Research Article Open Access

Beneficial effects of maltobionic acid on bone density in healthy Japanese adult women: A randomized double-blind placebo-controlled study

Daiki Suehiro[1], Ken Fukami[1], Tsuyoshi Takara[2]

[1]San-ei Sucrochemical Co., Ltd., 24-5, Kitahama-mac hi, Chita-city Aichi, 478-8503, Japan; [2]Medical Corporation Seishinkai, Takara Clinic, 9F Taisei Building, 2-3-2, Higashi-Gotanda, Shinagawa-ku, Tokyo 141-0022, Japan

Corresponding author: Daiki Suehiro, Research and Development department, San-ei Sucrochemical Co., Ltd. 24-5, Kitahama-machi, Chita-city Aichi, 478-8503, Japan

Submission Date: June 18th, 2019. **Acceptance Date:** September 27th, 2019. **Publication Date:** September 30th, 2019.

Citation: Daiki Suehiro, Ken Fukami, Tsuyoshi Takara. Beneficial effects of maltobionic acid on bone density in healthy Japanese adult women: A randomized double-blind placebo-controlled study. *Functional Foods in Health and Disease* 2019; 9(9): 606-627. DOI: https://doi.org/10.31989/ffhd.v9i9.629

ABSTRACT

Background: Osteoporosis is characterized by reduced bone mineral density (BMD) and increased fracture risk, with a higher incidence in post-menopausal women. This study aimed to evaluate the safety and efficacy of long-term ingestion of maltobionic acid on BMD in healthy Japanese women.

Methods: A randomized, double-blind, placebo-controlled, parallel-group study was conducted from February to December 2018. Thirty-eight healthy Japanese women aged 50–69 years who were at least 1 year past the onset of natural menopause were allocated to two groups (19 in each group) using a computerized random-number generator: one in which participants ingested 7 g of corn syrup containing maltobionic acid and another in which participants ingested 7 g of placebo (maltose syrup) per day for 24 weeks. BMD and bone metabolism parameters were measured dual-energy X-ray absorptiometry (DEXA) method and a peripheral blood test, respectively, whereas safety was evaluated via a physical examination, peripheral blood test, urinalysis, assessment of subjective symptoms, and a medical questionnaire.

Results: Of the 38 subjects, one subject discontinued the study halfway and 14 were excluded before the efficacy analysis because of conflicts with control criteria. Thus, the final study

population was 23 subjects (10 in the Test food group and 13 in the Placebo group). There were no adverse events related to consumption of the test food. Consumption of corn syrup solids containing maltobionic acid was maintained during the intervention period, and BMD, bone mineral content, and young adult mean values were found to be improved ($P < 0.05$). No safety concerns were observed during the intervention period.

Conclusion: These results indicate that the consumption of maltobionic acid may contribute to the prevention of osteoporosis.

Trial registration: UMIN-CTR ID: UMIN000031489; Foundation: San-ei Sucrochemical Co., Ltd.

Keywords: Maltobionic acid; bone mineral density; safety; long-term intake; osteoporosis; menopause

BACKGROUND

Osteoporosis is defined by WHO as "A disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk" [1]. With the aging society in Japan, the number of patients with osteoporosis is increasing annually, and the prevalence of osteoporosis was estimated to be approximately 13 million in 2015 [2, 3]. Osteoporotic fractures are associated with poorer quality of life [4] and increased risk of mortality [5]. Bone mineral density (BMD) is an important predictor of osteoporotic fractures [6], and sufficiency of everyday intake of nutrients such as calcium, vitamin D, and vitamin K are known to have a major impact on BMD. Calcium in particular is a major mineral component of bone. Calcium intake has been found to be significantly related to bone mineral content (BMC) and BMD [7-9]; appropriate intake of this mineral is, therefore, important for the prevention of osteoporosis. In addition to the significance of the amount of calcium consumed, its efficient absorption by the intestines is important for improving nutritional status. The absorbability of calcium is influenced by various factors including the form in which calcium is ingested and its associated properties, such as its solubility, and the food consumed with it (e.g., oxalic acid and phosphorus) [10, 11].

Maltobionic acid (4-O-α-D-Glucopyranosyl-D-gluconic acid: CAS No. 534–42-9), in which glucose is α-1,4-bonded to gluconic acid, is an indigestible disaccharide present in honey. Maltobionic acid also forms a stable salt with inorganic cations that maintains high water solubility even when ion-bound with calcium [12]. We have previously reported that maltobionic acid enhances calcium and magnesium absorption and increases the amount of calcium in rat femurs by maintaining the solubilized state of minerals throughout the intestinal tract [12, 13]. Furthermore, in a 24-week intervention study in postmenopausal women, the intake of calcium salts composed mainly of maltobionic acid was shown to maintain and increase BMD, with no safety concerns related to their long-term consumption [14]. However, there are no reports on BMD improvement resulting from intake of the mineral-free state of maltobionic acid or information on the safety of its long-term intake. In the present study, we investigated the safety and efficacy of the long-term ingestion of maltobionic acid on the BMD of healthy Japanese women.

METHODS

Study design and participants

This was a randomized double-blind placebo-controlled study. The participants were recruited by ORTHOMEDICO Inc. (Tokyo, Japan), which runs a clinical trial recruitment site known as Go106 (https: //www.go106.jp). Individuals interested in participating were given a full explanation of the study. Those who provided written consent underwent preliminary selection process. Individuals who met the following criteria were included: Japanese women aged 50–69 years who were at least 1 year past the onset of natural menopause. Those who met any of the following exclusion criteria were excluded: (a) history of treatment for malignant tumor, cardiac failure, or myocardial infarction; (b) presence of other diseases (arrhythmia, liver dysfunction, kidney dysfunction, cerebrovascular disease, rheumatism, diabetes, dyslipidemia, hypertension, or other chronic disease); (c) regular use of pharmaceutical drugs (including *kampo*) or supplements; (d) regular ingestion of foods for specialized health use or with functional claims; (e) ingestion at least once per week of calcium, vitamin D, vitamin K, magnesium, isoflavones (including daidzein, genistein, equol) and all other supplements, foods for specialized health use, foods with functional claims, and foods with nutritional function claims that may affect bone metabolism; (f) allergies (pharmaceuticals and foods related to the test foods in this study); (g) participation in another clinical study within 3 years of providing written consent to participate in the present study; or (h) any other reason the principal investigator found to disqualify the individual from participating in this study.

This study's protocol received approval from the Institutional Review Board of Takara Clinic (Tokyo, Japan) on February 20, 2018 (no. 1802-1712-ST01-03-TC). The study was conducted with full consideration of medical ethical principles and in accordance with the Declaration of Helsinki (2013) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects. Testing was mainly conducted by the Takara Clinic. This study was registered with the University Hospital Medical Information Network (UMIN000031489). Subjects were recruited from February 27 to April 28, 2018, and the study was conducted from May 28 to December 1, 2018.

