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Complete List of Authors:	Osuka, Yosuke; Tokyo Metropolitan Institute of Gerontology, Research Team for Promoting Independence of the Elderly Kojima, Narumi; Tokyo Metropolitan Institute of Gerontology, Research Team for Promoting Independence of the Elderly Wakaba, Kyohsuke; University of Tsukuba, Faculty of Health and Sport Sciences Miyauchi, Daiji; Kyowa Co., Ltd Tanaka, Kiyoji; University of Tsukuba, Faculty of Health and Sport Sciences Kim, Hunkyung; Tokyo Metropolitan Institute of Gerontology, Research Team for Promoting Independence of the Elderly
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Effects of resistance training and/or beta-hydroxy-beta-methylbutyrate supplementation on muscle mass, muscle strength, and physical performance in older women with reduced muscle mass: protocol for a randomized, double-blind, placebo-controlled trial

Yosuke Osuka[1], Narumi Kojima[1], Kyohsuke Wakaba[2], Daiji Miyauchi[3], Kiyoji Tanaka[2], and Hunkyung Kim[1]

[1]Research Team for Promoting Independence of the Elderly, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

[2]Faculty of Health and Sport Sciences, University of Tsukuba, Ibaraki, Japan

[3]Kyowa Co., Ltd., Tokyo, Japan.

Corresponding author

Yosuke Osuka, PhD

Research Team for Promoting Independence of the Elderly

Tokyo Metropolitan Institute of Gerontology

35-2, Sakae-cho, Itabshi-ku, Tokyo, 173-0015, Japan

E-mail: osuka@tmig.or.jp

Phone: +81-3-3964-3241

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ABSTRACT

Introduction

Resistance training (RT) and nutritional supplementation seem to have beneficial effects on muscle properties and physical performance in older adults. However, the reported effects of specific RT programs and supplementation prescriptions vary among studies. The present study aims to examine the acute and residual effects of RT and/or beta-hydroxy-beta-methylbutyrate (HMB) supplementation on muscle mass, muscle strength, and physical performance in older women with reduced muscle mass.

Methods and analysis

This is a randomized, double-blind, placebo-controlled trial. Older women fitting the eligibility criteria were recruited in February 2018 from a population-based sample identified via a screening conducted in October 2017. In March 2018, the 156 participants were randomly allocated to undergo one of four interventions (RT+HMB, RT+placebo, education+HMB, and education+placebo) for 12 weeks. Supervised RT consisted of body weight, elastic band, ankle weight, and machine-based exercises twice weekly at the Tokyo Metropolitan Institute of Gerontology. Each participant ingested HMB (1,200 mg) or placebo supplements once daily. Sessions of education not associated with sarcopenia treatment were conducted every two weeks. Post-intervention follow-up will be conducted for 12 weeks, until September 2018. The study includes assessments conducted in March (baseline), June (post-intervention), and September 2018 (follow-up). The primary outcome is the longitudinal change in muscle mass. Secondary outcomes include the longitudinal changes in muscle strength, physical performance, muscle thickness, muscle quality, blood counts, blood biochemistry, calf circumference, skin viscoelasticity, habitual dietary intake, habitual physical activity levels, functional capacity, and health-related quality of life. Intention-to-treat analyses will be conducted.

Ethics and dissemination

The study protocol was approved by the Ethics Committee of the Tokyo Metropolitan Institute of Gerontology, Japan. The study is being conducted according to the principles of the Declaration of Helsinki. The findings will be presented at international academic congresses and published in peer-reviewed international journals.

Registration

UMIN000028560; pre-results.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- To the best of our knowledge, this will be the first study to examine the effects of resistance training and/or HMB supplementation among older women with reduced muscle mass.
- The study will collect information about the residual effects of the intervention over an observation period of 12 weeks.
- The trial has a randomized, double-blind, placebo-controlled design, which supports the generalizability of the findings and minimizes the risk of selection, performance, and detection bias.
- The trial will involve resistance training using body weight, ankle weights, and elastic bands, which will not provide objective information regarding muscle loading but represents a more suitable prescription for older adults and is more feasible as an exercise routine to be performed on a daily basis.
- All participants will be older women, which limits generalization of the findings to men.

INTRODUCTION

Sarcopenia is a syndrome characterised by progressive and generalized loss of skeletal muscle mass and strength.[1] In older adults, sarcopenia is associated with adverse health outcomes such as increased risk of incident falls,[2] reduced performance in instrumental or basic activities of daily living, hospitalization, institutionalization, and mortality.[3 4] To reduce the social burden of sarcopenia, it is important to develop prevention and treatment programs aimed to extend healthy life expectancy especially in older people with high risk of sarcopenia.

Most sarcopenia prevention or treatment programs recommend a combination of resistance training (RT) and nutritional supplementation.[5-7] According to a meta-analysis of nine randomized control trials (RCTs) on the combined effect of protein supplementation and RT on muscle outcomes, such strategies may be effective for increasing fat-free mass among older adults.[8] Both the European Working Group on Sarcopenia in Older People and the International Working Group on Sarcopenia, which serve as international advisory committees on sarcopenia, have argued in favour of leucine and beta-hydroxy-beta-methylbutyrate (HMB) supplementation as complementary strategies for sarcopenia prevention or treatment.[9]

HMB is synthesized in skeletal muscle via transamination of leucine to alpha-ketoisocaproic acid (KIC) by aminotransferase.[10] It is estimated that only 5–10% of KIC is metabolized by KIC dioxygenase to produce HMB in the liver.[11] HMB supplementation is believed to exert beneficial effects on skeletal muscle by increasing protein synthesis through activation of the mammalian target of rapamycin and by decreasing protein catabolism through down-regulation of the ubiquitin proteasome pathway.[12] Wu et al. reviewed the results of seven RCTs on the effects of HMB supplementation on muscle outcomes and concluded that HMB may contribute to preservation of muscle mass in older adults.[13] However, to our knowledge, few studies have examined the combined effect of RT and HMB supplementation on skeletal muscle.[14 15] Additionally, the acute and residual effects of RT and/or HMB supplementation on muscle outcomes in older people with reduced muscle mass remain unclear. Thus, there is a need for studies on the combined effect of nutrition and exercise in older populations with physical frailty, and such studies should be designed as four-arm RCTs (exercise-only, nutrition-only, both, and none).[9]

The present study aims to examine the acute and residual effects of RT and/or HMB supplementation on muscle mass, muscle strength, and physical performance in older women with reduced muscle mass. We hypothesized that, compared to RT alone, HMB supplementation alone, and placebo, combined RT and HMB supplementation would provide higher benefit in terms of improving and maintaining muscle mass, muscle strength, and physical performance. These findings will provide new evidence regarding the effectiveness of non-pharmaceutical interventions for sarcopenia, and will be useful in the development of sarcopenia prevention and treatment programs in physically frail populations.

METHODS AND ANALYSIS

Study design, procedure, and ethics

In accordance with the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) and CONSORT (Consolidated Standards of Reporting Trials) statements, we designed a randomized, double-blind, placebo-controlled trial to be conducted between March and September 2018 at the Tokyo Metropolitan Institute of Gerontology (TMIG), Tokyo, Japan. The study protocol was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) on 7 August 2017 (trial registration no. UMIN000028560) and was approved by the Ethics Committee of the TMIG on 15 September 2017.

Older women fitting the eligibility criteria were recruited in February 2018, from among a population-based sample identified via a screening conducted in October 2017. After recruitment, a four-arm intervention was conducted between March and June 2018. The participants will be followed until September 2018 to observe the residual effects of the intervention. After the observation period, all interventions will be available to all participants, in agreement with ethical principles. Amendments to the protocol will be disclosed on the UMIN-CTR page of the trial. The study involves assessments conducted in March (baseline), June (post-intervention), and September 2018 (follow-up). Before the baseline assessment, all participants have received written and oral information from the researchers (YO, NK, and KH) regarding the study purpose, procedures, confidentiality of personal information, possible benefits, possible risks, and coping strategy for the risks. Only participants who provided written informed consent proceeded within the trial (assessments and interventions). The flow chart of the study is shown in Figure 1.

Participants

To select the study sample, a population-based comprehensive geriatric survey was conducted as screening assessment at the TMIG in October 2017. Invitation letters for the screening assessment were sent to 6,366 subjects randomly selected from the Basic Resident Register of the Itabashi ward, which is a special ward located in the northwest area of Tokyo. In total, 1,035 older women participated in the screening assessment. The inclusion criteria of this study were: (i) age $\geq$ 65 years; (ii) reduced muscle mass, defined as a skeletal muscle index <5.7 kg/m^2, per the sarcopenia diagnosis consensus issued by the Asian Working Group for Sarcopenia; and (iii) informed consent for undergoing screening. The exclusion criteria were: (i) exercise restriction issued by a medical doctor; (ii) use of other supplements known to increase muscle mass; (iii) impaired cardiac, kidney, or liver function; and (iv) judged as ineligible by a medical doctor. A sample of 328 women fitting the eligibility criteria received invitation letters regarding the study intervention, of whom 156 participated in the baseline assessment.

Randomization, allocation concealment, and blinding

A baseline assessment was conducted in March 2018. Afterwards, the participants were randomly allocated to four groups in a ratio of 1:1:1:1 based on a computer-generated randomization number. The allocation was conducted by two researchers from the University of Tsukuba (KW and KT). The allocation keys will be blinded from the researchers at the TMIG (YO, NK, and HK), participants, exercise trainer, analysists, and assessors until December 2018 in order to maintain allocation concealment. The researchers holding the allocation keys will not have contact with the participants.

Intervention

During the intervention and observational periods, all participants were instructed not to change their habitual dietary intake or physical activity levels (i.e., those reported during the baseline assessment in March 2018).

RT program

Participants allocated to the RT program took part in 60-min exercise sessions on two non-consecutive days per week for 12 weeks. All sessions took place at the TMIG, under the supervision of a well-experienced exercise trainer who was not one of the researchers associated with the study. Each exercise session consisted of a 5-min warm-up exercise, a 50-min RT exercise, and a 5-min cool-down exercise. Warm-up and cool-down exercises included stretching activities for the shoulders, trunk, chest, hips, and hamstrings. RT consisted of chair-based (weeks 1–12), elastic band (weeks 5–7), ankle weight (weeks 7–12), and machine-based exercises (weeks 9–12). The chair-based exercise included heel raise, toe raise, knee extension, hip adduction using a rubber ball, and knee lift exercises from the sitting position, as well as squats. Additionally, heel raise and knee lift exercises were performed progressively from a seated to a standing position, and lateral leg raise exercises were performed while standing behind the chair and holding onto the back of the chair. Elastic band exercises included knee lift, hip adduction, and arm rowing exercises. Knee extension exercises in sitting position, as well as heel raise, knee lift, and lateral leg raise exercises in standing position also were performed using ankle weights of 0.5, 0.75, 1.0, or 1.5 kg, according to the physical condition of each participant. In the last 4 weeks of the intervention, the participants performed five types of exercise (arm rowing, leg extension, hip lateral rotation, leg press, and trunk flexion) using dedicated RT machines (Mizuno Ltd., Tokyo, Japan). Each type of movement was performed in 1–3 sets of 8–10 repetitions, with the first four counts being performed with increasing strength and the last four with decreasing strength. The exercise trainer supervised the participants and provided verbal feedback to ensure sufficient tension was achieved in the target muscle groups. Exercise intensity was maintained at 12–14 points on the Borg Rate of Perceived Exertion scale.

This RT program was based on a program employed successfully in our previous studies.[16-18]

HMB and placebo supplementation

All participants were instructed to ingest active or placebo products provided by Kyowa Co., Ltd., Tokyo, Japan. The active products contain 30 mg of protein, 20 mg of fat, 3,500 mg of carbohydrates, 0.2 mg of sodium, 207 mg of calcium, and 1,200 mg of HMB. Placebo products do not include calcium or HMB, and the missing amounts are provided as carbohydrates. Both products are provided in powder form with the same flavour, appearance, and packaging. The two researchers (KW and KT) maintaining the allocation keys labelled the package with an identification code and sent the products to the researchers at the TMIG (YO, NK, and HK). The participants received the blinded product, which they were instructed mix with 200 mL of water and ingest once a day after any meal. The participants were instructed to record the ingestion of products in a diary.

