The Journal of Physical Therapy Science

Original Article

Effects of water exercise on body composition and components of metabolic syndrome in older females with sarcopenic obesity

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Abstract. [Purpose] Very few studies have been conducted on the benefits of water exercise for older adults with sarcopenic obesity. Whether the water exercise intervention is effective for improving sarcopenia and/or obesity remains unclear. This study aimed to investigate the effects of water exercise on body composition and components of metabolic syndrome in older females with sarcopenic obesity. [Participants and Methods] Participants (aged \geq 60 years) were divided into a water exercise group and a control group. Water-based strength and endurance exercises were performed three times a week for 12 weeks. Lean soft tissue mass, fat mass, and body fat percentage were measured by dual-energy x-ray absorptiometry. [Results] Two-way analysis of variance revealed significant interactions (time \times group) for total body fat percentage and leg body fat percentage. In the exercise group, leg body fat percentage significantly decreased after the intervention, but no significant change was observed in the control group. The components of metabolic syndrome showed no significant interactions in either group (time × group). [Conclusion] No significant changes were observed in the components of metabolic syndrome. However, 12-week water exercise may be effective for reducing fat mass in females with sarcopenic obesity. Key words: Body composition, Sarcopenic obesity, Water exercise

(This article was submitted Aug. 18, 2022, and was accepted Oct. 3, 2022)

INTRODUCTION

Sarcopenic obesity is defined as a combination of sarcopenia¹), an age-related loss of skeletal muscle mass and strength, and obesity²). Sarcopenic obesity has been reported to significantly increase all-cause mortality, increase the risk of physical dysfunction relative to sarcopenia alone³⁾, and have a closer association with metabolic syndrome than either sarcopenia or obesity alone⁴⁾.

Strategies to prevent or improve sarcopenic obesity include exercise interventions, such as resistance exercise and aerobic exercise. In general, high-intensity resistance exercise is required to increase skeletal muscle mass, even in older adults⁵). However, a previous systematic review on the effects of progressive resistance training has provided evidence that, although effective in improving the physical functioning of older adults, the use of progressive resistance training as a clinical intervention for older patients requires caution because adverse events have not been adequately reported⁶). In fact, 86 of the 121 studies reviewed did not address adverse events or reactions associated with the exercise training⁶. Moreover, previous studies have reported that the risk of falls increases with age and is higher in obese people and females than in non-obese people and males⁷).

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Water exercise is effective for middle-aged and older adults, even for those with reduced lower limb muscle strength, joint diseases with accompanying lower limb joint pain, and obesity^{8, 9)}. In a previous study, water exercise performed twice a week for 12 weeks significantly improved maximal oxygen uptake and lower limb muscle strength in postmenopausal females¹⁰⁾. Another study reported that 12-week water exercise significantly increased lower limb muscle strength and ankle plantar flexion in older adults aged ≥ 65 years¹¹⁾. Water exercise is thus considered an effective intervention for improving muscle strength and cardiopulmonary fitness in older adults¹²⁾. Moreover, in obese people, 13-week water exercise led to significant decreases in body weight and body fat percentage (BFP)¹³⁾. Furthermore, a study examining the effects of water exercise on the components of metabolic syndrome in patients with coronary artery disease¹⁴⁾ reported significant improvements in blood lipid profiles, such as reduced total cholesterol and triglycerides (TG).

At present, however, evidence is lacking regarding the benefits of water exercise for older adults with sarcopenic obesity, and whether the water exercise intervention is effective for improving sarcopenia and/or obesity remains unclear. Accordingly, the present study aimed to investigate the effects of water combined exercise on body composition and components of metabolic syndrome in Japanese females with sarcopenic obesity, with the hypothesis that water exercise can improve the components of metabolic syndrome, increase muscle mass, and decrease BFP in older sarcopenic obese patients.

PARTICIPANTS AND METHODS

Participants were recruited through a newspaper advertisement for an water exercise class and health and physical fitness tests in Kusatsu city. A total of 36 people participated in a briefing session. Exclusion criteria in this study were the following: 1) Less than 60 years.

2) Patients with secondary obesity due to adrenal gland disease, heart disease, severe liver dysfunction, cirrhosis, or severe renal dysfunction.

