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Daily Intake of D-β-Hydroxybutyric Acid (D-BHB) Reduces Body Fat in Japanese Adult Participants: A Randomized, Double-Blind, Placebo-Controlled Study

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Summary Currently, there is considerable interest in ketone metabolism owing to the benefits for human health. Conventionally, strict dietary restrictions on carbohydrates are required to increase plasma ketone levels, while supplementation with D-β-hydroxybutyric acid (D-BHB) can easily increase plasma ketone levels. We hypothesized that a daily intake of D-BHB could promote weight loss, especially through fat reduction. Herein, D-BHB (OKETOA™) was produced via a proprietary fermentation process from sugar. In this randomized, double-blind, placebo-controlled study, we assessed the safety and fat-reduction effects after 12 wk of daily ingestion of D-BHB (2.9 g) in 22 healthy Japanese adults and 22 control participants. Blood samples were collected pre- and post-treatment. Blood chemistry, anthropometric variables, and the body composition of the participants were investigated. Data analysis revealed that visceral fat at 12 wk significantly decreased by 9.0 cm^2 (p=0.037), as evidenced by analysis of covariance. Blood parameters and body condition showed no significant differences between the two groups, and the participants reported no adverse effects or discomfort. Furthermore, data were analyzed by regrouping the participants. After removing one suspicious diabetes participant, all others showed significant decreases in visceral fat, body weight, BMI, and fat weight. Additionally, those aged under 50 y old had significantly decreased abdominal circumference and body fat percentage, in addition to visceral fat, body weight, BMI, and fat weight. Overall, our findings indicate that daily D-BHB intake may reduce body fat without dieting or exercise intervention. This study was registered with the UMIN Clinical Trials Registry as UMIN000045322.

Key Words D-β-hydroxybutyric acid (D-BHB), ketogenic diet (KD), ketone body, lifestyle-related diseases, obesity

The obese population has increased rapidly in recent years, and obesity is a risk factor for lifestyle-related diseases (*1*). Body fat associated with obesity is classified as subcutaneous and visceral fat, with visceral fat being more strongly correlated with lifestyle-related diseases (*2*). Therefore, various types of diets have been developed and recommended for weight loss, such as low-carbohydrate diets (e.g., ketogenic diet [KD]), paleo-type diets, plant-forward diets, intermittent fasting, clean eating, traditional regional diets (e.g., Mediterranean diet), and other specifically designed diets (e.g., dietary approaches to stop hypertension, Mayo Clinic diet) (*3*). Among these, the KD is a high-fat, very low-carbohydrate, and adequate-protein diet (*4–6*). It has been clinically used since the early 1920s to control seizures in patients with epilepsy, especially those who do not respond adequately to antiepileptic medication (*6–8*). KD has received extensive interest not only for its effectiveness in weight loss, but also for its beneficial effects on several diseases, such as neurological disorders, obesity, type 2 diabetes mellitus, cancer, intestinal disorders, and respiratory compromise (*9–19*). KD causes a metabolic switch from glycolysis to ketosis by producing natural ketone bodies in the liver, namely D-β-hydroxybutyric acid (D-BHB), acetoacetate, and acetone from fat, of which D-BHB accounts for more than 70% of the total ketones produced (*20*). Meanwhile, the production of ketones in the liver requires strict dietary restrictions; thus, exogenous ketone supplements, such as 1,3-butanediol, medium-chain triglyceride (MCT), ketone salts, and ketone ester, may be more successful in inducing ketosis (*21*). However, there is limited evidence on how exogenous ketones participate it weight loss.

Recently, the racemic form of DL-BHB salt, which is primarily chemically synthesized, has been available in the market (*22*). This formulation contains equal amounts of D-BHB and L-BHB, which differ in efficacy and metabolism. The role of L-BHB in humans remains unclear (*23*). Hence, there are concerns regarding salt

