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Effect of resistance training and lemon myrtle extract on muscle size of older adults: A pilot randomized controlled trial

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ABSTRACT

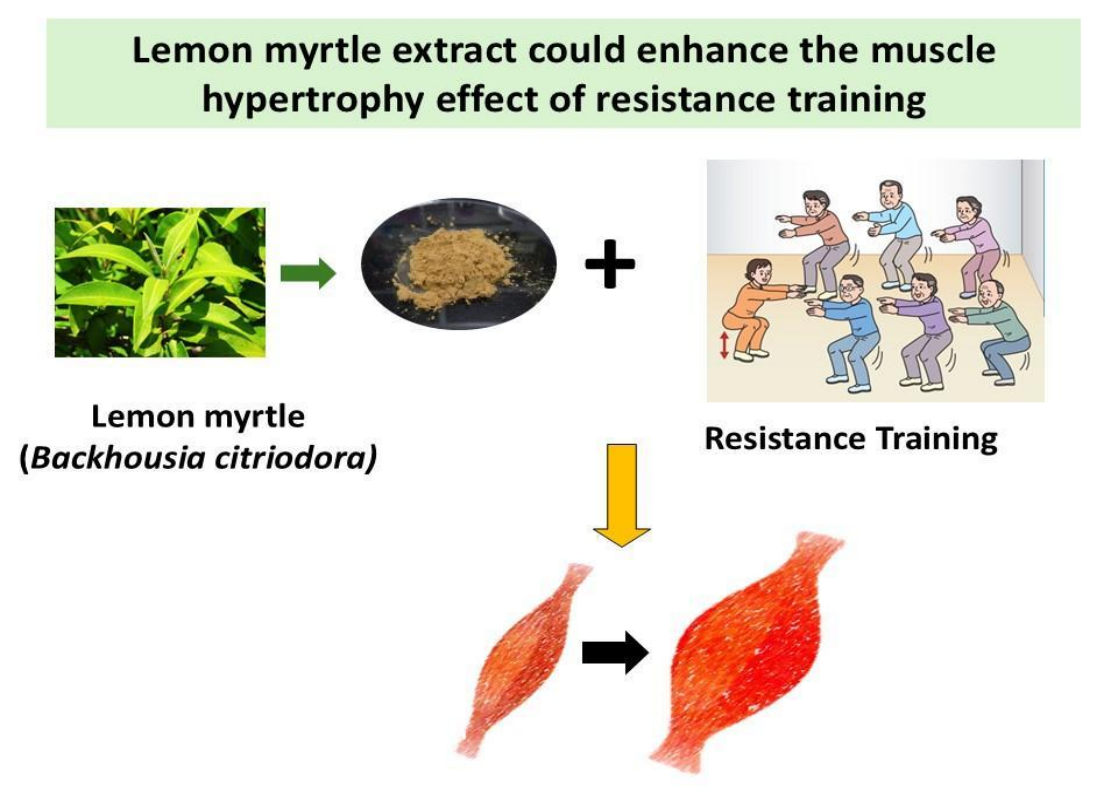
Background: Recent studies have shown that lemon myrtle leaf (LM) extract activates muscle satellite cells. This research examined the impact of combining LM extract supplementation with resistance training on muscle hypertrophy in older adults.

Methods: This randomized controlled trial allocated fourteen older adults (65-79 years) to either an LM supplement group (n=7) or a placebo group (n=7). Participants engaged in low-load resistance training twice weekly for 12 weeks while receiving their designated supplements. Muscle thickness at the anterior thigh (AT) was assessed using B-mode ultrasound at baseline, 6 weeks, and 12 weeks to evaluate muscle hypertrophy. Trial registration: UMIN000050432.

Results: A 12-week intervention yielded significant muscle thickness enhancements in both groups. Nonetheless, the LM group exhibited a substantially greater relative increase ($p < 0.05$) compared to the placebo group. Furthermore, the low-load resistance training program elicited a significant 7.4% increase in muscle thickness in the LM group at six weeks, whereas the placebo group displayed a negligible 0.1% change.

Conclusions: The present study suggests that intake of LM extract could enhance the muscle hypertrophy effect of resistance training and requires further investigation in the future.

