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The Effects of BCAA-Enriched Essential Amino Acid Mixture Intake on Appetite and GLP-1 Secretion: A Randomized, Double-Blind, Placebo-Controlled, Triple-Crossover Study

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Abstract

Background: Obesity and related metabolic disorders necessitate safe and effective strategies for appetite control. Previous reports have indicated that protein and specific amino acids can stimulate glucagon-like peptide-1 (GLP-1) secretion, thereby helping regulate food intake. We developed a BCAA-enriched essential amino acid mixture (BEAA®) with a known muscle mass improvement effect, but its influence on appetite and GLP-1 secretion has not been fully clarified. **Methods:** A randomized, double-blind, placebo-controlled, triple-crossover study was conducted to evaluate the impact of BEAA® on appetite and GLP-1 secretion in healthy subjects. Subjects were assigned to consume one of three products—BEAA® (6 g), BEAA® (3 g), or placebo—in a random order, separated by a one-week washout. Appetite-related parameters (hunger, fullness, prospective food intake, satisfaction) were assessed using a visual analog scale (VAS) at multiple time points up to 180 minutes post-intake. Blood samples were collected to measure active GLP-1 levels (ΔGLP-1) and calculate the net incremental area under the curve (niAUC). **Results:** Compared to the placebo, BEAA® (6 g) showed a trend toward lowering hunger scores at 90 and 120 minutes post-intake, though the differences were not statistically significant. GLP-1 levels (ΔGLP-1 at 30 minutes and niAUC) also trended higher with BEAA® (6 g) compared to the placebo but did not reach statistical significance. No significant differences were observed for BEAA® (3 g), and no serious adverse events were reported. **Conclusions:** A single 6 g dose of BEAA® demonstrated a tendency to induce GLP-1 secretion and main-

tain mild hunger in healthy adults. These findings suggest that BEAA® may serve not only as a supplement for muscle mass enhancement but also as a potential functional food for appetite regulation.

Keywords

Essential Amino Acid, Branched-Chain Amino Acid, BEAA, Appetite, GLP-1

1. Introduction

The prevalence of obesity is increasing globally, with approximately 2.5 billion adults classified as obese (BMI ≥ 25) in 2022, accounting for 43% of the global population [1]. This rise is accompanied by an increase in lifestyle-related diseases such as type 2 diabetes and cardiovascular disorders, making obesity a worldwide challenge [1]. An imbalance between energy intake and expenditure is a primary cause of obesity, highlighting the importance of controlling appetite to prevent excessive energy intake [2] [3]. Appetite is a complex physiological phenomenon influenced by subjective sensations like hunger and satiety with nutritional interventions targeting it under investigation [4]. Enhancing satiety and maintaining mild hunger can naturally extend the interval between meals, reducing daily energy intake [5].

Appetite regulation involves not only the central nervous system but also various hormones secreted from the gastrointestinal tract in response to nutrient intake. Among these, glucagon-like peptide-1 (GLP-1) plays a central role in appetite and metabolic regulation [6]. GLP-1 is secreted from endocrine cells (L cells) in the lower small intestine in response to carbohydrates, fats, and proteins [7]. Its physiological effects include promoting insulin secretion in a glucose-dependent manner, inhibiting glucagon secretion, delaying gastric emptying, and directly acting on the brain's appetite centers to suppress appetite and enhance satiety [6] [8]. Based on these effects, GLP-1 receptor agonists are widely used in treating type 2 diabetes and obesity [9].

Recent studies have reported diet-induced GLP-1 secretion, which is attracting attention as a safer approach to appetite control than GLP-1 receptor agonists [10] [11]. Previous studies have shown that among the three major nutrients, protein is a potent stimulator of GLP-1 secretion [12]. For instance, whey protein intake has been reported to enhance postprandial GLP-1 secretion [13]. Additionally, amino acids, the building blocks of proteins, are also thought to promote GLP-1 secretion. Animal studies have reported that essential amino acids (EAAs), like phenylalanine and tryptophan, and branched-chain amino acids (BCAAs), like valine, directly stimulate GLP-1 secretion through amino acid sensors such as calcium-sensing receptors (CaSR) expressed on L cells [14].