While there are no clear recommendations regarding HMB supplementation, Wu et al. suggested a daily dose of 3,000 mg for older adults.[13] However, as body weight is lower among sarcopenic Japanese older women than among western older women,[16 19 20] we set the active dose at 1,200 mg HMB daily. No adverse events associated with HMB supplementation have been reported.[21]

Health education program

Participants allocated to the health education groups took part in six 60-min sessions (once every two weeks for 12 weeks) of health education not related to sarcopenia prevention but focused specifically on dementia prevention, prevention of bank transfer fraud, music therapy, nutrition and general health, oral care, and social education, each given at the TMIG by an expert in that field.

Assessments

This study includes assessments conducted in March (baseline), June (post-intervention), and September 2018 (follow-up) at the TMIG. All assessors are TMIG employees who are not researchers and are blinded to group allocation.

Body composition

Body composition assessments involve measuring the following muscle mass indices: whole-body fat-free mass; upper-extremity and lower-extremity lean mass; appendicular lean mass, as the sum of the upper- and lower-extremity lean mass; and skeletal mass index, as the appendicular lean mass divided by the height squared (kg/m^2).[22] The body weight, fat mass, and percent fat are also obtained. Body composition measurements are conducted using the InBody720 device (Biospace Co., Ltd, Seoul, Korea), which has a validity for estimating appendicular lean mass in community-dwelling older populations in comparison of dual-energy X-ray absorptiometry

systems.[23]

Muscle strength

Knee extensor strength

Peak isometric knee extensor strength in the dominant leg is measured in a sitting position. The assessor places the hand-held dynamometer (μTas F-1; ANIMA, Tokyo, Japan) on the skin above the anterior ankle, at a level 5 cm above the tip of the lateral malleolus. The participants are instructed to extend the knee with maximum power starting from a knee joint angle of 90°.[24] After a few practice repetitions, the participants are measured twice, and the best result is retained.

Hip adductor strength

Peak isometric hip adduction strength is also measured in a sitting position. The assessor places the hand-held dynamometer (μTas F-1; ANIMA) at 3 cm proximal to the medial knee fissure and instructs the participant to exert maximal hip adduction force while compressing a 12-cm rod equipped with a dynamometer, keeping both knee joints at 90°.[25] After a few practice repetitions, the participants are measured twice, and the best result is retained.

Handgrip strength

Handgrip strength is measured using a hand-held Smedley-type dynamometer. The participants are instructed to stand naturally, grip the device with their dominant hand, and squeeze as hard as possible.[26] The best result of two trials is retained.

Physical performance

Usual and maximal gait speed

Using a stopwatch, the usual and maximal gait speed are measured as the time taken to walk 5 m (between markers set at 3 m and 8 m of an 11-m walking path)[26] at the usual or maximal speed, respectively. Usual gait speed is measured once. Maximal gait speed is measured twice, and the best result is retained.

Timed up-and-go

This test measures the time taken to stand up from the chair, walk to and around a marker placed 3 m away, return to the chair, and sit back down.[27] The participants are instructed to perform these movements as quickly as possible. The test is performed twice, and the best result is retained.

Five-repetition sit-to-stand

This test measures the time taken to stand up from the chair until full knee and hip extension, sit back down, and repeat this movement five times as quickly as possible.[28] The participants are instructed to fold their arms across the chest, stand-up completely, and make firm contact when sitting. After a few practice repetitions, the test is performed twice, and the best result is retained.

Muscle thickness and quality

Muscle thickness is measured using a B-mode ultrasound device (Mysono U6; Samsung Medison, Seoul, Korea). The participants are instructed to lie on the back in a bed and extend the hip and knee until fully relaxed. The assessor performs a transverse scan by placing a linear probe (5–12 MHz) on the skin surface at the midpoint between the lateral epicondyle and the ipsilateral greater trochanter of the femur, perpendicular to the longitudinal axis of the quadriceps femoris muscle. To measure the thickness of the rectus femoris and vastus intermedius, an electronic calliper is used. Quadriceps femoris thickness is defined as the sum between the thickness of the rectus femoris and that of the vastus intermedius. The muscle quality of the rectus femoris is evaluated on ultrasound images processed using dedicated software (Adobe Photoshop CS6 version 13.0; Adobe Systems, San Jose, CA, USA) and is expressed as the brightness of the image on a scale from 0 (black) to 256 (white). The protocol for assessing muscle thickness and quality is described in detail elsewhere.[29]

Blood biochemistry

Blood samples are collected from the antecubital vein. Analyses are carried out centrally in one laboratory (Health Sciences Research Institute, Inc., Kanagawa, Japan). Enzymatic methods are used to determine creatinine, cholesterol (total, high-density lipoprotein, low-density lipoprotein), triglyceride, blood glucose, and glycated haemoglobin levels. Reference methods recommended by the Japan Society of Clinical Chemistry are used to assess aspartate transaminase, alanine transaminase, lactate dehydrogenase, and creatinine kinase levels. Other assays include the urease method with glutamate dehydrogenase (for blood urea nitrogen), direct colorimetry (for Fe), flow cytometry (for white blood cells), erythrocyte fragility test (for red blood cells), sodium lauryl sulphate method (for haemoglobin), microhematocrit method (for haematocrit), latex agglutination turbidimetry (for cystatin C), nephelometric immunoassay (for high-sensitivity C-reactive protein), immunoradiometric assay (for insulin-like growth factor-1), and chemiluminescence immunoassay (for vitamin B12).

Anthropometric indices

The body mass index (kg/m^2) is calculated as the body weight divided by the body height squared. Plastic tape is used to measure the calf circumference in the non-dominant leg.

Skin viscoelasticity

Skin viscoelasticity is evaluated using the Cutometer® dual MPA 580 (Courage + Khazaka electronic GmbH, Cologne, Germany), which measures the elasticity of the upper layer of the skin using negative pressure to induce mechanical deformation; such measurements are useful for quantitative evaluation of age-related changes in skin elasticity.[30 31] The displacement of the skin at the aperture

of the Cutometer® probe is measured optically, and the resistance of the upper skin layer to the applied negative pressure and the ability to return to the original position are displayed as curves in real time (penetration depth as a function of time). Skin viscoelasticity is assessed for the skin of the cheek. The participants are instructed to refrain from using makeup on the day of the assessment. The test is performed five times with 10-sec intervals, and the average result is retained.

Habitual dietary intake

Habitual dietary intake is measured using a brief-type self-administered diet history questionnaire, which has been validated for estimating the monthly energy intake and the intake of each nutrient factor (protein, fat, carbohydrate, and calcium) in older adults.[32 33] These calculations are performed exclusively using software provided by Gender Medical Research Co., Ltd., Tokyo, Japan. Habitual dietary intake evaluations are conducted to determine whether the participants changed their dietary patterns over the course of the intervention.

Habitual physical activity

Habitual physical activity levels (metabolic equivalent-minutes/week) are assessed using the International Physical Activity Questionnaire, which has good reliability and validity for estimating daily physical activity levels.[34 35] We calculate total scores and the scores for each of the four physical activity domains (leisure time, domestic and gardening, occupational, and transport-related physical activity). Habitual physical activity evaluations are conducted to determine whether the participants changed their physical activity patterns over the course of the intervention.

Functional capacity

Functional capacity is assessed in terms of the instrumental self-maintenance (five items), intellectual activities (four items), and social roles (four items) subscales of the TMIG index of competence. The validity and reliability of the face-to-face assessment of the TMIG index of competence have been demonstrated previously.[36] Participants are asked whether or not they are able to perform the function described by each item, to which they may answer either “yes” (able to do so, 1 point) or “no” (unable to do so, 0 points), for a maximum total score of 13 points, with a higher score indicating better functional capacity.

Health-related quality of life (HR-QoL)

HR-QoL is assessed using the World Health Organization-Five Well-Being Index, which has good validity for assessing mental condition in older adults, and consists of five items representing mood in daily life over the preceding two weeks.[37] The participants are instructed to rate the frequency of the mood from 0 (never) to 5 (all the time), for a maximum score of 25 points, with a higher score

indicating better mental condition.

Assessment of adverse events and adherence

If any adverse events associated with RT or supplements occur during the intervention period, the information is recorded and the program discontinued immediately. We assess the adherence to the intervention including RT participation rate and adherence to the daily use of the assigned supplement (based on the participants' daily diary of study product intake). If a participant drops out from the program, the reasons are recorded.

Outcome measures

The primary outcome measure is muscle mass. Secondary outcome measures include muscle strength, physical performance, muscle thickness, muscle quality, blood counts, blood biochemistry, calf circumference, skin viscoelasticity, habitual dietary intake, habitual physical activity levels, functional capacity, and HR-QoL.

Sample size

A previous study on exercise and supplementation in community-dwelling older Japanese women with sarcopenia revealed a between-group difference of 3.1±3.2% in lower-extremity muscle mass.[16] Power calculation was conducted using PS Power and Sample Size Calculation version 3.0 (Vanderbilt University, Tennessee, USA).[38] Setting the two-sided α-error to 0.01 (Bonferroni correction for multiple comparisons as post-hoc tests) and power to 90%, the minimum sample size required to detect clinically important differences was 33 participants per group. Considering 15–18% attrition, we aimed to enrol at most 160 participants.

Statistical analysis

To promote the collection of quality data, the collected data will be double-checked by the assessors, and a statistician will check the range of values. Between-group comparisons of baseline characteristics will be conducted using one-way analysis of variance or the Kruskal-Wallis test for continuous variables, and the chi-square test for categorical variables. Continuous data will be expressed as mean and standard deviation or median and interquartile range. The longitudinal changes in main and secondary outcomes from baseline to post-intervention across four groups will form the primary focus of the analysis. Mixed-model repeated-measures analysis of variance with adjustment for baseline variables will be applied to test for interactions between group and time effect. For group and time interactions with $P < 0.05$, we will apply multiple comparison analyses to test five hypotheses, namely that longitudinal changes differ significantly as follows: for RT+HMB vs. RT+placebo, education+HMB, or education+placebo; for RT+placebo vs. education+placebo;

and for education+HMB vs. education+placebo. Bonferroni correction will be applied to adjust for these comparisons. The results will be presented as least-squares-adjusted means and standard errors. Within-group changes will be analysed using mixed-model repeated-measures analysis of variance. For subgroup analysis, the participants will be stratified according to sarcopenia status, as defined based on the Asian Working Group for Sarcopenia criteria. The intention-to-treat principle will be applied in all analyses. Missing data will be applied multiple imputation. All statistical analyses will be performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA) or Stata (StataCorp LLC, College Station, TX, USA). *P*-values < 0.05 will be considered to indicate significance.

ETHICS AND DISSEMINATION

The study protocol was approved by the Ethics Committee of the TMIG, and will be conducted in agreement with the Declaration of Helsinki. The findings of this study will be presented at international academic congresses and published in peer-reviewed international journals. Upon publication in a journal, we will also make the findings available on the TMIG homepage.

DISCUSSION

This study addresses the paucity of data regarding the effects of RT and/or HMB supplementation in older women with reduced muscle mass, and the findings will provide insight regarding the potential synergistic effect of RT and HMB supplementation on muscle mass, muscle strength, and physical performance. We will also examine the residual effects of such interventions over a 12-week observation period, and such findings may help understand the potential of non-pharmaceutical treatment for sarcopenia.