Keywords: sarcopenia, plant extract, muscle hypertrophy, lemon myrtle



INTRODUCTION

Skeletal muscles play a pivotal role in facilitating physical functions [1] and regulating systemic energy homeostasis through glucose and lipid metabolism [2]. Nonetheless, aging is associated with a decline in muscle size and strength, exacerbating systemic dysfunction [3-4]. Sarcopenia, characterized by age-related muscle loss, is a significant risk factor for various chronic diseases [5-8], motor disorders [9-10], and mortality [11]. Consequently, sarcopenia has emerged as a primary therapeutic target for improving aging outcomes in older adults. Exercise interventions, particularly resistance training and nutritional optimization, are essential for preventing and managing sarcopenia [12]. Our prior research demonstrated the efficacy of low-load resistance training in inducing muscle hypertrophy in middle-aged and older adults, presenting a viable strategy for mitigating sarcopenia [13].

Recently, skeletal muscle satellite cells (SCs) have attracted increasing attention as a factor that causes sarcopenia. In healthy adults, when skeletal muscles are stimulated by injury or exercise, SCs are activated, and the capacity for muscle restoration is increased. In contrast, older individuals with sarcopenia show decreased SC functionality and loss of muscle mass [14]. In a previous study, we identified a novel functional food ingredient that activated SCs in lemon myrtle (Backhousia citriodora) leaves. Lemon Myrtle belongs to

the genus Backhousia in the family Myrtaceae. It has several biological properties, including antimicrobial [15-16], anti-inflammatory [17-19], and antioxidant activities [18-21]. We found that the aqueous extract of lemon myrtle leaves (LM) and its active compound casuarinin activated skeletal muscle satellite cells in vitro and in vivo [22]. These findings suggest that LM may have potential health benefits as a functional food. Accordingly, we have been developing LM as a functional food product following the guidelines established by the Functional Food Center [23-24]. However, in recent years, research has indicated that activation of muscle satellite cells is a key mechanism underlying skeletal muscle hypertrophy induced by resistance exercise [25]. Building on this evidence, we hypothesized that LM extract can enhance the hypertrophic effects of resistance exercise through activation of muscle satellite cells. Despite the uncertain influence of LM on human skeletal muscle, this investigation examines the combined effects of LM supplementation and resistance training on muscle thickness in older adults.

METHODS

Participants: Japanese older men and women were recruited through a Contract Research Organization. Participants were eligible if they met the following criteria: (1) age ≥ 65 years and (2) perceived deterioration in muscle mass or strength, coupled with reduced ambulatory speed. Conversely, the exclusion criteria were as follows: (1) taking antidiabetic medications; (2) prohibited from exercising by a doctor; (3) symptoms that interfered with training, such as lower back pain or arthralgia; (4) taking supplements, functional foods, or medicines to improve symptoms related to muscle or arthrosis on a daily basis; (5) attending a health club on a daily basis; (6) considerable undernutrition; and (7) judged ineligible by a doctor.

In total, 35 participants agreed to participate in this study. They underwent a screening test (medical examination, blood test, 30-second chair stand test (CS-30), normal walking speed, maximum walking speed, body composition, and questionnaire survey). Following the screening process, 20 participants were selected and randomly assigned to two groups, stratified by age, sex, maximum walking speed, and CS-30 to control for potential biases.

Study design: A randomized, double-blind, placebo-controlled intervention study was meticulously designed to assess the efficacy of LM extract. The investigation adhered strictly to the principles outlined in the Declaration of Helsinki and was conducted under the diligent supervision of the principal investigator. The study protocol, accompanied by pertinent documentation, underwent rigorous review and received approval from the Juntendo University Graduate School of Health and Sports Science Institutional Review Board (Approval No. 2022-131). Participants received comprehensive explanations regarding essential study aspects, including purpose, methodology, potential adverse reactions, and informed consent was secured in writing from each individual. This clinical trial was duly registered with the UMIN Clinical Trials Registry (Identifier: UMIN 000050432). The study was conducted in Tokyo, Japan, between March 2023 and June 2023. Utilizing a randomized controlled intervention design, participants were randomly allocated to one of two groups: a resistance training and placebo intake group (placebo group) or a resistance training and LM intake group (LM group). The present study employed a blinded assessment protocol, wherein researchers evaluating each parameter remained unaware of group assignments. Body composition, muscle thickness, walking speed, and CS-30 were assessed at three distinct time points: baseline (0 weeks), mid-intervention (6 weeks), and post-intervention (12 weeks), concurrent

with the 12-week resistance training program conducted at Sports Oasis Yukigaya's training facility. Participants received either LM or placebo supplements throughout the intervention period. Furthermore, dietary patterns were evaluated utilizing the brief-type self-administered diet history questionnaire (BDHQ) at pre-intervention, mid-intervention, and post-intervention stages to monitor alterations in dietary quality and quantity.