We developed a unique EAA mixture enriched with BCAAs, known as BEAA®,

which has shown muscle mass enhancing effects in humans upon continuous intake of 3 g [15]. The mechanism of this effect is thought to involve BEAA® absorption from the gastrointestinal tract, transport to skeletal muscles via the bloodstream, and activation of the mammalian target of rapamycin (mTOR) signaling pathway, strongly promoting muscle protein synthesis. Considering the mechanism reported in previous studies where amino acids are recognized by L cells in the small intestine, inducing GLP-1 secretion [7], BEAA® intake is expected to induce GLP-1 secretion during absorption, maintaining mild hunger. However, most previous studies have focused on the effects of proteins like whey protein or single amino acids [13] [16], leaving the effects of BEAA® unclear.

Therefore, this study aims to clarify the effects of single-dose BEAA® intake on postprandial satiety, hunger, and blood GLP-1 levels compared to a placebo in healthy adults aged 20 - 65 with a BMI below 30, using a placebo-controlled, randomized, double-blind, triple-crossover trial.

2. Materials and Methods

2.1. Study Subjects and Setting

For this study, the principal investigator recruited paid volunteers according to selection criteria, comprising inclusion/exclusion criteria. The subjects were healthy adults who met the selection criteria and did not violate the exclusion criteria. The selection criteria were as follows: 1) Healthy Japanese males and females aged 20 to 64 years old; 2) Subjects without a smoking habit; 3) Subjects who do not habitually consume large amounts of alcohol; 4) Subjects whose BMI is less than 30; 5) Subjects with the capacity for self-judgment who voluntarily give written informed consent.

The exclusion criteria were as follows: 1) Subjects who contract or have a history of serious diseases (e.g., liver, kidney, digestive, heart, respiratory, endocrine, metabolic, skeletal muscle, and/or tendon disease); 2) Subjects who have a chronic disease and regularly use medications; 3) Subjects who contract or have a surgical history of digestive disease affecting digestion and absorption; 4) Subjects who have a declared allergic reaction to ingredients contained in test foods or loading foods; 5) Subjects who cannot stop consuming supplements and/or functional foods (including Food for Specified Health Uses, Foods with Function Claims, and enriched foods or nutritional supplements which contain proteins and/or amino acids); 6) Subjects who are judged as unsuitable for the current study through screening tests; 7) Subjects who are diagnosed as anemic by the screening tests and deemed unsuitable for frequent blood sampling; 8) Subjects who have had diarrhea within the week prior to the screening tests; 9) Subjects who cannot stop drinking from 2 days before each measurement; 10) Subjects who are pregnant, breastfeeding, or planning to become pregnant during this study; 11) Subjects who have a habit of skipping breakfast; 12) Subjects who have excessive alcohol intake of more than 20 g/day of pure alcohol equivalent for more than 4 days a week; 13) Subjects who are a shift worker and/or midnight-shift worker;

14) Subjects who are under treatment for or have a history of drug addiction and/or alcoholism; 15) Subjects who have donated over 200 mL of blood and/or blood components within the preceding month or over 400 mL of blood and/or blood components within the 3 months prior to the current study; 16) Subjects who are participating in or willing to participate in other clinical studies; 17) Subjects who are judged as unsuitable for the current study by the investigator for other reasons.

This study was subject to deliberation and approval (approval date: 19 December, 2024) by the Ethical Committee of Kobuna Orthopedics Clinic (Chairman: Toshio Kawada) and was approved according to the "Declaration of Helsinki October 2013, WMA Fortaleza General Assembly (Brazil), as amended" and the "Ethical Guidelines for Life Science and Medical Research Involving Human Subjects" (2021). This research was conducted under the supervision of a physician at Nihonbashi Cardiology Clinic. The study plan has been registered in the clinical trial registration system operated by the University Hospital Medical Information Network Research Center, with the registration ID UMIN000056748 (name of the trial registered: A Study on the Effect of Test Food on Appetite—A Randomized, Double-blind, Placebo-controlled, Triple-crossover Study).

2.2. Research Methods

This study was conducted as a placebo-controlled, randomized, double-blind, triple-crossover trial (allocation ratio: 1:1:1) with a pre-observation period (1 week) and three test sessions with BEAA® (6 g), BEAA® (3 g), and placebo intake (1 week washout period), with no methodological changes after the study began.