Literature reports regarding the individual effect of HMB supplementation on muscle properties and physical performance in older people are encouraging.[19 20 39] However, to our knowledge, only two studies have evaluated the combined effect of RT and HMB supplementation in older populations.[14 15] Specifically, Vukovich et al. enrolled 31 adults aged >70 years and reported significant muscle mass gain after 12 weeks of exercise with HMB supplementation but not after exercise with placebo (*P* for interaction, 0.08).[14] On the other hand, Stout et al. enrolled 36 adults aged >65 years and reported significant improvement in muscle quality, calculated as muscle strength relative to muscle mass, after 24 weeks of HMB supplementation alone (i.e., no exercise).[15] It remains unclear whether the combined program provides higher benefits. These previous studies had a relatively small sample size, which might increase β-error; moreover, the participants were relatively healthy and had almost normal muscle mass, suggesting that they may have benefited less from HMB supplementation. As completely controlled groups were not previously included, the potential

synergy between RT and HMB supplementation remains unclear. The present study will address these limitations and serve as the first investigation of the acute and residual independent and combined effects of RT and HMB supplementation, which will be conducted in a larger sample of older adults with reduced muscle mass. The randomized, double-blind, placebo-controlled design will minimize the risk of selection, performance, and detection bias, and will support the generalizability of the results. One limitation is that this study will involve RT using body weight, elastic bands, and ankle weights, which does not provide objective information regarding muscle loading; on the other hand, such RT programs are more suitable for older adults and will be more feasible in daily practice. Another limitation is that all participants will be older women, which will preclude generalization of our findings to men.

DISCLOSURES

Acknowledgments

We are grateful to the participants and the staff members of the Tokyo Metropolitan Institute of Gerontology.

Authors' contributions

YO and HK conceived the study, designed the study protocol, participated in the coordination of the study (recruitment and screening), and wrote the manuscript. NK participated in the coordination of the study and helped manage data collection. KW and TK contributed to designing the study protocol, conducted the randomization, and have been maintaining allocation concealment. DM contributed to designing the study protocol and negotiated a contract. All authors read and approved the final manuscript. HK is principal investigator in this trial.

Funding and competing interests

This study is financially supported by Kyowa Co., Ltd., Tokyo, Japan (no grant number) and is being conducted as a joint research effort of the Tokyo Metropolitan Institute of Gerontology, University of Tsukuba, and Kyowa Co., Ltd. The intervention products (HMB and placebo supplements) were provided by Kyowa Co., Ltd. DM is employed by Kyowa Co., Ltd. The funder is not involved in subject recruitment, intervention, data collection, data analysis, or preparation of the manuscript.

Patient consent

Obtained.

Ethics approval

Approved by the Ethics Committee of the Tokyo Metropolitan Institute of Gerontology on 15 September 2017.

Data sharing statement

Data collection is ongoing and will be handled anonymously. The results will be offered for publication in a peer-reviewed journal and, afterwards, will be available on the TMIG website.

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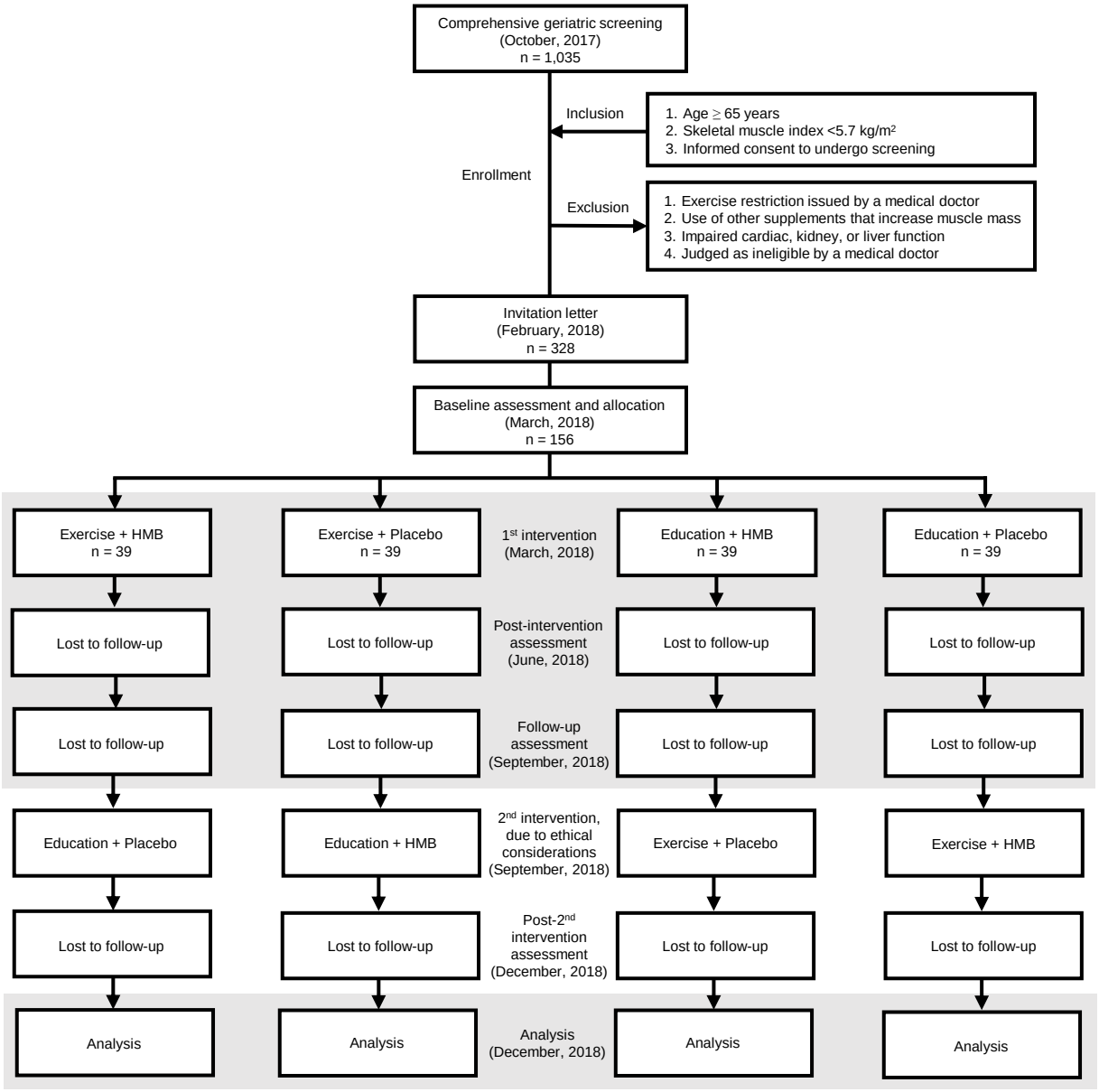
Figure legends

Figure 1. Study flowchart covering participant recruitment and enrolment, group allocation, intervention, observation, and data analysis

A second intervention will be provided after the follow-up assessment, in agreement with ethical principles. However, only data from the highlighted fields will be included in the analysis.


Comprehensive geriatric screening
(October, 2017)
n = 1,035
Inclusion
1. Age ≥ 65 years
2. Skeletal muscle index <5.7 kg/m²
3. Informed consent to undergo screening
Enrollment
Exclusion
1. Exercise restriction issued by a medical doctor
2. Use of other supplements that increase muscle mass
3. Impaired cardiac, kidney, or liver function
4. Judged as ineligible by a medical doctor
Invitation letter
(February, 2018)
n = 328
Baseline assessment and allocation
(March, 2018)
n = 156
Exercise + HMB
n = 39
Exercise + Placebo
n = 39
1st intervention
(March, 2018)
Education + HMB
n = 39
Education + Placebo
n = 39
Lost to follow-up
Lost to follow-up
Post-intervention
assessment
(June, 2018)
Lost to follow-up
Lost to follow-up
Lost to follow-up
Lost to follow-up
Follow-up
assessment
(September, 2018)
Lost to follow-up
Lost to follow-up
Education + Placebo
Education + HMB
2nd intervention,
due to ethical
considerations
(September, 2018)
Exercise + Placebo
Exercise + HMB
Lost to follow-up
Lost to follow-up
Post-2nd
intervention
assessment
(December, 2018)
Lost to follow-up
Lost to follow-up
Analysis
Analysis
Analysis
(December, 2018)
Analysis
Analysis

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Reference
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	pg. 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	pg. 2
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	pg. 14
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	pg. 1
	5b	Name and contact information for the trial sponsor	pg. 14
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	pg. 14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	pg. 3
	6b	Explanation for choice of comparators	pg. 3
Objectives	7	Specific objectives or hypotheses	pg. 3
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	pg. 3-4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	pg. 4, 5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	pg. 5, Fig. 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	pg. 6, 7; Fig. 1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	pg. 11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	pg. 7, 10, 11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	pg. 6
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	pg. 11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	pg. 5, Fig. 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	pg. 11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	pg. 11
Methods: Assignment of interventions (for controlled trials)			

Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	pg. 6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	pg. 6, 7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	pg. 6, 7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	pg. 6, 7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	pg. 6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	pg. 7-11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	pg. 7, 10, 11
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	pg. 11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	pg. 11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	pg. 11-12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	pg. 12
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	pg. 11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Approved; pg. 5, 12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	pg. 5
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	pg. 14
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	pg. 5
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	pg. 14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	pg. 14
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	pg. 11

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	pg. 14
	31b	Authorship eligibility guidelines and any intended use of professional writers	pg. 14
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	pg. 5
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Effects of resistance training and/or beta-hydroxy-beta-methylbutyrate supplementation on muscle mass, muscle strength, and physical performance in older women with reduced muscle mass: protocol for a randomized, double-blind, placebo-controlled trial

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Effects of resistance training and/or beta-hydroxy-beta-methylbutyrate supplementation on muscle mass, muscle strength, and physical performance in older women with reduced muscle mass: protocol for a randomized, double-blind, placebo-controlled trial

Yosuke Osuka[1], Narumi Kojima[1], Kyohsuke Wakaba[2], Daiji Miyauchi[3], Kiyoji Tanaka[2], and Hunkyung Kim[1]

[1]Research Team for Promoting Independence of the Elderly, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

[2]Faculty of Health and Sport Sciences, University of Tsukuba, Ibaraki, Japan

[3]Kyowa Co., Ltd., Tokyo, Japan.

Corresponding author

Yosuke Osuka, PhD

Research Team for Promoting Independence of the Elderly

Tokyo Metropolitan Institute of Gerontology

35-2, Sakae-cho, Itabshi-ku, Tokyo, 173-0015, Japan

E-mail: osuka@tmig.or.jp

Phone: +81-3-3964-3241

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ABSTRACT

Introduction

Resistance training (RT) and nutritional supplementation seem to have beneficial effects on muscle properties and physical performance in older adults. However, the reported effects of specific RT programs and supplementation prescriptions vary among studies. The present study aims to examine the acute and residual effects of RT and/or beta-hydroxy-beta-methylbutyrate (HMB) supplementation on muscle mass, muscle strength, and physical performance in older women with reduced muscle mass.

Methods and analysis

This is a randomized, double-blind, placebo-controlled trial. Older women fitting the eligibility criteria were recruited in February 2018 from a population-based sample identified via a screening conducted in October 2017. In March 2018, the 156 participants were randomly allocated to undergo one of four interventions (RT+HMB, RT+placebo, education+HMB, and education+placebo) for 12 weeks. Supervised RT consisted of body weight, elastic band, ankle weight, and machine-based exercises twice weekly at the Tokyo Metropolitan Institute of Gerontology. Each participant ingested HMB (1,200 mg) or placebo supplements once daily. Sessions of education not associated with sarcopenia treatment were conducted every two weeks. Post-intervention follow-up will be conducted for 12 weeks, until September 2018. The study includes assessments conducted in March (baseline), June (post-intervention), and September 2018 (follow-up). The primary outcome is the longitudinal change in muscle mass. Secondary outcomes include the longitudinal changes in muscle strength, physical performance, muscle thickness, muscle quality, blood counts, blood biochemistry, calf circumference, skin viscoelasticity, habitual dietary intake, habitual physical activity levels, functional capacity, and health-related quality of life. Intention-to-treat analyses will be conducted.

Ethics and dissemination

The study protocol was approved by the Ethics Committee of the Tokyo Metropolitan Institute of Gerontology, Japan. The study is being conducted according to the principles of the Declaration of Helsinki. The findings will be presented at international academic congresses and published in peer-reviewed international journals.