The target sample size was calculated based on the results of the previous study [14]. The primary outcome was BMD of the total anterior surface of the lumbar vertebrae, and the efficacy of the test food was evaluated by determining the difference in BMD between the time of screening to the completion of the 24-week intervention period (post-24W). In the previous study, the mean change at post-24W was 0.005 g/cm^2 for the Test food group and −0.017 g/cm^2 for the Placebo group, and the difference between the groups was 0.022 g/cm^2. The standard deviation was 0.019. A significance level (α) of 0.05 and a power (1-β) of 0.90 were set and it was finally determined that assigning approximately 17 subjects per group would be satisfactory. Thus, 19 subjects were included to account for dropout during the study period. Therefore, the target sample size was 34 subjects, and the actual sample size was 38 subjects in this study.

We decided on the sample size based on the BMD of the total anterior surface of the lumbar vertebrae, as described in a previous study [14].

Selection, randomization, and blinding

The 63 subjects who consented to participate in this study were determined by the principal investigator to be eligible for inclusion. The participants underwent bone density measurements when they were screened and tested, prior to ingesting the test substance. The results indicated that those with a total young adult mean (YAM) of 70–100% in the anterior surface of the lumbar vertebrae were eligible to participate. The lower limits of the BMD values for each individual were utilized. Then, to avoid major differences in age and mean ± standard deviation (SD) BMD (total left femoral neck and anterior surface of the lumbar vertebrae), 19 participants were placed in the Test food group and another 19 in the Placebo group. Group allotments were conducted by an intermediary study controller using StatLight #11 (Yukms Co., Ltd., Kanagawa, Japan). The allocation ratio for the groups was 1:1. The group allocation was unknown to the study participants, principal investigator, outcome assessors, and all other staff involved in this study; none of these individuals were involved in the allocation process.

Test food

The test food used in the intervention was corn syrup containing maltobionic acid (SourOligo, San-ei Sucrochemical Co., Ltd. Aichi, Japan). Corn syrup containing maltobionic acid comprises 40.3% maltobionic acid, 16.5% maltotronic acid, 13.2% other carbohydrates, and 30.0% moisture. The placebo food was a maltose syrup (contain 50.8% maltose, 12.5% maltotriose, 6.7% other carbohydrates, and 30.0% moisture; San-ei Sucrochemical Co., Ltd.). Prior to the start of the study, the institutional review board confirmed that the foods could not be distinguished based on odor or color. The study participants ingested 7 g per day after meals with cold water or warm water. The intervention period was 24 weeks.

Outcome measures

Examinations were conducted at baseline and at 12 and 24 weeks after initiating the intervention.

1. Primary outcome: X-ray examination (dual-energy X-ray absorptiometry method)

X-ray examinations consisted of dual-energy X-ray absorptiometry (DEXA) using a Discovery X-ray BMD measuring device (Hologic Inc., USA) that was used to measure total lumbar vertebral anterior surface (L2–L4), bone area, bone mineral content (BMC), T-score, YAM value Z score prior to ingestion, 12 weeks post-intervention (post-12W), and 24 W post-intervention (post-24W) via medical scanning. The T-score refers to the value for which the index was specified using the mean BMD value (reference value) of young age as 0 and standard deviations as 1SD. The YAM value represents the mean BMD of young adults (lumbar: 1.011 g /cm^2, femoral neck: 0.787 g/cm^2) as 100% and the bone density of the study participants as a percentage [15]. The Z-scores indicate values for which indices were specified using mean BMD values at the same ages as 0 and standard deviations as 1SD.

2. Secondary outcome: Peripheral blood test

Approximately 21 mL of venous blood and 13 mL of urine were collected from each study participant at Takara Clinic. The bone metabolism markers used included Tartrate-resistant acid phosphatase 5b (TRACP-5b), osteocalcin (OC), bone-specific alkaline phosphatase (BAP), type I collagen cross-linked N-telopeptides (u-NTx, s-NTx), and deoxypyridinoline (DPD). Measurements were conducted by LSI Medience Corporation (Tokyo, Japan).

3. Safety assessment items

3.1 Physical measurements and physiological testing

We measured height, weight, body mass index (BMI), body fat percentage, systolic blood pressure (BP), diastolic BP, and pulse. Height was measured only once following the conclusion of the participants' orientation meeting. The height measurement was conducted at ORTHOMEDICO Inc., while all other measurements were conducted at Takara Clinic.

3.2 Peripheral blood test

Approximately 15 mL of blood was collected from each study participant at Takara Clinic.
Hematological testing consisted of measurements of white blood count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Ht), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cell imaging using a Sysmex XE-2100 (Sysmex Corporation, Hyogo, Japan), HEG-L (Sysmex Corporation), and an optical microscope BX41 (Olympus Corporation, Tokyo, Japan). Measurements were conducted by LSI Medience Corporation.

Blood biochemistry testing consisted of measurements of the following: aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyl transferase (γ-GT), alkaline phosphatase (ALP), lactate dehydrogenase (LD), leukocyte alkaline phosphatase (LAP), total bilirubin (T-Bil), direct bilirubin (D-Bil), indirect bilirubin (I-Bil), cholinesterase (ChE), zinc sulfate turbidity test (ZTT), total protein (TP), urea nitrogen (UN), creatinine (CRE), uric acid (UA), creatine kinase (CK), sodium (Na), potassium (K), chlorine (Cl), calcium (Ca), inorganic phosphorus (IP), serum iron (Fe), serum amylase (AMY), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), glucose (Glu), glycated hemoglobin (HbA1c), and glucoalbumin (GA). These measurements were performed using an H7700 (Hitachi High-Technologies Corporation, Tokyo, Japan) and a JCA-BM9130 (JEOL Ltd., Tokyo, Japan. Measurements were conducted by LSI Medience Corporation.

3.3 Urinalysis

Approximately 50 mL of urine was collected from each study participant, and protein (Pro), glucose (Glu), urobilinogen (Uro), bilirubin (Bil), ketone bodies (Ket), pH, and occult blood (Bld) were measured. Measurements were conducted by LSI Medience Corporation.

4. Diet survey

We conducted a questionnaire survey of the participants' nutritional intake for a 1-month period proximate to the study at pre-ingestion, post-12W, and post-24W using a brief-type self-administered diet history questionnaire (BDHQ) [16, 17] that investigated the intake of 56 foods and beverages in the last month. Dietary intake in terms of energetics and selected nutrients was estimated based on computer algorithms.

Statistical analysis

All outcomes in this study were examined using outpatient testing at the pre-ingestion, post-12W, and post-24W time points, and intra- and inter-group comparisons were made. The intra-group comparisons were performed using Dunnett's test with the time points and study participants as the fixed factors using the measured values in the following comparisons: pre-ingestion vs. post-12W and pre-ingestion vs. post24W. Inter-group comparisons were performed by determining the amount of change at all time points in the Test food group versus Placebo group. The amount of change was determined by subtracting the pre-ingestion measured value from the post-12W or post-24W measured value. The pre-ingestion measured value and amount of change were analyzed using Student's *t*-test.

All statistical analyses were performed using two-sided testing, and the standard of significance was set at 5%. The software used was SPSS for Windows (ver. 23.0; IBM Japan, Tokyo, Japan) and Microsoft Excel 2007 and 2013 (Microsoft Japan, Tokyo, Japan). Redundancy with other time points or other items was given no consideration.