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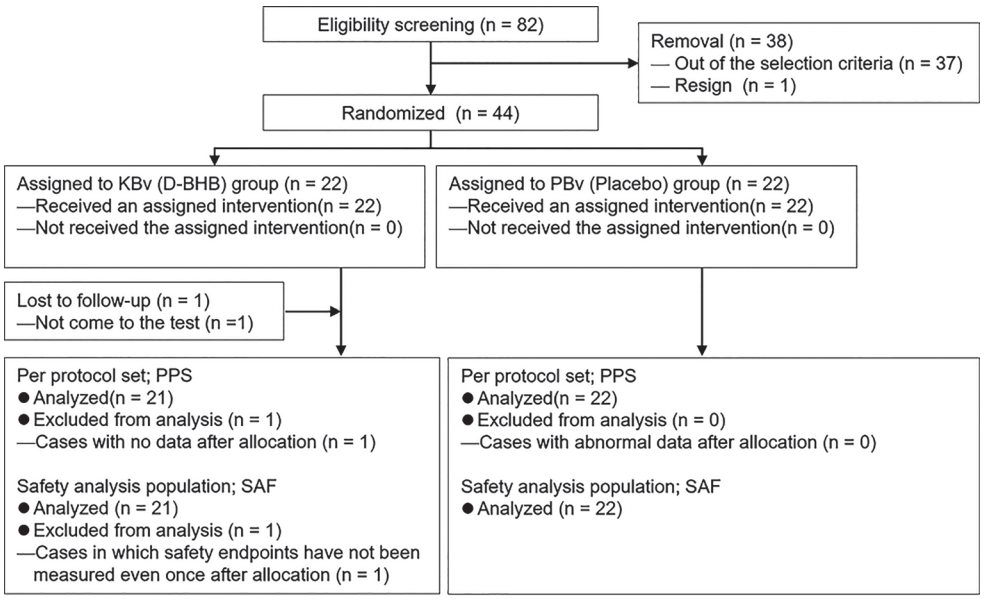

Fig. 1. Study flow chart.

overdose and effectiveness, among others. Thus, a safer, natural form of D-BHB is needed for exogenous intake. Therefore, we produced D-BHB (OKETOA^{TM}) by proprietary natural fermentation of sugar using *Halomonas* sp. KM-1 (*24*).

The American Diabetes Association Consensus Report 10 recommends diet, physical activity, and behavioral counseling for treating weight gain and obesity in patients with type 2 diabetes. However, there is no clear evidence that dietary supplements are effective for weight loss (*2*).

In this study, we hypothesized that daily intake of D-BHB could promote weight loss, especially through fat reduction, and assessed the weight-loss effects and safety of long-term D-BHB ingestion in Japanese adults with a regular diet and normal lifestyle via a randomized, double-blind, placebo-controlled study.

MATERIALS AND METHODS

D-BHB and placebo beverage preparation. D-BHB beverage (KBv) and placebo beverage (PBv) components were as follows. D-BHB (OKETOA^{TM}) was provided by Osaka Gas Chemical (Osaka, Japan). The content of D-BHB is based on previous studies (*22*, *25*, *26*). KBv contains 2.9 g D-BHB, 5,000 mg erythritol, 800 mg alanine, 400 mg acidifier (sodium citrate), 350 mg flavorings, and 110 mg sweetener (sucralose, acesulfame K) in 100 mL volume. PBv contains 5,000 mg erythritol, 800 mg alanine, 1,150 mg acidifier (citric acid, sodium citrate), 350 mg flavorings, and 110 mg sweetener (sucralose, acesulfame K) in 100 mL volume. These beverages were produced by API Co., Ltd. (Gifu, Japan).

Participants and clinical study. This randomized, double-blind, placebo-controlled study was conducted from November 2021 to February 2022 at Nerima Medical Association Minamimachi Clinic, Tokyo, Japan. Participants with body mass index (BMI; kg/m^2) ranging from 23.4 to 29.6 kg/m^2 were enrolled in the study. The treatment period was 12 wk, and the first day of the treatment period was defined as week zero. Physiological tests were performed 3–7 wk prior to intervention and 9–12 wk post-intervention. Before the intervention, the 44 participants were randomly assigned to test (22 participants) or placebo (22 participants) groups through stratified randomization with the stratification factors of sex and age (Fig. 1). Forty-three participants (21 KBv, 22 PBv) completed all intervention tests.

During the treatment period, the participants drank the test beverage after breakfast. A diet diary was kept 3 d before measurement day, for a total of 3 d, using a Calorie and Nutrition Diary (ORTHOMEDICO Inc., Tokyo, Japan). The number of steps taken was recorded every day throughout the test period. The primary endpoint was the visceral fat area. The secondary endpoints included abdominal subcutaneous fat area, total abdominal fat area, body weight, BMI, abdominal circumference, waist circumference, body fat percentage, fat mass, lean body mass, muscle mass, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride (TG), as well as original questionnaires post-intervention, which were compared with the corresponding baseline values. Blood samples were taken from the participants 12 wk pre- and post-intervention. At each time interval before blood sampling, the participants fasted for 6 h.