Trial registration

URL: https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000032688

STRENGTHS AND LIMITATIONS OF THIS STUDY

- To the best of our knowledge, this will be the first four-arm trial to examine the effects of resistance training and/or HMB supplementation among older adults with reduced muscle mass.
- The study will collect information about the residual effects of the intervention over an observation period of 12 weeks.
- The trial has a randomized, double-blind, placebo-controlled design, which supports the generalizability of the findings and minimizes the risk of selection, performance, and detection bias.
- In addition to machine-based exercises, the participants will also perform resistance training using body weight, ankle weights, and elastic bands, which will not provide objective information regarding muscle loading but which represents a more suitable prescription for older adults and a more feasible exercise routine to be performed on a daily basis.
- All participants will be older women, which limits generalization of the findings to men.

INTRODUCTION

Sarcopenia is a syndrome characterised by progressive and generalized loss of skeletal muscle mass and strength.[1] In older adults, sarcopenia is associated with adverse health outcomes such as increased risk of incident falls,[2] reduced performance in instrumental or basic activities of daily living, hospitalization, institutionalization, and mortality.[3 4] To reduce the social burden of sarcopenia, it is important to develop prevention and treatment programs aimed to extend healthy life expectancy especially in older people with high risk of sarcopenia.

Most sarcopenia prevention or treatment programs recommend a combination of resistance training (RT) and nutritional supplementation.[5-7] According to a meta-analysis of nine randomized control trials (RCTs) on the combined effect of protein supplementation and RT on muscle outcomes, such strategies may be effective for increasing fat-free mass among older adults.[8] Both the European Working Group on Sarcopenia in Older People and the International Working Group on Sarcopenia, which serve as international advisory committees on sarcopenia, have argued in favour of leucine and beta-hydroxy-beta-methylbutyrate (HMB) supplementation as complementary strategies for sarcopenia prevention or treatment.[9]

HMB is synthesized in skeletal muscle via transamination of leucine to alpha-ketoisocaproic acid (KIC) by aminotransferase.[10] It is estimated that only 5–10% of KIC is metabolized by KIC dioxygenase to produce HMB in the liver.[11] HMB supplementation is believed to exert beneficial effects on skeletal muscle by increasing protein synthesis through activation of the mammalian target of rapamycin and by decreasing protein catabolism through down-regulation of the ubiquitin proteasome pathway.[12] However, there are few studies on the combined effects of RT and HMB supplementation on muscle outcomes in older adults.[13] To our knowledge, two RCTs have examined such effects in elderly populations, but the participants were healthy older adults and the RCTs had a two-arm design.[14 15] Additionally, the residual effects of RT and/or HMB supplementation on muscle outcomes remain unclear. Thus, it is necessary to investigate the combined effect of RT and HMB supplementation in older populations with muscle wasting, and such studies should be designed as four-arm RCTs (RT-only, HMB supplementation-only, both, and none).[9]

The present study aims to examine the acute and residual effects of RT and/or HMB supplementation on muscle mass, muscle strength, and physical performance in older women with reduced muscle mass. We hypothesized that, compared to RT alone, HMB supplementation alone, and placebo, combined RT and HMB supplementation would provide higher benefit in terms of improving and maintaining muscle mass, muscle strength, and physical performance. These findings will provide new evidence regarding the effectiveness of non-pharmaceutical interventions for sarcopenia, and will be useful in the development of sarcopenia prevention and treatment programs in physically frail populations.

METHODS AND ANALYSIS

Study design, procedure, and ethics

In accordance with the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) and CONSORT (Consolidated Standards of Reporting Trials) statements, we designed a randomized, double-blind, placebo-controlled trial to be conducted between March and September 2018 at the Tokyo Metropolitan Institute of Gerontology (TMIG), Tokyo, Japan. The study protocol was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) on 7 August 2017 (trial registration no. UMIN000028560) and was approved by the Ethics Committee of the TMIG on 15 September 2017.

Older women fitting the eligibility criteria were recruited in February 2018, from among a population-based sample identified via a screening conducted in October 2017. After recruitment, a four-arm intervention was conducted between March and June 2018. The participants will be followed until September 2018 to observe the residual effects of the intervention. After the observation period, all interventions will be available to all participants, in agreement with ethical principles. Amendments to the protocol will be disclosed on the UMIN-CTR page of the trial. The study involves assessments conducted in March (baseline), June (post-intervention), and September 2018 (follow-up). Before the baseline assessment, all participants have received written and oral information from the researchers (YO, NK, and KH) regarding the study purpose, procedures, confidentiality of personal information, possible benefits, possible risks, and coping strategy for the risks. Only participants who provided written informed consent proceeded within the trial. The flow chart of the study is shown in Figure 1.

Participants

To select the study sample, a population-based comprehensive geriatric survey was conducted as screening assessment at the TMIG in October 2017. Invitation letters for the screening assessment were sent to 6,366 subjects randomly selected from the Basic Resident Register of the Itabashi ward, which is a special ward located in the northwest area of Tokyo. In total, 1,035 older women participated in the screening assessment. The inclusion criteria of this study were: (i) age ≥ 65 years; (ii) reduced muscle mass, defined as a skeletal muscle index <5.7 kg/m^2, per the sarcopenia diagnosis consensus issued by the Asian Working Group for Sarcopenia[16]; and (iii) informed consent for undergoing screening. The exclusion criteria were: (i) exercise restriction issued by a medical doctor; (ii) use of other supplements known to increase muscle mass; (iii) impaired cardiac, kidney, or liver function; and (iv) judged as ineligible by a medical doctor. A sample of 328 women fitting the eligibility criteria received invitation letters regarding the study intervention, of whom 156 participated in the baseline assessment, whereas the remaining 172 declined to participate due to personal reasons.

Randomization, allocation concealment, and blinding

A baseline assessment was conducted in March 2018. Afterwards, the participants were randomly

allocated to four groups in a ratio of 1:1:1:1 based on a computer-generated randomization number. The allocation was conducted by two researchers from the University of Tsukuba (KW and KT). The allocation keys will be blinded from the researchers at the TMIG (YO, NK, and HK), participants, exercise trainer, analysists, and assessors until December 2018 in order to maintain allocation concealment. The researchers holding the allocation keys will not have contact with the participants.

Intervention

During the intervention and observational periods, all participants were instructed not to change their habitual dietary intake or physical activity levels.

RT program

Participants allocated to the RT program took part in 60-min exercise sessions on two non-consecutive days per week for 12 weeks. All sessions took place at the TMIG, under the supervision of a well-experienced exercise trainer who was not one of the researchers associated with the study. Each exercise session consisted of a 5-min warm-up exercise, a 50-min RT exercise, and a 5-min cool-down exercise. Warm-up and cool-down exercises included stretching activities for the shoulders, trunk, chest, hips, and hamstrings. RT consisted of chair-based (weeks 1–12), elastic band (weeks 5–7), ankle weight (weeks 7–12), and machine-based exercises (weeks 9–12). The chair-based exercise included heel raise, toe raise, knee extension, hip adduction using a rubber ball, and knee lift exercises from the sitting position, as well as squats. Additionally, heel raise and knee lift exercises were performed progressively from a seated to a standing position, and lateral leg raise exercises were performed while standing behind the chair and holding onto the back of the chair. Elastic band exercises included knee lift, hip adduction, and arm rowing exercises. Knee extension exercises in sitting position, as well as heel raise, knee lift, and lateral leg raise exercises in standing position also were performed using ankle weights of 0.5, 0.75, 1.0, or 1.5 kg, according to the physical condition of each participant. In the last 4 weeks of the intervention, the participants performed five types of exercise (arm rowing, leg extension, hip lateral rotation, leg press, and trunk flexion) using dedicated RT machines (Mizuno Ltd., Tokyo, Japan). All exercises were performed as 1–3 sets of 8–10 repetitions with gradual loading. The number of sets and repetitions was increased based on the perceived exertion of each participant. Each movement was performed slowly, in 8 counts (increasing strength in the first 4 counts and decreasing strength in the last 4 counts). The exercise trainer supervised the participants and provided verbal feedback to ensure sufficient tension was achieved in the target muscle groups. Exercise intensity was maintained at 12–14 points on the Borg Rate of Perceived Exertion scale. This RT program was based on a program employed successfully in our previous studies.[17-19]

HMB and placebo supplementation

All participants were instructed to ingest active (calcium HMB) or placebo products provided by Kyowa Co., Ltd., Tokyo, Japan. The active products contain 30 mg of protein, 20 mg of fat, 3,500 mg of carbohydrates, 0.2 mg of sodium, 207 mg of calcium, and 1,200 mg of HMB. Placebo products do not include calcium or HMB, and the missing amounts are provided as carbohydrates. Both products are provided in powder form with the same flavour, appearance, and packaging. The two researchers (KW and KT) maintaining the allocation keys labelled the package with an identification code and sent the products to the researchers at the TMIG (YO, NK, and HK). The participants received the blinded product, which they were instructed to mix with 200 mL of water and ingest once a day after any meal. The participants were instructed to record the ingestion of products in a diary.

While there are no clear recommendations regarding HMB supplementation, Wu et al. suggested a daily dose of 3,000 mg for older adults.[20] However, as body weight is lower among sarcopenic Japanese older women than among western older women,[17 21 22] we set the active dose at 1,200 mg HMB daily based on the positive results we previously obtained in a similar population,[17] which used a daily HMB dosage similar to that used in other studies (0.03 vs. 0.03–0.04 g/kg of body weight).[21 22] No adverse events associated with HMB supplementation have been reported.[23]

Health education program

Participants allocated to the health education groups took part in six 60-min sessions (once every two weeks for 12 weeks) of health education not related to sarcopenia prevention but focused specifically on dementia prevention, prevention of bank transfer fraud, music therapy, nutrition and general health, oral care, and social education, each given at the TMIG by an expert in that field.

Assessments

This study includes assessments conducted in March (baseline), June (post-intervention), and September 2018 (follow-up) at the TMIG. All assessors are TMIG employees who are not researchers and are blinded to group allocation.

Body composition

Body composition assessments involve measuring the following muscle mass indices: whole-body fat-free mass; upper-extremity and lower-extremity lean mass; appendicular lean mass, as the sum of the upper- and lower-extremity lean mass; and skeletal mass index, as the appendicular lean mass divided by the height squared (kg/m^2).[24] The body weight, fat mass, and percent fat are also obtained. Body composition measurements are conducted using the InBody720 device (Biospace Co., Ltd, Seoul, Korea), which was validated against dual-energy X-ray absorptiometry systems for estimating appendicular lean mass in community-dwelling older populations,[25] providing minimal within-day coefficients of variation for all six frequencies (0–1.9%).[26]

Muscle strength

Knee extensor strength

Peak isometric knee extensor strength in the dominant leg is measured in a sitting position. The assessor places the hand-held dynamometer (μTas F-1; ANIMA, Tokyo, Japan) on the skin above the anterior ankle, at a level 5 cm above the tip of the lateral malleolus. The participants are instructed to extend the knee with maximum power starting from a knee joint angle of 90°.[27] After a few practice repetitions, the participants are measured twice, and the best result is retained.

Hip adductor strength

Peak isometric hip adduction strength is also measured in a sitting position. The assessor places the hand-held dynamometer (μTas F-1; ANIMA) at 3 cm proximal to the medial knee fissure and instructs the participant to exert maximal hip adduction force while compressing a 12-cm rod equipped with a dynamometer, keeping both knee joints at 90°.[28] After a few practice repetitions, the participants are measured twice, and the best result is retained.

Handgrip strength

Handgrip strength is measured using a hand-held Smedley-type dynamometer. The participants are instructed to stand naturally, grip the device with their dominant hand, and squeeze as hard as possible.[29] The best result of two trials is retained.

