Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 9 Issue 12 December 2025

Research Article

Effect of Agaro-Oligosaccharide Intake on Prevention of Muscle Strength Decline in Older Women: A Randomized Controlled Trial

Rina Kurasawa[1], Hiroyuki Hasegawa[2], Suguru Kurokawa[1,2], Koji Karasawa[3], Toshiyuki Ochi[3], Takahiro Kubota[4] and Ryoji Hirota[5]*

[1]Graduate School of Health Sciences, Matsumoto University, Nagano, Japan

[2]Faculty of Human Health Sciences, Matsumoto University, Nagano, Japan

[3]R&D Division, Ina Food Industry Co., Ltd., Nagano, Japan

[4]Department of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Niigata University of Pharmacy and Medical and Life Sciences, Niigata, Japan

[5]Department of Hygiene, Kitasato University School of Medicine, Kanagawa, Japan

***Corresponding Author:** Ryoji Hirota, Department of Hygiene, Kitasato University School of Medicine, Kanagawa, Japan.

DOI: 10.31080/ASMS.2025.09.2179

Received: October 14, 2025

Published: November 10, 2025

Abstract

Background: Frailty and sarcopenia are major health issues among older women. Agaro-oligosaccharides (AGO), low-molecular-weight carbohydrates derived from agarose, exhibit anti-inflammatory and antioxidant activities that may support muscle maintenance.

Methods: In this 12-week randomized, double-blind, placebo-controlled trial, 23 community-dwelling older women (AGO n = 12; placebo n = 11) received AGO (500 mg/day) or placebo. Outcomes included 10-m walking speed, grip strength, 30-second chair-stand test (CS-30), timed up-and-go (TUG), and lower-limb circumferences.

Results: At week 6, the AGO group showed a greater increase in left-hand grip strength than placebo (Δ = +1.2 ± 1.5 kg vs −0.5 ± 1.4 kg; p = 0.020) and a greater increase in right calf circumference (Δ = +0.6 ± 1.3 cm vs −1.1 ± 1.3 cm; p = 0.008). In the pre-frail subgroup (frailty score ≥4; n = 9), left-hand grip strength improved significantly with AGO (Δ = +1.8 ± 1.8 kg; p = 0.049). Between-group differences were not significant at week 12, although directions of change remained favorable in the AGO group. No serious adverse events occurred.

Conclusions: AGO supplementation (500 mg/day) was well tolerated and associated with short-term improvements in muscle strength and calf circumference among older women, with signals stronger in pre-frail participants. Larger, longer trials are warranted to confirm durability and clinical relevance.

Keywords: Agaro-Oligosaccharides; Sarcopenia; Frailty; Older Women; Randomized Controlled Trial; Muscle Strength

Abbreviations

AGO: Agaro-oligosaccharides; CS-30: Chair-Stand Test; TUG: Timed Up and Go

Introduction

Japan is among the most rapidly aging societies worldwide. Frailty and sarcopenia diminish QOL and elevate disability risk in older adults, representing an urgent public health challenge. Older women are particularly susceptible to age-related losses in muscle mass and strength, highlighting the need for effective, sustainable preventive strategies [10,11].

Nutritional approaches to frailty prevention have gained attention. Agaro-oligosaccharides (AGO) are low-molecular oligosaccharides produced from agarose via acid or enzymatic hydrolysis [6,14,20]. AGO have been reported to improve gut ecology [25,26], exert anti-inflammatory activity [5,10], and display antioxidant effects [15,24]. Importantly, low-molecular AGO can cross intestinal epithelia and enter the circulation in animals and humans, suggesting the potential for systemic actions [21,22].

In vivo studies indicate that AGO may strengthen the intestinal barrier, reduce inflammation, and mitigate oxidative stress—mechanisms plausibly linked to attenuation of muscle loss and weakness [13,24]. Human data remain limited: plasma absorption of low-molecular AGO after oral intake has been demonstrated in healthy adults [21], and improved gait speed has been reported in older men in a prior report [16]. However, effects of AGO on muscle function in older women are insufficiently characterized.

We conducted a randomized, double-blind, placebo-controlled trial to examine whether 12-week AGO intake (500 mg/day) attenuates muscle strength decline in community-dwelling women aged ≥65 years and to evaluate AGO as a feasible nutritional strategy for frailty prevention. Preliminary evidence in prefrail older men has suggested that AGO supplementation is feasible and may yield functional improvements under randomized allocation [17]. These findings provided a rationale for testing AGO in older women to evaluate sex-specific effects and confirm reproducibility in a randomized controlled setting.