Exclusion criteria of participants included: (i) undertreatment for or a history of malignancy, heart failure, or myocardial infarction; (ii) with implanted pacemakers or implantable cardioverter defibrillators; (iii) under treatment for the following chronic diseases: arrhythmia, hepatic disorder, renal disorder, cerebrovascular disorder, rheumatism, diabetes mellitus, dyslipidemia, hypertension, or other chronic diseases; (iv) who regularly consume food for a specified health use, food with functional labeling, or other food/beverages with possi-

ble functionality; (v) who regularly use pharmaceuticals (including herbal medicines) or supplements; (vi) with allergies (to pharmaceuticals and food related to the tested food); (vii) who have an exercise routine of at least three times a week; (viii) who have an irregular lifestyle such as night work shifts, etc.; (ix) with extreme dietary restrictions, such as eating no carbohydrates at all; (x) on a diet; (xi) pregnant, lactating, or intending to become pregnant during the study period; (xii) participated in another clinical trial in the 28 d prior to the date of consent or will participate during the study period; (xiii) any other person who is deemed by the investigator to be inappropriate for the study.

All participants consented to participate in the study with written consent after being briefed on the nature, purpose, and possible adverse effects of the study. The study was approved by the Seishinkai Medical Corporation Takara Clinic Ethics Committee (Approval No.: 2108-05240-0001-0C-TC). All procedures were performed in accordance with the Declaration of Helsinki of 1975 (revised in 2013; https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). This study was registered in advance with the UMIN Clinical Trials Registry (http://www.umin.ac.jp) as UMIN000045322.

Blood chemistry, anthropometric variables, and body composition of participants. The following blood chemistry parameters were measured on-site in the hospital using a JEOL Clinical Biochemistry Analyzer JCA-BM8060 (JEOL, Tokyo, Japan): TG, total cholesterol, HDL cholesterol, and LDL cholesterol. Systolic blood pressure, diastolic blood pressure, and pulse rate were measured by a Digital Automatic Blood Pressure Monitor HEM-6022 (OMRON, Kyoto, Japan). Body temperature was measured by non-contact thermometers (Dretec, Saitama, Japan). Bodyweight, body fat percentage, fat mass, lean body mass, and muscle mass were measured by a Multi-frequency Body Composition Analyzer MC-780A-N (Tanita, Tokyo, Japan).

Computed tomography. Computed tomography (CT) scans were performed to assess changes in the visceral fat area, subcutaneous fat area, and total fat area with weight loss. The scans were performed at the Minamimachi Clinic (Tokyo, Japan) with a SOMATOM Perspective (SIEMENS, Tokyo, Japan). Images of the coronal abdominal (navel) sections (same anatomical position) of the participants were analyzed before and after D-BHB intake using ImageJ version 1.52u (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2012).

Statistical analyses. The sample size was calculated based on the hypothesis that visceral fat would be reduced by 12 wk intake of BHB, assuming as a pilot study the example of MCT, which are expected to have a similar mechanism of action as BHB (25). The actual measured difference in visceral fat reduction after 12 wk of MCT intake between groups was -10.6 cm^2 (test food group: $N=33$, -12.2 ± 11.2, placebo group: $N=31$, -1.6 ± 12.8). Based on these results, the Cohen's d of the effect size between groups was estimated to be 0.897. The statistical significance level (α) was 5% two-sided, the statistical power ($1-\beta$) was 80%, and the sample size for the t-test was calculated to be 21 subjects per group. Twenty-two subjects per group was chosen to account for the 5% dropout.

Per protocol analysis set was used for the data analysis. Comparisons between groups were performed using the analysis of covariance (ANCOVA) method. Data are expressed as the means, standard deviation (SD), and standard error (SE), with baseline (at screening or pre-intervention) as the covariate. Differences with $p<0.05$ were considered statistically significant.

Table 1. Baseline participants characteristics.

	KBv (D-BHB)	PBv (Placebo)
Number	21	22
Men/women	8/13	9/13
Age (y)	46.6 (10.2)	45.8 (10.3)
Height (cm)	163.2 (8.0)	162.4 (7.1)
Body weight (kg)	68.8 (7.8)	67.1 (6.4)
BMI (kg/m^2)	25.8 (1.6)	25.4 (1.1)
Body fat ratio (%)	32.0 (7.4)	30.6 (8.6)
Waist circumference (cm)	93.8 (4.8)	91.8 (4.1)
Total cholesterol (mg/dL)	226.2 (28.1)	226.0 (34.9)
HDL cholesterol (mg/dL)	59.6 (16.3)	58.1 (15.7)
LDL cholesterol (mg/dL)	138.6 (28.6)	137.7 (33.3)
Triacylglycerol (mg/dL)	134.1 (73.3)	147.5 (124.6)
Glucose (mg/dL)	87.0 (7.1)	87.3 (15.9)

Values are shown as the mean (SD).
KBv, ketone (D-BHB) beverage; PBv, placebo beverage.