A statistician used computer-generated random numbers to assign subjects using block randomization (block size 3), adjusting for gender, age, and BMI. The three randomized sequences were then assigned to the BEAA® (6 g)-first sequence, the BEAA® (3 g)-first sequence, and the placebo-first sequence by a test food assignment officer, who had no direct involvement in the research. Furthermore, this officer created and sealed a table (key code) recording the assignment results. This key code remained sealed until disclosure after determining the subjects for analysis, ensuring blinding of all parties except the test food assignment officer. Additionally, the test food was individually packaged in aluminum pouches per meal to ensure blinding of both the subjects and the intervention implementers.

The sample size was calculated based on a study that reported the effects of spice-containing soup on appetite in healthy adults [17]. The target number of participants in this study was set at 15, calculated using a significance level of 0.05 and a power of 0.90, based on the change in satiety scores 40 minutes after meal loading as measured by the Japanese version of the Appetite Sensations Questionnaire.

Subjects were instructed to consume a prescribed meal for dinner by 9:00 PM the day before each test. This meal consisted of 1 to 2 servings of Sato Rice (Sato Foods Co., Ltd., Niigata, Japan) (the same number of servings per subject each

time) alongside Kikubari Gozen Beef Sukiyaki-Style with 4 Side Dishes (Nichirei Foods Co., Ltd., Tokyo, Japan), for a total calorie count between 520 and 814 kcal. Afterward, subjects were allowed only water until arrival at the hospital on the test day, with no other food or drink permitted. Water intake was standardized as much as possible for each test. On the test day, after arrival at the hospital, subjects consumed the prescribed test meal with 100 mL of water within 5 minutes. The test meal consisted of a charcoal-grilled aged red salmon hand-rolled rice ball (Seven-Eleven Japan Co., Ltd., Tokyo, Japan), with intake quantities standardized for each subject across observation periods. Immediately after the test meal was consumed, the test food was dissolved in 400 mL of water with 8 g of skim milk powder (Morinaga Milk Industry Co., Ltd., Tokyo, Japan) and consumed within 10 minutes. After consuming the test food, subjects drank 50 mL of water.

In Period I, the BEAA® (6 g)-first sequence consumed the test food BEAA® (6 g), the BEAA® (3 g)-first sequence consumed BEAA® (3 g), and the placebo-first sequence consumed the placebo. In Period II, the BEAA® (6 g)-first sequence consumed BEAA® (3 g), the BEAA® (3 g) -first sequence consumed the placebo, and the placebo-first sequence consumed BEAA® (6 g). In Period III, the BEAA® (6 g)-first sequence consumed the placebo, the BEAA® (3 g)-first sequence consumed BEAA® (6 g), and the placebo-first sequence consumed BEAA® (3 g). Additionally, during the study period, participants were instructed not to consume excessive alcohol, not to drink alcohol before exercise, and to maintain their usual lifestyle prior to the study, refrain from whole blood or component blood donation, avoid barium stomach examinations, prohibit the use or intake of supplements or health foods, refrain from participating in studies involving the intake of other foods, the use of pharmaceuticals, or the application of cosmetics, and refrain from travel both domestically and internationally. These precautions were explained to the subjects throughout the study period. Additionally, participants were instructed to avoid alcohol consumption within 2 days prior to each examination, to go to bed around midnight and get sufficient sleep the night before each examination, to avoid caffeine intake (e.g., green tea, coffee) the day before each examination, to avoid breakfast or lunch containing strong spices or high oil content, and to refrain from strenuous exercise from the time of waking on the day before each examination until the examination was completed. Participants were also prohibited from leaving the examination venue or sleeping during these periods. Furthermore, research subjects were instructed that, except in emergencies, they must obtain permission from the principal investigator or sub-investigator before using any medication.

2.3. Intake of Test Food

BEAA® intake was set at 3 g based on previous reports showing muscle mass enhancement [15], with an exploratory 6 g dose. The BEAA® (6 g) test food contained an EAA mixture (Toyo Shinyaku Co., Ltd., Saga, Japan) with added flavor and sweeteners. The BEAA® (3 g) and placebo foods were indistinguishable in ap-

pearance and taste, with the EAA mixture partially or entirely replaced with dextrin. The daily intake of BEAA® (6 g), BEAA® (3 g), and the placebo was designed to be 6.8 g.

The nutritional composition per daily intake is shown in Table 1. The amount of total EAA in BEAA® (6 g) was 6.0 g, while that in BEAA® (3 g) was 3.0 g.