3) Females who are pregnant or suspected of being pregnant

4) Patients who are going to the hospital for orthopedics or have limited exercise

Of the 36 participants, 12 attended the water exercise class, and 24 underwent health and physical fitness tests. In this study, we divided into the water exercise group (n=9) and control group (n=11), because nine of those who attended the water exercise group and 11 in the control group met the criteria for sarcopenic obesity. One person who was taking medications for hypercholesterolemia and allergic rhinitis was included in the water exercise group, but no side effects (i.e., weight gain or loss) were observed. All participants gave written informed consent before participation. This study was approved by the Ethics Committee of Ritsumeikan University (IRB-BKC-2018-060-2).

The exercise intervention was performed for 12 weeks (3 times/week) with the guidance of an instructor at a fitness facility. The average water temperature was 30°C, and the water level was fixed at below or near the xiphoid process.

Table 1 shows the combined water exercise protocol. The training intensity was set to 13 to 15 points on the 6 to 20 point of the ratings perceived exertion (RPE). The intensity was set at 13 and was progressed to 15 by week 4. Heart rate immediately after aerobic exercise was monitored once a week and increased from 102.0 ± 12.0 beats/minute in the first week to 123.0 ± 11.7 beats/minute the final week. Participants of both groups maintained their daily dietary intake and physical activity throughout the 12-week period.

	Exercise	Time	Sets	
Warm up	Underwater walking and stretching	7.5 min	×1 set	
Resistance exercise	Hip flexion and extension (right)	1 min		
	Hip flexion and extension (left)	1 min		
	Rest	30 s		
	Knee flexion and extension (right)	1 min	×3 sets	
	Knee flexion and extension (left)	1 min		
	Rest	30 s		
		Total 5 min		
Aerobic exercise	Stride walk	3 min		
	Kick walk	3 min		
	Twist walk	3 min	×1 set	
	Lateral walk	3 min		
	Jogging	3 min		
		Total 15 min		
Cool down	Underwater walking and stretching	7.5 min	×1 set	

Table 1	Water	evercise	protocol
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Participants underwent physical fitness tests in the laboratory before and after intervention in the early morning after fasting for ≥ 8 hours. Height, body weight, and abdominal circumference were measured, and body mass index (BMI) was calculated from the measured height and body weight. Resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) and ankle-brachial pulse wave velocity (baPWV) were also measured (form PWV/ABI; Omron Korin Co., Ltd., Kyoto, Japan).

Fasting (>8 h) blood samples were collected from participants in the seated position by venipuncture in tubes with or without ethylene diamine tetraacetic acid (for plasma or serum). Serum total cholesterol was measured by cholesterol dehydrogenase spectrophotometry (UV method), serum high-density lipoprotein cholesterol (HDL-C) was measured by a direct method, and TG was measured by an enzyme method (GK-GOP/free glycerol elimination). Fasting plasma blood glucose level was analyzed by the hexokinase UV method, and glycohemoglobin level (hemoglobin A1c; HbA1c) was analyzed by the latex immunoturbidimetric method. Blood tests were outsourced to Medic Inc, Shiga, Japan. The components of metabolic syndrome examined in this study included abdominal circumference, TG, HDL-C, fasting blood glucose level, SBP, and DBP¹⁵⁾.

Body composition was measured by dual energy X-ray absorptiometry (DXA; Lunar Prodigy, GE Healthcare Japan). Lean soft tissue mass (LSTM), body fat mass, and BFP of the total body, arms, trunk, and legs were measured to determine sarcopenia, and differences in training effects were examined by site.

Obesity was defined as a BFP of <30% as measured by DXA. A previous study on DXA-measured body composition in Asians reported that a BFP of roughly 30% corresponded to the Japanese obesity standard (BMI=25)¹⁶). Sarcopenia was defined as a skeletal muscle index (SMI, height-adjusted appendicular skeletal muscle mass) of <6.12 kg/m² as measured by DXA^{17, 18}). At present, no clear criteria for sarcopenic obesity exist, and sarcopenic obesity is defined by a number of different measurement methods for sarcopenia and obesity¹⁹). The Japanese Association on Sarcopenia and Frailty recommends using the criteria of the Asian Working Group for Sarcopenia (AWGS)²⁰). However, we could not use this criteria, because the prevalence of sarcopenia according to the AWGS criteria in Asian older adults is very low (4–11%)²¹).