RESULTS

Baseline participant characteristics

Among the 82 participants randomized to treatment, 39 who did not satisfy the inclusion criteria were excluded; thus, 44 participants participated in the trial, among which one participant did not attend the last examination, and thus, 43 completed the study (Fig. 1). The percentages of individuals in the KBv and PBv groups who ingested the test beverage were 100.0% (2.1%) and 99.7% (2.1%) (mean (SD)), respectively. The per protocol analysis set was identical to the safety analysis population (Fig. 1). Therefore, the remaining 43 participants were analyzed. The baseline characteristics of the participants are listed in Table 1. There were no significant differences between the groups.

Anthropometric variables and body composition

Anthropometric data are shown in Table 2. When we analyzed all test data, visceral fat area post-intervention had decreased significantly in the KBv test group from pre-intervention, and visceral fat area post-intervention increased in the PBv group from pre-intervention ($p=0.037$). When we compared the test KBv and placebo PBv groups using ANCOVA, the mean difference in change in visceral fat from baseline was -9.0 cm^2 (SE

Table 2. Anthropometric measurements of study participants.

	D-BHB (n=21)		Placebo (n=22)		Difference vs. PBv	
	Week 0	Week 12	Week 0	Week 12	Δ	p
Visceral fat area (cm^2)	111.0 (23.6)	104.8 (22.0)	111.8 (25.6)	114.5 (26.8)	−9.0 (4.2)	0.037*
Subcutaneous fat area (cm^2)	238.0 (58.0)	245.1 (60.4)	219.2 (64.5)	222.5 (68.0)	4.5 (7.9)	0.572
Total fat area (cm^2)	349.0 (54.0)	350.0 (59.5)	330.9 (61.8)	337.0 (61.5)	−3.0 (9.9)	0.766
Body weight (kg)	68.8 (7.8)	68.7 (8.2)	67.1 (6.4)	67.7 (7.0)	−0.8 (0.5)	0.084
BMI (kg/m^2)	25.8 (1.6)	25.7 (1.7)	25.4 (1.1)	25.6 (1.3)	−0.3 (0.2)	0.127
Abdominal circumference (cm)	93.8 (4.8)	91.6 (5.1)	91.8 (4.1)	91.2 (4.7)	−1.3 (1.0)	0.173
Waist circumference (cm)	84.0 (7.0)	84.4 (6.7)	84.0 (4.2)	84.4 (4.4)	0.0 (0.8)	0.979
Body fat percentage (%)	32.0 (7.4)	31.9 (7.3)	30.6 (8.6)	31.4 (7.6)	−0.9 (0.5)	0.100
Fat mass (kg)	21.9 (5.1)	21.7 (5.2)	22.3 (7.7)	21.2 (5.1)	−0.7 (1.4)	0.623
Lean body mass (kg)	47.0 (8.7)	47.0 (8.7)	46.5 (8.1)	46.6 (8.0)	−0.1 (0.3)	0.640
Muscle mass (kg)	44.3 (8.4)	44.4 (8.4)	43.5 (8.0)	44.0 (7.6)	−0.5 (0.6)	0.395

D-BHB and placebo values are expressed as the mean (SD), whereas change is expressed as the mean (SE).
Difference vs. PBv is presented as the mean (SD) difference between pre- and post-intervention values for KBv and PBv groups.
Significant different from week 0 to 12; *$p<0.05$ analyzed by ANCOVA.
KBv, ketone (D-BHB) beverage; PBv, placebo beverage.

Table 3. Anthropometric measurements of study participants removing suspicious diabetes.