Physical performance

Usual and maximal gait speed

Using a stopwatch, the usual and maximal gait speed are measured as the time taken to walk 5 m (between markers set at 3 m and 8 m of an 11-m walking path)[29] at the usual or maximal speed, respectively. Usual gait speed is measured once. Maximal gait speed is measured twice, and the best result is retained.

Timed up-and-go

This test measures the time taken to stand up from the chair, walk to and around a marker placed 3 m away, return to the chair, and sit back down.[30] The participants are instructed to perform these movements as quickly as possible. The test is performed twice, and the best result is retained.

Five-repetition sit-to-stand

This test measures the time taken to stand up from the chair until full knee and hip extension, sit back down, and repeat this movement five times as quickly as possible.[31] The participants are instructed to fold their arms across the chest, stand-up completely, and make firm contact when sitting. After a few practice repetitions, the test is performed twice, and the best result is retained.

Muscle thickness and quality

Muscle thickness is measured using a B-mode ultrasound device (Mysono U6; Samsung Medison, Seoul, Korea). The participants are instructed to lie on the back in a bed and extend the hip and knee until fully relaxed. The assessor performs a transverse scan by placing a linear probe (5–12 MHz) on the skin surface at the midpoint between the lateral epicondyle and the ipsilateral greater trochanter of the femur, perpendicular to the longitudinal axis of the quadriceps femoris muscle. To measure the thickness of the rectus femoris and vastus intermedius, an electronic calliper is used. Quadriceps femoris thickness is defined as the sum of the thickness of the rectus femoris and that of the vastus intermedius. The muscle quality of the rectus femoris is evaluated on ultrasound images processed using dedicated software (Adobe Photoshop CS6 version 13.0; Adobe Systems, San Jose, CA, USA) and is expressed as the brightness of the image on a scale from 0 (black) to 256 (white). Muscle echo intensity was shown to correlate significantly with interstitial fibrous tissue in muscle biopsy samples.[32] The protocol for assessing muscle thickness and quality is described in detail elsewhere.[33]

Blood biochemistry

Blood samples are collected from the antecubital vein. Analyses are carried out centrally in one laboratory (Health Sciences Research Institute, Inc., Kanagawa, Japan). Enzymatic methods are used to determine creatinine, cholesterol (total, high-density lipoprotein, low-density lipoprotein), triglyceride, blood glucose, and glycated haemoglobin levels. Reference methods recommended by the Japan Society of Clinical Chemistry are used to assess aspartate transaminase, alanine transaminase, lactate dehydrogenase, and creatinine kinase levels. Other assays include the urease method with glutamate dehydrogenase (for blood urea nitrogen), direct colorimetry (for Fe), flow cytometry (for white blood cells), erythrocyte fragility test (for red blood cells), sodium lauryl sulphate method (for haemoglobin), microhematocrit method (for haematocrit), latex agglutination turbidimetry (for cystatin C), nephelometric immunoassay (for high-sensitivity C-reactive protein), immunoradiometric assay (for insulin-like growth factor-1), and chemiluminescence immunoassay (for vitamin B12).

Anthropometric indices

The body mass index (kg/m^2) is calculated as the body weight divided by the body height squared. Plastic tape is used to measure the calf circumference in the non-dominant leg.

Skin viscoelasticity

Skin viscoelasticity is evaluated using the Cutometer® dual MPA 580 (Courage + Khazaka electronic GmbH, Cologne, Germany), which measures the elasticity of the upper layer of the skin using negative pressure to induce mechanical deformation; such measurements are useful for quantitative evaluation of age-related changes in skin elasticity.[34 35] The displacement of the skin at the aperture of the

Cutometer® probe is measured optically, and the resistance of the upper skin layer to the applied negative pressure and the ability to return to the original position are displayed as curves in real time. Skin viscoelasticity is assessed for the skin of the cheek. The participants are instructed to refrain from using makeup on the day of the assessment. The test is performed five times with 10-sec intervals, and the average result is retained.

Habitual dietary intake and physical activity

Habitual dietary intake is measured using a brief-type self-administered diet history questionnaire, which has been validated for estimating the monthly energy intake and the intake of each nutrient factor in older adults.[36 37] These calculations are performed exclusively using software provided by Gender Medical Research Co., Ltd., Tokyo, Japan. Habitual physical activity levels are assessed using the International Physical Activity Questionnaire, which has good reliability and validity for estimating daily physical activity levels.[38 39] We calculate total scores and the scores for each of the four physical activity domains (leisure time, domestic and gardening, occupational, and transport-related physical activity). These evaluations are conducted to determine whether the participants changed their lifestyle patterns during the intervention period.

Functional capacity

Functional capacity is assessed in terms of the instrumental self-maintenance (five items), intellectual activities (four items), and social roles (four items) subscales of the TMIG index of competence. The validity and reliability of the face-to-face assessment of the TMIG index of competence have been demonstrated previously.[40] Participants are asked whether or not they are able to perform the function described by each item, to which they may answer either “yes” (able to do so, 1 point) or “no” (unable to do so, 0 points), for a maximum total score of 13 points, with a higher score indicating better functional capacity.

Health-related quality of life (HR-QoL)

HR-QoL is assessed using the World Health Organization-Five Well-Being Index, which has good validity for assessing mental condition in older adults, and consists of five items representing mood in daily life over the preceding two weeks.[41] The participants are instructed to rate the frequency of the mood from 0 (never) to 5 (all the time), for a maximum score of 25 points, with a higher score indicating better mental condition.

Assessment of adverse events and adherence

Any RT- or supplement-related adverse events occurring during the intervention period are recorded. The affected participants are free to discontinue the trial or continue other interventions (i.e.,

participants with HMB- or placebo-related side effects may continue to perform RT, whereas participants with RT-related injuries may continue to take HMB or placebo). We assess the adherence to the intervention, including RT participation rate and adherence to the daily use of the assigned supplement (based on the participants' daily diary of study product intake). If a participant drops out from the program, the reasons are recorded.

Outcome measures

The primary outcome measure is muscle mass. Secondary outcome measures include muscle strength, physical performance, muscle thickness, muscle quality, blood counts, blood biochemistry, calf circumference, skin viscoelasticity, habitual dietary intake, habitual physical activity levels, functional capacity, and HR-QoL.

Sample size

This study is designed to detect a moderate effect size ($f = 0.25$) for significant interactions between two factors (RT vs education and HMB vs placebo) regarding the longitudinal changes in outcomes. Detecting significant interactions would confirm our hypothesis that, compared to RT alone, HMB supplementation alone, and placebo, combined RT and HMB supplementation provides higher benefit in muscle mass, muscle strength, and physical performance. Setting the two-sided α-error to 0.05 and power to 80%, the minimum sample size required was 31 participants per group. Considering approximately 20% attrition, we aimed to enrol at most 160 participants. The power calculation was conducted using G*Power version 3.1.9.2 for Windows (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany).[42]

Statistical analysis

To promote the collection of quality data, the collected data will be double-checked by the assessors, and a statistician will check the range of values. Between-group comparisons of baseline characteristics will be conducted using one-way analysis of variance (ANOVA) or the Kruskal-Wallis test for continuous variables, and the chi-square test for categorical variables. Continuous data will be expressed as mean and standard deviation or median and interquartile range. Differences in longitudinal mean changes in main and secondary outcomes from baseline to post-intervention will form the primary focus of the analysis. Two-way ANOVA for the changes in outcomes will be applied to test for interactions between two factors (RT vs education and HMB vs placebo). The main effects of RT (RT+HMB and RT+placebo vs education+HMB and education+placebo) and HMB (RT+HMB and education+HMB vs RT+placebo and education+placebo) will also be tested. Within-group changes will be analysed using paired t-tests, while between-group differences in the change in outcomes will be compared using one-way ANOVA. The Scheffe method will be applied for

relationships showing significance on one-way ANOVA. The results will be presented as means and deviations. For subgroup analysis, the participants will be stratified according to sarcopenia status, as defined based on the Asian Working Group for Sarcopenia criteria. The intention-to-treat principle will be applied in all analyses. Missing data will be treated via multiple imputation, in which the Markov Chain Monte Carlo approach will be applied to generate 20 imputed data sets based on the baseline characteristics and the outcome variables. All statistical analyses will be performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). *P*-values < 0.05 will be considered to indicate significance.

Patient and public involvement

This study will be done without participant involvement. Participants will be not invited to comment on the study design and will be not consulted to develop participant relevant outcomes or interpret the results. Participants will be not invited to contribute to the writing or editing of this paper for readability or accuracy.

ETHICS AND DISSEMINATION

The study protocol was approved by the Ethics Committee of the TMIG, and will be conducted in agreement with the Declaration of Helsinki. The findings of this study will be presented at international academic congresses and published in peer-reviewed international journals. Upon publication in a journal, we will also make the findings available on the TMIG homepage.

DISCUSSION

This study addresses the paucity of data regarding the effects of RT and/or HMB supplementation in older women with reduced muscle mass, and the findings will provide insight regarding the potential synergistic effect of RT and HMB supplementation on muscle mass, muscle strength, and physical performance. We will also examine the residual effects of such interventions over a 12-week observation period, as such findings may help understand the potential of non-pharmaceutical treatment for sarcopenia.

Literature reports regarding the individual effect of HMB supplementation on muscle properties and physical performance in older people are encouraging.[21 22 43] However, to our knowledge, only two studies have evaluated the combined effect of RT and HMB supplementation in older populations.[14 15] Specifically, Vukovich et al. enrolled 31 adults aged >70 years and reported significant muscle mass gain after 12 weeks of exercise with HMB supplementation but not after exercise with placebo (*P* for interaction, 0.08).[14] On the other hand, Stout et al. enrolled 36 adults aged >65 years and reported significant improvement in muscle quality, calculated as muscle strength relative to muscle

mass, after 24 weeks of HMB supplementation alone (i.e., no exercise).[15] It remains unclear whether the combined program provides higher benefits. These previous studies had a relatively small sample size, which might increase β-error; moreover, the participants were relatively healthy and had almost normal muscle mass, suggesting that they may have benefited less from HMB supplementation. As completely controlled groups were not previously included, the potential synergy between RT and HMB supplementation remains unclear. The present study will address these limitations and serve as the first investigation of the acute and residual, independent and combined effects of RT and HMB supplementation, which will be conducted in a larger sample of older adults with reduced muscle mass. The randomized, double-blind, placebo-controlled design will minimize the risk of selection, performance, and detection bias, and will support the generalizability of the results. One limitation is that, in addition to machine-based RT, the intervention involves body weight, elastic band, and ankle weight RT, which do not provide objective information regarding muscle loading; on the other hand, such RT programs are more suitable for older adults and will be more feasible in daily practice. Another limitation is that all participants will be older women, which will preclude generalization of our findings to men.

DISCLOSURES

Acknowledgments

We are grateful to the participants and the staff members of the Tokyo Metropolitan Institute of Gerontology.

Authors' contributions

YO and HK conceived the study, designed the study protocol, participated in the coordination of the study (recruitment and screening), and wrote the manuscript. NK participated in the coordination of the study and helped manage data collection. KW and TK contributed to designing the study protocol, conducted the randomization, and have been maintaining allocation concealment. DM contributed to designing the study protocol and negotiated a contract with the provider of the intervention products. All authors read and approved the final manuscript. HK is principal investigator in this trial.

Funding

This study is financially supported by Kyowa Co., Ltd., Tokyo, Japan (no grant number) and is being conducted as a joint research effort of the Tokyo Metropolitan Institute of Gerontology, University of Tsukuba, and Kyowa Co., Ltd.

Competing interests

The intervention products (HMB and placebo supplements) were provided by Kyowa Co., Ltd. DM is employed by Kyowa Co., Ltd. The funder is not involved in subject recruitment, intervention, data collection, data analysis, or preparation of the manuscript.

Patient consent

Obtained.

Ethics approval

Approved by the Ethics Committee of the Tokyo Metropolitan Institute of Gerontology on 15 September 2017.

Data sharing statement

Data collection is ongoing and will be handled anonymously. The results will be offered for publication in a peer-reviewed journal and, afterwards, will be available on the TMIG website.

