Research Article **Open Access**

Effects of γ-aminobutyric acid (GABA) intake in combination with exercise on muscle strength in humans with decreased mobility: a randomized, double-blind, placebo-controlled, parallel-group study

Eriko Uehara[1]*, Hideki Hokazono[1], Akihito Tomonaga[2] and Mitsuhiko Fukagawa[3]

[1]Research & Development Laboratory, Sanwa Shurui Co., Ltd., 2231-1 Yamamoto, Usa, Oita 879-0495, Japan; [2]Tana Orthopedic Surgery, Kaiseikai Medical Corporation, Daini East Building 2F, 15-7 Tana-cho, Aoba-ku, Yokohama, Kanagawa 227-0064, Japan; [3]Kitashinyokohama Orthopedic Surgery, Kaiseikai Medical Corporation, Kitashinyokohama Medical Building 3F, 1-2-3 Kitashinyokohama, Kohoku-ku, Yokohama, Kanagawa 223-0059, Japan.

***Corresponding author:** Eriko Uehara, Ph.D., Research & Development Laboratory, Sanwa Shurui Co., Ltd., 2231-1 Yamamoto, Usa, Oita 879-0495, Japan.

Submission Date: March 18th, 2024; **Acceptance Date:** April 11th, 2024; **Publication Date:** April 12th, 2024

Please cite this article as: Uehara E., Hokazono H., Tomonaga A., Fukagawa M. Effects of γ-aminobutyric acid (GABA) intake in combination with exercise on muscle strength in humans with decreased mobility: a randomized, double-blind, placebo-controlled, parallel-group study. *Functional Foods in Health and Disease* 2024; 4(4): 248-269.
DOI: https://doi.org/10.31989/ffhd.v14i4.1334

ABSTRACT

Background: Aging-related declines in skeletal muscle mass, muscle strength, and physical function are related to instability, falls, and frailty in older people, resulting in the need for nursing care.

Objective: To investigate the effect of oral γ-aminobutyric acid (GABA) intake and exercise on muscle parameters in healthy subjects whose muscle strength is beginning to decline with age.

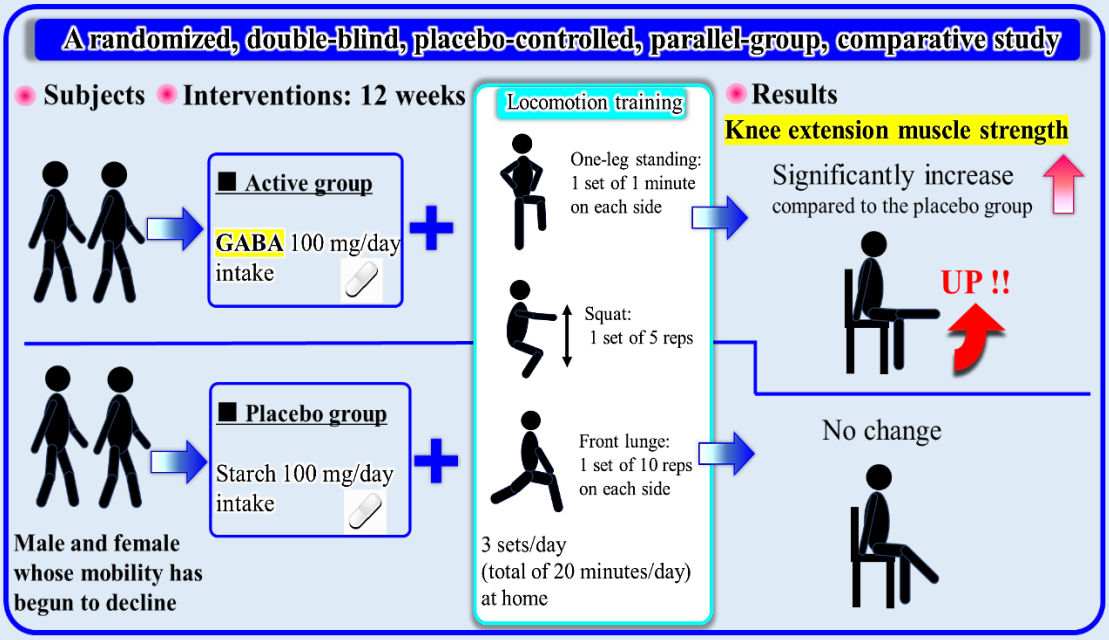
Methods: A randomized, double-blind, placebo-controlled, parallel-group comparative study was conducted. Fifty subjects (over 40 years old) were randomly divided into the GABA (100 mg/day) or placebo food intake group. Subjects orally consumed the respective study substance every day for 12 weeks. They performed daily "locomotion training" as devised by the Japanese Orthopedic Society. Muscle mass, fat mass, and knee extension muscle strength were measured.

Results: The two groups had no significant differences in muscle and fat mass. Compared with the placebo food group, the GABA group showed a significant increase in muscle strength for the stronger leg (either left or right) during pre-dose Week 0 (at Week 6, $p = 0.02$). In post hoc subgroup analysis by sex, when compared with the placebo food intake group, the GABA food intake group showed significant improvement in knee extension muscle strength of the right leg at Weeks 6 ($p = 0.001$) and 12 ($p = 0.007$), the left leg at Week 6 ($p = 0.02$), the stronger of the left and right legs at pre-dose Week 0 (at Week 6, $p = 0.001$), and the weaker of the left and right legs at pre-dose Week 0 (at Week 12, $p = 0.013$) in males.

Conclusions: These results suggest that GABA intake combined with daily exercise is effective for maintaining knee extension muscle strength, which decreases with age. Furthermore, there were no safety issues with the intake of GABA-containing food during this study.

Trial registration: **UMIN-CTR:** UMIN000050152.

Keywords: γ-Aminobutyric acid, GABA, Muscle mass, Knee extension muscle strength, Aging



INTRODUCTION

At a conference in the United States in 1988, Dr. Rosenberg proposed the term “sarcopenia” to describe age-related muscle changes [1]. In 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) defined the diagnostic criteria for sarcopenia as a condition in which decreased skeletal muscle mass is essential and accompanied by either decreased muscle strength or physical function [2]. In 2018, a new consensus paper (EWGSOP2) was published by EWGSOP.

EWGSOP2 defined sarcopenia as a progressive and systemic skeletal muscle disease. The significant change in EWGSOP2 from EWGSOP is that decreased muscle strength is an essential diagnostic item instead of skeletal muscle mass. Muscle strength is currently the most reliable indicator of muscle function. Specifically, if decreased muscle strength is observed, there is a high possibility of sarcopenia. To assess for evidence of sarcopenia, EWGSOP states that muscle strength (knee flexion/extension) can be used in research studies [3].

Sarcopenia is closely related to staggering, falls and even frailty in older people, which in turn leads to the need for nursing care [4]. Sarcopenia is significantly associated with a higher risk for all-cause mortality among nursing home residents [5]. The onset of sarcopenia often begins in middle age due to an unbalanced diet or malnutrition in association with a lack of physical activity [6]. Sarcopenia affects approximately 13% of the world's population aged over 60 years [7].

Gamma-aminobutyric acid (GABA) is an amino acid found in plants and vertebrates and produced in the human body [8]. GABA activates vagal afferents and modulates brain function [9]. GABA is metabolized to succinic semialdehyde by GABA transaminase, and it is then either reduced to gamma-hydroxybutyrate or oxidized to succinate and finally converted to CO_2 and water via the citric acid cycle [10]. Various physiological effects of GABA have been reported, including improvement of sleep quality [11], anti-stress [12], and suppression of blood pressure elevation [13]. Germinated brown rice rich in GABA may be recommended as an economical, nutritious, and anti-inflammatory preoperative feed [14]. The Functional Food Center has defined "functional foods" as follows: Natural or processed foods that contain biologically active compounds, which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms [15]. Nedachi et al. reported that GABA is a functional food that promotes sleep, and a mixture of GABA and lafma can improve sleep quality [16].

In the study by Choi et al., in which middle-aged females who abstained from any regular exercise consumed GABA-enriched fermented sea tangle (*Laminaria japonica*) containing 54.5 ± 0.071 mg GABA (GFST) for 8 weeks, lean body mass as measured by Dual Energy X-ray Absorptiometry (DEXA) was significantly increased compared to the placebo group [17]. In a study in which healthy males (26–48 years old) ingested 10 g of whey protein + 100 mg of GABA daily and performed resistance training at 60% of their maximum strength at a fitness club (twice a week) for 12 weeks, lean body mass (measured using DEXA) significantly increased compared to placebo [18]. Therefore, we investigated the effects of GABA intake and light-intensity exercise at home in combination on muscle quantity and strength in healthy males and females aged 40 and older in which muscle strength was beginning to decline, as determined by the "2-step test", in a placebo-controlled, double-blind, randomized, parallel-group, comparison study.

MATERIALS AND METHODS

Study design and ethical statement: We designed a randomized, double-blind, placebo-controlled, parallel-group, comparative study protocol approved by the Medical Station Clinic Research Ethics Committee (Tokyo, Japan, IRB number: 20000022) on January 26, 2023. The study was conducted in accordance with the "Declaration of Helsinki (revised in October 2013)" and in compliance with the "Ethical Guidelines for Medical and Biological

Research Involving Human Subjects (Ministry of Education, Culture, Sports, Science and Technology/Ministry of Health, Labour and Welfare, March 23, 2021)", and "Guidance on Ethical Guidelines for Life Science and Medical Research Involving Human Subjects (Ministry of Education, Culture, Sports, Science and Technology/Ministry of Health, Labour and Welfare/Ministry of Economy, Trade and Industry, April 16, 2021)".