	KBv (D-BHB) (n=21)		PBv (Placebo) (n=21)		Difference vs. PBv	
	Week 0	Week 12	Week 0	Week 12	Δ	p
Visceral fat area (cm^2)	111.0 (23.6)	104.8 (22.0)	111.3 (26.1)	114.7 (27.4)	−9.6 (4.2)	0.028*
Subcutaneous fat area (cm^2)	238.0 (58.0)	245.1 (60.4)	215.8 (64.0)	221.4 (69.5)	1.8 (7.8)	0.813
Total fat area (cm^2)	349.0 (54.0)	350.0 (59.5)	327.1 (60.5)	336.1 (62.9)	−6.3 (9.8)	0.528
Body weight (kg)	68.8 (7.8)	68.7 (8.2)	67.3 (6.5)	68.1 (7.0)	−1.0 (0.4)	0.030*
BMI (kg/m^2)	25.8 (1.6)	25.7 (1.7)	25.3 (1.1)	25.6 (1.4)	−0.4 (0.2)	0.025*
Abdominal circumference (cm)	93.8 (4.8)	91.6 (5.1)	91.8 (4.2)	91.2 (4.8)	−1.4 (1.0)	0.164
Waist circumference (cm)	84.0 (7.0)	84.4 (6.7)	83.9 (4.3)	84.4 (4.5)	0.0 (0.8)	0.985
Body fat percentage (%)	32.0 (7.4)	31.9 (7.3)	30.4 (7.8)	31.1 (7.6)	−0.8 (0.5)	0.105
Fat mass (kg)	21.9 (5.1)	21.7 (5.2)	20.3 (4.9)	21.1 (5.2)	−0.9 (0.4)	0.046*
Lean body mass (kg)	47.0 (8.7)	47.0 (8.7)	47.0 (8.1)	47.0 (7.9)	0.0 (0.3)	0.956
Muscle mass (kg)	44.3 (8.4)	44.4 (8.4)	44.4 (7.8)	44.4 (7.6)	0.0 (0.2)	0.956

D-BHB and placebo values are expressed as the mean (SD), whereas change is expressed as the mean (SE).
Difference vs. PBv is presented as the mean (SD) difference between pre- and post-intervention values for KBv and PBv groups.
Significant different from week 0 to 12; *$p<0.05$ analyzed by ANCOVA.
KBv, ketone (D-BHB) beverage; PBv, placebo beverage.

4.2, 95% CI− 17.4, 95% CI+ −0.6, p=0.037). There were no significant differences in other parameters among groups.

We further analyzed the data by regrouping the participants and removing one suspicious diabetes participant from the PBv group whose pre-intervention blood analysis showed 155 mg/dL of glucose, 8.5% HbA1c 8.5% (NGSP value), and glycoalbumin 22.9% in the serum (*26*, *27*). In the group (KBv: 21, PBv: 21) (Table 3), we found that the visceral fat area, body weight, BMI, and fat weight post-intervention had decreased significantly in the test KBv group following intervention (p=0.028, p=0.030, p=0.025, and p=0.046), respectively. The test KBv group showed a decrease in the visceral fat area, body weight, BMI, and fat weight by 9.6 cm^2 (SE 4.2, 95% CI− 18.1, 95% CI+ −1.1, p=0.028), 1.0 kg (SE 0.4, 95% CI− 1.8, 95% CI+ −0.1, p=0.030), 0.4 kg/m^2 (SE 0.2, 95% CI− 0.7, 95% CI+ 0.0, p=0.025), and 0.9 kg (SE 0.4, 95% CI− 1.8, 95% CI+ 0.0, p=0.046), respectively, compared with the placebo PBv group (Table 3).

We also analyzed the data by regrouping the participants based on age (<50 y and ≥50 y) and compared the degrees of change following intervention in the individual items between KBv and PBv (Table 4). In the under-50-y-old group (KBv: 13, PBv: 14), visceral fat

Table 4. Anthropometric measurements of study participants under 50 y old.

	KBv (D-BHB) (n=13)		PBv (Placebo) (n=14)		Difference vs. PBv	
	Week 0	Week 12	Week 0	Week 12	Δ	p
Visceral fat area (cm^2)	113.2 (25.6)	104.6 (24.9)	111.2 (18.8)	118.2 (20.2)	−15.3 (5.2)	0.007**
Subcutaneous fat area (cm^2)	239.7 (60.0)	240.9 (63.1)	193.4 (57.2)	200.7 (62.6)	−6.7 (9.0)	0.466
Total fat area (cm^2)	353.0 (54.4)	345.5 (62.3)	304.6 (60.6)	319.0 (67.2)	−21.7 (12.8)	0.103
Body weight (kg)	70.0 (8.0)	69.4 (8.6)	68.4 (6.5)	69.4 (7.0)	−1.6 (0.6)	0.013*
BMI (kg/m^2)	25.6 (1.3)	25.4 (1.7)	25.5 (1.2)	25.9 (1.5)	−0.5 (0.2)	0.012*
Abdominal circumference (cm)	93.1 (3.8)	90.1 (5.0)	91.0 (4.5)	90.6 (5.2)	−2.6 (1.1)	0.024*
Waist circumference (cm)	84.6 (7.2)	83.4 (6.2)	84.2 (3.4)	84.8 (4.1)	−1.7 (0.9)	0.074
Body fat percentage (%)	30.7 (7.4)	30.1 (7.1)	28.4 (8.1)	29.4 (8.2)	−1.5 (0.7)	0.027*
Fat mass (kg)	21.3 (4.8)	20.6 (4.7)	19.3 (5.5)	20.4 (5.9)	−1.7 (0.6)	0.011*
Lean body mass (kg)	48.7 (9.1)	48.8 (9.2)	49.1 (7.8)	49.0 (7.7)	0.2 (0.4)	0.698
Muscle mass (kg)	46.0 (8.8)	46.1 (8.9)	46.4 (7.5)	46.3 (7.4)	0.2 (0.4)	0.676