Table 1. Analysis of nutrient composition values of test food.

	BEAA® (6 g)	BEAA® (3 g)	Placebo
Energy (kcal)[a]	27.0	26.5	26.0
Protein (g)[b]	6.0	3.0	0.0
Fat (g)	0.0	0.0	0.0
Carbohydrate (g)	0.8	3.7	6.6
Salt equivalent (g)	0.004	0.002	0.000
Total EAA (g)	6.0	3.0	0.0
Total (g)	6.8	6.8	6.8

[a]Calorie conversion factor: protein, 4; fat, 9; carbohydrate, 4; [b]Nitrogen-to-protein conversion factor: 8.82(BEAA® (6 g), BEAA® (3 g)), 6.25 (Placebo).

2.4. Evaluation Items

The primary outcome was appetite scores (hunger, fullness, prospective food intake, satisfaction) obtained using the Japanese version of the Appetite Sensations Questionnaire [18] [19], assessed seven times (pre-intake (PRE), immediately post-intake (POST), and 30, 60, 90, 120, and 180 minutes post-intake) with a 100 mm visual analog scale (VAS). Secondary outcomes included changes in active GLP-1 levels (ΔGLP-1) and niAUC, assessed six times (pre-intake (PRE) and 30, 60, 90, 120, and 180 minutes post-intake). Active GLP-1 was measured using the GLP-1 Active form (high sensitivity) Assay Kit (Immuno-Biological Laboratories, Fujioka, Japan). No changes to the study outcomes were made after the study began.

Participants were provided with food diaries and participant logs. They were instructed to record the items outlined below daily from one week before Period I until the day before Period III. Alcohol intake was calculated separately.

Survey items: 1) Presence of physical changes; 2) Bedtime; 3) Presence of changes in living conditions; 4) Presence of menstruation (female subjects only); 5) Use of pharmaceuticals (excluding nutritional drinks; including newly designated quasi-drugs and quasi-drugs with new scope); 6) Dietary content (including supplements, health foods, drinks, and alcohol).

2.5. Statistical Analysis

The analysis population was defined as the Per-Protocol Set (PPS). For appetite scores and ΔGLP-1, the change from baseline was calculated for each test food

intake. A paired t-test was performed among BEAA® (6 g) intake (BEAA (6 g)), BEAA® (3 g) intake (BEAA (3 g)), and placebo intake (PLA). niAUC was calculated using the trapezoidal rule based on the ΔGLP-1 values from pre-intake to 180 minutes post-intake for each test food. Paired t-tests were also performed between BEAA (6 g) and BEAA (3 g) and PLA. Furthermore, to test for carryover effects, analysis of variance (ANOVA) using a general linear model was performed, with group and test food as fixed factors, and pre-intake appetite scores (hunger, fullness, prospective food intake, satisfaction) and GLP-1 levels of each subject as random factors, to examine time effects and sequence effects.

All tests were two-tailed with a significance level of 5%. Statistical analyses were performed using IBM SPSS Statistics 28. Subject background characteristics are presented as means ± standard deviations, and other data as means ± standard errors. No additional analyses were performed.

3. Results

3.1. Analysis Subjects

The number of subjects enrolled in this study was 15 (10 males, 5 females). As there were no dropouts after randomization, the study commenced with all 15 subjects. The assigned interventions were implemented for the 15 subjects in each group. During the study period, 1 subject (female) in the BEAA® (3 g)-first sequence met the discontinuation criteria (failure to attend scheduled visits for reasons unrelated to the study) and was thus excluded, resulting in 14 subjects completing the study. Additionally, after study completion, 2 subjects met exclusion criteria, resulting in 12 subjects (9 males, 3 females) for analysis. The reason for exclusion was violation of study precautions during the study period (1 subject in the BEAA® (6 g)—first sequence, 1 subject in the BEAA® (3 g)—first sequence).

The period from subject recruitment to completion of follow-up was January to February 2025. The study was terminated upon completion of follow-up for all subjects. Subject characteristics for the analysis population in this study are shown in Table 2, while a flowchart illustrating the process from enrollment to analysis is given in Figure 1.

Table 2. Subject characteristics.

	All Subjects Analyzed (n = 12)
Male/Female	9/3
Age (years)	31.4 ± 3.6
Height (cm)	168.6 ± 8.8
Weight (kg)	62.5 ± 8.9
BMI (kg/m^2)	21.9 ± 1.7

Values are expressed as means ± SDs.