Before starting the study, we registered the trial with the University Hospital Medical Information Network (UMIN)-Clinical Trials Registry operated by the UMIN Center (ID: UMIN000050152).

Before participating in the study, we handed prospective participants an explanation document and consent form approved by the ethics review committee, explained the study sufficiently, and allowed them to ask questions and have enough time to decide about participating. The medical director obtained each subject's voluntary consent in writing.

This study was conducted at Kaiseikai Medical Corporation Kaiseikai Tana Orthopedic Surgery (Yokohama, Japan), Kaiseikai Medical Corporation Kitashinyokohama Orthopedics and Surgery (study site, Yokohama, Japan), and LSI Medience Corporation (laboratory, Tokyo, Japan).

Subjects: After obtaining informed consent for study participation from subjects, the following demographics were reviewed or confirmed: sex, age, medical history, surgical history, history of present illness, allergy, drinking habit and the amount, smoking habit and the amount, status of lifestyle and exercise habit, use status for drugs, health foods, and status of participation in other clinical trials or studies. The principal investigator decided the selection according to the inclusion and exclusion criteria based on reviewing the results of the pre-dose test and demographic data.

Subjects were required to meet all the following inclusion criteria and none of the exclusion criteria. The Japanese Orthopedic Society has developed a "2-step test" to examine the degree of the locomotive syndrome, and the society's official locomotive syndrome prevention awareness website [19] introduces the 2-step test method and guide movie. The "2-step test" is introduced on Japan's Ministry of Health, Labour and Welfare website [20]. The 2-step test is a reliable and excellent method for assessing walking ability [21]. The test examines step size and height, muscle strength, balance ability, and lower limb flexibility. The value is calculated as two-step length (cm)/height (cm). The Japanese Orthopedic Society defines people with a 2-step value of 0.9 or more and less than 1.1 as locomotive syndrome level 2; these individuals have a progressive decline in their mobility and are at high risk of being unable to live independently. People with locomotive syndrome level 2 who are experiencing pain may have a musculoskeletal disease; thus, it is recommended that people consult an orthopedic specialist. People with a 2-step value of 1.1 or more and less than 1.3 are defined as locomotive syndrome level 1, which means that their mobility has begun to decline, but improvement is expected with diet and exercise [19]. This study targeted males and females with a 2-step value of 1.1 or more and less than 1.3, that is, locomotive syndrome level 1.

Inclusion criteria: The study participants were healthy males and females aged 40 years or older who were aware of a decline in muscle strength due to aging. They scored 1.1 or more and less than 1.3 on the 2-step test conducted at the pre-dose Week 0 test. They also agreed to participate in the research of their own volition after

fully understanding the purpose and content of the study.

Exclusion criteria: Participants excluded from the study were individuals with a history of a disease that may affect the evaluation of the study. Those who could not perform exercise training due to joint/muscular symptoms or diseases, and those who find it difficult to perform daily activities or walk due to severe pain in their knees or lower back. Individuals diagnosed with sarcopenia. Individuals with a history of musculoskeletal impairment and injury (low back pain, etc.) who are at risk of recurrence due to exercise training. Those who regularly consumed foods high in protein and amino acids may influence the evaluation of the study. Individuals who may regularly use pharmaceuticals that may affect the evaluation of the study (including topical medications such as poultices). Individuals who engage in regular exercise. Individuals who engage in excessive exercise. Individuals with a history of a disease related to motor function, such as a fracture, sprain, or muscle strain, within one year before the pre-dose Week 0 test. Individuals who use canes or supports and use them during the study period. Individuals who regularly engaged in activities that may affect evaluation effectiveness (e.g., massage, chiropractic, hot springs). Individuals with a disease related to joints or muscles currently being treated or a disease that is judged to require treatment. Individuals with serious diseases such as nervous system diseases, malignant tumors, diabetes, liver diseases, kidney diseases, heart diseases, etc., or those with a history of such diseases. Those who are currently being treated for or have a history of mental illness (depression, sleep disorders, etc.) or those who are strongly suspected of having one. Individuals whose lifestyle has become irregular multiple times during the study period due to day/night shift work or night shifts or persons engaged in manual labor such as transporting heavy objects. Individuals who drink large amounts of alcohol (more than 60 g of alcohol per day). Individuals at risk of developing allergies related to the study. The principal investigator judged individuals to be inappropriate as subjects based on the clinical test values, physical measurements, and physical test values of the preliminary test. Those who have participated in other clinical studies within one month before obtaining consent to participate in this study or those who plan to participate in other clinical studies after obtaining consent to participate in this study. Individuals who are pregnant or breastfeeding or who plan to do so during the study period. Individuals were judged to be unsuitable as subjects based on the answers to the lifestyle questionnaire. Individuals who cannot follow the procedures for various tests conducted during the study period (e.g., entering online diaries). Any other individuals the principal investigator considered inappropriate as study participants.

Target sample size and rationale: In a study by Choi et al. in which middle-aged women who abstained from exercise (number of subjects = 21) took 54.5 ± 0.071 mg of GABA or placebo food [17] and in a study in which men who regularly engaged in muscle training (number of subjects = 21) took 10 g of whey protein + 100 mg of GABA or 10 g of whey protein alone [18], lean body mass significantly increased compared to the placebo group. Based on these studies, the number of subjects was calculated with a significance level of 5% and a power to detect 80%. In this study, we increased the number of subjects per group to account for dropouts and decided on 25 subjects per group, 50 subjects in total (Figure 1).

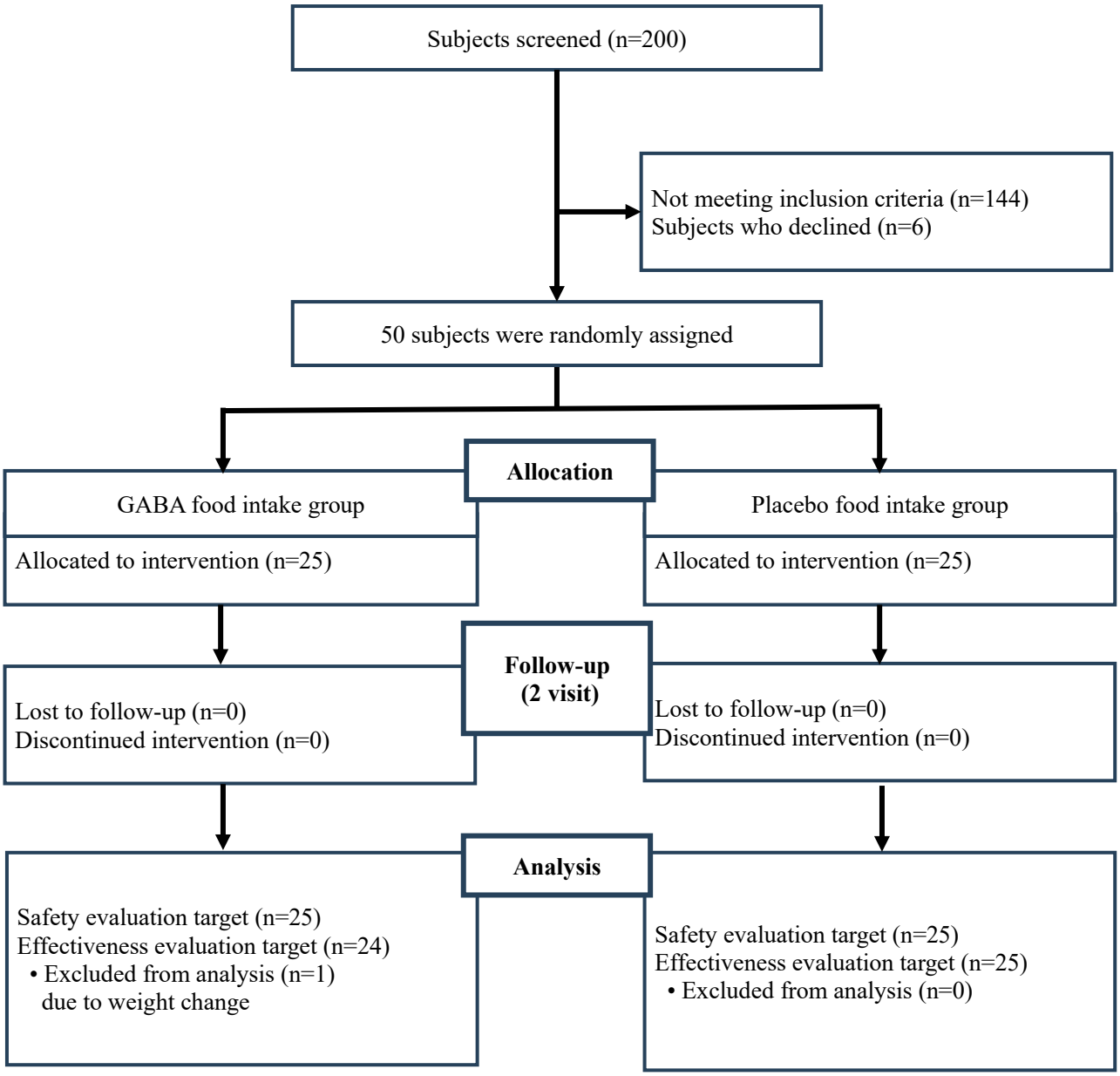


Figure 1. Flow chart of the study.