D-BHB and placebo values are expressed as the mean (SD), while change is expressed as the mean (SE).
Difference vs. PBv is presented as the mean (SD) difference between pre- and post-intervention values for KBv and PBv groups.
Significant difference from weeks 0 to 12; ** $p<0.01$, * $p<0.05$ analyzed by ANCOVA.
KBv, ketone (D-BHB) beverage; PBv, placebo beverage.

Table 5. Baseline characteristics of study participants.

	KBv (D-BHB) (n=21)		PBv (Placebo) (n=22)	
	Week 0	Week 12	Week 0	Week 12
Total cholesterol (mg/dL)	226.2 (28.1)	232.7 (29.2)	226.0 (34.9)	225.8 (28.5)
HDL-cholesterol (mg/dL)	59.6 (16.3)	62.1 (16.8)	58.1 (15.7)	61.8 (17.0)
LDL-cholesterol (mg/dL)	138.6 (28.6)	142.3 (32.9)	137.7 (33.3)	135.5 (28.7)
TG (triglyceride) (mg/dL)	134.1 (73.3)	155.4 (135.8)	147.5 (124.6)	155.0 (122.2)
Systolic blood pressure (mmHg)	122.3 (17.3)	121.1 (14.7)	123.1 (11.6)	124.6 (13.3)
Diastolic blood pressure (mmHg)	82.3 (9.1)	82.4 (11.7)	81.2 (9.1)	82.7 (10.1)
Pulse rate (bpm)	81.0 (10.1)	83.7 (10.9)	75.6 (9.4)	76.2 (7.8)
Body temperature (°C)	36.3 (0.2)	35.9 (0.5)	36.3 (0.2)	36.1 (0.4)

Values are shown as the mean (SD). KBv, ketone (D-BHB) beverage; PBv, placebo beverage; D-BHB, D-β-hydroxybutyric acid.

area, body weight, BMI, abdominal circumference, body fat percentage, and fat weight had decreased significantly post-intervention compared to pre-intervention in the KBv test participants, compared to that of the PBv placebo participants (p=0.007, p=0.013, p=0.012, p=0.024, p=0.027, and p=0.011, respectively). Compared to the PBv group, the KBv group showed a decrease in visceral fat area, body weight, BMI, abdominal circumference, body fat percentage, and fat weight of 15.3 cm^2 (SE 5.2, 95% CI− −26.1, 95% CI+ −4.5, p=0.007), 1.6 kg (SE 0.6, 95% CI− −2.7, 95% CI+ −0.4, p=0.013), 0.5 kg/m^2 (SE 0.2, 95% CI− −1.0, 95% CI+ −0.1, p=0.012), 2.6 cm (SE 1.1, 95% CI− −4.9, 95% CI+ −0.4, p=0.024), 1.5% (SE 0.7, 95% CI− −2.9, 95% CI+ −0.2, p=0.027), and 1.7 kg (SE 0.6, 95% CI− −2.9, 95% CI+ −0.4, p=0.011), respectively.

The baseline levels of total cholesterol, HDL cholesterol, LDL cholesterol, triacylglycerol, and glucose are listed in Table 1. There were no significant differences between the groups in all variables measured. The results of the nutrition survey and physical activity are summarized in Table 5. There were no significant differences between the groups.

Adverse events

No participants withdrew from the trial because of adverse effects or discomfort due to the beverage. During the test period, three cases of headache (2 KBv and 1 PBv), one cases of atheroma (KBv), one case of flatulence (KBv), one case of cough (KBv), two cases of dysmenorrhea (PBv), and one case of cold (PBv) were reported. No major adverse effects were noted. Therefore, the physician responsible concluded that no adverse events related to the trial had occurred.