Maximal voluntary contraction (MVC) was measured to determine the relative intensity of knee extension exercise using knee extension equipment (S-16216; Takei Kiki Kogyo Co., Ltd., Niigata, Japan)²²⁾. Participants were seated on a chair with their trunk vertically bent at both hips and knees at 90 degrees. The knee extension equipment was fixed to the legs of the chair. MVC was measured twice for 2–3 seconds with a 1-min rest between each measurement. The higher value of the two measurements was used as the isometric muscle strength.

To assess exercise function, grip strength, vertical jump, one-leg stand, timed up and go, 2-step value, and usual walking speed were measured. Grip strength was measured using a grip strength meter (GRIP-D; Takei Kiki Kogyo Co., Ltd.) twice each on the left and right sides. The mean value of each higher value was adopted. Vertical jump was measured using a digital vertical jump measuring device (Jump-MD; Takei Kiki Kogyo Co., Ltd.). The measurement was performed twice, and the higher value was adopted as the measurement value. Five-meter walking speed was measured as the time required to walk 5 m between 3 m and 8 m lines on a walking path 11 m in length. As a measure of balance, the one-leg stand test was performed with eyes open, and the time it took for the feet to touch the ground was measured. The measurement was performed twice and the maximum value was adopted as the measurement value, with 2 minutes being the longest. As a measure of mobility, the timed up and go test was performed to assess the time (seconds) it took to get up from a chair, walk 3 m, return to the chair, and sit down. The measurement was performed twice and the higher value was calculated as the distance of 2 steps (from the start line, with both toes aligned) divided by height²³. The measurement was performed twice, and the higher value of the two measurements was adopted.

Physical activity was measured before and after the exercise intervention to examine whether changes in daily life had any impact. The average number of steps taken per day was measured with a 3-axis accelerometer (HJA-350IT; Omron Healthcare Co., Ltd., Kyoto, Japan). Participants wore the accelerometer on the hip during waking hours (starting from when they woke up and ending at bedtime), except during water exercise. Data were collected for 7 days or more (at least 10 hours for each day) including Saturdays and Sundays.

A brief self-administered diet history questionnaire (BDHQ) was used to collect dietary intake data. The BDHQ asks about weekly dietary habits during the last month. The validity of the questionnaire has been confirmed in a previous study²⁴.

All data are presented as mean \pm SD. Primary outcomes were body composition (total body/arm/trunk/leg LSTM, body fat mass, and BFP) and the components of metabolic syndrome (abdominal circumference, TG, HDL-C, fasting blood glucose, SBP, and DBP). The main effect and interaction (time \times group) between the water exercise group and the control group were analyzed by repeated two-way ANOVA. The post-hoc test (Bonferroni) was performed only when the interaction was significant. In addition, the effect size (η^2) over time was calculated to determine the effect that was not affected by the sample size. The IBM SPSS statistics software package (version 24) was used for all statistical analyses, with the p-value set to 5%.

RESULTS

All 9 participants in the water exercise group completed the 12-week exercise intervention and participated in pre- and post-intervention measurements (Table 1). The participation rate in the water exercise group was 95.7%. In the control group, all 11 participants underwent pre- and post-intervention measurements. A significant time main effect was observed for the average number of steps per day before and after intervention, with no significant interaction (time × group, Table 2).

Table 3 shows changes in muscle mass and fat mass. No change in SMI $\geq 6.12 \text{ kg/m}^2$ was observed after intervention in both groups, and there was no difference in the prevalence of sarcopenia (100%) between the two groups. No significant time main effect or interaction (time × group) was observed for total body and regional LSTM, SMI, total body fat mass, trunk fat mass, arm fat mass, arm BFP, and trunk BFP.

Table 3 also shows changes in the components of metabolic syndrome. In both groups, the components of metabolic syndrome showed no significant interaction (time \times group). A significant time main effect was observed for lipid intake (p<0.05), but no significant time main effect or interaction was observed for other variables of nutritional intake.