All participants were instructed to engage in a low-load resistance training program using their own body weight and to avoid changing their dietary patterns and lifestyle habits throughout the intervention period. The participants started taking the tablets and taking the course in the resistance training program mentioned below. During the intervention period, they took the tablets daily and participated in the training program twice a week at Sports Oasis Yukigaya.

Resistance training: The resistance training program was implemented over 12 weeks, with two sessions per week, at Sports Oasis Yukigaya's training gymnasium. Conducted by certified sports instructors from Sports Oasis Inc., the program comprised 23 classes. The training regimen consisted of four standardized exercises: squats, bilateral split squats, push-ups, and crunches. All exercises involved the participants' body weights. The order of each exercise, number of repetitions per set, and rest time between sets are listed in Table 1.

Table 1. Training program

Week	1-2			3-4			5-6			7-8			9-12	
Exercises	Order	Sets		Order	Sets		Order	Sets		Order	Sets		Order	Sets
Squat	1	2		1	2		1	3		1	3		3	3
Split squat (right leg)	2	2		2	2		2	2		2	3		1	3
Split squat (left leg)	3	2		3	2		3	2		3	3		2	3
Push-up	4	2		4	2		4	3		4	3		4	3
Crunch	5	2		5	2		5	3		5	3		5	3
Repetitions per set	6			8			8			8			8	
CON-ECC (seconds)	3-3			3-3			3-3			3-3			3-3	
Interval (seconds)	60			60			60			60			60	
Frequency (days/week)	2			2			2			2			2	

CON-ECC: A measure of the duration (in seconds) of both the concentric phase and eccentric phase for each repetition. Interval; rest time between each set.

Test supplement: The lemon myrtle extract used in this study was commercially prepared (Lemon Myrtle UP™, Kaneka Corporation, Osaka, Japan). The preparation was produced by extracting lemon myrtle leaves using boiling water and powdering with dextrin. The casuarinin content, a type of ellagitannin, was certified according to its specifications. The total weight of a LM tablet was 250 mg that consisted of 1.25 mg of casuarinin derived from

LM (Lemon Myrtle UP™) and excipients (crystalline cellulose, maltitol, silicon dioxide, and calcium stearate). The total weight of a placebo tablet was 250 mg that contained only excipients (crystalline cellulose, maltitol, silicon dioxide, and calcium stearate) and caramel color. LM tablet and placebo tablet were indistinguishable in appearance.

During the intervention period, each participant ingested two LM or placebo tablets daily with water for 12 weeks.

Body composition: Body composition parameters, including body weight, fat mass, and percentage body fat (% fat), were quantified utilizing bio-electrical impedance analysis (BIA) with the InBody 270 body composition analyzer (InBody Japan Inc., Tokyo, Japan). Subsequently, Body Mass Index (BMI) was calculated using the standard formula: body weight (kg) divided by height squared (m^2).

Muscle thickness: Muscle thickness measurements were acquired through B-mode ultrasound imaging (LOGIQ e, GE HealthCare Japan, Tokyo, Japan) with a 5-18 MHz scanning head. The measurement site was the anterior aspect of the thigh (AT), specifically the midpoint between the greater trochanter and the lateral condyle of the femur. Participants were instructed to remain seated for a minimum of 30 minutes preceding the measurement and to assume a supine position during the assessment, in accordance with the protocol outlined by Ozaki et al. [13].

Physical functions: Physical function was evaluated using two established assessments: the 10-m walk test and the 30-second chair stand test (CS-30). The walking performance assessment involved timing participants as they walked at maximum speed along a 10-m corridor (1-m width, hard-surfaced floor), with two trials completed. The CS-30 test required participants to perform as many sit-to-stand cycles as possible within 30 seconds, using a chair with a 40-cm seat height and keeping their arms crossed across the chest. The subjects were instructed to sit on a 40-cm stool with both arms crossed against the chest and stand up when the participant's bottom touched the stool as many times as possible in 30 seconds, as outlined by Jones [26]. The test was initiated in the sitting position. The number of stands was recorded as a score.

Brief Diet History Questionnaire (BDHQ): Since it was necessary to correctly evaluate the effect of resistance training or active ingredients to assess the change in food intake during the study period, we chose to administer a brief self-administered diet history questionnaire (BDHQ), which is widely used in Japan. The Brief-Type Self-Administered Diet History Questionnaire (BDHQ) is a comprehensive, four-page instrument designed to assess dietary habits and food intake frequency during the preceding month. The questionnaire employs a selective format and requires approximately 15 minutes to complete. Subsequent nutrient intake calculations were based on standardized Japanese food composition tables.