RESULTS

Analysis of subjects

Figure 1 shows a follow-up flow chart for the study participants. Of the 63 individuals who consented to participation in this study, 25 were excluded during interviews with the principal investigator or because of the inclusion/exclusion criteria. Finally, 38 individuals were enrolled in this study. We excluded 15 potential candidates: 1 subject who dropped out of the study, 3 subjects who violated compliance, and 4 subjects whose BMD of the total lumbar spine fell outside the 2SD range for any of the percentage changes, 2 who exhibited a percent change in serum total protein that exceeded the mean ± 2SD range, and 5 with abnormally high urinary NTx (> 89.0 nmol BCE/mmol･Cr). Among bone metabolism markers associated with osteoclasts, urinary NTx is the ultimate product of metabolism of bone during resorption and is positively correlated with decreased bone density [18]. The total protein in the blood is negatively correlated with bone density, and it seems to show the effect on the bone density [19]. Therefore, at the case review meeting after the completion of the study, we excluded 5 subjects who showed abnormally high values (> 89.0 nmol BCE/mmol･Cr) of urine NTx before and after ingestion of the test food and 2 subjects who showed an outlier value for any of the percentage changes in each test, from pre-ingestion onward, for total protein in blood.

Thus, the final subject population consisted of per-protocol sets of 13 subjects in the Placebo group (mean age, 55.8 ± 4.4 years) and 10 subjects in the Test food group (mean age, 58.2 ± 5.4 years). The subject population for the safety assessment items consisted of study subjects who underwent at least one intervention. There were 17 subjects (mean age, 55.9 ± 4.2 years) in the Placebo group and 18 subjects in the Test food group (mean age, 57.3 ± 4.6 years). The results of the dietary survey by BDHQ showed no significant differences in nutrient intake between Test food group and Placebo group, and no study participants had an intake rate of less than 90%.

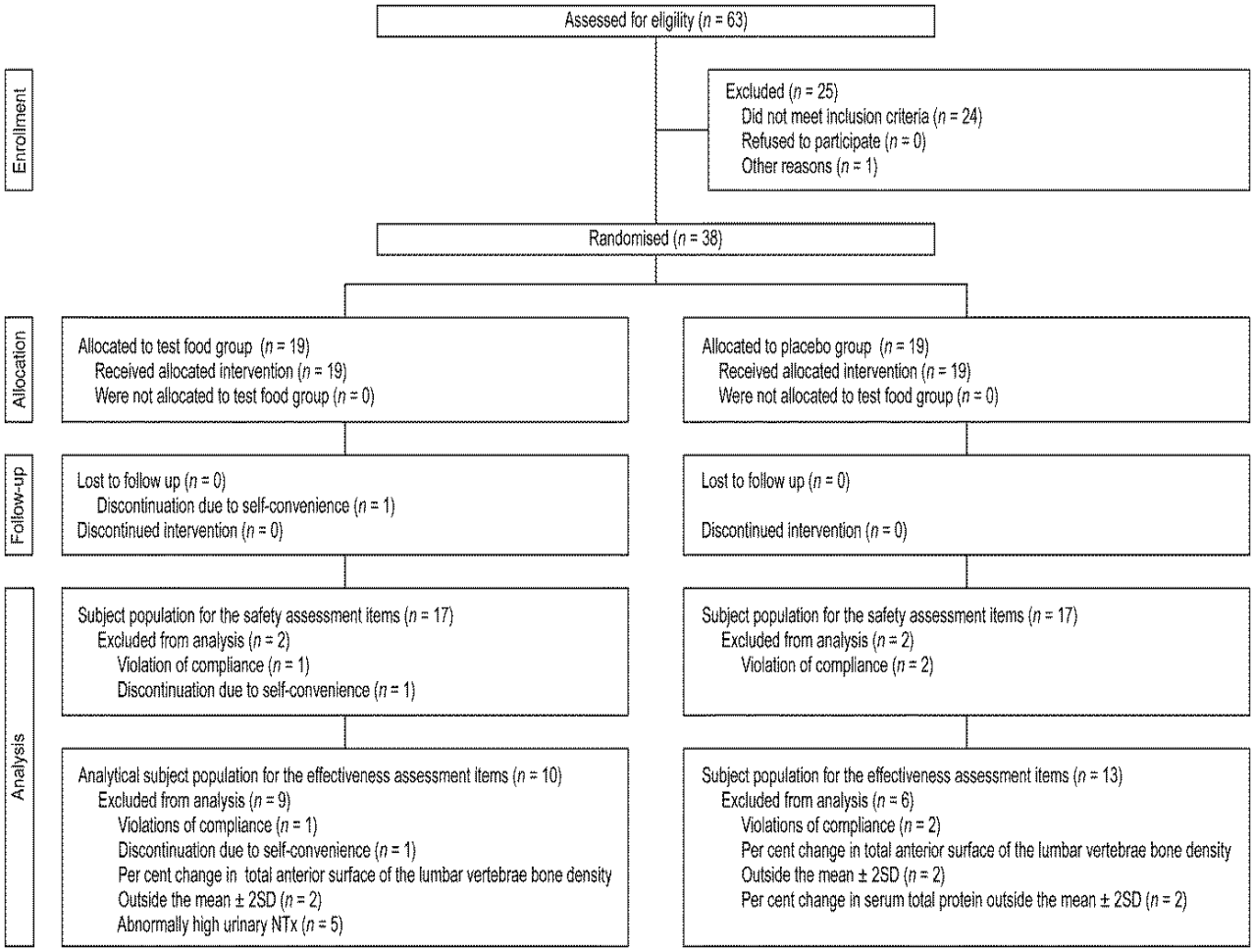

Figure 1. Follow-up flow chart for the study participants

Efficacy assessment

The background characteristics of the 23 subjects in the subject population for the efficacy analysis are shown in Table 1. The efficacy assessment items (BMD measurements for lumbar vertebrae anterior surface) are shown in Table 2. The peripheral blood test results are shown in Table 3.

Table 1. Characteristics of subjects

		Test food group (n = 10)		Placebo group (n = 13)	
Age	years	58.2	± 5.4	55.8	± 4.4
Body height	Cm	158.5	± 4.8	156.0	± 5.0
Body weight	Kg	56.5	± 9.3	52.9	± 8.8
BMI	kg/m^2	22.6	± 3.9	21.8	± 4.3
Body fat percentage	%	27.7	± 7.8	26.4	± 5.8
BP	mmHg	115.1	± 15.3	123.0	± 14.7
Diastolic BP	mmHg	70.5	± 12.6	76.3	± 12.7
Pulse	Bpm	78.6	± 7.3	75.3	± 8.6

Values are mean ± SD

Table 2. Changes in anterior surface of lumbar vertebrae (L2, L3, L4, L2-L4)

