects of the KEβHB supplement in the absence of other nutritional stimulants (e.g., standard meal), the metabolism of the KEβHB supplement when consumed with other food components is not known. Thus, the optimal dose required to achieve ketosis when the KEβHB supplement is taken along with standard meals by individuals with prediabetes and its metabolic effects need to be investigated in future studies. Last, we did not investigate the effect of dietary fiber intake, which is known to affect absorption of nutrients in the gut (51). Future studies should assess the effect modification by dietary fiber and other lifestyle factors that may influence the absorption and metabolic fate of KEβHB.

In conclusion, a single KEβHB-supplemented drink acutely increased blood βHB concentrations, mimicking a state of nutritional ketosis, and lowered the plasma glucose response in adults with prediabetes. These findings highlight the need for additional research on the effect of repeated ingestions of KEβHB supplements in prediabetes, including the optimal dose, timing, and duration to achieve maximal benefit. Physiological pathways associated with GIP may underlie the glucose-lowering effect of KEβHB supplementation in prediabetes.

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