Statistical analysis: Values are presented as mean ± standard deviation (SD). Baseline parameters were statistically evaluated using Fisher's exact test or unpaired t-tests. Comparisons within groups of the normal and maximum walking speeds and CS-30 scores were performed using paired t-test. Comparison between groups for percent change in anterior thigh muscle thickness at 6 and 12 weeks using unpaired t-test; p-value corrected by the Bonferroni method to account for multiplicity. Statistical significance was defined as $p < 0.05$. Analyses were conducted using Bell Curve for Excel (Social Survey Research Information Co., Ltd., Japan).

Safety assessment: Participants kept diaries to record

their subjective symptoms, medication, and lifestyle habits during intervention period. They were checked and interviewed with the content of their diaries in detail every 4 weeks by investigators to follow their health status. Medical interviews, blood pressure and pulse, and blood tests were examined and recorded at pre- and post-intervention. Detailed information on blood pressure, pulse rates, and blood biochemical parameters is provided in Table S1.

RESULTS

A participant flowchart is shown in Figure 1. A total of 35 older adults were screened, and 20 participants were selected for this study. Of these, two participants in the placebo group dropped out owing to medical problems, sciatica and arrhythmia, respectively, during the intervention, and 18 participants completed the training. Doctors judged these problems occurred independently and were not related to this intervention. The details of adverse events and safety assessment are described in Table S2. To ensure data integrity, four participants were excluded from analysis due to inadequate adherence to the exercise program. Reasons for exclusion included: two participants missing three consecutive sessions (n = 2), one participant concurrently engaging in external exercises (n = 1), and one participant experiencing substantial weight loss (n = 1) before the study began.