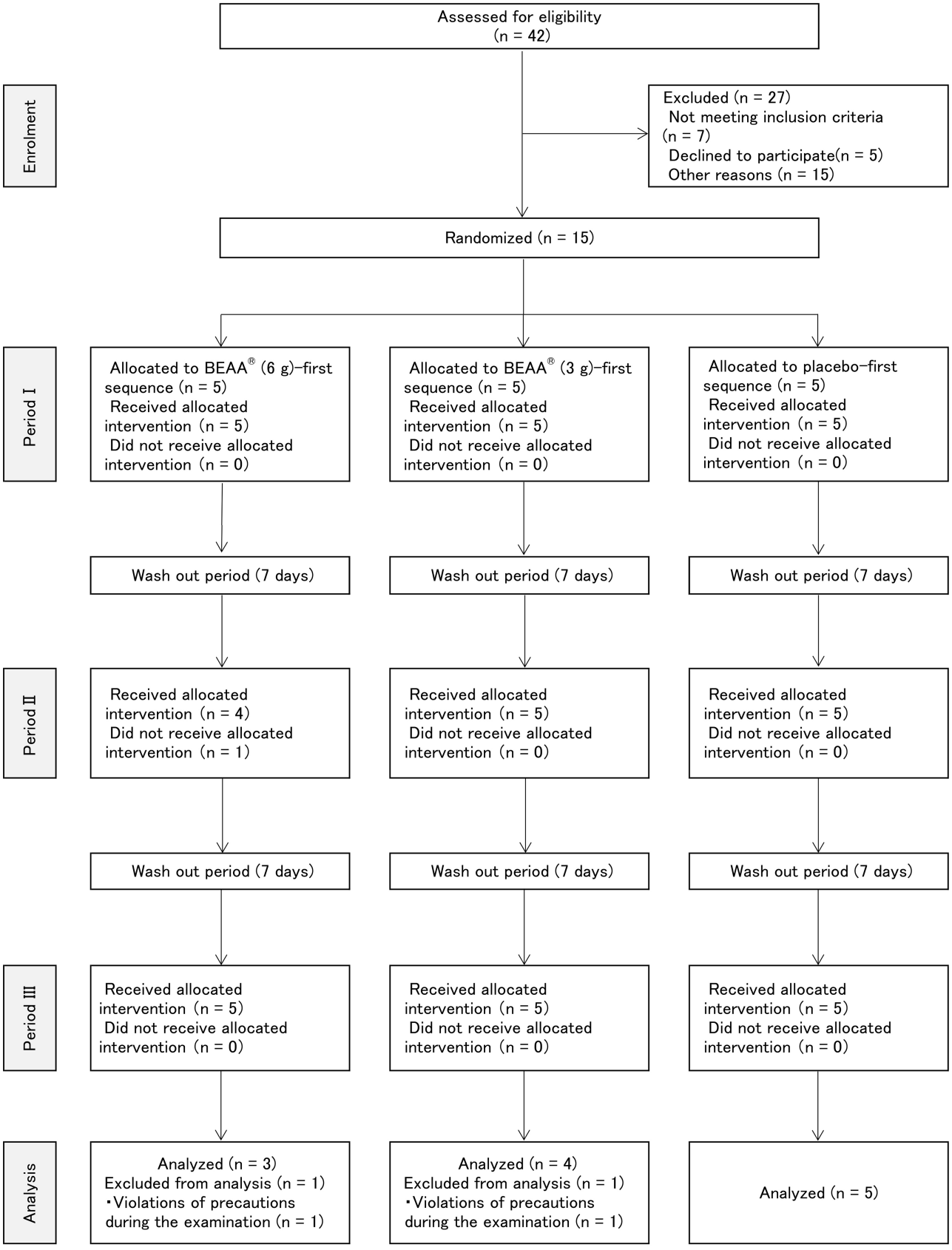

Figure 1. A flow diagram of the study design.

3.2. Analysis Results

Figure 2 shows the time-course changes in VAS appetite scores (hunger, fullness, prospective food intake, satisfaction) post-intake. BEAA (6 g) showed a trend toward lower hunger scores at 90 and 120 minutes compared to PLA (90 minutes, P = 0.096; 120 minutes, P = 0.081), but no significant differences were found among BEAA (6 g), BEAA (3 g), and PLA. No significant differences were observed in fullness, prospective food intake, or satisfaction scores.