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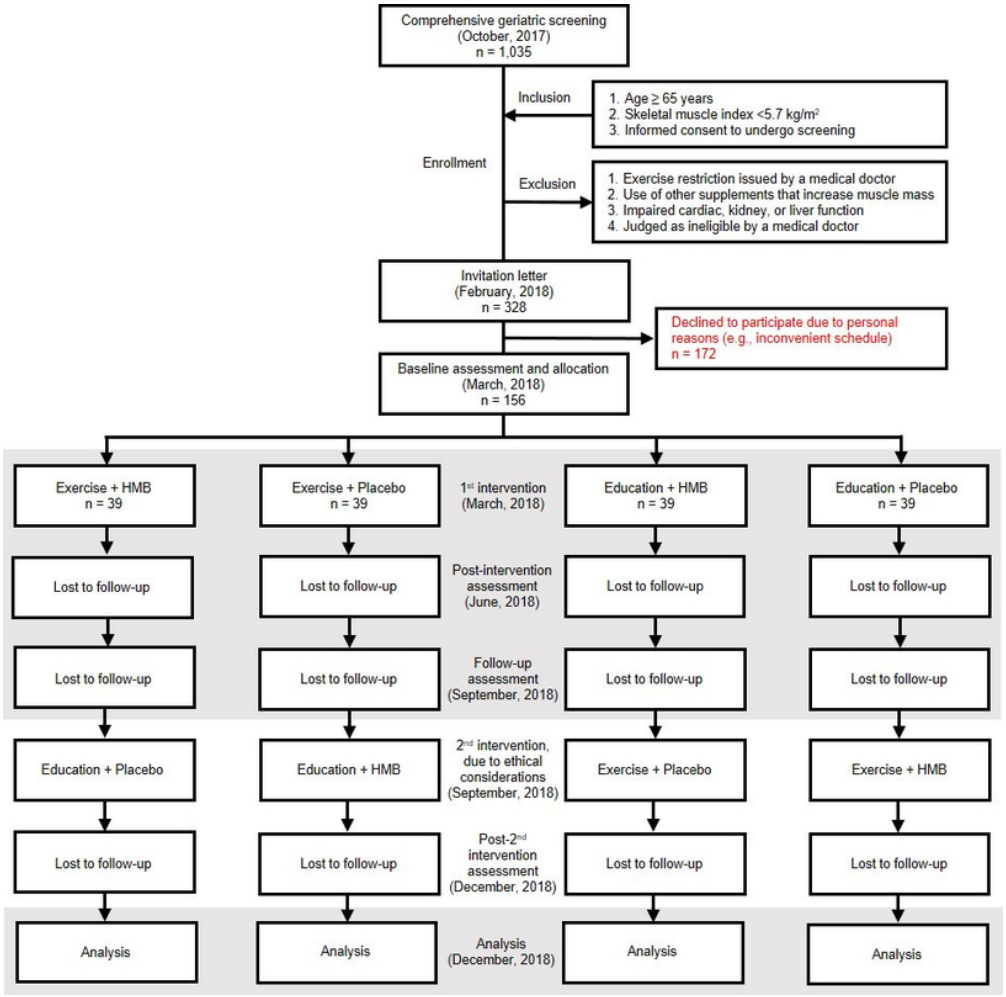
FIGURE LEGENDS

Figure 1. Study flowchart covering participant recruitment and enrolment, group allocation, intervention, observation, and data analysis

A second intervention will be provided after the follow-up assessment, in agreement with ethical principles. However, only data from the highlighted fields will be included in the analysis.



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A second intervention will be provided after the follow-up assessment, in agreement with ethical principles. However, only data from the highlighted fields will be included in the analysis.

68x68mm (300 x 300 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Reference
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	pg. 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	pg. 2
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	pg. 14
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	pg. 1
	5b	Name and contact information for the trial sponsor	pg. 14
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	pg. 14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	pg. 3
	6b	Explanation for choice of comparators	pg. 3
Objectives	7	Specific objectives or hypotheses	pg. 3
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	pg. 3-4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	pg. 4, 5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	pg. 5, Fig. 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	pg. 6, 7; Fig. 1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	pg. 11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	pg. 7, 10, 11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	pg. 6
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	pg. 11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	pg. 5, Fig. 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	pg. 11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	pg. 11
Methods: Assignment of interventions (for controlled trials)			

Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	pg. 6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	pg. 6, 7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	pg. 6, 7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	pg. 6, 7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	pg. 6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	pg. 7-11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	pg. 7, 10, 11
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	pg. 11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	pg. 11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	pg. 11-12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	pg. 12
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	pg. 11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Approved; pg. 5, 12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	pg. 5
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	pg. 14
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	pg. 5
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	pg. 14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	pg. 14
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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Effects of resistance training and/or beta-hydroxy-beta-methylbutyrate supplementation on muscle mass, muscle strength, and physical performance in older women with reduced muscle mass: protocol for a randomized, double-blind, placebo-controlled trial

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Effects of resistance training and/or beta-hydroxy-beta-methylbutyrate supplementation on muscle mass, muscle strength, and physical performance in older women with reduced muscle mass: protocol for a randomized, double-blind, placebo-controlled trial

Yosuke Osuka[1], Narumi Kojima[1], Kyohsuke Wakaba[2], Daiji Miyauchi[3], Kiyoji Tanaka[2], and Hunkyung Kim[1]

[1]Research Team for Promoting Independence of the Elderly, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
[2]Faculty of Health and Sport Sciences, University of Tsukuba, Ibaraki, Japan
[3]Kyowa Co., Ltd., Tokyo, Japan.

Corresponding author
Yosuke Osuka, PhD
Research Team for Promoting Independence of the Elderly
Tokyo Metropolitan Institute of Gerontology
35-2, Sakae-cho, Itabshi-ku, Tokyo, 173-0015, Japan
E-mail: osuka@tmig.or.jp
Phone: +81-3-3964-3241

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ABSTRACT

Introduction

Resistance training (RT) and nutritional supplementation seem to have beneficial effects on muscle properties and physical performance in older adults. However, the reported effects of specific RT programs and supplementation prescriptions vary among studies. The present study aims to examine the acute and residual effects of RT and/or beta-hydroxy-beta-methylbutyrate (HMB) supplementation on muscle mass, muscle strength, and physical performance in older women with reduced muscle mass.

Methods and analysis

This is a randomized, double-blind, placebo-controlled trial. Older women fitting the eligibility criteria were recruited in February 2018 from a population-based sample identified via a screening conducted in October 2017. In March 2018, the 156 participants were randomly allocated to undergo one of four interventions (RT+HMB, RT+placebo, education+HMB, and education+placebo) for 12 weeks. Supervised RT consisted of body weight, elastic band, ankle weight, and machine-based exercises twice weekly at the Tokyo Metropolitan Institute of Gerontology. Each participant ingested HMB (1,200 mg) or placebo supplements once daily. Sessions of education not associated with sarcopenia treatment were conducted every two weeks. Post-intervention follow-up will be conducted for 12 weeks, until September 2018. The study includes assessments conducted in March (baseline), June (post-intervention), and September 2018 (follow-up). The primary outcome is the longitudinal change in muscle mass. Secondary outcomes include the longitudinal changes in muscle strength, physical performance, muscle thickness, muscle quality, blood counts, blood biochemistry, calf circumference, skin viscoelasticity, habitual dietary intake, habitual physical activity levels, functional capacity, and health-related quality of life. Intention-to-treat analyses will be conducted.

Ethics and dissemination

The study protocol was approved by the Ethics Committee of the Tokyo Metropolitan Institute of Gerontology, Japan. The study is being conducted according to the principles of the Declaration of Helsinki. The findings will be presented at international academic congresses and published in peer-reviewed international journals.

Trial registration

URL: https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000032688

STRENGTHS AND LIMITATIONS OF THIS STUDY

- To the best of our knowledge, this will be the first four-arm trial to examine the effects of resistance training and/or HMB supplementation among older adults with reduced muscle mass.
- The study will collect information about the residual effects of the intervention over an observation period of 12 weeks.
- The trial has a randomized, double-blind, placebo-controlled design, which supports the generalizability of the findings and minimizes the risk of selection, performance, and detection bias.
- In addition to machine-based exercises, the participants will also perform resistance training using body weight, ankle weights, and elastic bands, which will not provide objective information regarding muscle loading but which represents a more suitable prescription for older adults and a more feasible exercise routine to be performed on a daily basis.
- All participants will be older women, which limits generalization of the findings to men.

INTRODUCTION

Sarcopenia is a syndrome characterised by progressive and generalized loss of skeletal muscle mass and strength.[1] In older adults, sarcopenia is associated with adverse health outcomes such as increased risk of incident falls,[2] reduced performance in instrumental or basic activities of daily living, hospitalization, institutionalization, and mortality.[3 4] To reduce the social burden of sarcopenia, it is important to develop prevention and treatment programs aimed to extend healthy life expectancy, especially in older people with high risk of sarcopenia.

Most sarcopenia prevention or treatment programs recommend a combination of resistance training (RT) and nutritional supplementation.[5-7] According to a meta-analysis of nine randomized control trials (RCTs) on the combined effect of protein supplementation and RT on muscle outcomes, such strategies may be effective for increasing fat-free mass among older adults.[8] Both the European Working Group on Sarcopenia in Older People and the International Working Group on Sarcopenia, which serve as international advisory committees on sarcopenia, have argued in favour of leucine and beta-hydroxy-beta-methylbutyrate (HMB) supplementation as complementary strategies for sarcopenia prevention or treatment.[9]

HMB is synthesized in skeletal muscle via transamination of leucine to alpha-ketoisocaproic acid (KIC) by aminotransferase.[10] It is estimated that only 5–10% of KIC is metabolized by KIC dioxygenase to produce HMB in the liver.[11] HMB supplementation is believed to exert beneficial effects on skeletal muscle by increasing protein synthesis through activation of the mammalian target of rapamycin and by decreasing protein catabolism through down-regulation of the ubiquitin proteasome pathway.[12] However, there are few studies on the combined effects of RT and HMB supplementation on muscle outcomes in older adults.[13] To our knowledge, two RCTs have examined such effects in elderly populations, but the participants were healthy older adults and the RCTs had a two-arm design.[14 15] Additionally, the residual effects of RT and/or HMB supplementation on muscle outcomes remain unclear. Thus, it is necessary to investigate the combined effect of RT and HMB supplementation in older populations with muscle wasting, and such studies should be designed as four-arm RCTs (RT-only, HMB supplementation-only, both, and none).[9]

The present study aims to examine the acute and residual effects of RT and/or HMB supplementation on muscle mass, muscle strength, and physical performance in older women with reduced muscle mass. We hypothesized that, compared to RT alone, HMB supplementation alone, and placebo, combined RT and HMB supplementation would provide higher benefit in terms of improving and maintaining muscle mass, muscle strength, and physical performance. These findings will provide new evidence regarding the effectiveness of non-pharmaceutical interventions for sarcopenia, and will be useful in the development of sarcopenia prevention and treatment programs in physically frail populations.

METHODS AND ANALYSIS

Study design, procedure, and ethics

In accordance with the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) and CONSORT (Consolidated Standards of Reporting Trials) statements, we designed a randomized, double-blind, placebo-controlled trial to be conducted between March and September 2018 at the Tokyo Metropolitan Institute of Gerontology (TMIG), Tokyo, Japan. The study protocol was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) on 7 August 2017 (trial registration no. UMIN000028560) and was approved by the Ethics Committee of the TMIG on 15 September 2017.

Older women fitting the eligibility criteria were recruited in February 2018, from among a population-based sample identified via a screening conducted in October 2017. After recruitment, a four-arm intervention was conducted between March and June 2018. The participants will be followed until September 2018 to observe the residual effects of the intervention. After the observation period, all interventions will be available to all participants, in agreement with ethical principles. Amendments to the protocol will be disclosed on the UMIN-CTR page of the trial. The study involves assessments conducted in March (baseline), June (post-intervention), and September 2018 (follow-up). Before the baseline assessment, all participants received written and oral information from the researchers (YO, NK, and KH) regarding the study purpose, procedures, confidentiality of personal information, possible benefits, possible risks, and coping strategy for the risks. Only participants who provided written informed consent proceeded within the trial. The flow chart of the study is shown in Figure 1.