Randomization: The allocation manager created an allocation table using random numbers and assigned allocation numbers to the study foods. The allocation table was sealed by the allocation manager and kept sealed until the allocation table was opened. After fixing the subjects for analysis and their data, the allocation manager opened the allocation table and disclosed the information. If a serious adverse event requiring emergency treatment occurred, the allocation table would have been opened as necessary, and only the minimum required information would have been disclosed; however, no serious adverse event occurred.

Study foods: The active food was a hard capsule containing 100 mg of GABA. The raw material for GABA was fermented barley GABA 90% (Sanwa Shurui Co., Ltd., Oita, Japan). The placebo food contained starch instead of GABA and was visually indistinguishable from the

active food. Other ingredients contained in the capsule were starch, milk calcium, microcrystalline cellulose, cyclic oligosaccharides, and sucrose esters.

Blinding: This was a double-blind study, so the subjects and investigators were blinded to the treatment. The manufacturer numbered the study food packaging; neither the subjects nor the investigators knew what number belonged to the active or placebo food.

Interventions: After the pre-dose Week 0 test, subjects consumed their assigned study food, one capsule daily, with water or lukewarm water every day for 12 weeks. If the subject was unable to consume the study food on that day, carryover to the next day was prohibited.

From the start of intake until the day before the 12th week test, the subjects performed "locomotion training" as the Japanese Orthopedic Society devised for about 20 minutes a day at home [22]. "Locomotion training" consists of three types of training: one-leg standing, 1 set of 1 minute on each side; squat, 1 set of 5 reps; and front lunge, 1 set of 10 reps on each side. The subjects performed "locomotion training" that stimulated the lower body muscles at 3 sets per day. The intensity levels for exercise are expressed in metabolic equivalents (METs). One MET is the amount of oxygen consumed while sitting at rest, equal to 3.5 mL O_2 per kg body weight x min [23]. The METs of the lower body exercises given in this study were 2.11±0.44 for one-leg standing [24], 6.5±1.1 for squats, and 8.5±0.1 for front lunges [25]. Prolonged walking corresponds to 2METs [26]. For adults aged 41 to 60, walking at 130 steps/min corresponds to 6 METs [27]. Running at 134 m/min corresponds to 8 METs [28].

Six and twelve weeks after the start of intake, predetermined tests and interviews were carried out. The subjects completed an online diary every day from 3 days before the start of intake until the day before the 12th-week test. The following instructions were provided to the subjects. Activity measurements were carried out three days before pre-dose Week 0, the 6th and 12th-week test dates.

Study period: Subjects were asked not to travel, go on business overseas, or consume healthy foods (protein, amino acids, etc.) that may affect the study evaluation. Subjects were asked not to change their lifestyle habits before participating in the trial, such as eating, drinking, exercising, bedtime, and smoking. Subjects were asked to avoid excessive exercise, activities that strain their muscles (such as carrying heavy objects), dietary restrictions, and overeating. Subjects were asked to avoid activities that may affect the effectiveness of the evaluation (massage, chiropractic, hot springs, etc.). Subjects were asked to record when they took medicine or received treatment in their diaries. Subjects were asked not to donate blood. Subjects were asked to consume the specified amount of study food daily and record it in the daily diary. Subjects were asked to perform a specified amount of exercise training daily and record it in the daily diary. Subjects were asked to confirm whether there were any significant changes in their exercise status from the three days before the test, their sleep status from the day before, and their daily living conditions (transportation, work situation, etc.) on the day of the test. The day before the test, subjects were asked to go to bed by midnight and get enough sleep and rest. If subjects lost the study food, they were asked to contact the medical facility support organization to replenish the missing food. Subjects were asked not to

drink alcohol from the day before the test until the end of the test on the day of the test. Subjects were asked to fast for at least 10 hours the night before the test and to limit fluid intake from the time they woke up to the time they arrived at the test site. The water consumed had to be the same, 200–500 mL on each test day. On the test day, subjects were asked to come to the test site without ingesting the study food. Subjects were asked to visit the test site without locomotion training on the test day.

Test items and timing: After obtaining informed consent for study participation from subjects, a lifestyle questionnaire, height measurement, and a 2-step test were conducted at only pre-dose Week 0.

The following tests were conducted to evaluate the effectiveness of test food intake. Primary outcomes: body composition and lean body mass measurement using Bioelectrical Impedance Analysis (BIA); secondary outcomes: Timed Up and Go (TUG) test, grip strength measurement, knee extension muscle strength measurement, growth hormone level measurement, self-awareness questionnaire; and other evaluation items: activity level measurement was conducted at pre-dose Week 0, Week 6, and Week 12. All tests began between 9:00 and 11:00 a.m. and ended approximately in the morning. InBody770 (InBody Japan Co., Ltd., Tokyo, Japan) was used to measure body composition and lean body mass. Kinesis QTUG (Kinesis Health Technologies Ltd., Dublin, Ireland) was used for TUG test measurements. During the test, the subject got up from a chair, walked 3 m, returned, and sat down again. An obstacle (height: 0.1 m, depth: 0.1 m, width: 1.0 m) was placed in the center of the walking path, and subjects were instructed to step over it without touching it. TUG recording time was measured. Falls risk estimate and frailty estimate are each values that indicate the risk of falling and the frailty level based on an elderly population model [29]. Grip strength was measured twice, the first with the right hand and then with the left hand, using a Smedley grip dynamometer YOII (Tsutsumi Co., Ltd., Tokyo, Japan), and the maximum value for each was recorded. Measurements were taken for both hands and hands with higher and lower pre-dose Week 0 test values. In this study, we assumed that the hand with the higher grip strength value at Week 0 was “the dominant hand,” and the hand with the lower grip strength value at Week 0 was “the non-dominant hand.” The maximum isometric knee extension muscle strength of both legs was measured using a knee extension strength meter (Tension Meter D, Takei Kiki Kogyo Co., Ltd., Niigata, Japan). The maximum value was divided by the body weight on the test day and multiplied by 100 to calculate the body weight percentage. The values were calculated for the left and right legs and the leg with the higher and lower pre-dose Week 0 test values. In this study, we assumed that the leg with the higher knee extension muscle strength value at Week 0 was “the dominant leg,” and the leg with the lower knee extension muscle strength value at Week 0 was “the non-dominant leg.” After arriving at the test site and resting for 30 minutes, blood was drawn, and growth hormone levels were measured by the Electro Chemiluminescence Immunoassay (ECLIA) method [30]. The fall anxiety scale [31] was used for the self-awareness questionnaire. The scale is a questionnaire that asks subjects to answer 4-choice questions about their fear of falling for each of the ten activities. The responses of “No anxiety at all,” “A little anxiety,” “Anxiety,” and “A lot of anxiety” were scored 1, 2, 3, and 4 points, respectively,

and the total was calculated. Using an activity monitor (MY CALORY® MC-500, Yamasa Tokei Keiki Co., Ltd., Tokyo, Japan), total calories consumed and active calories were recorded for three days before the test, and the average value for the three days was used.

To evaluate the safety of the test food, a health check, keeping a diary (subjective symptoms, every day), body composition and lean body mass measurement, physical examinations (blood pressure and pulse rate), and laboratory tests (blood and urine tests, excluding Week 6) were conducted at pre-dose Week 0, Week 6, and Week 12. The principal investigator determined the subject's subjective symptoms and objective findings. A list of symptoms, date of onset and resolution, severity, seriousness, treatment, outcome, causal relationship with the study food, and principal investigator's comments were created. In principle, follow-up was to be performed as necessary until the abnormal findings of the adverse event resolved or showed a recovery trend. An online diary was used to record the intake of study foods, changes in physical condition, medication usage, and exercise training performance.

If the test could not be performed on the scheduled test date due to the subject's circumstances, the test date was changed to within seven days before or after the scheduled test date at the discretion of the principal investigator. If the test date was delayed, the study food could be consumed until the day before.

Analysis set and statistical analysis methods: After completion of the final examination, the analysis set was confirmed, and the data was locked. After confirming that the data was locked, the allocation manager opened the study food allocation table at the direction of the principal investigator.

Among the subjects who completed all the prescribed research schedules and test contents, those who met the following criteria were excluded from the efficacy analysis subjects. In the safety evaluation, subjects who completed all of the prescribed study schedule and study content were included in the analysis. Regarding adverse events, subjects who had consumed the study food at least once were targeted. Those whose intake rate of research foods was less than 80%. Those who exhibit significant behavior that impairs the reliability of test results, such as missing diary records. Those found to have met the exclusion criteria after being included in the study or those found unable to comply with the restrictions during the research period. Those for whom there was a clear reason why their exclusion was considered appropriate.

IBM SPSS Statistics version 27 (International Business Machines Corporation, Armonk, NY, USA) or Microsoft Excel for Microsoft 365 MSO version 2208 (Microsoft Corporation, Redmond, WA, USA) were used for data tabulation and analysis.