DISCUSSION

We hypothesized that a daily intake of D-BHB promotes weight loss and exhibits fat reduction effects. Thus, we assessed the safety and fat reduction effects of D-BHB (2.9 g) daily ingestion for 12 wk in 21 Japanese adults and 22 control participants including one suspicious diabetes participant, without dieting or exercise intervention, and observed visceral fat reduction of 9.0 cm^2 (SE 4.2, 95% CI− 17.4, 95% CI+ −0.6, p=0.037) (Table 2). After we obtained the pre-intervention

blood analysis data, one suspicious diabetes participant was found in the PBv group (*27*, *28*); therefore, the participant was removed, and we formed another group (KBv; 21, PBv; 21). In this group, we observed significant reductions in visceral fat, body weight, BMI, and fat weight by as much as 9.6 cm^2, 1.0 kg, 0.4 kg/m^2, 0.9 kg (p=0.028, p=0.030, p=0.025, and p=0.046), respectively (Table 3). These data obtained by excluding diabetic patients were considered to be reliable results.

In this investigation, we requested the participants not to change their lifestyle during the intervention period, and baseline characteristics of study participants did not change before and after intervention (Table 5); thus, the daily total energy consumption was assumed that it was not changed. However, significant reductions in visceral fat, body weight, BMI, and fat weight were observed in this investigation (Table 3).

Endogenous ketones have conventionally been thought to be substances synthesized from fat in the liver under severe carbohydrate restriction or starvation conditions (*20*); however, recently, in contrast to the conventional theory, ketones are known to be selectively used as an energy source in the body under normal conditions. Thus, ketones are produced and consumed in the human body even under normal conditions (*29*). Recent studies have shown that exogenous ketones reduce blood glucose levels (*30*) and fat (*22*) concentrations in humans. In particular, the reduction in blood glucose levels have been confirmed to be independent of insulin secretion (*31*). Thus, fat may be changed to ketone bodies to compensate total energy requirement.

The observed fat reduction with unchanging total energy intake suggests that the excess energy consumption may be due to the conversion of carbohydrate to ketone bodies via fat, rather than direct and efficient energy production from carbohydrate and fat as is dictated by conventional theory. Unfortunately, at present, there is no evidence regarding the direct enhancement of fat consumption, but we would like to pursue this topic in future studies.

Furthermore, weight loss by exogenous ketones was observed only in animal models. Caminhotto et al. reported that D-BHB treated rats exhibited visceral fat mass reduction (−16%). However, the difference was not statistically significant (p=0.0529) (*32*). Thus, to the best of our knowledge, this is the first study to elucidate the effect of exogenous ketones on weight loss in humans.

Obesity is considered one of the most significant problems of the 21st century. In particular, visceral fat accumulation, a key feature of abdominal obesity, can cause the pathogenesis and development of metabolic syndromes, including type 2 diabetes mellitus, dyslipidemia, and hypertension (*33*). Some studies have reported that both regular aerobic exercise and the consumption of a hypocaloric diet are associated with a substantial reduction in the visceral fat area independent of age, biological sex, or ethnicity (*34*), and visceral fat reduction induced greater beneficial effects on parameters of metabolic syndrome than subcutaneous fat reduction (*35*).

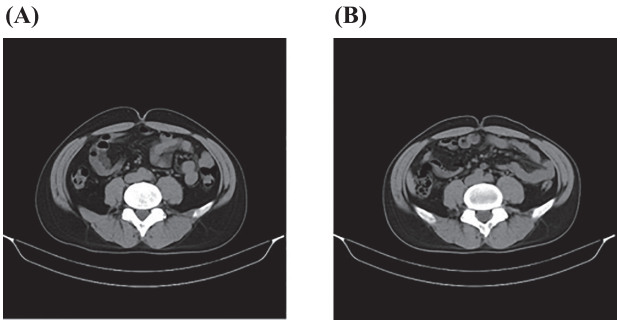

Fig. 2. Computed tomography scans of the coronal abdomen (navel; same anatomical position) of one participant analyzed before (A) and after (B) D-BHB intervention. Visceral fat area was reduced following daily consumption of 2.9 g of D-BHB with no changes in diet and no exercise. An example of this participant's visceral fat loss by 25.4 cm^2 in 12 wk is shown. D-BHB, D-β-hydroxybutyric acid.

Additionally, as seen in Table 4, the under-50-y-old sub-group with high basal metabolism subjected to KBv intervention showed significant reductions in visceral fat area of 15.3 (5.2) cm^2 (mean (SD)), body fat weight of 1.7 (0.6) kg, body fat percentage of 1.5 (0.7) %, body weight of 1.6 (0.6) kg, and BMI of 0.5 (0.2) kg/m^2.