Participants

To select the study sample, a population-based comprehensive geriatric survey was conducted as a screening assessment at the TMIG in October 2017. Invitation letters for the screening assessment were sent to 6,366 subjects randomly selected from the Basic Resident Register of the Itabashi ward, which is a special ward located in the northwest area of Tokyo. In total, 1,035 older women participated in the screening assessment. The inclusion criteria of this study were: (i) age ≥ 65 years; (ii) reduced muscle mass, defined as a skeletal muscle index <5.7 kg/m^2, per the sarcopenia diagnosis consensus issued by the Asian Working Group for Sarcopenia[16]; and (iii) informed consent for undergoing screening. The exclusion criteria were: (i) exercise restriction issued by a medical doctor; (ii) use of other supplements known to increase muscle mass; (iii) impaired cardiac, kidney, or liver function; and (iv) judged as ineligible by a medical doctor. A sample of 328 women fitting the eligibility criteria received invitation letters regarding the study intervention, of whom 156 participated in the baseline assessment, whereas the remaining 172 declined to participate due to personal reasons.

Randomization, allocation concealment, and blinding

A baseline assessment was conducted in March 2018. Afterwards, the participants were randomly

allocated to four groups in a ratio of 1:1:1:1 based on a computer-generated randomization number. The allocation was conducted by two researchers from the University of Tsukuba (KW and KT). The allocation keys will be blinded from the researchers at the TMIG (YO, NK, and HK), participants, exercise trainer, analysists, and assessors until December 2018 in order to maintain allocation concealment. The researchers holding the allocation keys will not have contact with the participants.

Intervention

During the intervention and observational periods, all participants were instructed not to change their habitual dietary intake or physical activity levels.

RT program

Participants allocated to the RT program took part in 60-min exercise sessions on two non-consecutive days per week for 12 weeks. All sessions took place at the TMIG, under the supervision of a well-experienced exercise trainer who was not one of the researchers associated with the study. Each exercise session consisted of a 5-min warm-up exercise, a 50-min RT exercise, and a 5-min cool-down exercise. Warm-up and cool-down exercises included stretching activities for the shoulders, trunk, chest, hips, and hamstrings. RT consisted of chair-based (weeks 1–12), elastic band (weeks 5–7), ankle weight (weeks 7–12), and machine-based exercises (weeks 9–12). The chair-based exercise included heel raise, toe raise, knee extension, hip adduction using a rubber ball, and knee lift exercises from the sitting position, as well as squats. Additionally, heel raise and knee lift exercises were performed progressively from a seated to a standing position, and lateral leg raise exercises were performed while standing behind the chair and holding onto the back of the chair. Elastic band exercises included knee lift, hip adduction, and arm rowing exercises. Knee extension exercises in sitting position, as well as heel raise, knee lift, and lateral leg raise exercises in standing position also were performed using ankle weights of 0.5, 0.75, 1.0, or 1.5 kg, according to the physical condition of each participant. In the last 4 weeks of the intervention, the participants performed five types of exercise (arm rowing, leg extension, hip lateral rotation, leg press, and trunk flexion) using dedicated RT machines (Mizuno Ltd., Tokyo, Japan). All exercises were performed as 1–3 sets of 8–10 repetitions with gradual loading. The number of sets and repetitions was increased based on the perceived exertion of each participant. Each movement was performed slowly, in 8 counts (increasing the load throughout the first 4 counts and decreasing it throughout the last 4 counts). The exercise trainer supervised the participants and provided verbal feedback to ensure sufficient tension was achieved in the target muscle groups. Exercise intensity was maintained at 12–14 points on the Borg Rate of Perceived Exertion scale. This RT program was based on a program employed successfully in our previous studies.[17-19]

HMB and placebo supplementation

All participants were instructed to ingest active (calcium HMB) or placebo products provided by Kyowa Co., Ltd., Tokyo, Japan. The active products contain 30 mg of protein, 20 mg of fat, 3,500 mg of carbohydrates, 0.2 mg of sodium, 207 mg of calcium, and 1,200 mg of HMB. Placebo products do not include calcium or HMB, and the missing amounts are provided as carbohydrates. Both products are provided in powder form with the same flavour, appearance, and packaging. The two researchers (KW and KT) maintaining the allocation keys labelled the package with an identification code and sent the products to the researchers at the TMIG (YO, NK, and HK). The participants received the blinded product, which they were instructed to mix with 200 mL of water and ingest once a day after any meal. The participants were instructed to record the ingestion of products in a diary.

While there are no clear recommendations regarding HMB supplementation, Wu et al. suggested a daily dose of 3,000 mg for older adults.[20] However, as body weight is lower among Japanese than among western elderly women,[17 21 22] we set the active dose at 1,200 mg HMB daily based on the positive results we previously obtained in a similar population of sarcopenic women[17] taking HMB at a daily dosage similar to that used in other studies (0.03 vs. 0.03–0.04 g/kg of body weight).[21 22] No adverse events associated with HMB supplementation have been reported.[23]

Health education program

Participants allocated to the health education groups took part in six 60-min sessions (once every two weeks for 12 weeks) of health education not related to sarcopenia prevention but focused specifically on dementia prevention, prevention of bank transfer fraud, music therapy, nutrition and general health, oral care, and social education, each given at the TMIG by an expert in that field.

Assessments

This study includes assessments conducted in March (baseline), June (post-intervention), and September 2018 (follow-up) at the TMIG. All assessors are TMIG employees who are not researchers and are blinded to group allocation.

Body composition

Body composition assessments involve measuring the following muscle mass indices: whole-body fat-free mass; upper-extremity and lower-extremity lean mass; appendicular lean mass, as the sum of the upper- and lower-extremity lean mass; and skeletal mass index, as the appendicular lean mass divided by the height squared (kg/m^2).[24] The body weight, fat mass, and percent fat are also obtained. Body composition measurements are conducted using the InBody720 device (Biospace Co., Ltd, Seoul, Korea), which was validated against dual-energy X-ray absorptiometry systems for estimating appendicular lean mass in community-dwelling older populations,[25] providing minimal within-day coefficients of variation for all six frequencies (0–1.9%).[26]

Muscle strength

Knee extensor strength

Peak isometric knee extensor strength in the dominant leg is measured in a sitting position. The assessor places the hand-held dynamometer (μTas F-1; ANIMA, Tokyo, Japan) on the skin above the anterior ankle, at a level 5 cm above the tip of the lateral malleolus. The participants are instructed to extend the knee with maximum power starting from a knee joint angle of 90°.[27] After a few practice repetitions, the participants are measured twice, and the best result is retained.

Hip adductor strength

Peak isometric hip adduction strength is also measured in a sitting position. The assessor places the hand-held dynamometer (μTas F-1; ANIMA) at 3 cm proximal to the medial knee fissure and instructs the participant to exert maximal hip adduction force while compressing a 12-cm rod equipped with a dynamometer, keeping both knee joints at 90°.[28] After a few practice repetitions, the participants are measured twice, and the best result is retained.

Handgrip strength

Handgrip strength is measured using a hand-held Smedley-type dynamometer. The participants are instructed to stand naturally, grip the device with their dominant hand, and squeeze as hard as possible.[29] The best result of two trials is retained.

Physical performance

Usual and maximal gait speed

Using a stopwatch, the usual and maximal gait speed are measured as the time taken to walk 5 m (between markers set at 3 m and 8 m of an 11-m walking path)[29] at the usual or maximal speed, respectively. Usual gait speed is measured once. Maximal gait speed is measured twice, and the best result is retained.

Timed up-and-go

This test measures the time taken to stand up from the chair, walk to and around a marker placed 3 m away, return to the chair, and sit back down.[30] The participants are instructed to perform these movements as quickly as possible. The test is performed twice, and the best result is retained.

Five-repetition sit-to-stand

This test measures the time taken to stand up from the chair until full knee and hip extension, sit back down, and repeat this movement five times as quickly as possible.[31] The participants are instructed to fold their arms across the chest, stand-up completely, and make firm contact when sitting. After a few practice repetitions, the test is performed twice, and the best result is retained.

Muscle thickness and quality

Muscle thickness is measured using a B-mode ultrasound device (Mysono U6; Samsung Medison, Seoul, Korea). The participants are instructed to lie on the back in a bed and extend the hip and knee until fully relaxed. The assessor performs a transverse scan by placing a linear probe (5–12 MHz) on the skin surface at the midpoint between the lateral epicondyle and the ipsilateral greater trochanter of the femur, perpendicular to the longitudinal axis of the quadriceps femoris muscle. To measure the thickness of the rectus femoris and vastus intermedius, an electronic calliper is used. Quadriceps femoris thickness is defined as the sum of the thickness of the rectus femoris and that of the vastus intermedius. The muscle quality of the rectus femoris is evaluated on ultrasound images processed using dedicated software (Adobe Photoshop CS6 version 13.0; Adobe Systems, San Jose, CA, USA) and is expressed as the brightness of the image on a scale from 0 (black) to 256 (white). Muscle echo intensity was shown to correlate significantly with interstitial fibrous tissue in muscle biopsy samples.[32] The protocol for assessing muscle thickness and quality is described in detail elsewhere.[33]

Blood biochemistry

Blood samples are collected from the antecubital vein. Analyses are carried out centrally in one laboratory (Health Sciences Research Institute, Inc., Kanagawa, Japan). Enzymatic methods are used to determine creatinine, cholesterol (total, high-density lipoprotein, low-density lipoprotein), triglyceride, blood glucose, and glycated haemoglobin levels. Reference methods recommended by the Japan Society of Clinical Chemistry are used to assess aspartate transaminase, alanine transaminase, lactate dehydrogenase, and creatinine kinase levels. Other assays include the urease method with glutamate dehydrogenase (for blood urea nitrogen), direct colorimetry (for Fe), flow cytometry (for white blood cells), erythrocyte fragility test (for red blood cells), sodium lauryl sulphate method (for haemoglobin), microhematocrit method (for haematocrit), latex agglutination turbidimetry (for cystatin C), nephelometric immunoassay (for high-sensitivity C-reactive protein), immunoradiometric assay (for insulin-like growth factor-1), and chemiluminescence immunoassay (for vitamin B12).

Anthropometric indices

The body mass index (kg/m^2) is calculated as the body weight divided by the body height squared. Plastic tape is used to measure the calf circumference in the non-dominant leg.

Skin viscoelasticity

Skin viscoelasticity is evaluated using the Cutometer® dual MPA 580 (Courage + Khazaka electronic GmbH, Cologne, Germany), which measures the elasticity of the upper layer of the skin using negative pressure to induce mechanical deformation; such measurements are useful for quantitative evaluation of age-related changes in skin elasticity.[34 35] The displacement of the skin at the aperture of the

Cutometer® probe is measured optically, and the resistance of the upper skin layer to the applied negative pressure and the ability to return to the original position are displayed as curves in real time. Skin viscoelasticity is assessed for the skin of the cheek. The participants are instructed to refrain from using makeup on the day of the assessment. The test is performed five times with 10-sec intervals, and the average result is retained.

Habitual dietary intake and physical activity

Habitual dietary intake is measured using a brief-type self-administered diet history questionnaire, which has been validated for estimating the monthly energy intake and the intake of each nutrient factor in older adults.[36 37] These calculations are performed exclusively using software provided by Gender Medical Research Co., Ltd., Tokyo, Japan. Habitual physical activity levels are assessed using the International Physical Activity Questionnaire, which has good reliability and validity for estimating daily physical activity levels.[38 39] We calculate total scores and the scores for each of the four physical activity domains (leisure time, domestic and gardening, occupational, and transport-related physical activity). These evaluations are conducted to determine whether the participants changed their lifestyle patterns during the intervention period.