Figure 3 shows the time-course changes in ΔGLP-1 and the niAUC for GLP-1. BEAA (6 g) showed a trend toward higher ΔGLP-1 at 30 minutes compared to PLA (P=0.097), but no significant differences were found among BEAA (6 g), BEAA (3 g), and PLA. The niAUC for GLP-1 was higher with BEAA (6 g) compared to PLA (P=0.089), but no significant differences were found among BEAA (6 g), BEAA (3 g), and PLA.

3.3. Adverse Events

No serious adverse events were observed during the study. All recorded events were deemed unrelated to the test food by the principal investigator.

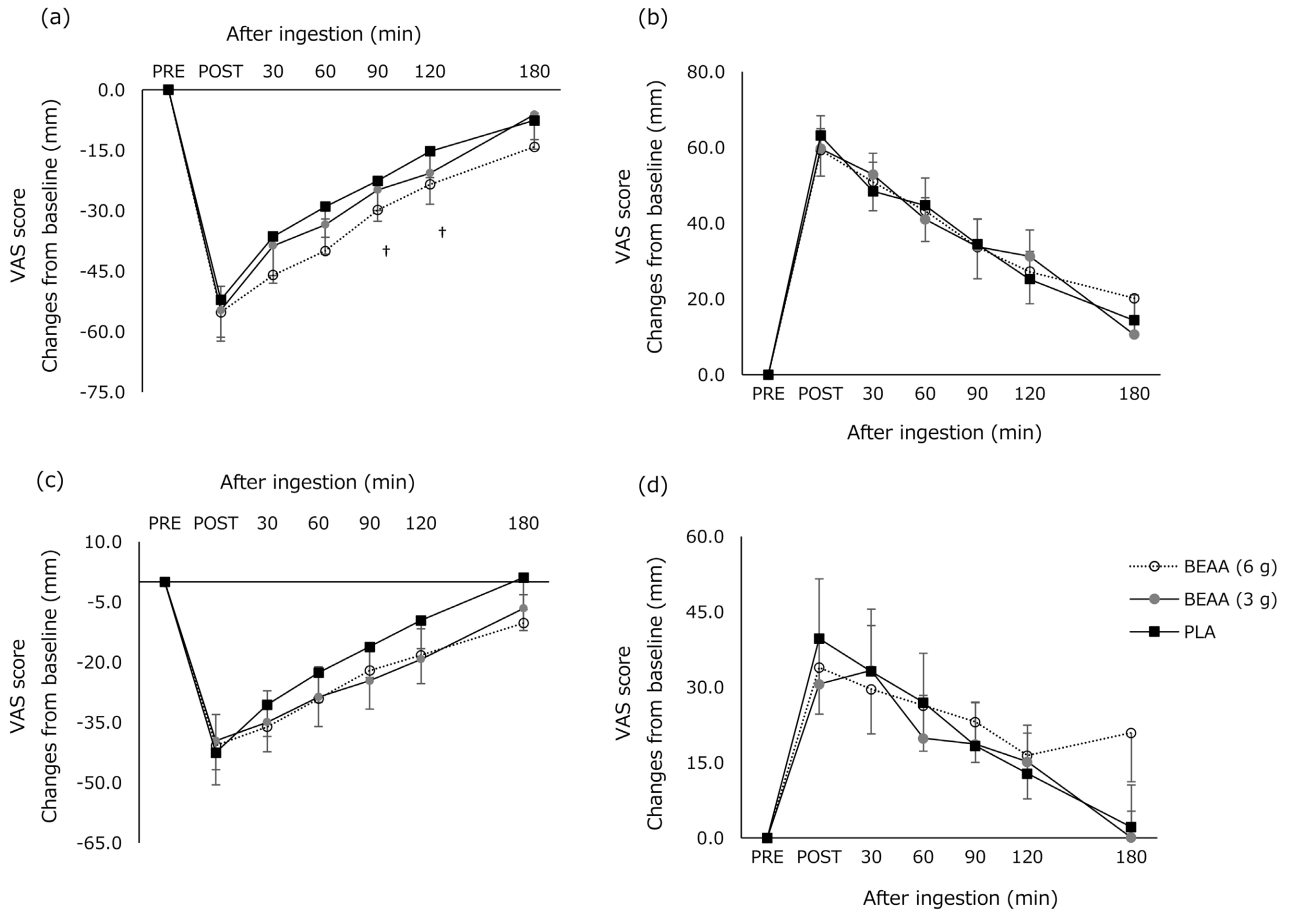

Figure 2. Time-course changes in VAS appetite scores (hunger (a), fullness(b), prospective food intake (c), satisfaction (d)). Values are expressed as means ± SEs. † BEAA (6 g) vs. PLA (P < 0.1).