Each subject's demographics (age, height, body weight, body mass index, systolic and diastolic blood pressure, pulse rate, 2-step test value, muscle mass, and body fat mass) at pre-dose Week 0 were calculated (average ± standard deviation). Comparisons between groups were performed using the two-sample *t*-test, and between sexes were performed using the chi-square test. For the primary and secondary outcomes, the actual values at pre-dose Week 0 and the amount of change from pre-dose Week 0 to weeks 6 and 12 for each group were calculated (average ± standard deviation). For the primary outcomes, comparisons between groups at each time point were performed using the two-sample *t*-test. Intragroup comparisons in the mass of change from pre-dose Week 0 to each time point were performed using the one-sample *t*-test. Among the secondary outcomes, the TUG test, grip strength,

and growth hormone value were evaluated similarly to the primary outcomes. Among the secondary outcomes, knee extension muscle strength and self-awareness questionnaire were compared between groups at each time point using the Mann-Whitney U test. Intragroup comparisons in the amount of change from pre-dose Week 0 to each time point were performed using the Wilcoxon signed-rank sum test. If a significant difference between groups was found in the actual values measured in pre-dose Week 0, an analysis of covariance was performed (excludes self-awareness questionnaire). However, if an interaction was observed, analysis of covariance was not performed. It is known that there are sex differences in body composition and lean body mass [32], as well as knee extension muscle strength [33]. Therefore, as a post-hoc analysis of this study, we conducted a stratified analysis by males. In the post-hoc analysis, body composition, lean body mass, and knee extension muscle strength were analyzed in males in the same manner as in males and females. The testing was two-sided, with a significant level of 5%. The number of adverse events was tallied for each group, and a list was created. If the same adverse event occurred multiple times in the same subject, it was treated as one event. In the safety evaluation test, the measured values at each time point in each group were compared using a one-sample *t*-test. Even those considered outliers were evaluated among the test and measured values. If the cause was clearly determined to be a measurement error, that time point was excluded from the analysis. If there were missing values, no imputation was performed, and they were not included in the analysis as missing values. The intake status of drugs/health foods and study food intake rate were tabulated and evaluated. The study food intake rate was calculated by dividing the number of intakes by the number of intakes that should be taken. There was no deviation from the original protocol.

RESULTS

Subjects: Of the 200 subjects who provided written informed consent, 150 were excluded from the pre-dose test, and 50 subjects who met the inclusion criteria were selected (Figure 1).

Subjects analyzed*:* All 50 subjects ingested the study food and completed the prescribed study schedule and content; thus, all 50 subjects, 25 in the active food intake group (group A) and 25 in the placebo food intake group (group P), were included in the safety evaluation analysis.

The subjects for the efficacy analysis were considered before opening the allocation table. One subject (ID 494017: group A) was excluded from the analysis because it was thought that weight loss during Weeks 6 and 12 may have affected the test results. Four subjects (ID 494030: group A; ID 494062: group A; ID 494068: group P; ID 494185: group P) lacked activity measurement data before the Week 6 or Week 12 test, so the days of missing data were excluded from the calculations. One person (ID 494048: group A) developed acute lower back pain before the Week 6 test, received acupuncture at an osteopathic clinic, and discontinued exercise training, so Week 6 test results were not submitted. It was decided to treat these as missing values. Forty-nine subjects (24 in group A, 25 in group P) were included in the efficacy analysis (Figure 1).

Recruitment and trial period*:* Recruitment began on January 31, 2023, the study started on May 9, 2023, and the follow-up was completed on August 13, 2023.

Subject demographics: Tables 1 and 2 show the backgrounds of the full analysis set of 50 subjects and the per-protocol set of 49 subjects, respectively. In both sets, there were no significant differences between groups in any pre-dose Week 0 values, and no imbalance was observed.

Table 1. Subject demographics (Full Analysis Set).

		Group A				Group P				*p*-value
Number of subjects			25				25			
Male			8				12			0.39
Female			17				13			
Age	(years)	57.0	±	6.2		59.1	±	8.6		0.33
Height	(cm)	161.01	±	6.80		163.02	±	8.15		0.35
Body weight	(kg)	56.88	±	8.35		58.12	±	8.93		0.61
Body mass index	(kg/m^2)	21.91	±	2.72		21.79	±	2.23		0.87
Systolic blood pressure	(mmHg)	114.6	±	12.4		115.2	±	13.5		0.85
Diastolic blood pressure	(mmHg)	73.6	±	10.5		74.0	±	8.2		0.86
Pulse rate	(beats/min)	72.0	±	11.8		72.4	±	9.3		0.89
2-step value		1.19	±	0.05		1.19	±	0.06		0.94
Muscle mass	(kg)	38.49	±	6.07		40.30	±	7.84		0.37
Body fat mass	(kg)	16.06	±	5.07		15.37	±	4.12		0.60

Values at pre-dose Week 0. "Group A" and "Group P" refer to the GABA and placebo food intake groups, respectively. Values are expressed as mean ± standard deviation (excluding number of subjects and sex). Comparisons between groups were performed using the two-sample *t*-test, and between sexes were performed using the chi-square test.

Table 2. Subject demographics (Per Protocol Set).

		Group A				Group P				*p*-value	
Number of subjects			24				25				
Male			7				12			0.24	
Female			17				13				
Age	(years)	57.0	±	6.4		59.1	±	8.6		0.34	
Height	(cm)	160.84	±	6.89		163.02	±	8.15		0.32	
Body weight	(kg)	56.89	±	8.53		58.12	±	8.93		0.62	
Body mass index	(kg/m^2)	21.96	±	2.77		21.79	±	2.23		0.82	
Systolic blood pressure	(mmHg)	114.6	±	12.7		115.2	±	13.5		0.86	
Diastolic blood pressure	(mmHg)	73.4	±	10.7		74.0	±	8.2		0.81	
Pulse rate	(beats/min)	71.9	±	12.0		72.4	±	9.3		0.85	
2-step value		1.19	±	0.05		1.19	±	0.06		0.99	
Muscle mass	(kg)	38.38	±	6.17		40.30	±	7.84		0.35	
Body fat mass	(kg)	16.20	±	5.13		15.37	±	4.12		0.54	

Values at pre-dose Week 0. "Group A" and "Group P" refer to the GABA and placebo food intake groups, respectively. Values are expressed as mean ± standard deviation (excluding number of subjects and sex). Comparisons between groups were performed using the two-sample *t*-test, and between sexes were performed using the chi-square test.

Study food intake rate: The intake rate of the study food was 100% for 48 subjects and 98.8% for two subjects.

Primary outcomes (body composition and lean body mass measurement): Table 3 shows the actual muscle and body fat mass values measured at pre-dose Week 0 and the changes at Weeks 6 and 12. No significant differences existed between groups in the mass of change at Week 6 and 12 for any item. In the intragroup comparison with pre-dose Week 0, body fat mass decreased significantly in group A at Week 6 and 12 and in group P at Week 12. There were no significant differences in other items at any time point evaluated.

Table 3. Primary outcomes.

	Group	Actual value	Change in mass	
		Pre-dose Week 0	Week 6	Week 12
Muscle mass (kg)	A	38.38 ± 6.17	0.28 ± 0.91	0.28 ± 0.80
	P	40.30 ± 7.84	0.08 ± 0.91	0.34 ± 0.96
p-value (two-sample *t*-test)		0.35	0.45	0.81
Body fat mass (kg)	A	16.20 ± 5.13	-0.54 ± 1.23*	-0.93 ± 1.55**
	P	15.37 ± 4.12	-0.29 ± 1.29	-0.65 ± 1.16**
p-value (two-sample *t*-test)		0.54	0.49	0.48

Values are expressed as mean ± standard deviation. "A" and "P" refer to the GABA and placebo food intake groups, respectively. Comparisons between groups were performed using the two-sample *t*-test. Significant differences in the one-sample *t*-test of intragroup changes relative to pre-dose Week 0 are expressed as $^{**}p < 0.01$, $^{*}p < 0.05$. The numbers of subjects were A: 24 (23 at Week 6 only) and P: 25.

Secondary outcomes and other evaluation items (TUG test, grip strength, knee extension muscle strength, growth hormone value, self-awareness questionnaire, and activity level measurement)

TUG test: There were no significant differences between groups in the amount of change at Week 6 and 12 for any item. In an intragroup comparison with pre-dose Week 0, TUG recording time significantly decreased at Week 6 and 12 in both groups. The fall risk estimates for group P decreased significantly in Week 12. Frailty estimates significantly decreased in Group A in Week 12 and Group P in Week 6. There were no significant differences in other items at any time point evaluated.

Grip strength: For all items, there were significant differences between the groups in the actual values measured in pre-dose Week 0; thus, an analysis of covariance was conducted. However, an interaction was observed at Week 12 on the left hand, so covariance analysis was not performed. No significant differences between groups were found in the amount of change at Weeks 6 and 12 for any of the items analyzed by covariance analysis. In the intragroup comparison with pre-dose Week 0, there was a significant increase in right-hand and left-hand active groups at Weeks 6 and 12. The dominant hand value significantly decreased in Week 6 in Group P. The non-dominant hand significantly increased in Week 6 and 12 in both groups. There were no significant differences in other items at any time point evaluated.

Knee extension muscle strength: Table 4 shows the knee extension muscle strength value divided by the body weight on the test day and multiplied by 100. The amount

of change at Week 6 for the dominant leg (group A: 11.17 ± 8.01%, group P: 4.86 ± 7.40%, $p = 0.02$) was significantly increased in group A compared to group P. In the intragroup comparison with pre-dose Week 0, all items significantly increased in both groups at Week 6 and 12.

Table 4. Secondary outcomes (knee extension muscle strength).