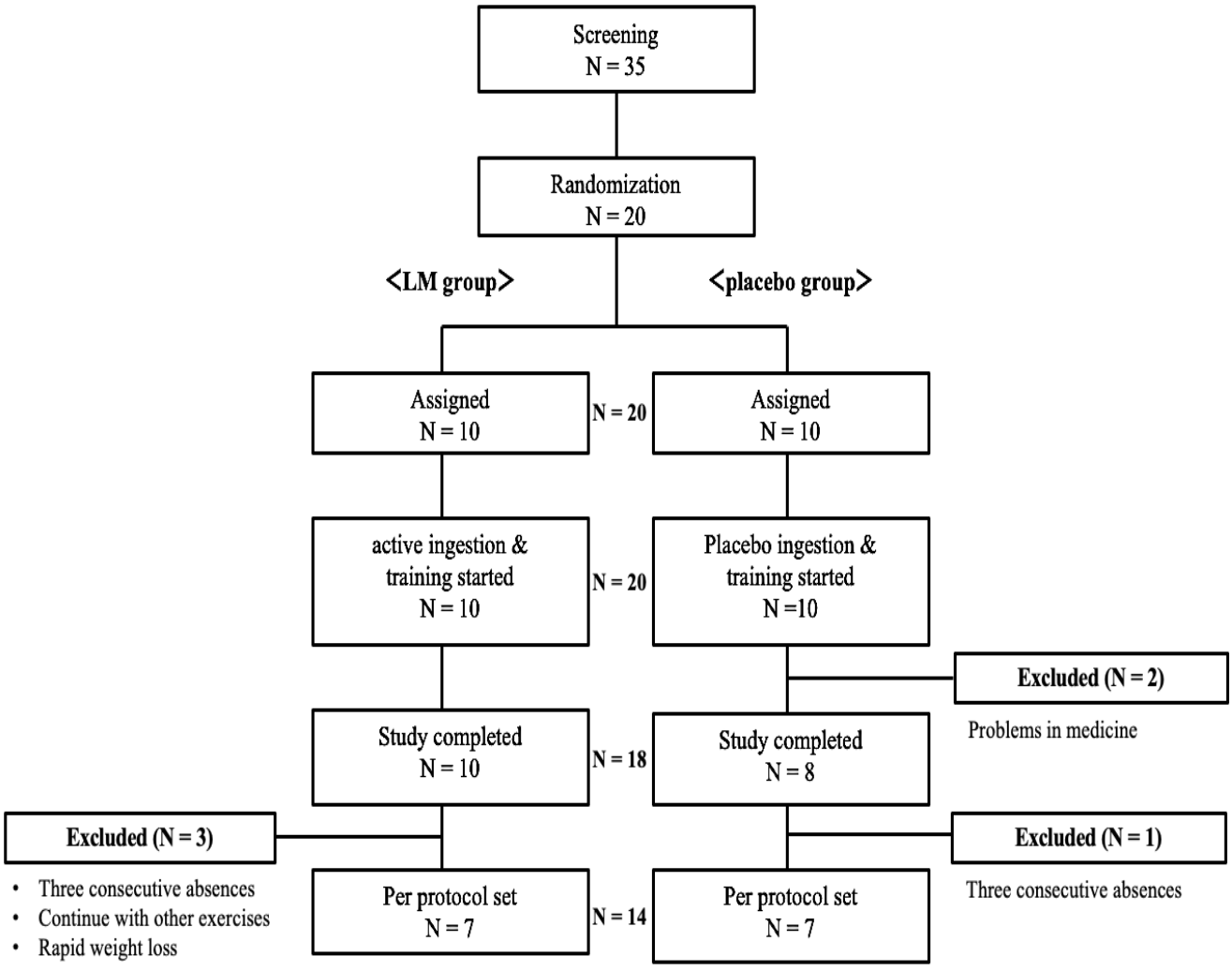


Figure 1. Flowchart showing the distribution of participants through the intervention.

Before intervention: The baseline characteristics of participants are summarized in Table 2. Comparative analysis revealed no statistically significant differences between the Leucine Metabolite (LM) and placebo groups regarding age, body weight, height, body mass index (BMI), muscle thickness, physical function, or dietary intake parameters (daily total energy, protein, fat, carbohydrate, and total calorie consumption) prior to intervention.

Table 2. Baseline characteristics of the participants

	LM	Placebo	*p*-value[1]
Participants (number) [Men: Women]	7 [3:4]	7 [4:3]	1.000
Physical parameters			
Age (year)	72.1 ± 3.8	72.7 ± 4.6	0.805
Body weight (kg)	58.13 ± 11.83	61.43 ± 13.86	0.640
Height (cm)	158.64 ± 8.94	160.37 ± 8.41	0.716
BMI (kg/m^2)	22.91 ± 3.22	23.7 ± 3.83	0.685
Muscle thickness			
Anterior thigh (mm)	23.94 ± 5.65	27.54 ± 6.98	0.310
Physical functions			
Normal walking speed (m/sec)	1.32 ± 0.16	1.34 ± 0.08	0.729
Maximum walking speed (m/sec)	1.74 ± 0.23	1.76 ± 0.11	0.777
CS-30 (times)	20.7 ± 4.2	20.3 ± 4.0	0.849
Food intake			
Energy (kcal/day)	1946.96 ± 601.26	2175.85 ± 486.55	0.449
Protein (g/day)	97.64 ± 36.76	88.58 ± 24.44	0.597
Fat (g/day)	66.21 ± 22.07	66.53 ± 17.18	0.976
Carbohydrates (g/day)	231.32 ± 74.76	282.14 ± 72.89	0.222

Values are presented as the means ± SD. BMI, body mass index; CS-30, the 30-s chair stand test. [1] Comparison between groups using Fisher's exact test or unpaired Student's t-test.

After intervention: After 12 weeks of intervention, both groups showed similar levels of improvement in the normal and maximum walking speeds and CS-30 scores (Table 3). Over the 12-week study period, both groups demonstrated notable gains in adductor thigh (AT) muscle thickness. Importantly, the LM group exhibited a significantly greater relative increase in muscle thickness compared to the placebo group, with a p-value of 0.044 (Figure 2). In addition, at six weeks, increased muscle thickness was observed in the LM group (7.4 ± 4.9 %) but not in the placebo group (0.1 ± 3.7%) (Figure 2). No significant differences in daily total energy, protein, fat, and carbohydrate intake were observed between the LM and placebo groups before and after the intervention (data not shown).

Table 3. Effects of exercise and LM administration on physical functions

			LM (n=7)[1]	Placebo (n=7)[1]	*p*-value[2]
Physical functions					
	Normal walking speed (m/sec)	baseline	1.32 ± 0.16	1.34 ± 0.08	0.729
		6 weeks	1.65 ± 0.18*	1.45 ± 0.18	0.054
		12 weeks	1.65 ± 0.19*	1.57 ± 0.19**	0.425
	Maximum walking speed (m/sec)	baseline	1.74 ± 0.23	1.76 ± 0.11	0.777
		6 weeks	1.99 ± 0.22*	2.00 ± 0.23*	0.888
		12 weeks	2.15 ± 0.23**	2.15 ± 0.30*	0.992
	CS-30 (times)	baseline	20.7 ± 4.2	20.3 ± 4.0	0.849
		6 weeks	22.0 ± 3.2	23.1 ± 2.9	0.495
		12 weeks	23.4 ± 3.6	25.4 ± 4.4	0.370

Values are expressed as mean ± standard deviation (SD). The 30-s chair stand test is denoted as CS-30. Statistical significance within groups versus baseline is indicated by: *$p < 0.05$ and **$p < 0.01$ (paired Student's t-test). Intergroup comparisons were conducted using unpaired Student's t-tests.