	Exercise (n=9)		Control		
	Pre	Post	Pre	Post	Effect size (η^2)
Age (years)	67.6 ± 5.2	67.8 ± 5.1	66.9 ± 5.4	67.2 ± 5.5	0.004
Height (cm)	155.9 ± 3.8	156.1 ± 3.7	153.9 ± 8.0	153.9 ± 8.0	0.029
Weight (kg)	53.1 ± 5.9	52.6 ± 5.6	55.6 ± 7.4	55.6 ± 8.3	0.039
BMI (kg/m ²)	21.9 ± 3.0	21.7 ± 2.9	23.5 ± 2.6	23.4 ± 2.2	0.097
baPWV (cm/s)	$1,\!653.8\pm 300.6$	$1,\!553.0\pm237.5$	$1,\!603.8\pm228.4$	$1,\!507.8\pm215.2$	0.082
Daily steps (steps/day)	$5,\!263.7\pm3,\!240.9$	$6,\!457.8 \pm 2,\!261.8$	$4,\!066.7\pm2,\!388.3$	$4,\!930.9 \pm 2,\!402.1$	0.048
Energy intake (kcal/day)	$1,\!597.8\pm 530.5$	$1,740.7 \pm 561.1$	$1,\!745.2\pm 652.2$	$1,\!646.0\pm544.7$	0.001

Table 2. Changes in physical characteristics and nutritional status in the two groups of participants

A significant time main effect was observed for the average number of steps per day before and after intervention, with no significant interaction (time \times group).

BMI: body mass index; baPWV: ankle-brachial pulse wave velocity. Mean \pm SD.

Table 3.	Changes	in body	composition and	l components of	metabolic syndrome i	n the two groups	s of participants
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	Exercise (n=9)		Control	- Effect - (-2)	
	Pre	Post	Pre	Post	- Effect size (η ²)
Body composition					
Lean soft tissue mass (kg)					
Total	32.3 ± 2.2	32.3 ± 2.3	32.6 ± 3.4	32.5 ± 3.7	0.00
Trunk	15.8 ± 1.0	12.2 ± 3.1	16.0 ± 1.2	12.1 ± 3.2	0.39
Legs	10.2 ± 1.0	10.1 ± 0.9	10.5 ± 1.2	10.4 ± 1.4	0.01
Arms	3.1 ± 0.3	3.1 ± 0.2	3.3 ± 0.4	3.3 ± 0.4	0.06
SMI (kg/m ²)	5.5 ± 0.5	5.4 ± 0.5	5.8 ± 0.3	5.7 ± 0.3	0.15
Fat mass (kg)					
Total	18.9 ± 4.5	18.4 ± 4.6	20.8 ± 5.1	21.2 ± 5.2	0.06
Trunk	11.0 ± 2.9	10.9 ± 3.1	10.7 ± 3.4	11.2 ± 4.0	0.00
Legs	5.3 ± 1.4	5.0 ± 1.3	7.3 ± 1.8	7.2 ± 1.6	0.33
Arms	1.8 ± 0.4	1.7 ± 0.5	2.0 ± 0.5	1.9 ± 0.4	0.07
Body fat percentage (%)					
Total	35.3 ± 4.7	34.7 ± 5.2	37.2 ± 4.9	37.8 ± 4.5	0.07
Trunk	39.7 ± 5.5	39.2 ± 6.2	39.0 ± 5.1	40.0 ± 5.3	0.00
Legs	32.7 ± 5.7	$31.6\pm5.6^{\ast}$	39.3 ± 6.7	39.3 ± 6.6	0.27
Arms	34.2 ± 3.2	33.6 ± 4.7	35.5 ± 5.6	35.8 ± 4.0	0.04
Components of metabolic syndrome					
Waist circumference (cm)	86.0 ± 6.8	84.4 ± 7.7	87.1 ± 11.1	87.0 ± 10.0	0.01
TG (mg/dL)	137.3 ± 45.5	139.7 ± 51.3	107.6 ± 38.6	99.3 ± 40.9	0.17
HDL-C (mg/dL)	62.1 ± 7.9	62.9 ± 8.7	76.2 ± 22.6	76.4 ± 17.2	0.19
FBG (mg/dL)	101.9 ± 24.6	107.1 ± 36.7	109.1 ± 33.2	98.8 ± 13.9	0.00
HbA1c (%)	5.8 ± 0.8	6.0 ± 1.5	5.8 ± 0.7	5.6 ± 0.4	0.01
SBP (mmHg)	127.9 ± 19.9	123.4 ± 20.4	136.4 ± 17.6	134.7 ± 15.8	0.13
DBP (mmHg)	72.9 ± 8.9	70.3 ± 7.5	80.0 ± 7.6	75.5 ± 6.2	0.01

A significant time main effect was observed for lipid intake (p<0.05), but no significant time main effect or interaction was observed for body composition and components of metabolic syndrome.