	Group	Actual value	Amount of change	
		Pre-dose Week 0	Week 6	Week 12
Right leg (%)	A	39.45 ± 13.23	12.29 ± 8.21**	13.85 ± 11.07**
	P	42.51 ± 16.26	9.24 ± 9.12**	14.73 ± 10.33**
p-value (Mann-Whitney U test)		0.52	0.27	0.65
Left leg (%)	A	37.31 ± 12.04	12.67 ± 9.12**	14.99 ± 12.72**
	P	41.78 ± 16.33	7.54 ± 7.19**	13.97 ± 8.38**
p-value (Mann-Whitney U test)		0.38	0.07	0.73
Dominant leg (%)	A	40.93 ± 12.97	11.17 ± 8.01**	11.86 ± 13.41**
	P	44.70 ± 16.58	4.86 ± 7.40**	12.49 ± 9.77**
p-value (Mann-Whitney U test)		0.41	0.02	0.61
Non-dominant leg (%)	A	35.83 ± 11.86	13.79 ± 9.87**	16.98 ± 10.59**
	P	39.59 ± 15.59	11.92 ± 8.92**	16.21 ± 9.02**
p-value (Mann-Whitney U test)		0.37	0.72	0.93

Values are expressed as mean ± standard deviation. "A" and "P" refer to the GABA and placebo food intake groups, respectively. The leg with the higher knee extension muscle strength value at Week 0 was "the dominant leg," and the leg with the lower knee extension muscle strength value at Week 0 was "the non-dominant leg." Comparisons between groups were performed using the Mann-Whitney U test. Significant differences in the Wilcoxon signed-rank sum test of intragroup changes relative to pre-dose Week 0 are expressed as $^{**}p < 0.01$, $^{*}p < 0.05$. The numbers of subjects were A: 24 (23 in Week 6 only) and P: 25.

Growth hormone value: Because the actual values measured in pre-dose Week 0 were significantly different between groups, an analysis of covariance was performed. There were no significant differences between groups in the amount of change at 6 and 12 weeks for any item. In the intragroup comparison with pre-dose Week 0, all items had no significant differences at any time point evaluated.

Self-awareness questionnaire (fall anxiety scale): Regarding Question 9, "Reaching into a cupboard or drawer," there was a significant difference between groups in the actual values measured in pre-dose Week 0. There were no significant differences between groups in the amount of change at Week 6 and 12 for any item. In the within-group comparison with pre-dose Week 0, the total score for all questions in group P decreased significantly at Week 12, and the score for question 7, "Going up and down stairs" in group A decreased significantly at Week 6. Other items did not differ significantly at any time point evaluated.

Activity level: There were no significant differences in all items between groups and in the intragroup comparison at any time point evaluated.

Post hoc subgroup analysis by sex: It is known that there are sex differences in body composition and lean body mass [32], as well as knee extension muscle strength [33], which showed a significant difference between groups in the per-protocol set. Therefore, as a post-hoc analysis of this study, we conducted a stratified analysis by males. The backgrounds of the 7 group A and 12 group P subjects are shown in Table 5. There were no significant differences between the groups in pre-dose Week 0 values, and no imbalance was observed in pre-dose Week 0 values.

Table 5. Subject demographics (male).

		Group A				Group P				*p*-value	
Number of subjects			7				12			-	
Age	(years)	60.1	±	8.3		60.8	±	8.1		0.86	
Height	(cm)	168.70	±	4.99		169.61	±	5.48		0.72	
Body weight	(kg)	63.79	±	7.05		63.94	±	8.34		0.97	
Body mass index	(kg/m^2)	22.44	±	2.61		22.18	±	2.35		0.83	
Systolic blood pressure	(mmHg)	117.1	±	13.5		118.5	±	15.0		0.85	
Diastolic blood pressure	(mmHg)	71.3	±	14.5		77.1	±	7.8		0.27	
Pulse rate	(beats/min)	69.4	±	15.4		74.6	±	11.4		0.41	
2-step value		1.17	±	0.05		1.20	±	0.06		0.33	
Muscle mass	(kg)	46.37	±	3.45		47.08	±	5.61		0.77	
Body fat mass	(kg)	14.74	±	4.66		14.07	±	3.58		0.73	

Value at pre-dose Week 0. "Group A" and "Group P" refer to the GABA and placebo food intake groups, respectively. Values are expressed as mean ± standard deviation (excluding number of subjects). Comparisons between groups were performed using the two-sample *t*-test.

Table 6 shows the actual muscle and body fat mass values measured at pre-dose Week 0 and the changes at Week 6 and 12 in males. There were no significant differences between the groups in changes in body composition and lean body mass at Week 6 and 12. In an intra-group comparison with pre-dose Week 0, body fat mass decreased significantly in group A at Week 6 and in group P at Week 12. No significant changes were observed in lean body mass at any time point evaluated.

Table 7 shows the percent knee extension muscle strength value, divided by the body weight on the test day and multiplied by 100. The amount of change in the right leg at Week 6 (group A: 20.44 ± 6.20%, group P: 6.59 ± 8.82%, p = 0.001) and at Week 12 (group A: 22.33 ± 9.52%, group P: 11.05 ± 11.98%, p = 0.007), the amount of change in the left leg at Week 6 (group A: 17.67 ± 10.78%, group P: 5.76 ± 6.81%, p = 0.02), the amount of change in the dominant leg at Week 6 (group A: 18.70 ± 8.27%, group P: 2.43 ± 7.61%, p = 0.001), and the amount of change in the non-dominant leg at Week 12 (group A: 23.57 ± 9.23%, Group P: 13.84 ± 10.75%, p = 0.013) showed significant increases in group A compared with group P. In the intragroup comparison with pre-dose Week 0, the right leg, left leg, and the non-dominant leg increased significantly at Week 6 and 12 in both groups. There was a significant increase in Group A in Week 6 and 12 and Group P in Week 12 with the dominant leg. No significant changes were observed in other items at any time point.

Table 6. Post hoc subgroup analysis of primary outcomes (males).

	Group	Actual value	Change in mass	
		Pre-dose Week 0	Week 6	Week 12
Muscle mass (kg)	A	46.37 ± 3.45	0.53 ± 1.02	0.23 ± 0.96
	P	47.08 ± 5.61	0.12 ± 1.11	0.53 ± 1.13
p-value (two-sample *t*-test)		0.77	0.43	0.57
Body fat mass (kg)	A	14.74 ± 4.66	-1.43 ± 1.46*	-1.61 ± 1.86
	P	14.07 ± 3.58	-0.68 ± 1.38	-1.12 ± 1.13**
p-value (two-sample *t*-test)		0.73	0.28	0.47

Values are expressed as mean ± standard deviation. "A" and "P" refer to the GABA and placebo food intake groups, respectively. Comparisons between groups were performed using the two-sample *t*-test. Significant differences in the one-sample *t*-test of intragroup changes relative to pre-dose Week 0 are expressed as $^{**}p < 0.01$, $^{*}p < 0.05$. The number of subjects was A: 7 and P: 12.

Table 7. Post hoc subgroup analysis of secondary outcomes (knee extension muscle strength, males).

	Group	Actual value	Amount of change	
		Pre-dose Week 0	Week 6	Week 12
Right leg (%)	A	49.21 ± 13.07	20.44 ± 6.20*	22.33 ± 9.52*
	P	48.93 ± 17.28	6.59 ± 8.82*	11.05 ± 11.98**
p-value (Mann-Whitney U test)		1.00	0.001	0.007
Left leg (%)	A	48.14 ± 12.30	17.67 ± 10.78*	21.07 ± 17.28*
	P	47.88 ± 17.22	5.76 ± 6.81*	12.19 ± 9.59**
p-value (Mann-Whitney U test)		0.95	0.02	0.23
Dominant leg (%)	A	52.17 ± 10.88	18.70 ± 8.27*	19.83 ± 16.95*
	P	51.48 ± 17.15	2.43 ± 7.61	9.40 ± 10.62**
p-value (Mann-Whitney U test)		0.97	0.001	0.096
Non-dominant leg (%)	A	45.19 ± 13.27	19.41 ± 10.75*	23.57 ± 9.23*
	P	45.33 ± 16.76	9.93 ± 9.32**	13.84 ± 10.75**
p-value (Mann-Whitney U test)		0.84	0.12	0.013

Values are expressed as mean ± standard deviation. "A" and "P" refer to the GABA and placebo food intake groups, respectively. The leg with a higher knee extension muscle strength value at Week 0 was "the dominant leg," and the leg with a lower knee extension muscle strength value at Week 0 was "the non-dominant leg." Comparisons between groups were performed using the Mann-Whitney U test. Significant differences in the Wilcoxon signed-rank sum test of intragroup changes relative to pre-dose Week 0 are expressed as $^{**}p < 0.01$, $^{*}p < 0.05$. Number of subjects were A: 7 and P: 12.

Safety test: Two adverse events occurred in 2 of 25 subjects (8.0%) in group A, but none in group P before studying food intake. During the intake period, 11 adverse events occurred in 8 of 25 subjects (32.0%) in group A and 12 in 6 of 25 subjects (24.0%) in group P. The breakdown of adverse events after starting intake was 2 cases of muscle pain, 2 cases of new coronavirus infection, 1 case of acute lower back pain, 1 case of abdominal pain, 1 case of hip pain, 1 case of knee pain, 1 case of gastrocnemius muscle spasm, 1 case of nasal discharge, and 1 case of heatstroke in group A; 2 cases of knee pain, 1 case of gastrocnemius muscle spasm, 1 case of cold symptoms, 1 case of eczema (scalp), 1 case of headache, 1 case of wrist bruise, 1 case of lower back pain, 1 case of abdominal pain/diarrhea, 1 case of diarrhea, 1 case of calf pain, and 1 case of cough in group P. Some of the adverse events were self-reported as caused by locomotion training and were related to this study, but in both cases, causes other than the study food could be identified. Thus, as determined by the principal investigator, there was no correlation to the study food. Regarding other adverse events, the principal investigator determined that there was no relationship to the study food. No drugs or healthy foods were taken during the trial period that would affect the results.