Materials and Methods

Study design, registration, and ethics

This 12-week, double-blind, randomized, placebo-controlled clinical trial was conducted between July and October 2024 in Nagano Prefecture, Japan.

Ethics approval and trial registration

This 12-week, double-blind, randomized, placebo-controlled clinical trial was conducted between July and October 2024 in Nagano Prefecture, Japan. The protocol was approved by the Research Ethics Committee of Matsumoto University (Approval No. 156) and registered in the UMIN Clinical Trials Registry (UMIN000055055). All participants provided written informed consent before enrollment.

Study tablets

The agaro-oligosaccharide (AGO)-containing tablets and placebo tablets used in this study were manufactured and provided by Ina Food Industry Co., Ltd. (Ina, Nagano, Japan) according to the formulation shown in Table 1. Both types of tablets were identical in appearance and taste. The AGO tablets were designed to contain 500 mg of AGO per daily dose (six tablets), whereas the placebo tablets contained the same excipients without AGO.

Ingredient	AGO tablets	Placebo tablets
Agaro-oligosaccharides	51	–
Dextrin	12	62.5
Sorbitol	34	34
Sucrose fatty acid ester	3	3
Sodium citrate	–	0.5
Total	**100**	**100**

Table 1: Composition of agaro-oligosaccharide (AGO) and placebo tablets (%, w/w).

Participants

Community-dwelling women aged ≥65 years were recruited through public health outreach in Matsumoto City, Saku City, and surrounding areas.

Inclusion criteria were: (1) age ≥65 years; (2) independence in activities of daily living including unassisted ambulation; and (3) no use of nutritional supplements targeting muscle function within the prior 3 months.

Exclusion criteria were: (1) severe chronic disease (e.g., advanced cardiovascular disease, renal failure); (2) recent musculoskeletal injury; (3) cognitive impairment that would preclude informed consent or adherence; (4) known allergy to agar/AGO; and (5) concurrent participation in another clinical trial.

At baseline, frailty status was assessed using the Japan Geriatrics Society Frailty Checklist. Participants with scores ≥4 (pre-frail/frail) were identified a priori for subgroup analyses.

Randomization, allocation concealment, and blinding

Participants were randomized (1:1) to AGO or placebo using concealed allocation. Participants, investigators, and assessors were blinded throughout the trial.

Outcome measures

- **10-meter walking speed (m/s):** Measured at habitual pace using a clearly marked 10-m course with acceleration and deceleration zones at either end. Timing was performed with a handheld stopwatch; the mean of repeated trials was recorded when feasible.
- **Anthropometrics and Blood Pressure:** Height and weight measured with calibrated instruments; BMI calculated as kg/m^2. Seated blood pressure measured using an automated sphygmomanometer after ≥5 minutes of rest.
- **Pain Assessment:** Self-reported knee and low-back pain using a 0–10 visual analogue/numeric rating scale (higher scores indicating greater pain).
- **Physical Function and Muscle Strength:** Handgrip strength (left/right): Assessed with a Smedley-type dynamometer; the best of two trials per hand was recorded.
- **30-second chair-stand test (CS-30):** Number of full stands completed in 30 seconds from a standard chair without using arms.
- **Timed Up and Go (TUG):** Time to stand from a chair, walk 3 m, turn, return, and sit.
- **Isometric Knee Extensor Strength:** Measured using a leg-press dynamometer when available, following standardized positioning and instruction.
- **Limb Circumferences:** Mid-thigh and calf circumferences measured with a non-stretchable tape at consistent anatomical landmarks.

Visit schedule and assessments

Participants were assessed at baseline (week 0), week 6, and week 12. All measurements were performed by trained staff using standardized protocols and the same devices across visits when possible. Adverse events and concomitant medications were reviewed at each visit.

Sample size calculation

- The sample size was calculated based on the effect estimates reported in Kurasawa., *et al.* (2025).
- A parallel-group, double-blind, placebo-controlled RCT was planned with 1:1 allocation.
- The primary endpoint was the change in 10-meter walking speed from baseline to week 12.
- Based on preliminary data, a between-group mean difference of $\Delta = 0.33$ m/s was assumed, with group standard deviations $SD_1 = 0.10$ and $SD_2 = 0.24$.