SMI: skeletal mass index; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; FBG: fasting blood glucose; HbA1c: hemoglobin A1c; SBP: systolic blood pressure; DBP: diastolic blood pressure. Mean \pm SD. *p<0.05 vs. Pre.

Significant interaction (time × group) was observed for Total BFP and Leg BFP. Leg BFP in the water exercise group was significantly decreased after the intervention ($31.6 \pm 5.6\%$) compared to before the intervention ($32.7 \pm 5.7\%$), but no significant difference was observed in the control group. The effect size (η^2) was moderate at 0.072 (≥ 0.06) for total body BFP, and large at 0.265 (≥ 0.14) for leg BFP.

No significant interaction (time \times group) was observed for MVC, but a significant time main effect was observed in both exercise and control groups (Table 4).

DISCUSSION

The present study investigated the effects of 12-week water combined exercise on body composition and components of metabolic syndrome in females with sarcopenic obesity. The main results are as follows: 1) A significant interaction (time \times group) was observed for total body BFP and leg BFP; in the water exercise group, leg BFP was significantly decreased after the intervention, whereas no significant change was observed in the control group, 2) total and regional LSTM showed no significant time main effect or interaction (time \times group), 3) components of metabolic syndrome showed no significant time main effect or interaction (time \times group). The present study is the first to demonstrate the effects of water exercise on body composition and components of metabolic syndrome in older females with sarcopenic obesity, revealing body fat loss to be the main effect of the exercise intervention.

A previous study found no significant changes in BMI before and after a 48-week underwater walking intervention (27.3 vs. 26.4 kg/m²), while significant decreases in waist circumference (89.4 vs. 87.1 cm) and lower limb lean mass (13.8 vs. 13.5 kg) were observed after the intervention²⁵⁾. In that study, 72 participants were randomized to land-walking, waterwalking, and control groups in a supervised center-based program. The exercise groups trained 3 times a week at a matched intensity, with an increase in heart rate reserve from 40-45% to 55-65%. The authors speculated that the water-walking intervention might have led to more beneficial changes in body composition and lean mass distribution due to the effect of water resistance, relative to non-exercise (control) and land-walking interventions. Another study that compared changes in body composition in females after 8 weeks of underwater walking training²⁶ found a significant decrease in body cell mass relative to baseline (36.2 vs. 33.6%), but no changes in BMI (27.8 vs. 28.1 kg/m²) and total BFP (33.8 vs. 34.3%). These findings suggest that underwater walking may be beneficial for abdominal or total body fat loss in healthy middle-aged and older females, but no effect is expected on muscle mass changes. Meanwhile, there is also a report that 6 months of aquaaerobic training led to significant decreases in body composition elements including BMI (26.2 vs. 24.9 kg/m²), total BFP (35.3 vs. 33.7%), and total lean mass (45.9 vs. 42.4 kg) in middle-aged overweight females²⁷⁾. At present, it remains unclear whether water exercise is beneficial for older females with sarcopenia/obesity. In the present study, leg BFP, but not BMI or total/regional LSTM, was significantly decreased after 12 weeks of water exercise in older females with sarcopenic obesity. Trunk LSTM tended to be decreased, but this may be related to a decrease in visceral fat since trunk LSTM measured by DXA includes visceral fat mass²⁸⁾. The probable reason why muscle mass in present study did not decrease may be related to the fact that the subjects in present study were originally subjects with low muscle mass.

In a previous study that examined the effects of 12-week water exercise (3 times/week for 60 minutes) on muscle strength in healthy older adults aged \geq 65 years, significant increases in the peak torque of hip extension, hip flexion, and ankle plantar flexion were observed¹¹). Similarly, in a study targeting older females aged 60–75 years, 12-week water exercise (3 times/ week for 70 minutes) significantly increased the muscle strength of knee extension and flexion²⁹. These previous studies reporting improved muscle strength after water exercise used an intervention period of 12 weeks, with an exercise frequency of 3 times/week at an intensity of 12 to 16 RPE, which are similar to the conditions used in the present study. While no changes in LSTM were observed after the intervention in the present study, a significant time main effect of MVC was observed in both groups, suggesting that water exercise may help improve qualitative factors, such as the central nervous system, of muscle strength in sarcopenic obese females.