In body composition, lean body mass measurement, and physical examination, some items showed significant changes in the post-intake tests compared to pre-dose Week 0; however, all changes were minor. Therefore, it was determined by the principal investigator that there was no clinical problem. There were some significant changes in the hematology, blood biochemistry, and urinalysis tests compared to pre-dose Week 0, but all changes were within standard values. Some subjects showed positive protein (qualitative) and occult blood reaction (qualitative) results in urine tests. Still, as both were thought to be due to menstruation or physiological fluctuations, the principal investigator determined that there was no clinical problem.

DISCUSSION

In this study, when healthy people over 40 years of age who were aware of a decline in muscle strength and who objectively showed a decrease in muscle strength using the 2-step test ingested GABA in combination with lower body exercise, no significant difference was observed in lean body mass and growth hormone levels. On the other hand, in the study by Choi et al. in which middle-aged females with a habit of aerobic exercise (once or twice a week) were asked to abstain from regular exercise and ingested GFST for eight weeks, lean body mass significantly increased compared to the placebo group [17]. In our study, lean body mass was measured using the BIA method, whereas the study by Choi et al. employed the DEXA method. The difference in measurement principle may have influenced the difference in lean body mass results. In the study by Choi et al. [17], total fat mass was about 36 kg in the GFST intake group and 34 kg in the placebo group at pre-dose Week 0. On the other hand, in this study, total fat mass was 16.2 kg in the GABA intake group and 15.4 kg in the placebo group at pre-dose Week 0, suggesting that differences in the body composition at pre-dose Week 0 may have affected muscle mass of change. In our study, subjects performed locomotion training (standing on one leg, squats, and heel raises) during the test period. One-leg standing (2.1 METs), squats (6.5 METs), and front lunges (8.5 METs) performed by subjects in this study were equivalent to prolonged walking, walking at a speed of 130 steps/min, and running at a speed of 134 m/min, respectively [24–28]. When 93 elderly people performed the locomotion training for three months, a noticeable decreasing trend in Body Mass Index (BMI) was observed [34]. BMI is calculated as [weight (kg)] ÷ [height (m) squared] and is used to determine obesity and underweight status (thinness). Locomotion training may

have influenced body composition. GABA intake promotes growth hormone secretion [35]. Growth hormones play a vital role in the growth and maintenance of skeletal muscle, stimulating increases in muscle protein synthesis [36]. In the study by Choi et al. [17], growth hormone levels were also significantly increased in the GABA intake group, suggesting that the increase in growth hormone levels caused the increase in lean body mass. Further, in the study by Choi et al. [17], growth hormone levels were measured by a commercially available kit, Immulite 2000 (Siemens AG, Muenchen, Germany). On the other hand, in our study, growth hormone concentrations were measured by the ECLIA method, and growth hormone levels were not significantly increased in the GABA intake group. The difference in measurement method may have affected the results.

In this study, the GABA intake group significantly increased knee extension muscle strength compared to the placebo group at Week 6. Locomotion training improves knee extension muscle strength in older adults [37]. In this study, the combination of GABA intake and locomotion training significantly improved knee extension muscle strength compared to locomotion training alone. In older people, lack of exercise and hormonal changes lead to decreased repair and regeneration ability due to reduced muscle satellite cells, resulting in muscle protein breakdown that exceeds synthesis. Furthermore, mitochondrial dysfunction causes metabolic abnormalities, leading to quantitative and qualitative abnormalities in skeletal muscle [4]. Muscle quality can be expressed as the ratio of muscle strength to muscle mass [38]. The effect of muscle strength on quality of life is more important than muscle mass. More specifically, muscle strength overwhelms muscle mass as a determinant of quality of life [39]. A positive correlation exists between 6-minute walking distance and knee extension muscle strength [40]. On the other hand, experiments in C2C12 myoblasts showed that GABA promotes muscle cell proliferation, myoblast determination protein 1 (MyoD), and peroxisome proliferator-activated receptor γ coactivator (PGC)-1α expression and suppresses myostatin expression [41]. MyoD functions to transdifferentiate non-muscle cells into muscle cells [42] and is integral to skeletal muscle development [43]. Increased expression of PGC-1α in the skeletal muscle of aged wild-type mice protects against the development of mitochondrial dysfunction and sarcopenia [44]. Myostatin, a member of the transforming growth factor-β superfamily, is an attractive target for muscle disease therapy because of its role as a negative regulator of muscle growth and strength [45]. Orally ingested GABA passes into the blood and reaches peak concentration after 30 minutes [11]. In a study in which mice were administered GABA, a substrate for homocarnosine synthesis in skeletal muscle, homocarnosine levels were significantly increased in the GABA-treated groups compared to the GABA-free group. In other words, orally administered GABA reached the skeletal muscles [46]. GABAA receptors are expressed on C2C12 myoblasts [47]. From the above, it was thought that orally ingested GABA could reach the muscle tissue, increase the number of muscle cells, and promote muscle growth and strength gains. This was suggested to be the mechanism of the significant increase in knee extension muscle strength in the GABA intake group in our study.

In post hoc subgroup analysis by sex, for males, in knee extension muscle strength, the GABA intake group showed a significant increase compared to the placebo group in the right leg at Week 6 and 12, in the left leg at Week 6, in the dominant leg at Week 6, and in the non-dominant leg at Week 12. The rate of decline in knee extension muscle strength due to aging is greater in males than in females [48]. Testosterone is the primary male hormone responsible for regulating sex differentiation, producing male sex characteristics [49]. The mean serum

testosterone concentrations were 13.8, 10.3, and 5.6 pg/mL in males aged 20–30, 40–59, and 60+ years, respectively. In contrast, concentrations were 0.23, 0.20, and 0.20 in females aged 20–30, 40–59, and 60+ years, respectively [50]. One week of treatment with testosterone propionate increased GABAA receptor sensitivity [51]. GABAA receptors are expressed on C2C12 myoblasts [47]. Therefore, this study suggested that GABA intake had a greater effect on increasing knee extension muscle strength in males whose GABAA receptor sensitivity was increased due to the action of testosterone.

Physiologists have reported that human muscle strength increases from morning to evening [52]. In this study, each test was conducted in the morning, so it was assumed that the measured knee extension muscle strength was relatively low from a circadian rhythm perspective. Since the tests were conducted at similar times, it was thought that there was little variation in knee extension muscle strength between tests. Therefore, in this study, it was suggested that there is no need to consider the influence of circadian rhythm on the measured knee extension muscle strength.

We considered the effects of exercise and GABA intake on muscle mass, muscle quality, and muscle strength. Choi et al. found that GFST intake without training resulted in a significant increase in muscle mass compared to the group without training that did not ingest GFST; however, there was no significant difference in knee extension muscle strength [17]. On the contrary, in the present study, GABA intake with locomotion training did not result in a significant difference in muscle mass compared to the group with locomotion training that did not ingest GABA. Yet, there was a significant increase in the knee extension muscle strength. With aging, muscle protein synthesis decreases [53], and fat infiltrates muscle cells, resulting in poor muscle quality and, consequently, a decline in muscle strength [54]. Muscle density is more strongly related to muscle strength than muscle size [55], and an increase in intramuscular lipids contributes to sarcopenia [56]. Particularly in obese people, the increase in intramuscular lipids is remarkable [54]. When older adults who had been doing muscle training stopped training for a long time, muscle fibers decreased, and intramuscular lipids increased [57]. Based on these previous studies, obese people and older adults who do not exercise or have stopped exercising exhibit decreases in muscle mass, muscle density, and muscle strength. Total fat at pre-dose week 0 was high in both groups in the study by Choi et al. [17], suggesting a tendency towards obesity. In addition, before participating in the study, the subjects had been doing aerobic exercise once or twice a week, but they abstained from exercise during the test period. In other words, it was assumed that muscle protein decreased, and muscle lipids increased in the group that did not ingest GFST. We speculated that muscle protein significantly increased in the GFST intake group, but since they also abstained from exercise, intramuscular lipids also increased, resulting in no significant difference in muscle strength between the groups. Exercise increases muscle protein synthesis in older adults [58]. Moreover, resistance training in aged rats was demonstrated to reduce intramuscular lipid accumulation, increase MyoD expression, and decrease myostatin expression [57]. In other words, resistance training increases muscle mass and muscle strength. Based on these reported findings, muscle mass and strength decrease with age but increase with exercise. Therefore, in the present study, muscle protein synthesis increased, and intramuscular lipids decreased in both groups because they performed locomotion training, and this could be the reason for the lack of significant differences in muscle mass between the groups. GABA promotes the proliferation of myoblasts, increases their expression of MyoD, and decreases their expression of

myostatin [41], suggesting that GABA increases the number of myotubes and myofibers. In the present study, we speculated that in the GABA intake group, intramuscular lipids decreased due to training, and the number of myotubes and myofibers increased due to training and GABA intake, resulting in an overall increase in muscle density. This could explain why the GABA intake group significantly increased knee extension muscle strength in the group comparison.