With a two-sided $\alpha = 0.05$ and 90% power, the required number of participants per group (n) was calculated using the two-sample t-test formula (unequal variances):

$$n = 2(Z_{1-}\alpha/_2 + Z_{1-}\beta)^2\sigma^2/\Delta^2$$

where $Z_{1-a}/_2 = 1.96$ for $\alpha = 0.05$ (two-tailed), $Z_{1-\beta} = 1.28$ for 90% power, $\Delta = 0.33$ m/s, and $\sigma = 0.18$ m/s (pooled SD).

Substituting these values yields $n = 7$ per group.

Allowing for 15% attrition, the target enrollment was 9 participants per group (total N = 18).

Using ANCOVA with baseline walking speed as a covariate would be expected to yield equal or greater power for the same N.

AGO-containing tablets and matching placebos were provided by Ina Food Industry Co., Ltd. Each daily dose (six tablets) contained 500 mg of AGO, whereas placebo tablets contained identical excipients without AGO.

Statistical analysis

Baseline characteristics were summarized using descriptive statistics. For each outcome, change scores (Δ) were computed as the difference from baseline to week 6 and to week 12. Between-group comparisons of Δ values used Welch's t-tests (unequal variances). Normality was examined prior to parametric testing. The primary analysis was conducted on all randomized participants with available data at each time point; a pre-specified subgroup analysis was performed among participants with baseline frailty scores ≥4 (pre-frail/frail). Two-sided $p < 0.05$ was considered statistically significant. Analyses were conducted using EZR (version 1.68), a graphical interface for R designed for medical statistics. Although an a priori calculation suggested n=9 per group, the study remained modest in size and may have been underpowered for functional endpoints.

6. Results

6.1 Participant characteristics

The study flow is shown in the flowchart (Figure 1). A total of 23 participants were randomized (AGO n = 12; placebo n = 11). One participant in the AGO group withdrew for personal reasons, whereas all other participants completed follow-up assessments with tablet intake on more than 95% of study days. Baseline characteristics including age, BMI, blood pressure, frailty score, grip strength, gait speed, and lower-limb circumference were comparable between groups (Table 2; Table 3 for the pre-frail subset).

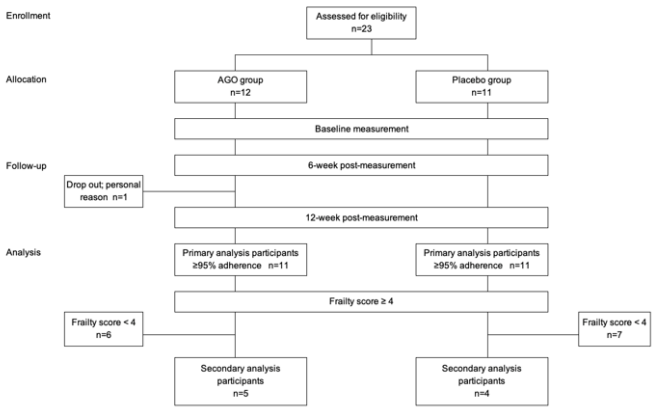

Figure 1: CONSORT flow diagram of participant recruitment, randomization, follow-up, and analysis.

		Group A (n = 11)		Group P (n = 11)		p-value
Age	(years)	77.7	(4.5)	79.5	(5.6)	0.433
Height	(cm)	150.3	(4.5)	151.3	(8.7)	0.747
Weight	(kg)	54.0	(5.4)	51.3	(11.8)	0.501
SBP (Systolic Blood Pressure)	(mmHg)	143.8	(24.0)	143.4	(14.6)	0.958
DBP (Diastolic Blood Pressure)	(mmHg)	83.1	(18.3)	78.5	(10.5)	0.483
Knee pain	(score)	1.2	(2.5)	1.5	(2.4)	0.797
Low back pain	(score)	1.5	(1.9)	2.2	(3.1)	0.515
Student's t-test; Mean (SD).						

Table 2: Baseline characteristics (all participants).

		Group A (n = 5)	Group P (n = 4)	p-value
Age	(years)	77.6 (1.9)	78.3(6.7)	0.840
Height	(cm)	150.4 (2.3)	150.5 (4.8)	0.967
Weight	(kg)	51.8 (3.5)	53.1 (9.0)	0.765
SBP (Systolic Blood Pressure)	(mmHg)	146.0 (17.5)	147.8 (15.4)	0.880
DBP (Diastolic Blood Pressure)	(mmHg)	81.0 (11.9)	80.8 (7.3)	0.972
Knee pain	(score)	1.0 (1.4)	3.3 (3.4)	0.217
Low back pain	(score)	0.8 (1.3)	4.8 (3.9)	0.067
Student's t-test; Mean (SD).				