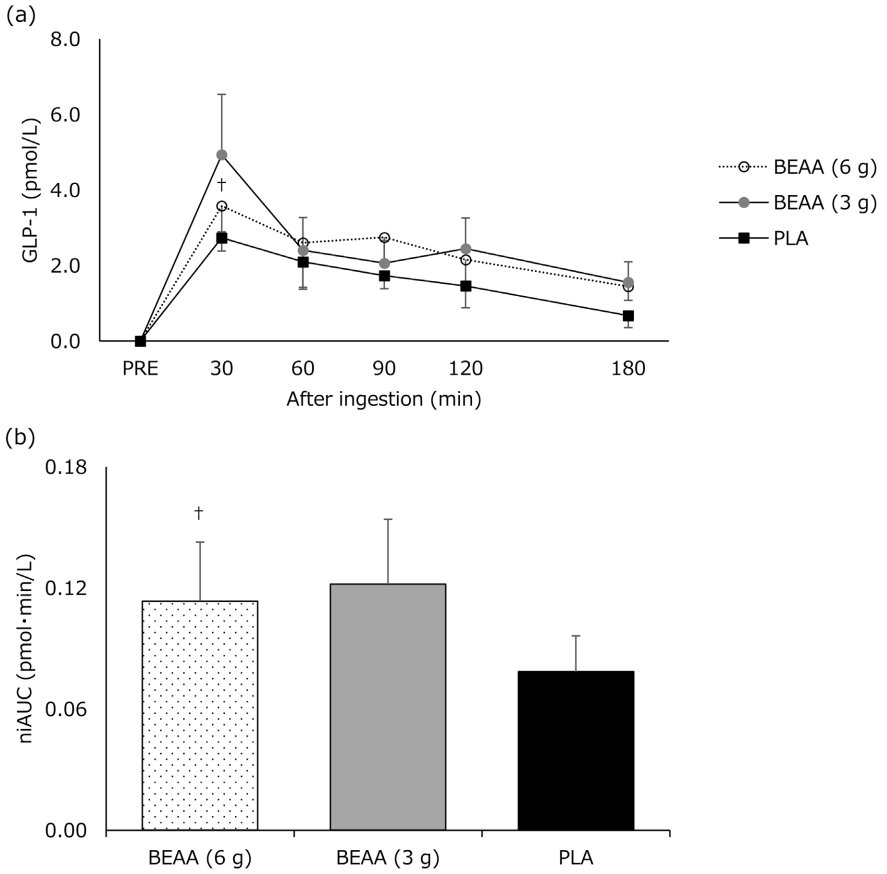

Figure 3. Time-course changes in ΔGLP-1 (a) and niAUC for GLP-1 (b). Values are expressed as means ± SEs. † BEAA (6 g) vs. PLA ($P < 0.1$).

4. Discussion

This study examined the appetite-suppressing effects of BEAA® in healthy adults aged 20 - 65 with a BMI below 30 using a placebo-controlled, randomized, double-blind, triple-crossover trial. The VAS hunger score showed a trend toward lower values with BEAA (6 g) at 90 and 120 minutes post-intake compared to PLA, though the differences were not statistically significant. Additionally, ΔGLP-1 at 30 minutes and niAUC for GLP-1 showed a trend toward higher values with BEAA (6 g) compared to PLA, though the differences were not statistically significant. No significant trends or differences were observed with BEAA (3 g). Since it was confirmed that there was no carryover effect in the assessment of carryover effects, the washout period is considered to have been appropriate.