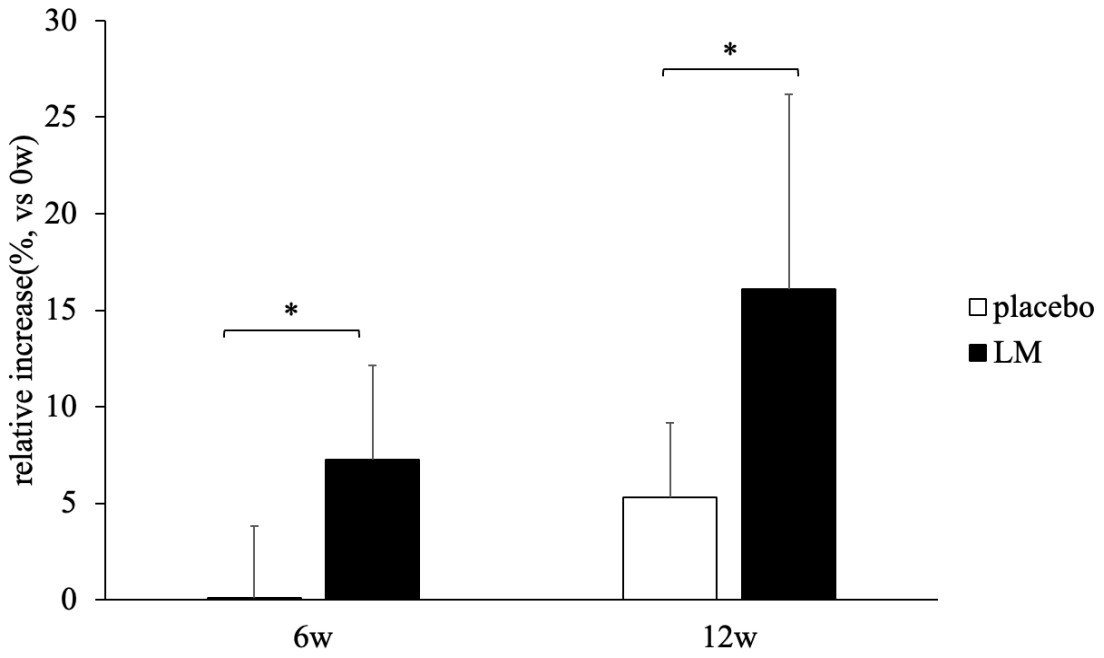


Figure 2. Relative increase of muscle thickness of AT in the LM group and placebo group. Data was analyzed using an unpaired t-test. n=14. *: $p < 0.05$ vs placebo group.

DISCUSSION

This research study sought to explore the potential benefits of combining a 12-week bodyweight-based resistance training regimen with a daily intake of Leucine Metabolite (LM) extract on enhancing muscle size and combating age-related muscle loss in older adults. In this study, resistance training intervention with a placebo increased muscle size. Interestingly, the combination of

resistance training and LM extract intake further increased the muscle size, suggesting that the intake of LM extract enhanced the muscle hypertrophy effect of resistance training. In this rigorously designed study, participants were randomly assigned to receive either LM extract or a placebo supplement, with neither researchers nor participants aware of group assignments, allowing for an unbiased comparison of the effects.