Regarding safety, the principal investigator determined that no adverse events were related to the study food. In addition, significant differences were observed in some items in body composition/lean body mass measurements, physical examinations, and clinical examinations when comparing pre-dose Week 0 and after intake in each group; however, these were all minor changes. Therefore, the causal relationship with the active food was determined to be "not related." Based on the above, it was concluded that GABA-containing foods can be safely consumed under the conditions of this study.

CONCLUSION

In this study, healthy males and females assessed as locomotive syndrome level 1 and over the age of 40 ingested GABA and underwent exercise training focused on the lower body, which resulted in a significant increase in knee extension muscle strength at Week 6. In particular, knee extension muscle strength in males significantly improved at Week 6 and 12. This study supports previous research on the vital role of an increased GABA intake in promoting growth hormone secretion, resulting in increased muscle protein synthesis and lean body mass. Since knee extension muscle strength is an essential biomarker for sarcopenia, this study suggests that GABA is a functional food ingredient that can be beneficial in alleviating sarcopenia. The results indicate that the combination of oral intake of GABA and exercise focusing on the lower body in subjects over 40, especially males, is effective in maintaining the ability to stand up from a lying position or sitting in a chair, the ability to sit in a chair, and the ability to walk.

List of Abbreviations: Active food: GABA-containing food, Group A: Active food intake group, Group P: Placebo food intake group, Placebo food: GABA-free food, Pre-dose Week 0: Tests performed before starting intake, TUG test: Timed Up & Go test, Week 6: Test conducted six weeks after starting intake, Week 12: Test conducted 12 weeks after starting intake, xx ± yy: Mean value (or least squares mean value) ± standard deviation (or standard error).

Funding: The present study was funded by Sanwa Shurui Co., Ltd.

Competing Interests: Uehara and Hokazono are employees of Sanwa Shurui Co., Ltd. The other authors have no conflicts of interest to disclose. Sanwa Shurui Co., Ltd. concluded an outsourcing contract with EP Mediate Co., Ltd. to outsource the trial. EP Mediate Co., Ltd. concluded an operating agreement with Kaiseikai Medical Corporation Tana Orthopedic Surgery, Kaiseikai Medical Corporation Kitashinyokohama Orthopedic Surgery (study site), and LSI Medience Corporation (laboratory) and conducted the study. The compensation based on these operating agreements was a legitimate business compensation for the implementation of the research and did not affect the study results.

Author Contributions: Conceptualization: E.U. and H.H. Data curation: A.T. and M.F. Formal analysis: A.T. and M.F. Funding acquisition: E.U. and H.H. Investigation A.T. and M.F. Methodology: E.U. and H.H. Project administration: E.U. and H.H. Resources: E.U. Supervision: H.H. Visualization: E.U. Writing-original draft E.U. Writing-review and editing: H.H., A.T. and M.F.

REFERENCES

1. Rosenberg IH: Summary comments: epidemiological and methodological problems in determining nutritional status of older persons. Am J Clin Nutr 1989, 50: 1231–1233.
2. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Marin FC, et al.: Sarcopenia: European consensus on definition and diagnosis. Age Ageing 2010, 39:412–423. DOI: https://doi.org/10.1093/ageing/afq034
3. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, Cooper C, et al.: Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 2019, 48: 16–31. DOI: https://doi.org/10.1093/ageing/afy169
4. Nishikawa H, Fukunishi S, Asai A, Yokohama K, Nishiguchi S, Higuchi K: Pathophysiology and mechanisms of primary sarcopenia (Review). Int J Mol Med 2021, 48: 156. DOI: https://doi.org/10.3892/ijmm.2021.4989
5. Zhang X, Wang C, Dou Q, Zhang W, Yang Y, Xie X: Sarcopenia as a predictor of all-cause mortality among older nursing home residents: a systematic review and meta-analysis. BMJ Open 2018, 8: e021252. DOI: https://doi.org/10.1136/bmjopen-2017-021252
6. Vogele D, Otto S, Sollmann N, Haggenmüller B, Wolf D, Beer M, Schmidt SA: Sarcopenia- definition, radiological diagnosis, clinical significance. RöFo 2023, 195: 393–405 . DOI: https://doi.org/10.1055/a-1990-0201
7. Dent E, Woo J, Scott D, Hoogendijk EO: Sarcopenia measurement in research and clinical practice. Eur J Intern Med 2021, 90: 1–9. DOI: https://doi.org/10.1016/j.ejim.2021.06.003
8. De Bie TH, Witkamp RF, Balvers MG, Jongsma MA: Effects of γ-aminobutyric acid supplementation on glucose control in adults with prediabetes: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 2023, 118: 708–719. DOI: https://doi.org/10.1016/j.ajcnut.2023.07.017
9. Nakamura U, Nohmi T, Sagane R, Hai J, Ohbayashi K, Miyazaki M, Yamatsu A, et al.: Dietary gamma-aminobutyric acid (GABA) induces satiation by enhancing the postprandial activation of vagal afferent nerves. Nutrients 2022, 14: 2492. DOI: https://doi.org/10.3390/nu14122492
10. Oketch-Rabah HA, Madden EF, Roe AL, Betz JM: United States Pharmacopeia (USP) safety review of gamma-aminobutyric acid (GABA). Nutrients 2021, 13: 2742. DOI: https://doi.org/10.3390/nu13082742
11. Yamatsu A, Yamashita Y, Pandharipande T, Maru I, Kim M: Effect of oral γ-aminobutyric acid (GABA) administration on sleep and its absorption in humans. Food Sci Biotechnol 2016, 25: 547–551. DOI: https://doi.org/10.1007/s10068-016-0076-9
12. Nakamura H, Takishima T, Kometani T, Yokogoshi H: Psychological stress-reducing effect of chocolate enriched with gamma-aminobutyric acid (GABA) in humans: assessment of stress using heart rate variability and salivary chromogranin. A Int J Food Sci Nutr 2009, 60: 106–113. DOI: https://doi.org/10.1080/09637480802558508
13. Shimada M, Hasegawa T, Nishimura C, Kan H, Kanno T, Nakamura T, Matsubayashi T: Anti-hypertensive effect of γ-aminobutyric acid (GABA)-rich chlorella on high-normal blood pressure and borderline hypertension in placebo-controlled double-blind study. Clin Exp Hypertens 2009, 31: 342–354. DOI: https://doi.org/10.1080/10641960902977908
14. Thacker P, Mandalika S, Shah R, Doctor N: Effect of preoperative feeding with germinated brown rice supplement on clinical outcome of orthopaedic and gastrointestinal surgery patients. Bioact Compd Health Dis 2021, 4: 149–166. DOI: https://doi.org/10.31989/bchd.v4i7.799
15. Martirosyan D, Kanya H, Nadalet C: Can functional foods reduce the risk of disease? Advancement of functional food definition and steps to create functional food products. Funct Foods Health Dis 2021, 11: 213–221. DOI: https://www.doi.org/10.31989/ffhd.v11i5.788
16. Nedachi T, Haketa K, Harakawa S, Miura N, Wakame K: Effect of combining sleep-promoting food intake and electric field application on sleep in healthy participants: A pilot study. Funct Foods Health Dis 2021, 11: 659–672. DOI: https://www.doi.org/10.31989/ffhd.v11i12.861
17. Choi WC, Reid SNS, Ryu JK, Kim Y, Jo YH, Jeon HB: Effects of γ-aminobutyric acid-enriched fermented sea tangle (*Laminaria japonica*) on brain derived neurotrophic factor-related muscle growth and lipolysis in middle aged women. Algae 2016, 31: 175–187. DOI: https://doi.org/10.4490/algae.2016.31.6.12
18. Sakashita M, Nakamura U, Horie N, Yokoyama Y, Kim M, Fujita S: Oral supplementation using gamma-aminobutyric acid and whey protein improves whole body fat-free mass in men after resistance training. J Clin Med Res 2019, 11: 428–434. DOI: https://doi.org/10.14740/jocmr3817
19. The Japanese Orthopedic Society's official locomotive syndrome prevention awareness website, "2-step test" (in Japanese) [https://locomo-joa.jp/check/test/two-step] Retrieved April 8, 2024.
20. The Japanese Ministry of Health, Labour and Welfare website, "Locomotive degree test" (in Japanese)

[https://www.mhlw.go.jp/content/000656490.pdf] Retrieved April 8, 2024.