		Group	***n***	**Pre-ingestion**	**Post-12W**	**Post-24W**	**Pre-ingestion vs. Post-12W**	**Pre-ingestion vs. Post-24W**	
							Amount of change	**Amount of change**	
Bone area (L2)	cm^2	Test food	10	13.34 ± 1.07	13.65 ± 0.97	13.53 ± 0.92	0.31 ± 0.44	0.19 ± 0.48	#
		Placebo	13	13.79 ± 0.94	13.76 ± 1.00	13.47 ± 0.86	-0.03 ± 0.49	-0.33 ± 0.65	
BMC (L2)	g	Test food	10	11.09 ± 1.44	11.11 ± 1.64	11.21 ± 1.55	0.02 ± 0.38	0.13 ± 0.46	#
		Placebo	13	11.88 ± 1.62	11.76 ± 1.87	11.36 ± 1.73	-0.12 ± 0.55	-0.52 ± 0.73	
BMD (L2)	g/cm^2	Test food	10	0.83 ± 0.10	0.81 ± 0.11	0.83 ± 0.10	-0.02 ± 0.02	0.00 ± 0.02	
		Placebo	13	0.86 ± 0.09	0.85 ± 0.09	0.84 ± 0.10	-0.01 ± 0.02	-0.02 ± 0.03	
T-score (L2)	-	Test food	10	-1.78 ± 0.93	-1.95 ± 0.99	-1.82 ± 0.94	-0.17 ± 0.23	-0.04 ± 0.16	
		Placebo	13	-1.50 ± 0.84	-1.58 ± 0.84	-1.68 ± 0.98	-0.08 ± 0.20	-0.18 ± 0.32	
YAM value (L2)	%	Test food	10	81.70 ± 9.66	79.90 ± 10.45	81.40 ± 9.94	-1.80 ± 2.39	-0.30 ± 1.95	
		Placebo	13	84.31 ± 8.72	83.54 ± 8.75	82.69 ± 10.23	-0.77 ± 2.13	-1.62 ± 3.25	
Z score (L2)	-	Test food	10	1.09 ± 1.02	0.99 ± 1.02	1.15 ± 1.00	-0.10 ± 0.24	0.06 ± 0.16	
		Placebo	13	1.08 ± 0.69	1.02 ± 0.73	0.97 ± 0.87	-0.06 ± 0.21	-0.12 ± 0.30	
Bone area (L3)	cm^2	Test food	10	14.97 ± 1.18	14.97 ± 1.07	15.11 ± 1.14	0.00 ± 0.49	0.14 ± 0.49	
		Placebo	13	14.60 ± 0.93	14.53 ± 0.96	14.55 ± 0.93	-0.07 ± 0.47	-0.04 ± 0.40	
BMC (L3)	g	Test food	10	12.54 ± 1.78	12.51 ± 1.49	12.76 ± 1.70	-0.03 ± 0.66	0.22 ± 0.42	#
		Placebo	13	12.94 ± 1.79	12.74 ± 1.91	12.66 ± 2.00	-0.20 ± 0.66	-0.28 ± 0.64	
BMD (L3)	g/cm^2	Test food	10	0.84 ± 0.09	0.84 ± 0.09	0.85 ± 0.10	0.00 ± 0.02	0.01 ± 0.02	
		Placebo	13	0.89 ± 0.11	0.88 ± 0.10	0.87 ± 0.11	-0.01 ± 0.02	-0.02 ± 0.03	
T-score (L3)	-	Test food	10	-1.92 ± 0.86	-1.93 ± 0.81	-1.84 ± 0.92	-0.01 ± 0.19	0.08 ± 0.15	#
		Placebo	13	-1.48 ± 0.97	-1.57 ± 0.95	-1.64 ± 1.03	-0.09 ± 0.22	-0.16 ± 0.26	
YAM value (L3)	%	Test food	10	80.10 ± 9.09	80.00 ± 8.52	80.80 ± 9.46	-0.10 ± 2.28	0.70 ± 1.57	#
		Placebo	13	84.62 ± 10.16	83.62 ± 9.84	82.92 ± 10.76	-1.00 ± 2.31	-1.69 ± 2.63	
Z score (L3)	-	Test food	10	0.70 ± 0.97	0.74 ± 0.92	0.85 ± 1.01	0.04 ± 0.20	0.15 ± 0.16	#
		Placebo	13	0.84 ± 0.89	0.78 ± 0.87	0.75 ± 0.95	-0.05 ± 0.24	-0.09 ± 0.28	

		Group	*n*	Pre-ingestion	Post-12W	Post-24W	Pre-ingestion vs. Post-12W	Pre-ingestion vs. Post-24W	
							Amount of change	Amount of change	
Bone area (L4)	cm^2	Test food	10	14.74 ± 2.55	15.77 ± 2.25	15.73 ± 2.45	1.03 ± 2.63	0.99 ± 2.72	
		Placebo	13	15.70 ± 1.41	15.79 ± 1.18	15.68 ± 1.20	0.09 ± 0.81	-0.01 ± 0.64	
BMC (L4)	g	Test food	10	12.57 ± 2.24	13.19 ± 1.86	13.37 ± 1.97	0.62 ± 1.88	0.80 ± 1.93	
		Placebo	13	13.62 ± 2.16	13.63 ± 1.73	13.57 ± 2.01	0.02 ± 1.07	-0.05 ± 0.58	
BMD (L4)	g/cm^2	Test food	10	0.86 ± 0.08	0.84 ± 0.10	0.85 ± 0.08	-0.01 ± 0.04	0.00 ± 0.03	
		Placebo	13	0.87 ± 0.11	0.86 ± 0.10	0.86 ± 0.11	0.00 ± 0.04	0.00 ± 0.02	
T-score (L4)	-	Test food	10	-1.72 ± 0.73	-1.85 ± 0.84	-1.73 ± 0.68	-0.13 ± 0.32	-0.01 ± 0.24	
		Placebo	13	-1.61 ± 0.99	-1.63 ± 0.87	-1.65 ± 0.95	-0.02 ± 0.33	-0.05 ± 0.20	
YAM value (L4)	%	Test food	10	81.30 ± 7.67	80.00 ± 9.19	81.20 ± 7.51	-1.30 ± 3.47	-0.10 ± 2.56	
		Placebo	13	82.31 ± 10.76	82.08 ± 9.55	82.23 ± 10.20	-0.23 ± 3.49	-0.08 ± 2.22	
Z score (L4)	-	Test food	10	0.36 ± 0.83	0.26 ± 0.90	0.38 ± 0.85	-0.10 ± 0.32	0.02 ± 0.26	
		Placebo	13	0.18 ± 0.96	0.21 ± 0.87	0.23 ± 0.94	0.02 ± 0.33	0.05 ± 0.21	
Bone area (L2-4)	cm^2	Test food	10	43.05 ± 3.81	44.39 ± 3.55	44.37 ± 4.01	1.34 ± 2.44	1.31 ± 3.13	
		Placebo	13	44.09 ± 2.50	44.08 ± 2.62	43.70 ± 2.61	-0.01 ± 0.89	-0.39 ± 1.00	
BMC (L2-4)	g	Test food	10	36.50 ± 4.54	36.81 ± 4.56	37.35 ± 4.52	0.31 ± 2.37	0.85 ± 2.64	#
		Placebo	13	38.44 ± 4.99	38.13 ± 5.09	37.58 ± 5.41	-0.30 ± 1.07	-0.86 ± 1.15	
BMD (L2-4)	g/cm^2	Test food	10	0.84 ± 0.09	0.83 ± 0.09	0.84 ± 0.08	-0.01 ± 0.02	0.00 ± 0.01	#
		Placebo	13	0.87 ± 0.10	0.86 ± 0.09	0.86 ± 0.10	-0.01 ± 0.02	-0.01 ± 0.02	
T-score (L2-4)	-	Test food	10	-1.44 ± 0.72	-1.52 ± 0.76	-1.43 ± 0.70	-0.08 ± 0.13	0.01 ± 0.07	
		Placebo	13	-1.17 ± 0.81	-1.22 ± 0.78	-1.27 ± 0.85	-0.05 ± 0.15	-0.10 ± 0.16	
YAM value (L2-4)	%	Test food	10	83.30 ± 8.60	82.10 ± 9.05	83.40 ± 8.38	-1.20 ± 1.48	0.10 ± 0.99	#
		Placebo	13	86.23 ± 9.55	85.46 ± 9.17	84.77 ± 10.16	-0.77 ± 1.48	-1.46 ± 1.71	
Z score (L2-4)	-	Test food	10	0.00 ± 0.67	-0.08 ± 0.65	0.05 ± 0.65	-0.08 ± 0.11	0.05 ± 0.08	
		Placebo	13	0.00 ± 0.64	-0.03 ± 0.62	-0.02 ± 0.70	-0.03 ± 0.14	-0.02 ± 0.13	