In our preceding investigation [27], we demonstrated that a 12-week low-load resistance training regimen yielded a significant 10% increase in adductor thigh (AT) muscle thickness. This program encompassed nine essential exercises - squats, split squats, push-ups, heel raises, crunches, hip lifts, seated rows, shoulder presses, and arm curls - with a progressive increase in repetitions per set from 8 to 15. In contrast, the placebo group in this study showed a 5% increase in adductor thigh (AT) muscle thickness after 12 weeks of low-load resistance training with four exercises (6-8 repetitions/set).The squats, push-ups, and crunches in this training may represent low-intensity training, as the squats, push-ups, and crunches were reported to be 3.6, 3.0, and 1.9 metabolic equivalents (METs) in older adults, respectively, when estimated on the basis of METs for physical activity [28]. Furthermore, the higher ratio of female participants might result in a greater hypertrophic effect in our previous study. That is 57% for placebo group in the present study, whereas 71% in our previous study [27]. Given the established relationship between relative muscle load and the body mass-to-lower body strength ratio, body mass-based resistance training may offer advantageous muscle hypertrophy outcomes in older women, who characteristically exhibit a reduced lower extremity muscle strength-to-body mass ratio [29]. However, the LM group showed a 15% increase in muscle size after 12 weeks of training. An increase in the muscle size in the LM group was also observed in week 6. Notably, the intervention yielded no significant modifications in daily nutritional intake, specifically regarding total energy, protein, fat, and carbohydrate consumption. These data suggest that intake of LM extract enhances the muscle hypertrophy effect of resistance training and may be useful for the prevention of sarcopenia.

According to medical interview and blood test at pre and post intervention for participants, there were no clinically problematic abnormalities. In addition, we checked subject diaries for 12 weeks intervention period. The number of adverse events was similar between the groups (10 in the LM group vs. 13 in the placebo group) and all adverse events were classified as unrelated to the training and LM supplementation. Therefore, there was no concern on health when older adults orally administrate 250 mg of LM extracts per day for 12 weeks. The present study possessed several limitations. Primarily, the absence of a non-exercise control group necessitates a cautious interpretation of the training effects. Nevertheless, informed by our preceding investigation [27], it is reasonable to infer that the exercise program employed in this study elicited muscle hypertrophic effects. Additionally, participants were instructed to adhere to their customary dietary patterns and lifestyle habits throughout the study duration. Second, the results were descriptive and did not address the mechanisms underlying muscle hypertrophy in the LM group. Based on previous studies, LM may affect muscle satellite cells, which play important roles in muscle hypertrophy. However, this has not been demonstrated in humans. Third, one of the primary limitations of this study is restricted to be relatively small and including only older adults who perceived deterioration in muscle mass or strength, coupled with reduced ambulatory speed. Research has consistently shown that a multifaceted approach, integrating exercise and nutrition, yields the most effective outcomes in addressing sarcopenia. To build upon these findings,

subsequent studies should strive to enroll larger participant cohorts or multigenerational participants.

CONCLUSIONS

In conclusion, the present study suggests that intake of LM extract could enhance the muscle hypertrophy effect of resistance training and requires further investigation in the future.

SUPPLEMENTARY MATERIALS

Table S1. Physiological, blood biochemistry, and hematological parameters for the study population