The mechanism by which BEAA® may induce GLP-1 secretion likely involves its absorption in the gastrointestinal tract, particularly reaching the lower small intestine, which is rich in GLP-1-producing L cells, thereby directly activating receptors on L cell surfaces. L cells can sense extracellular amino acids through receptors like CaSR [20] [21]. Valine, a BCAA, strongly induces GLP-1 secretion via CaSR [14], with other basic and aromatic amino acids serving as strong agonists of CaSR [22]. The valine, lysine, histidine, phenylalanine, and tryptophan in

BEAA® likely stimulate these receptors, inducing GLP-1 secretion. Thus, the synergistic action of multiple amino acids in BEAA® may have resulted in the observed GLP-1 secretion trend.

The trend toward maintaining mild hunger with BEAA® may be due to increased blood GLP-1 levels. GLP-1 delays gastric emptying, prolonging physical gastric fullness and contributing to mild hunger maintenance [6]. Additionally, GLP-1 acts on the brain's appetite control center—the hypothalamus—activating central appetite-suppressing signals and reducing hunger [8] [23]. The observed trend of increased GLP-1 levels at 30 minutes post-intake and decreased VAS hunger scores at 90 and 120 minutes suggests that the hunger-reducing effect may be due to increased GLP-1 levels.

While BEAA (6 g) showed a trend toward maintaining mild hunger, no effects were observed on fullness, prospective food intake, or satisfaction. This may be because the small amount of BEAA® utilized did not sufficiently trigger factors contributing to fullness, such as gastric expansion, while endocrine responses like GLP-1 secretion occurred, showing some effect on hunger [24]. Additionally, while BEAA (6 g) showed trends toward maintaining mild hunger and inducing GLP-1 secretion, BEAA (3 g) demonstrated no effects on hunger or GLP-1 secretion. Previous reports have shown that subcutaneous administration of 9.9 g of a single amino acid affects hunger and appetite-related hormone secretion [16], suggesting that even with the synergistic action of multiple amino acids in BEAA®, the intake amount used herein may not have been sufficient to yield effects.

The results of this study suggest that BEAA® intake may offer a new means of controlling appetite and preventing overeating. For example, consuming BEAA® before meals or as a snack may naturally reduce subsequent meal intake or suppress unnecessary snacking, thereby potentially limiting excessive calorie intake. This could represent an ideal nutritional intervention for weight management.

The safety of BEAA® was also confirmed in this study. No adverse events related to BEAA® intake were observed, suggesting no safety issues with single-dose BEAA® intake.

However, this study has some limitations. First, the study targeted healthy adults with a BMI below 30, so the effects on obese individuals or those with type 2 diabetes are unknown. Additionally, as this study involved single-dose intake, the long-term effects of BEAA® on food intake and body weight remain unclear. Future research should involve long-term BEAA® intake trials to verify changes in actual food intake and body weight.

5. Conclusion

A single 6 g dose of BEAA® showed a trend toward inducing GLP-1 secretion and maintaining mild hunger in healthy adults. These results suggest that BEAA® may play a new role as a functional food for appetite regulation, in addition to its muscle mass-enhancing effects. In the future, BEAA® is expected to become an effective and safe tool for obesity prevention and improvement.

Authors' Contributions

Conceptualization, T.M. and T.K.; methodology, Y.H., S.M., and Y.U.; validation, S.M.; formal analysis, N.O.; investigation, Y.H. and Y.U.; writing—original draft preparation, Y.H and S.M.; writing—review and editing, T.M.; visualization, N.O. and S.M.; supervision, K.T. and Y.I.; project administration, K.F., T.O., and T.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee of Kobuna Orthopedics Clinic (approval date: 19 December 2024; approval number: MK-2412-04).

Informed Consent Statement

Informed consent was obtained from all subjects involved in this study.

Data Availability Statement

The data used in this manuscript are not publicly available because of commercial restrictions, but they can be made available on reasonable request.

Conflicts of Interest

The test food used in this study was provided by Toyo Shinyaku Co., Ltd. This study was commissioned to K.S.O., Inc. by Toyo Shinyaku Co., Ltd. Eight of the authors (Y.H., S.M., Y.U., N.O., K.F., T.O., T.K., and K.T.) are employees of and receive their salaries from Toyo Shinyaku Co., Ltd. Y.I., a physician affiliated with Nihonbashi Cardiology Clinic, conducted this study as the principal investigator under contract with K.S.O., Inc. T.M. is a professor emeritus at Kyoto University and supervised this study on behalf of Toyo Shinyaku Co., Ltd.

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