L2-L4, total anterior surface of the lumbar vertebrae; BMC, bone mineral content; BMD, bone mineral density; YAM, young adult mean. Values are mean ± SD. $^{\#}P < 0.05$ (vs. Placebo group)

Table 3. Changes in bone metabolism markers

		Group	*n*	Pre-ingestion	Post-12W	Post-24W	Pre-ingestion vs. Post-12W	Pre-ingestion vs. Post-24W
							Amount of change	Amount of change
TRACP-5b	mU/dL	Test food	10	439.20 ± 105.20	378.90 ± 83.90	359.30 ± 60.10	-60.30 ± 65.90	-79.90 ± 74.70
		Placebo	13	447.50 ± 127.20	372.00 ± 104.50	386.10 ± 126.10	-75.50 ± 100.50	-61.40 ± 59.50
OC	ng/mL	Test food	10	15.00 ± 5.30	18.20 ± 9.30	15.70 ± 6.60	3.20 ± 4.80	0.60 ± 2.10
		Placebo	13	16.00 ± 4.50	17.40 ± 5.20	17.30 ± 6.40	1.40 ± 2.50	1.30 ± 3.80
BAP	μg/L	Test food	10	13.60 ± 3.40	12.80 ± 3.00	13.50 ± 2.10	-0.80 ± 2.20	-0.10 ± 1.80
		Placebo	13	13.20 ± 3.40	12.80 ± 3.30	13.90 ± 5.10	-0.50 ± 1.20	0.60 ± 3.20
s-NTx	nmol BCE/L	Test food	10	16.90 ± 6.60	17.90 ± 6.30	15.80 ± 4.20	1.00 ± 2.10	-1.10 ± 3.60
		Placebo	13	16.70 ± 3.50	17.00 ± 4.00	17.50 ± 4.50	0.30 ± 3.30	0.80 ± 3.10
u-NTx	nmol BCE/mmol·Cr	Test food	10	46.70 ± 14.90	42.10 ± 16.10	47.90 ± 17.40	-4.60 ± 16.10	1.20 ± 21.60
		Placebo	13	52.00 ± 12.80	52.90 ± 18.40	53.40 ± 16.40	0.90 ± 15.80	1.40 ± 16.50
DPD	nmol/mmol·Cr	Test food	10	5.50 ± 0.90	5.60 ± 0.90	5.40 ± 0.80	0.10 ± 0.80	-0.10 ± 1.00
		Placebo	13	6.50 ± 2.40	7.00 ± 2.40	7.10 ± 3.50	0.50 ± 1.60	0.70 ± 2.40

TRACP-5b, tartrate-resistant acid phosphatase 5b; OC, osteocalcin; BAP, bone-specific alkaline phosphatase; NTx, type I collagen cross-linked N-telopeptides; DPD, deoxypyridinoline
Values are mean ± SD; $^{\#}P < 0.05$（vs. Placebo group)

1. Anterior surface of lumbar vertebrae

Investigation of the amount of change indicated that at post-24W, the bone area of L2 (Test food: +0.19 ± 0.48 cm^2, Placebo: −0.33 ± 0.65 cm^2: $P = 0.048$), BMC of L2 (Test food: +0.13 ± 0.46 g, Placebo: −0.52 ± 0.73 g: $P = 0.023$), BMC of L3 (Test food: +0.22 ± 0.42 g, Placebo: −0.28 ± 0.64 g: $P = 0.042$), BMD of L3 (Test food: +0.08 ± 0.017 g/cm^2, Placebo: −0.018 ± 0.027 g/cm^2: $P = 0.016$), T-score of L3 (Test food: +0.08 ± 0.15, Placebo: −0.16 ± 0.26: $P = 0.017$), YAM of L3 (Test food: +0.70 ± 1.57 %, Placebo: −1.69 ± 2.63: $P = 0.019$), Z-score of L3 (Test food: +0.15 ± 0.16, Placebo: −0.09 ± 0.28: $P = 0.024$), BMC of the total anterior surface of the lumbar vertebrae (Test food: +0.85 ± 2.64 g, Placebo: −0.86 ± 1.15 g: $P = 0.048$), BMD of the total anterior surface of the lumbar vertebrae (Test food: +0.002 ± 0.009 g/cm^2, Placebo: −0.013±0.018 g/cm^2: $P = 0.033$), and YAM of the total anterior surface of the lumbar vertebrae (Test food: +0.10 ± 0.99%, Placebo: −1.46 ± 1.71%: $P = 0.018$) in the Test food group were significantly higher than in the Placebo group.

2. Peripheral blood test (bone metabolism markers)

Although there were no significant differences in urinary DPD, values tended to be consistently lower in the Test food group than in the Placebo group from pre-intake to 24 weeks post-intake.

Safety assessment

The physical measurements and physiological test results for all subjects are presented in Table 4. The peripheral blood test results and urinalysis results are presented in Tables 5 and 6, respectively. The results of the physical measurements, physiological tests, and peripheral blood tests indicate that, from pre-ingestion to post-24W, the values were within the normal range or slightly outside the normal range. The urinalysis results indicated that the items for which values were outside standard values at post-12W included Pro (in 1 subject) in the Test food group versus included Pro (in 1), and Bld (in 1) in the Placebo group. At post-24W, values were outside the standard range for Pro (in 2), and Bld (in 1) in the Test food group versus Bld (in 2) in the Placebo group.