		Active (n=7)	Placebo (n=7)	p -value[1]
Physiological parameters				
Systolic blood pressure (mmHg)	baseline	129.3 ± 5.7	130.6 ± 12.8	0.812
	12 weeks	136.3 ± 8.6	131.4 ± 6.0	0.243
Diastolic blood pressure (mmHg)	baseline	73.6 ± 7.3	76.1 ± 10.5	0.604
	12 weeks	72.1 ± 6.5	73.0 ± 6.9	0.816
Pulse (bpm)	baseline	79.7 ± 11.2	77.9 ± 15.2	0.800
	12 weeks	70.7 ± 12.1*	73.9 ± 9.3	0.596
Blood biochemical parameters				
TG (mg/dL)	baseline	101.3 ± 25.7	111.7 ± 27.3	0.476
	12 weeks	85.3 ± 27.2	92.6 ± 16.6*	0.557
T-Cho (mg/dL)	baseline	217.9 ± 27.8	235.1 ± 18.2	0.194
	12 weeks	223.7 ± 22.5	210.0 ± 26.0**	0.312
HDL-Cho (mg/dL)	baseline	75.9 ± 16.8	63.3 ± 13.6	0.149
	12 weeks	76.3 ± 14.7	60.7 ± 13.0	0.057
LDL-Cho (mg/dL)	baseline	126.1 ± 25.5	151.4 ± 22.2	0.071
	12 weeks	127.7 ± 24.7	126.0 ± 23.9**	0.897
Glu (mg/dL)	baseline	91.7 ± 9.2	94.9 ± 6.3	0.470
	12 weeks	94.7 ± 11.6	95.3 ± 9.9	0.923
HbA1c (%)	baseline	5.47 ± 0.30	5.5 ± 0.4	1.000
	12 weeks	5.63 ± 0.43	5.5 ± 0.4	0.512
AST (U/L)	baseline	22.1 ± 4.3	21.3 ± 4.2	0.713
	12 weeks	22.9 ± 4.0	20.1 ± 2.7	0.163
ALT (U/L)	baseline	16.1 ± 6.6	13.0 ± 4.9	0.331
	12 weeks	18.1 ± 4.9	12.7 ± 3.9	0.037
γ-GT (U/L)	baseline	31.1 ± 23.0	28.9 ± 17.7	0.839
	12 weeks	32.4 ± 23.7	29.0 ± 18.0	0.765
ALP (U/L)	baseline	65.1 ± 10.9	59.7 ± 24.2	0.598
	12 weeks	68.7 ± 13.2*	59.0 ± 24.6	0.375
LDH (U/L)	baseline	194.7 ± 32.3	190.4 ± 20.2	0.771
	12 weeks	201.7 ± 35.2	195.7 ± 24.2	0.717
T-Bill (mg/dL)	baseline	1.03 ± 0.18	0.81 ± 0.22	0.069
	12 weeks	1.14 ± 0.42	0.87 ± 0.16	0.133
TP (g/dL)	baseline	7.06 ± 0.35	7.14 ± 0.39	0.670
	12 weeks	7.13 ± 0.34	7.20 ± 0.38	0.719
ALB (g/dL)	baseline	4.26 ± 0.24	4.17 ± 0.21	0.484
	12 weeks	4.30 ± 0.28	4.19 ± 0.27	0.457
UA (mg/dL)	baseline	4.86 ± 0.97	5.17 ± 1.23	0.606
	12 weeks	4.91 ± 1.03	5.16 ± 1.27	0.701
BUN (mg/dL)	baseline	13.20 ± 3.66	17.14 ± 3.68	0.068
	12 weeks	15.09 ± 2.29	14.90 ± 2.11	0.877
CRE (mg/dL)	baseline	0.69 ± 0.18	0.83 ± 0.13	0.116
	12 weeks	0.71 ± 0.18*	0.85 ± 0.19	0.171
Hematological parameters				
WBC (/mL)	baseline	4742 ± 850	5800 ± 1070	0.063
	12 weeks	4642 ± 969	6114 ± 2327	0.148
RBC ($\times 10^4$/mL)	baseline	415.1 ± 25.3	421.6 ± 73.1	0.830
	12 weeks	424.0 ± 26.9	413.0 ± 46.9	0.600
Hb (g/dL)	baseline	12.97 ± 0.80	12.54 ± 2.70	0.694
	12 weeks	13.10 ± 0.64	12.07 ± 2.41	0.296
Ht (%)	baseline	40.47 ± 2.43	38.89 ± 7.85	0.619
	12 weeks	41.79 ± 2.20**	38.69 ± 6.41	0.249
PLT ($\times 10^4$/mL)	baseline	23.94 ± 4.00	30.00 ± 7.62	0.087
	12 weeks	23.30 ± 4.53	29.13 ± 6.23	0.068

Values are presented as the means ± SD.

[1]Comparison between groups using unpaired Student's t-test.

*P < 0.05 and **P < 0.01 versus baseline within the group (paired Student's t -test).

Table S2. The details of adverse events

Adverse event (Case)	LM (n = 10)	Placebo (n = 10)
muscle pain	9	5
cough	0	2
abdominal pain	0	1
arrhythmia	0	1
headache	0	1
malaise	1	0
sciatica	0	1
stiff shoulder	0	1
toothache	0	1
Total	10	13

Abbreviations: LM, lemon myrtle leaf; AT, the anterior aspect of the thigh; SC, satellite cell; CS-30, 30-second chair stand test; BDHQ, the brief-type self-administered diet history questionnaire; BIA, bioelectrical impedance analysis; BMI, body mass index; SD, standard division; METs, metabolic equivalents.

Competing Interests: AN, SH and YT are employees of the Kaneka Corporation (Osaka, Japan), which funded this study. They were responsible for the test supplements but were not involved in the investigation, formal analysis, or data curation. The results of this study are as follows: clearly and without fabrication, falsification, or inappropriate data manipulation.

Authors' Contributions: A.N., S.H., Y.T., and S.M. research concepts; S.S., H.O., and S.M. designed research; A.N., S.H., and Y.T. test supplements; S.S., Shi.M., H.O., and S.M. performed experiments; S.S., H.O., and S.M. analyzed data; S.S., H.O., and S.M. interpreted results of experiments; S.S. and S.M. prepared figures; S.S., H.O., and S.M. drafted manuscript; S.S., A.N., S.H., Shi.M., H.O., and S.M. edited and revised manuscript; S.S., A.N., S.H., Y.T., Shi.M., H.O., and S.M. approved final version of manuscript.

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