In general, adipose tissue mass increases with age in response to a chronic positive calorie balance, reduced physical activity, and lower basal metabolic rate (*36*). Thus, the under-50-y-old participants have a higher potential for undergoing a reduction in adipose tissue mass with BHB intervention. In our study, D-BHB reduced visceral fat by 9.6 cm^2 (Table 3). Furthermore, the group of under 50-y-old participants showed the highest reduction in visceral fat of 15.3 cm^2 (Table 4).

In Japan, the Consumer Affairs Agency launched the "Food with Functional Labeling" system in April 2015 to increase the number of food products with clearly labeled specific nutritional functions and health benefits and to enable consumers to make more informed choices about food products with health functions (https://www.e-expo.net/pdf/news2015/20151228_caa01.pdf). A list of functional foods for visceral fat reduction from the Consumer Affairs Agency was consulted and prepared (https://www.fld.caa.go.jp/caaks/cssc01/) (Table S1, Supplemental Online Material). This list includes 22 functional ingredients (44 tested examples, including varying doses) that reduce visceral fat, with visceral fat reductions ranging from 1.3 to 13.9 cm^2. In our study, D-BHB reduced visceral fat by 9.6 cm^2 (Table 3). Figure 2 is a CT scan from a participant showing visceral fat reduction of 25.4 cm^2 after 12 wk. Therefore, these data provide initial evidence for the effectiveness of D-BHB in reducing visceral fat compared to the other known visceral fat-reducing foods, regardless of food and exercise control.

MCTs, composed of a mixture of C8 caprylic acid and C10 capric acid, are efficiently digested into free fatty acids, directly absorbed, and rapidly metabolized by the

liver. The acute production of excess acetyl-CoA drives the production of acetoacetic acid and D-BHB, which are both secreted into the systemic circulation (*37*). Nosaka et al. reported that MCT causes fat and body weight reduction in humans. In their study, MCT was compared with long-chain triglycerides as a placebo. The two groups of participants ingested test margarine (14 g/d) containing 5 g/d of MCTs or long-chain triglycerides. The participants on the MCT diet demonstrated significant decreases in visceral fat [−12.2 (11.2) cm^2 vs −1.6 (12.8) cm^2; MCT vs LCT, mean (SD)], subcutaneous fat [−38.2 (29.9) cm^2 vs −22.6 (19.3) cm^2], body fat weight [−3.8 (2.4) kg vs −2.4 (1.7) kg], body weight [−4.2 (2.8) kg vs −2.9 (2.0) kg], and BMI [−1.5 (1.0) kg/m^2 vs −1.0 (0.7) kg/m^2] after 12 wk, respectively (*25*).

In this study, BHB participants removing suspicious diabetes showed significant decreases in visceral fat [9.6 (4.2) cm^2], body fat weight [0.9 (0.4) kg], body weight [1.0 (0.4) kg], and BMI [0.4 (0.2) kg/m^2] after 12 wk, respectively, as shown in Table 3. These results are consistent with the MCT results above. Thus, the mechanism underlying the action of D-BHB in weight loss might be similar to that of MCT, which is mainly mediated by D-BHB. Therefore, our results clearly suggested that D-BHB has a notable visceral fat reduction effect compared to the other known visceral fat-reducing functional foods, regardless of food and exercise control.

Regarding safety, we reported no adverse effects on blood chemistry parameters or psychological well-being. Blood values and body condition were not significantly different among groups. Additionally, it is difficult to overdose on MCT because of gastric distress due to the MCT oil supplementation (*38*).

In conclusion, our results indicated that body fat was reduced significantly following a daily oral intake of 2.9 g of D-BHB, regardless of food and exercise control (Table 3). The reduction in visceral fat by D-BHB was superior to those by other visceral fat-reducing functional foods (Table S1). Exogenous D-BHB intake may serve as a superior diet supplement for weight loss without additional dieting or exercise intervention. The results of this study could be beneficial to researchers and clinicians related to this field and lay the foundation for future studies on ketone metabolism and supplementation.

Authorship

Conceptualization: JT, SK, TG, and YK; investigation: SK; writing—original draft: SK; formal analysis: YK; writing—review and editing: YK, JT and TG; supervision: JT.

Disclosure of state of COI

SK, YK, and JT are employee of OSAKA GAS Co., Ltd. (Osaka, Japan). TG declared no competing interests.

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Supporting information

Supplemental online material is available on J-STAGE.

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