Functional capacity

Functional capacity is assessed in terms of the instrumental self-maintenance (five items), intellectual activities (four items), and social roles (four items) subscales of the TMIG index of competence. The validity and reliability of the face-to-face assessment of the TMIG index of competence have been demonstrated previously.[40] Participants are asked whether or not they are able to perform the function described by each item, to which they may answer either “yes” (able to do so, 1 point) or “no” (unable to do so, 0 points), for a maximum total score of 13 points, with a higher score indicating better functional capacity.

Health-related quality of life (HR-QoL)

HR-QoL is assessed using the World Health Organization-Five Well-Being Index, which has good validity for assessing mental condition in older adults and consists of five items representing mood in daily life over the preceding two weeks.[41] The participants are instructed to rate the frequency of the mood from 0 (never) to 5 (all the time), for a maximum score of 25 points, with a higher score indicating better mental condition.

Assessment of adverse events and adherence

Any RT- or supplement-related adverse events occurring during the intervention period are recorded. The affected participants are free to discontinue the trial or continue other interventions (i.e.,

participants with HMB- or placebo-related side effects may continue to perform RT, whereas participants with RT-related injuries may continue to take HMB or placebo). We assess the adherence to the intervention, including RT participation rate and adherence to the daily use of the assigned supplement (based on the participants' daily diary of study product intake). If a participant drops out from the program, the reasons are recorded.

Outcome measures

The primary outcome measure is muscle mass. Secondary outcome measures include muscle strength, physical performance, muscle thickness, muscle quality, blood counts, blood biochemistry, calf circumference, skin viscoelasticity, habitual dietary intake, habitual physical activity levels, functional capacity, and HR-QoL.

Sample size

This study is designed to detect a moderate effect size ($f = 0.25$) for significant interactions between two factors (RT vs education and HMB vs placebo) regarding the longitudinal changes in outcomes. Detecting significant interactions would confirm our hypothesis that, compared to RT alone, HMB supplementation alone, and placebo, combined RT and HMB supplementation provides higher benefit regarding muscle mass, muscle strength, and physical performance. Setting the two-sided α-error to 0.05 and power to 80%, the minimum sample size required is 31 participants per group. Considering approximately 20% attrition, we aimed to enrol at most 160 participants. The power calculation was conducted using G*Power version 3.1.9.2 for Windows (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany).[42]

Statistical analysis

To promote the collection of quality data, the collected data will be double-checked by the assessors, and a statistician will check the range of values. Between-group comparisons of baseline characteristics will be conducted using one-way analysis of variance (ANOVA) or the Kruskal-Wallis test for continuous variables, and the chi-square test for categorical variables. Continuous data will be expressed as mean and standard deviation or median and interquartile range. Differences in longitudinal mean changes in main and secondary outcomes from baseline to post-intervention will form the primary focus of the analysis. Two-way ANOVA for the changes in outcomes will be applied to test for interactions between two factors (RT vs education and HMB vs placebo). The main effects of RT (RT+HMB and RT+placebo vs education+HMB and education+placebo) and HMB (RT+HMB and education+HMB vs RT+placebo and education+placebo) will also be tested. Within-group changes will be analysed using paired t-tests, while between-group differences in the change in outcomes will be compared using one-way ANOVA. The Scheffe method will be applied for

relationships showing significance on one-way ANOVA. For subgroup analysis, the participants will be stratified according to sarcopenia status, as defined based on the Asian Working Group for Sarcopenia criteria. The intention-to-treat principle will be applied in all analyses. Missing data will be treated via multiple imputation, in which the Markov Chain Monte Carlo approach will be applied to generate 20 imputed data sets based on the baseline characteristics and the outcome variables. All statistical analyses will be performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). *P*-values < 0.05 will be considered to indicate significance.

Patient and public involvement

This study will be conducted without participant involvement. Participants will be not invited to comment on the study design, will not be consulted to define relevant outcomes or interpret the results, and will not be invited to contribute to the writing or editing of this paper for readability or accuracy.

ETHICS AND DISSEMINATION

The study protocol was approved by the Ethics Committee of the TMIG, and will be conducted in agreement with the Declaration of Helsinki. The findings of this study will be presented at international academic congresses and published in peer-reviewed international journals. Upon publication in a journal, we will also make the findings available on the TMIG homepage.

DISCUSSION

This study addresses the paucity of data regarding the effects of RT and/or HMB supplementation in older women with reduced muscle mass, and the findings will provide insight regarding the potential synergistic effect of RT and HMB supplementation on muscle mass, muscle strength, and physical performance. We will also examine the residual effects of such interventions over a 12-week observation period, as such findings may help understand the potential of non-pharmaceutical treatment for sarcopenia.

Literature reports regarding the individual effect of HMB supplementation on muscle properties and physical performance in older people are encouraging.[21 22 43] However, to our knowledge, only two studies have evaluated the combined effect of RT and HMB supplementation in older populations.[14 15] Specifically, Vukovich et al. enrolled 31 adults aged >70 years and reported significant muscle mass gain after 12 weeks of exercise with HMB supplementation but not after exercise with placebo (*P* for interaction, 0.08).[14] On the other hand, Stout et al. enrolled 36 adults aged >65 years and reported significant improvement in muscle quality, calculated as muscle strength relative to muscle mass, after 24 weeks of HMB supplementation alone (i.e., no exercise).[15] It remains unclear whether

the combined program provides higher benefits. These previous studies had a relatively small sample size, which might increase β-error; moreover, the participants were relatively healthy and had almost normal muscle mass, suggesting that they may have benefited less from HMB supplementation. As completely controlled groups were not previously included, the potential synergy between RT and HMB supplementation remains unclear. The present study will address these limitations and serve as the first investigation of the acute and residual, independent and combined effects of RT and HMB supplementation, which will be conducted in a larger sample of older adults with reduced muscle mass. The randomized, double-blind, placebo-controlled design will minimize the risk of selection, performance, and detection bias, and will support the generalizability of the results. One limitation is that, in addition to machine-based RT, the intervention involves body weight, elastic band, and ankle weight RT, which does not provide objective information regarding muscle loading; on the other hand, such RT programs are more suitable for older adults and will be more feasible in daily practice. Another limitation is that all participants will be older women, which will preclude generalization of our findings to men.

DISCLOSURES

Acknowledgments

We are grateful to the participants and the staff members of the Tokyo Metropolitan Institute of Gerontology.

Authors' contributions

YO and HK conceived the study, designed the study protocol, participated in the coordination of the study (recruitment and screening), and wrote the manuscript. NK participated in the coordination of the study and helped manage data collection. KW and TK contributed to designing the study protocol, conducted the randomization, and have been maintaining allocation concealment. DM contributed to designing the study protocol and negotiated a contract with the provider of the intervention products. All authors read and approved the final manuscript. HK is principal investigator in this trial.

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Competing interests

The intervention products (HMB and placebo supplements) were provided by Kyowa Co., Ltd. DM is employed by Kyowa Co., Ltd. The funder is not involved in subject recruitment, intervention, data collection, data analysis, or preparation of the manuscript.

Patient consent

Obtained.

Ethics approval

Approved by the Ethics Committee of the Tokyo Metropolitan Institute of Gerontology on 15 September 2017.

Data sharing statement

Data collection is ongoing and will be handled anonymously. The results will be offered for publication in a peer-reviewed journal and, afterwards, will be available on the TMIG website.

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FIGURE LEGENDS

Figure 1. Study flowchart covering participant recruitment and enrolment, group allocation, intervention, observation, and data analysis

A second intervention will be provided after the follow-up assessment, in agreement with ethical principles. However, only data from the highlighted fields will be included in the analysis.

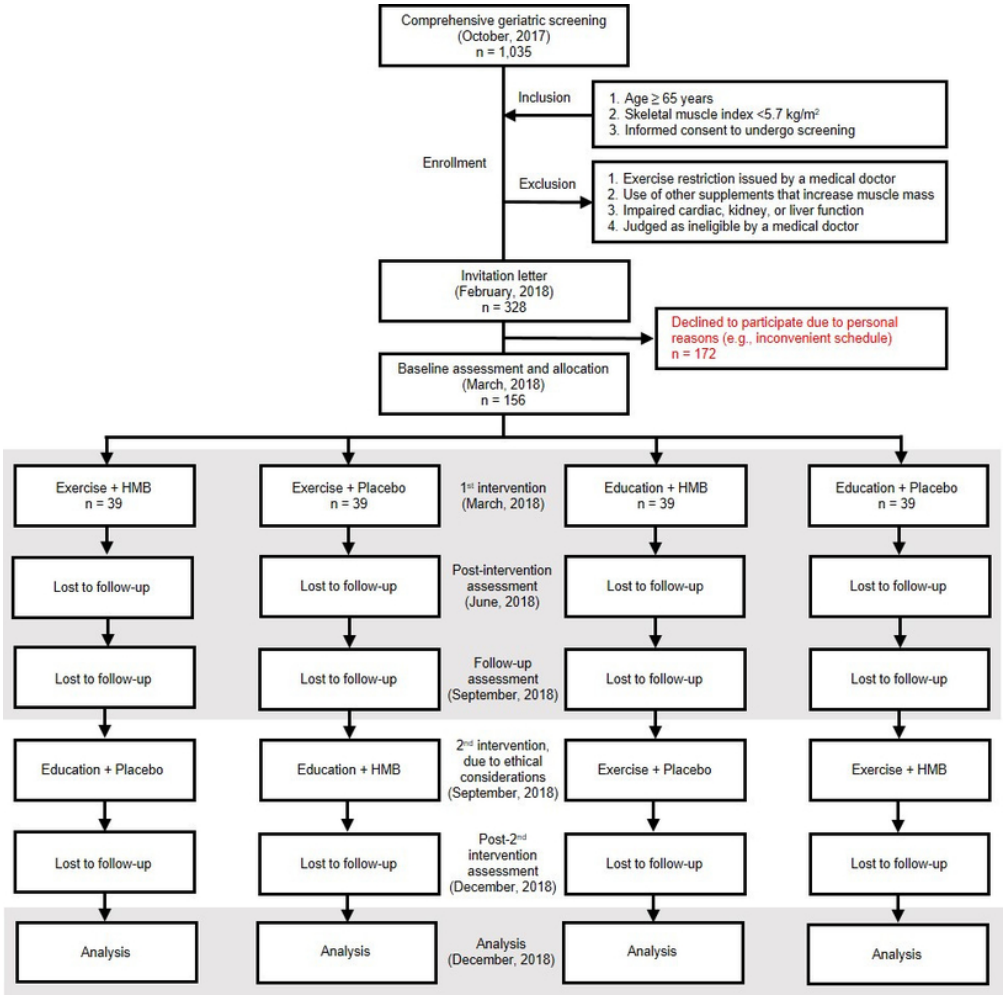

Figure 1. Study flowchart covering participant recruitment and enrolment, group allocation, intervention, observation, and data analysis
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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Reference
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	pg. 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	pg. 2
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	pg. 14
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	pg. 1
	5b	Name and contact information for the trial sponsor	pg. 14
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	pg. 14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	pg. 3
	6b	Explanation for choice of comparators	pg. 3
Objectives	7	Specific objectives or hypotheses	pg. 3
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	pg. 3-4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	pg. 4, 5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	pg. 5, Fig. 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	pg. 6, 7; Fig. 1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	pg. 11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	pg. 7, 10, 11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	pg. 6
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	pg. 11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	pg. 5, Fig. 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	pg. 11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	pg. 11
Methods: Assignment of interventions (for controlled trials)			

Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	pg. 6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	pg. 6, 7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	pg. 6, 7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	pg. 6, 7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	pg. 6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	pg. 7-11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	pg. 7, 10, 11
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	pg. 11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	pg. 11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	pg. 11-12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	pg. 12
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	pg. 11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Approved; pg. 5, 12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	pg. 5
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	pg. 14
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	pg. 5
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	pg. 14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	pg. 14
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	pg. 11

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	pg. 14
	31b	Authorship eligibility guidelines and any intended use of professional writers	pg. 14
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	pg. 5
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.