Table 4.	Changes in	exercise	runction i	in the two	o groups of	i participants	

	Exercise (n=9)		Contro	Effect	
	Pre	Post	Pre	Post	Effect size
Handgrip strength (kg)	23.7 ± 3.7	23.5 ± 2.9	22.8 ± 3.6	21.8 ± 4.2	0.034
Vertical jump (cm)	25.4 ± 3.2	25.3 ± 4.7	20.9 ± 5.8	21.7 ± 5.1	0.062
Usual gait speed (m/s)	1.5 ± 0.2	1.6 ± 0.2	1.4 ± 0.1	1.5 ± 0.1	0.033
2 steps length (cm/cm)	1.2 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	0.032
One leg stand (s)	36.6 ± 25.0	44.6 ± 20.7	50.5 ± 19.6	46.3 ± 19.9	0.042
MVC (N•m)	91.9 ± 48.1	$144.0\pm49.9\texttt{*}$	83.9 ± 41.5	$110.9\pm43.7\texttt{*}$	0.059

Significant interaction (time × group) was observed for Total and leg body fat percentage. Leg body fat percentage in the water exercise group was significantly decreased after the intervention $(31.6 \pm 5.6\%)$ compared to before the intervention $(32.7 \pm 5.7\%)$, but no significant difference was observed in the control group. MVC: maximal voluntary contraction. Mean \pm SD. *p<0.05 vs. Pre.

In the present study, 12-week water exercise (3 times/week for 45 minutes) did not show a significant main effect or interaction (time × group) with components of metabolic syndrome (Table 2). Among previous studies that examined the effects of water exercise on components of metabolic syndrome, one study targeting older people with type 2 diabetes found a significant reduction in HbA1c after 12-week water aerobic exercise (3 times/week for 30 minutes at a 70% maximum heart rate)³⁰⁾. In middle-aged and older adults with hypertension, SBP and DBP were significantly decreased after 12-week water exercise (3 times/week for 60 minutes), but significant changes were also observed in the control group³¹). Barakat et al. compared cross-sectional results from 3 experimental studies examining the effects of land, in-water, and mixed form (land + in-water) exercises during healthy pregnancy on maternal and newborn outcomes. They reported that total maternal weight gain differed between the land exercise and control groups (11.7 vs. 13.4 kg), as well as the percentage of pregnant females with excessive weight gain (20.6% vs. 37.9%); however, no differences were observed between the water exercise group and the control group. While the beneficial effects of water exercise on body composition in healthy older females have been suggested based on limited evidence from water-based studies, the impact of water exercise on metabolic profile and body weight remains unclear³²). Colado et al. investigated the effects of 24-week aquatic exercise on markers of cardiovascular health³³) and concluded that aquatic exercise can significantly decrease body fat and DBP, but not total cholesterol, HDL-cholesterol, LDL-cholesterol, TG, and blood glucose. In the present study, water exercise had no effect on improving the components of metabolic syndrome in sarcopenic obese females, possibly because the participants were healthy community-dwelling older adults with normal (or close to normal) values of metabolic syndrome risk factors. Further studies will be needed to accumulate evidence regarding the effects of water exercise on cardiometabolic risks in older females with sarcopenia and sarcopenic obesity.

This study has some limitations. First, the present study was not a randomized controlled study. In addition, the sample size was relatively small, with only 9 people allocated to the water exercise group. However, the effect size (η^2) of leg BFP, i.e., the primary outcome of this study, was large at 0.265. Second, since male participants were not included, it is unclear whether similar results would be obtained for men. Given that clear gender differences exist in body composition and components of metabolic syndrome^{34, 35}, a future study is warranted to clarify the effects of water exercise in men, including those with sarcopenic obesity.

In this study, no significant changes were observed in the components of metabolic syndrome. However, 12-week water exercise may be effective for reducing fat mass in females with sarcopenic obesity. Exercise prescription in water exercise for older sarcopenic obesity females may be effective for weight loss but not for muscle mass gain, so additional resistance training may be advisable.

Authors' contribution

Study concept and design: Dr. KS and Ms. SQ. Acquisition of subjects and/or data: Ms. KK and Dr. NH. Analysis and interpretation of data: Dr. MM and Dr. MI. Preparation of manuscript: Dr. KS and Ms. SQ.

Funding

This work was supported by the Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (K Sanada, #24300239).

Conflicts of interest

None.

ACKNOWLEDGEMENTS

We thank the participants in this study.

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