Table 4. Physical parameters (safety assessment)

		Group	*n*	Pre-ingestion				Post-12W			Post-24W		
Age	Years	Test food	17	57.3	±	4.6							
		Placebo	17	55.9	±	4.2							
Body height	Cm	Test food	17	159.8	±	4.3	#						
		Placebo	17	156.0	±	5.6							
Body weight	Kg	Test food	17	56.5	±	9.6		55.9	±	10.0	56.3	±	10.1
		Placebo	17	52.8	±	8.3		52.4	±	7.8	53.1	±	7.6
BMI	kg/m^2	Test food	17	22.2	±	3.8		21.9	±	4.0	22.1	±	4.0
		Placebo	17	21.8	±	4.1		21.7	±	3.9	21.9	±	3.9
Body fat percentage	%	Test food	17	26.2	±	7.6		26.6	±	7.0	26.0	±	7.7
		Placebo	17	26.2	±	6.1		26.2	±	5.6	25.9	±	5.6
Systolic blood pressure	mmHg	Test food	17	119.5	±	17.4		118.7	±	22.1	118.4	±	14.9
		Placebo	17	120.7	±	15.2		116.5	±	16.0	117.4	±	11.7
Diastolic blood pressure	mmHg	Test food	17	74.2	±	13.6		73.3	±	14.4	74.2	±	10.9
		Placebo	17	75.1	±	11.8		74.3	±	10.4	72.4	±	7.7
Pulse rate	Bpm	Test food	17	76.5	±	8.4		73.4	±	8.8	74.2	±	9.2
		Placebo	17	73.6	±	9.2		72.2	±	5.6	71.4	±	5.8

Values are mean ± SD; $^{\#}P < 0.05$ （vs. Placebo group）

Table 5. Peripheral blood test (safety assessment) results

	Reference value		Group	*n*	Pre-ingestion	Post-12W	Post-24W
WBC	3300-9000	/μL	Test food	17	5388.2 ± 1540.7	5411.8 ± 1311.4	5311.8 ± 1251.4
			Placebo	17	4735.3 ± 926.0	4758.8 ± 1308.7	4658.8 ± 993.8
RBC	380-500	$\times 10^4/\mu L$	Test food	17	450.0 ± 27.0	442.1 ± 25.4	452.4 ± 26.2
			Placebo	17	447.9 ± 24.1	439.0 ± 23.4	441.6 ± 31.5
Hb	11.5-15.0	g/dL	Test food	17	13.6 ± 0.9	13.3 ± 0.9	13.7 ± 0.8
			Placebo	17	13.4 ± 1.2	13.2 ± 1.0	13.4 ± 1.2
Ht	34.8-45.0	%	Test food	17	43.0 ± 2.8	42.3 ± 2.2	43.4 ± 2.5
			Placebo	17	42.1 ± 3.4	41.7 ± 2.7	41.9 ± 3.2
PLT	14.0-34.0	$\times 10^4/\mu L$	Test food	17	25.1 ± 5.9	24.1 ± 5.2	25.1 ± 5.0
			Placebo	17	25.8 ± 5.9	25.7 ± 7.5	25.1 ± 4.6
MCV	85-102	fL	Test food	17	95.6 ± 3.0	95.6 ± 2.9	96.1 ± 3.4
			Placebo	17	94.0 ± 5.6	94.9 ± 5.2	94.8 ± 4.5
MCH	28.0-34.0	pg	Test food	17	30.2 ± 1.1	30.1 ± 1.2	30.3 ± 1.2
			Placebo	17	29.9 ± 2.0	30.1 ± 1.7	30.4 ± 1.7
MCHC	30.2-35.1	%	Test food	17	31.7 ± 0.9	31.5 ± 0.8	31.5 ± 1.1
			Placebo	17	31.8 ± 1.1	31.7 ± 0.8	32.0 ± 1.0
Neu	40.0-75.0	%	Test food	17	56.5 ± 8.4	57.5 ± 7.2	57.1 ± 6.0
			Placebo	17	57.8 ± 7.7	56.5 ± 6.4	57.1 ± 7.8
Lym	18.0-49.0	%	Test food	17	36.6 ± 7.7	35.3 ± 6.8	35.3 ± 5.3
			Placebo	17	35.3 ± 7.4	35.9 ± 6.6	35.6 ± 7.1
Mon	2.0-10.0	%	Test food	17	4.3 ± 1.1	4.6 ± 0.9	4.8 ± 1.0
			Placebo	17	4.2 ± 1.4	4.5 ± 0.9	4.6 ± 1.1

	Reference value		Group	*n*	Pre-ingestion	Post-12W	Post-24W
Eos	0.0-8.0	%	Test food	17	2.0 ± 1.2	1.9 ± 1.2	2.2 ± 1.6
			Placebo	17	2.1 ± 1.0	2.3 ± 1.1	2.1 ± 1.4
Bas	0.0-2.0	%	Test food	17	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.4
			Placebo	17	0.6 ± 0.4	0.7 ± 0.4	0.7 ± 0.4
Neu level		/μL	Test food	17	3113.3 ± 1176.1	3169.9 ± 1029.9	3063.7 ± 893.2
			Placebo	17	2757.7 ± 775.0	2723.8 ± 955.5	2699.4 ± 891.6
Lym level		/μL	Test food	17	1916.6 ± 557.1	1864.4 ± 413.2	1857.1 ± 468.7
			Placebo	17	1646.4 ± 394.6	1672.3 ± 485.2	1619.0 ± 353.5
Mon level		/μL	Test food	17	226.7 ± 78.4	249.1 ± 68.8	247.7 ± 51.4
			Placebo	17	201.2 ± 73.3	217.5 ± 83.0	213.8 ± 57.9
Eos level		/μL	Test food	17	100.6 ± 47.2	98.8 ± 53.7	111.1 ± 85.8
			Placebo	17	102.9 ± 56.2	114.2 ± 68.0	96.8 ± 69.3
Bas level		/μL	Test food	17	31.0 ± 12.5	29.6 ± 15.7	32.1 ± 15.7
			Placebo	17	27.2 ± 24.0	31.0 ± 17.9	30.0 ± 17.4
AST	10-40	U/L	Test food	17	20.6 ± 4.0	20.6 ± 4.9	21.3 ± 4.0
			Placebo	17	21.1 ± 4.5	21.5 ± 4.3	21.4 ± 4.3
ALT	5-45	U/L	Test food	17	17.2 ± 5.6	17.8 ± 8.1	18.4 ± 6.5
			Placebo	17	16.9 ± 6.9	17.8 ± 8.8	17.1 ± 8.1
γ-GT	<30	U/L	Test food	17	23.1 ± 12.5	26.1 ± 18.4	25.7 ± 16.1
			Placebo	17	22.0 ± 8.7	33.4 ± 29.2	27.6 ± 21.8
ALP	100-325	U/L	Test food	17	208.7 ± 37.9	206.4 ± 49.6	213.4 ± 48.9
			Placebo	17	191.3 ± 42.9	194.3 ± 63.4	194.4 ± 59.6
LD	120-240	U/L	Test food	17	188.9 ± 31.6	191.8 ± 34.1	191.4 ± 32.8
			Placebo	17	195.1 ± 21.9	205.8 ± 26.5	192.2 ± 20.6