21. Ishigaki T, Ogawa T, Miyashita T, Hirata K, Kishida K, Chibana T, Shinomiya T, et al.: Reliability and validity of the two-step test as an assessment of gait performance in the home setting and identifying the cutoff values for independent gait (in Japanese). Phys Ther 2021, 48: 261–270.
22. The Japanese Orthopedic Society's official locomotive syndrome prevention awareness website, "locomotion training" (in Japanese). https://locomo-joa.jp/check/locotre Retrieved April 8, 2024
23. Jetté M, Sidney K, Blümchen G: Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity. Clin Cardiol 1990, 13: 555–565. DOI: https://doi.org/10.1002/clc.4960130809
24. Aramaki Y: Relationship between postural control and respiratory movement during one-leg standing in healthy males. J Phys Ther Sci 2023, 35: 479–482. DOI: https://doi.org/10.1589/jpts.35.479
25. Haramura M, Takai Y, Yamamoto M, Kanehisa H: Differences in cardiorespiratory and metabolic responses between body mass-based squat and lunge exercises with relation to muscular activity with relation to muscular activity level (in Japanese). Jpn J Phys Fitness Sports Med 2017, 66: 101–110. DOI: https://doi.org/10.7600/jspfsm.66.101
26. Franklin BA, Pamatmat A, Johnson S, Scherf J, Mitchell M, Rubenfire M: Metabolic cost of extremely slow walking in cardiac patients: implications for exercise testing and training. Arch Phys Med Rehab 1983, 64: 564–565.
27. Tudor-Locke C, Aguiar EJ, Han H, Ducharme SW, Schuna JM Jr, Barreira TV, Moore CC, et al.: Walking cadence (steps/min) and intensity in 21-40 year olds: CADENCE-adults. Int J Behav Nutr Phys Act 2019, 16: 8. DOI: https://doi.org/10.1186/s12966-019-0769-6
28. Ministry of Health, Labour and Welfare in Japan: Unit of physical activity/exercise (in Japanese). https://www.mhlw.go.jp/shingi/2006/11/s1109-5g.html
29. Podsiadlo D, Richardson S: The timed "Up and Go": a test of basic functional mobility for frail elderly persons. J Am Geriatr Soc 1991, 39: 142–148. DOI: https://doi.org/10.1111/j.15325415.1991.tb01616.x
30. Koyama S, Mori Y, Tomoda M, Miura H, Kawashima M, Koezuka N, Odagiri E: Clinical performance evaluation of human growth hormone measurement kit "Ecrusis reagent hGH" using electrochemiluminescence immunoassay (ECLIA method, in Japanese). Jpn J Med Pharm Sci 2012, 68: 899–910.
31. Ministry of Health, Labour and Welfare in Japan: Reference material 3-2: Tinetti's fall anxiety scale. Nursing care prevention manual (revised edition: March 2012, in Japanese). https://www.mhlw.go.jp/topics/2009/05/dl/tp0501-sankou3-2.pdf
32. Bredella MA: Sex differences in body composition. Adv Exp Med Biol 2017, 1043: 9–27. DOI: https://doi.org/10.1007/978-3-319-70178-32
33. Kristensen MT, Hulsbæk S, Faber LL, Kronborg L: Affiliations expand knee extension strength measures indicating probable sarcopenia is associated with health-related outcomes and a strong predictor of 1-year mortality in patients following hip fracture surgery. Geriatrics (Basel) 2021, 6: 8. DOI: https://doi.org/10.3390/geriatrics6010008
34. Arai T, Fujita H, Marutani K, Yoshizawa S, Asahi R, Morita Y, Yoshizawa S, e al.: The investigation of improvement effect on physical function and its sustainability after 6 months by "LOCOMO CALL" program as municipal nursing care prevention project (in Japanese). J Jpn Osteoporos Soc 2018, 4: 531–540.
35. Cavagnini F, Benetti G, Invitti C, Ramella G, Pinto M, Lazza M, Dubini A, et al.: Effect of γ-aminobutyric acid on growth hormone and prolactin secretion in man: influence of pimozide and domperidone. J Clin Endocr Metab 1980, 51: 789–792. DOI: https://doi.org/10.1210/jcem-51-4-789
36. De Palo EF, Gatti R, Antonelli G, Spinella P: Growth hormone isoforms, segments/fragments: does a link exist with multifunctionality? Clin Chim Acta 2006, 364:77–81. DOI: https://doi.org/10.1016/j.cca.2005.06.010
37. Aoki K, Sakuma M, Endo N: The impact of exercise and vitamin D supplementation on physical function in community-dwelling elderly individuals: a randomized trial. J Orthop Sci 2018, 23: 682–687. DOI: https://doi.org/10.1016/j.jos.2018.03.011
38. Wu M, Wei Y, Lv J, Guo Y, Pei P, Li J, Du H, et al.: Associations of muscle mass, strength, and quality with all-cause mortality in China: a population-based cohort study. Chin Med J (Engl.) 2022, 135: 1358–1368. DOI: https://doi.org/10.1097/CM9.0000000000002193
39. Chen MJ, Ko PS, Lee MC, Su SL, Yu S: Gender difference in appendicular muscle strength: determinant of the quality of life in the older Taiwanese. Aging (Albany NY) 2022, 14: 7517–7526. DOI: https://doi.org/10.18632/aging.204297
40. Nakao H, Yoshikawa T, Mimura T, Hara T, Nishimoto K, Fujimoto S: Influence of lower-extremity muscle force, muscle mass and

asymmetry in knee extension force on gait ability in community-dwelling elderly women. J Phys Ther Sci 2006, 18: 73–79. DOI: https://doi.org/10.1589/jpts.18.73

41. Uehara E, Hokazono H: GABA (γ-aminobutyric acid) promotes cell proliferation, increases MyoD and PGC-1α expression, and decreases myostatin expression in C2C12 myoblasts. Int J Food Sci Nutr 2022, 7: 8–12.
42. Battistelli C, Garbo S, Maione R: MyoD-induced trans-differentiation: a paradigm for dissecting the molecular mechanisms of cell commitment, differentiation, and reprogramming. Cells 2022, 11: 3435. DOI: https://doi.org/10.3390/cells11213435
43. Li H, Zhu C, Tao Z, Xu W, Song W, Hu Y, Zhu W, et al.: MyoD and Myf6 gene expression patterns in skeletal muscle during embryonic and posthatch development in the domestic duck (Anas platyrhynchos domestica). J Anim Breed Genet 2014, 131: 194–201. DOI: https://doi.org/10.1111/jbg.12057
44. Dillon LM, Rebelo AP, Moraes CT: The role of PGC-1 coactivators in aging skeletal muscle and heart. IUBMB Life 2012, 64: 231–241. DOI: https://doi.org/10.1002/iub.608
45. Muramatsu H, Kuramochi T, Katada H, Ueyama A, Ruike Y, Ohmine K, Shida-Kawazoe M, et al.: Novel myostatin-specific antibody enhances muscle strength in muscle disease models. Sci Rep 2021, 11: 2160. DOI: https://doi.org/10.1038/s41598-021-81669-8
46. Mizuno T, Matsui Y, Tomida M, Suzuki Y, Nishita Y, Tange C, Shimokata H, et al.: Differences in the mass and quality of the quadriceps with age and sex and their relationships with knee extension strength. J Cachexia Sarcopenia 2021, 12: 900–912. DOI: https://doi.org/10.1002/jcsm.12715
47. Kumrungsee T, Arima T, Sato K, Komaru T, Sato M, Oishi Y, Egusa A, et al.: Dietary GABA induces endogenous synthesis of a novel imidazole peptide homocarnosine in mouse skeletal muscles. Amino Acids 2020, 52: 743–753. DOI: https://doi.org/10.1007/s00726-020-02848-x
48. Nassar GN, Leslie SW: Physiology, Testosterone. StatPearls. January 2, 2023.
49. Cauley JA: Estrogen and bone health in men and women. Steroids 2015, 99: 11–15. DOI: https://doi.org/10.1016/j.steroids.2014.12.010
50. Bitran D, Kellogg CK, Hilvers RJ. Treatment with an anabolic-androgenic steroid affects anxiety-related behavior and alters the sensitivity of cortical GABAA receptors in the rat. Horm Behav 1993, 27: 568–583. DOI: https://doi.org/10.1006/hbeh.1993.1041
51. Hidaka Y, Chiba-Ohkuma R, Karakida T, Onuma K, Yamamoto R, Fujii-Abe K, Saito MM, et al.: Combined effect of midazolam and bone morphogenetic protein-2 for differentiation induction from C2C12 myoblast cells to osteoblasts. Pharmaceutics 2020, 12: 218. DOI: https://doi.org/10.3390/pharmaceutics12030218
52. Douglas CM, Hesketh SJ, Esser KA: Time of Day and Muscle Strength: A Circadian Output? Physiology (Bethesda) 2021, 36: 44–51. DOI: https://doi.org/10.1152/physiol.00030.2020
53. Burd NA, Gorissen SH, van Loon LJC: Anabolic resistance of muscle protein synthesis with aging. Exerc Sport Sci Rev 2013, 41: 169–173. DOI: https://doi.org/10.1097/JES.0b013e318292f3d5
54. Pereira AZ, Uezima CB, Zanella MT, do Prado RR, Gonzalez MC, Zheng J, Heymsfield SB: Muscle echogenicity and changes related to age and body mass index. JPEN J Parenter Enteral Nutr 2021, 45: 1591–1596. DOI: https://doi.org/10.1002/jpen.2030
55. Wang L, Yin L, Zhao Y, Su Y, Sun W, Chen S, Liu Y, et al.: Muscle density, but not size, correlates well with muscle strength and physical performance. J Am Med Dir Assoc 2021, 22: 751–759. e2. DOI: https://doi.org/10.1016/j.jamda.2020.06.052
56. Ribeiro MBT, Guzzoni V, Hord JM, Lopes GN, Marqueti RC, de Andrade RV, Selistre-de-Araujo, et al.: Resistance training regulates gene expression of molecules associated with intramyocellular lipids, glucose signaling and fiber size in old rats. Sci Rep 2017, 7: 8593. DOI: https://doi.org/10.1038/s41598-017-09343-6
57. Taaffe DR, Henwood TR, Nalls MA, Walker DG, Lang TF, Harris TB: Alterations in muscle attenuation following detraining and retraining in resistance trained older adults. Gerontology 2009, 55: 217–223. DOI: https://doi.org/10.1159/000182084
58. Giallauria F, Cittadini A, Smart NA, Vigorito C: Resistance training and sarcopenia. Monaldi Arch Chest Dis 2016, 84:738. DOI: https://doi.org/10.4081/monaldi.2015.738