	Reference value		Group	*n*	Pre-ingestion	Post-12W	Post-24W
LAP	37-61	U/L	Test food	17	50.9 ± 5.5	52.8 ± 7.0	51.3 ± 6.7
			Placebo	17	50.9 ± 6.7	56.0 ± 12.5	52.2 ± 11.1
T-Bil	0.2-1.2	mg/dL	Test food	17	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3
			Placebo	17	0.9 ± 0.3	0.9 ± 0.3	0.8 ± 0.4
D-Bil	0.0-0.2	mg/dL	Test food	17	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
			Placebo	17	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
I-Bil	0.2-1.0	mg/dL	Test food	17	0.7 ± 0.3	0.7 ± 0.2	0.8 ± 0.3
			Placebo	17	0.8 ± 0.2	0.8 ± 0.3	0.8 ± 0.3
ChE	200-452	U/L	Test food	17	343.1 ± 69.2	323.0 ± 73.6	340.8 ± 72.9
			Placebo	17	317.7 ± 53.1	319.2 ± 61.9	322.0 ± 67.6
ZTT	2.0-12.0	U	Test food	17	6.8 ± 2.1	7.1 ± 2.2	7.1 ± 1.9
			Placebo	17	5.6 ± 2.5	6.2 ± 2.7	6.3 ± 3.1
TP	6.7-8.3	g/dL	Test food	17	7.1 ± 0.3	7.0 ± 0.3	6.9 ± 0.4
			Placebo	17	7.0 ± 0.3	6.9 ± 0.3	6.7 ± 0.3
UN	8.0-20.0	mg/dL	Test food	17	12.4 ± 3.1	13.6 ± 2.8	14.5 ± 3.7
			Placebo	17	12.8 ± 2.2	13.9 ± 2.9	13.2 ± 2.3
CRE	0.47-0.79	mg/dL	Test food	17	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
			Placebo	17	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
UA	2.5-7.0	mg/dL	Test food	17	4.6 ± 0.9	4.4 ± 0.7	4.6 ± 0.7
			Placebo	17	4.2 ± 0.8	4.2 ± 0.7	4.2 ± 0.8
CK	40-150	U/L	Test food	17	97.5 ± 48.1	96.1 ± 47.1	101.0 ± 48.6
			Placebo	17	96.1 ± 33.5	92.2 ± 35.3	98.6 ± 38.6
Na	137-147	mEq/L	Test food	17	142.5 ± 1.5	141.1 ± 2.4	141.0 ± 1.7
			Placebo	17	142.4 ± 1.7	140.7 ± 2.4	141.2 ± 2.0
K	3.5-5.0	mEq/L	Test food	17	4.1 ± 0.3	3.9 ± 0.2	4.1 ± 0.3
			Placebo	17	4.2 ± 0.5	3.9 ± 0.4	4.1 ± 0.3

	Reference value		Group	*n*	Pre-ingestion	Post-12W	Post-24W
Cl	98-108	mEq/L	Test food	17	101.8 ± 1.4	100.9 ± 2.9	101.4 ± 2.5
			Placebo	17	102.5 ± 1.4	101.6 ± 2.3	102.2 ± 1.8
Ca	8.4-10.4	mg/dL	Test food	17	9.2 ± 0.2	8.9 ± 0.3	9.1 ± 0.3
			Placebo	17	9.1 ± 0.3	9.1 ± 0.3	9.0 ± 0.3
IP	2.5-4.5	mg/dL	Test food	17	3.7 ± 0.6	4.0 ± 0.5	3.5 ± 0.4
			Placebo	17	3.7 ± 0.4	3.9 ± 0.5	3.3 ± 0.5
Fe	40-180	μg/dL	Test food	17	104.2 ± 19.9	103.6 ± 32.0	113.5 ± 31.8
			Placebo	17	95.2 ± 29.7	106.8 ± 32.6	102.5 ± 35.5
AMY	40-122	U/L	Test food	17	80.1 ± 35.8	77.2 ± 32.7	84.5 ± 40.5
			Placebo	17	77.6 ± 26.2	79.0 ± 30.9	80.8 ± 37.7
TC	120-219	mg/dL	Test food	17	229.6 ± 36.8	222.3 ± 43.1	235.3 ± 42.2
			Placebo	17	242.9 ± 30.3	242.8 ± 31.5	248.7 ± 31.1
HDL-C	40-95	mg/dL	Test food	17	75.6 ± 18.1	72.7 ± 18.4	75.8 ± 20.3
			Placebo	17	83.6 ± 20.2	83.2 ± 21.3	87.6 ± 21.8
LDL-C	65-139	mg/dL	Test food	17	134.2 ± 30.7	130.5 ± 36.0	134.5 ± 34.2
			Placebo	17	143.2 ± 29.0	144.0 ± 31.6	141.9 ± 30.5
TG	30-149	mg/dL	Test food	17	95.3 ± 56.6	102.6 ± 55.8	96.2 ± 55.9
			Placebo	17	77.4 ± 34.2	79.4 ± 37.5	77.1 ± 39.1
Glu	70-109	mg/dL	Test food	17	83.9 ± 10.8	85.9 ± 6.5	83.4 ± 8.4
			Placebo	17	86.8 ± 9.6	85.4 ± 8.5	86.4 ± 10.4
HbA1c	4.6-6.2	%	Test food	17	5.6 ± 0.2	5.6 ± 0.2	5.5 ± 0.2
			Placebo	17	5.5 ± 0.2	5.4 ± 0.2	5.4 ± 0.3
GA	12.3-16.5	%	Test food	17	14.4 ± 1.7	15.0 ± 1.7	14.7 ± 1.5
			Placebo	17	14.2 ± 1.3	14.8 ± 1.3	14.5 ± 1.4

Values are mean ± SD

$^{\#}P < 0.05$（vs. Placebo group）

Table 6. Changes in urinalysis parameters (safety assessment)

	Reference value	Group	*N*	Pre-ingestion	Post-12W	Post-24W
Protein	(-)	Test food	17	(-): *n*=17	(-): *n*=16, (±): *n*=1	(-): *n*=15, (±): *n*=2
		Placebo	17	(-): *n*=17	(-): *n*=17	(-): *n*=17
Glucose	(-)	Test food	17	(-): *n*=17	(-): *n*=17	(-): *n*=17
		Placebo	17	(-): *n*=17	(-): *n*=17	(-): *n*=17
Urobilinogen	(±)	Test food	17	(±): *n*=17	(±): *n*=17	(±): *n*=17
		Placebo	17	(±): *n*=17	(±): *n*=17	(±): *n*=17
Bilirubin	(-)	Test food	17	(-): *n*=17	(-): *n*=17	(-): *n*=17
		Placebo	17	(-): *n*=17	(-): *n*=17	(-): *n*=17
Ketone	(-)	Test food	17	(-): *n*=16, (+): *n*=1	(-): *n*=17	(-): *n*=17
		Placebo	17	(-): *n*=17	(-): *n*=17	(-): *n*=17
pH	5.0-7.5	Test food	17	(5.0-7.5): *n*=16, (8.0): *n*=1	(5.0-7.5): *n*=17	(5.0-7.5): *n*=17
		Placebo	17	(5.0-7.5): *n*=17	(5.0-7.5): *n*=17	(5.0-7.5): *n*=17
Occult blood	(-)	Test food	17	(-): *n*=17	(-): *n*=17	(-): *n*=16, (±): *n*=1
		Placebo	17	(-): *n*=17	(-): *n*=16, (±): *n*=1	(-): *n*=15, (±): *n*=2

The number of subjects with each result is shown.

DISCUSSION

Osteoporosis is characterized by disrupted homeostasis of bone metabolism and leads a prolonged state of bone resorption. Women show a higher prevalence of osteoporosis than men, and it has been estimated that 70% of osteoporosis patients over the age of 40 are female [2]. Postmenopausal women exhibit accelerated osteoclast differentiation associated with estrogen deficiency, which leads to decreased bone density by increasing the rate of bone turnover [20]. In addition, the intestinal absorption rate of calcium decreases rapidly from immediately after menopause [21]. Therefore, it is important to take precautions against osteoporosis both before and after menopause in women [22, 23].

The present study aimed to investigate whether the ingestion of maltobionic acid elicited improvement in BMD in women aged 50–69 years who were at least 1 year past the onset of natural menopause. BMC of the total anterior surface of the lumbar vertebrae continued to decline by -0.30 g at post-12W and -0.88 g at post-24W of ingestion in the Placebo group compared with that observed pre-ingestion. In contrast, in the Test food group, the corresponding values increased by +0.31 g at post-12W and +0.85 g at post-24W of ingestion, compared with those observed pre-ingestion in the Placebo group. Intake of maltobionic acid contained in the test food increased calcium absorption and the amount of calcium in the femur when tested in a rat model [13]. Therefore, it is presumed that the intestinal absorption of calcium was enhanced by the ingestion of maltobionic acid, and that the efficient absorption of calcium via the intestine, without being excreted, contributed to the maintenance and increase in BMC.

Among the bone metabolism markers analyzed in this study, TRACP-5b, DPD, s-NTx, and u-NTx are bone resorption markers, and OC and BAP are bone formation markers. In particular, DPD, which is a bone resorption marker, is widely used as a non-invasive marker in medical practice to diagnose osteoporosis and confirm therapeutic effects [24, 25]. In the current study, subjects in the Test food group showed consistently lower DPD values from pre-ingestion to post-24W than subjects in the Placebo food group. Maltobionic acid contained in the test food has been confirmed to inhibit the differentiation of osteoclasts in an *in vitro* study (unpublished data). This suggests that maltobionic acid suppressed osteoclast differentiation and bone resorption, thereby attenuating the release of DPD. The findings indicate that not only was the absorption of calcium enhanced by the intake of maltobionic acid, but homeostasis of bone metabolism was also improved by suppressing bone resorption, which contributed to the maintenance of bone density. Of note, the primary outcome of this study, i.e. BMD at the front of the lumbar spine, is widely used as a diagnostic indicator of osteoporosis and fracture risk [15]. BMD of the total anterior surface of the lumbar vertebrae was significantly higher in the Test food group than in the Placebo group from pre-ingestion to post-24W. Soy isoflavones are widely used as therapeutic agents for osteoporosis because they have hormone-like effects and improve bone metabolism [26, 27]. An interventional study examining the effects of intake of isoflavones in postmenopausal women identified an increased BMD of 0.015 g/cm^2 24 weeks after ingestion, suggesting that they exhibit efficacy for the treatment of osteoporosis in this population [29]. Thus, the amount of change 0.015 g/cm^2 in bone mineral density is clinically meaningful. BMD of the total anterior surface of the lumbar vertebrae in this study decreased consistently in the Placebo group up to post-24W, with a reduction of 0.013 g/cm^2. In contrast, an increase in BMD of +0.002 g/cm^2 was observed in the

test food group post-24W compared with that before ingestion. Therefore, it is believed that the ingestion of the test food inhibited BMD loss (around 0.015 g/cm^2, which is considered clinically meaningful) that may occur in postmenopausal women. The same results were confirmed in the intervention test with calcium salts mainly composed of maltobionic acid [14], indicating that maltobionic acid contributes to maintenance and improvement of bone density, regardless of whether they are ingested as mineral salts or in a mineral-free state. However, further studies are necessary to elucidate the mechanism by which the intake of maltobionic acid improves bone density. This study has several limitations. First, despite the results suggesting that maltobionic acid inhibits bone resorption, no differences were seen in the bone resorption markers TPACP-5b or u-NYx and s-NTx. A detailed assessment of the effect on bone resorption requires a review of the duration of the intervention and timing of the measurements. Second, because the BDHQ used in the dietary survey is a questionnaire that collects information on the dietary habit followed in the previous month, the effect of the intake of calcium on BMD may not have been accurately reflected. Although calcium intake before and during the intervention period did not differ significantly between the groups, future studies should aim to examine the effect of the test food on improving bone density in more detail by including dietary surveys that record the subjects' daily meals.

The safety of the test food utilized in this study was assessed through physical measurements, physiological testing, urinalysis, and peripheral blood tests conducted at the pre-ingestion, post-12W, and post-24W time points. Some of the urinalysis results were false positives, and there were no significant inter-group differences; therefore, we concluded that the changes were of no medical significance. Although some items among the physical measurements, physiological tests, and peripheral blood tests showed significant intra- and inter-group differences, all values were within the standard range or only slight changes were observed. Based on these findings, we concluded that these changes were of no medical significance. Based on the safety assessment results, we found no clinically meaningful changes or adverse events that were thought to have been caused by ingestion of the test food over the 24-week period. Thus, we concluded that the test food did not pose any safety-related concerns in the study population.

CONCLUSION

Ingestion of the test food—corn syrup containing maltobionic acid—over a 24-week period led to the maintenance and increase of BMD, BMC, and YAM values in Japanese women aged 50–69 years who were at least 1 year past the onset of natural menopause. The results indicate that maltobionic acid can contribute to the prevention of osteoporosis by maintaining and increasing bone density in this age group. The results of this study additionally demonstrate the safety of its long-term ingestion over a 24-week period. Therefore, continuous intake of maltobionic acid in healthy Japanese adult women was found to improve bone mineral density and may contribute to the prevention of osteoporosis.

List of Abbreviations: BAP, bone-specific alkaline phosphatase; BDHQ, brief-type self-administered diet history questionnaire; BMC, bone mineral content; BMD, bone mineral density; DEXA, dual-energy X-ray absorptiometry method; DPD, deoxypyridinoline; NTx, type I collagen

cross-linked N-telopeptides; OC, osteocalcin; SD, standard deviation; TRACP-5b, tartrate-resistant acid phosphatase 5b; YAM, young adult mean

Competing Interests: Daiki Suehiro and Ken Fukami are employees of San-ei Sucrochemical Co., Ltd. San-ei Sucrochemical Co., Ltd. is the supplier of a food product containing maltobionic acid.

Author Contributions: Daiki Suehiro, Ken Fukami, and Tsuyoshi Takara designed the research protocol. Daiki Suehiro and Ken Fukami provided test and placebo foods. Tsuyoshi Takara is was the principal investigator of for this study and analyzed the physical data for evaluation of adverse events. Daiki Suehiro wrote the manuscript. Ken Fukami and Tsuyoshi Takara reviewed and edited the manuscript. Daiki Suehiro had primary responsibility for the final content. All authors read and approved the final version of the manuscript.

Acknowledgements and Funding: The authors thank the staff of Takara Clinic and ORTHOMEDICO Inc. who provided valuable assistance with the study and clinical testing.

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