Table 3: Characteristics of women with pre-frailty.

6.2 Primary and secondary outcomes (all participants)

At week 6, the AGO group showed a significantly greater increase in left-hand grip strength (Δ = +1.2 ± 1.5 kg) compared with the placebo group (Δ = −0.5 ± 1.4 kg; p = 0.020). Similarly, right lower-limb circumference increased significantly in the AGO group (Δ = +0.6 ± 1.3 cm) versus placebo (Δ = −1.1 ± 1.3 cm;, p = 0.008). No significant between-group differences were observed for gait speed, CS-30, TUG, or pain VAS, though gait speed showed a favorable trend in the AGO group (Δ = +0.6 ± 0.8 m/s vs +1.0 ± 1.3 m/s; p = 0.473).

At week 12, the improvements in grip strength and limb circumference were no longer significant between groups (left-hand grip strength Δ = 1.2 ± 1.6 kg in AGO vs −0.1 ± 2.1 kg in placebo; p = 0.136), although the direction of change remained favorable in the AGO group (Figure 2 A–B; Table 4).

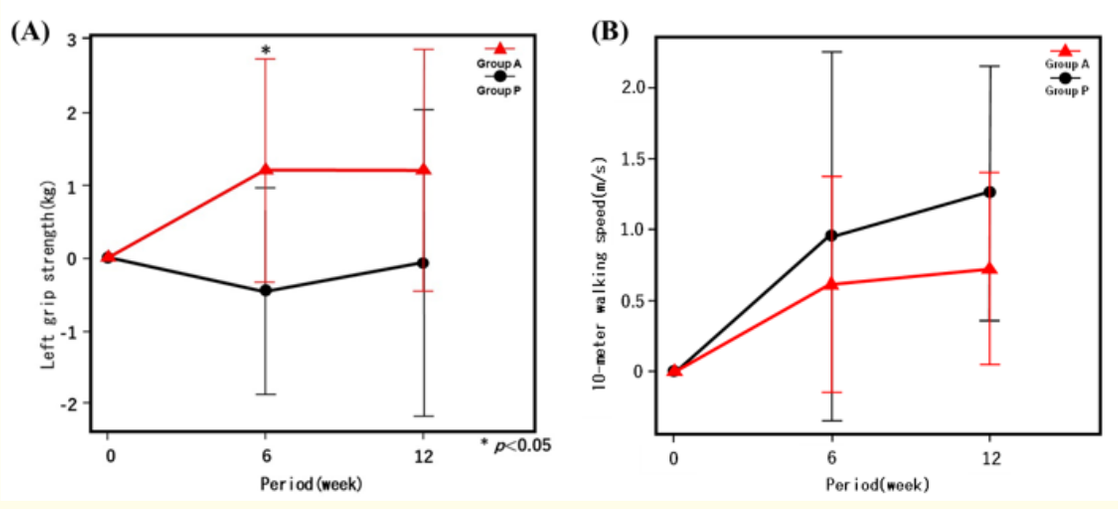

Figure 2: Overall cohort (all women).

(A) Left-hand grip strength (kg) and (B) 10-meter walking speed (m/s) measured at baseline, week 6, and week 12. Participants received agaro-oligosaccharides (AGO; 500 mg/day; n = 11) or placebo (n = 11) for 12 weeks. Data are presented as mean ± SD. Between-group comparisons were performed on change from baseline (Δ) using Welch's t-test (two-sided α = 0.05). Asterisks indicate significant differences versus placebo at the same time point (*p < 0.05). Grip strength was assessed using a Smedley-type dynamometer (best of two trials), and gait speed was measured over a marked 10-m course with acceleration and deceleration zones excluded.

Abbreviations: AGO, agaro-oligosaccharides; Δ, change from baseline; SD, standard deviation.

		Week of measurement	Group A (n = 11)		Group P (n = 11)		p-value
10-meter walking speed	(m/s)	6	0.6	(0.8)	1.0	(1.3)	0.473
	(m/s)	12	0.8	(0.6)	1.3	(0.9)	0.141
Right grip strength	(kg)	6	-0.8	(2.0)	-0.5	(1.7)	0.706
	(kg)	12	0.0	(2.1)	-0.4	(1.2)	0.641
Left grip strength	(kg)	6	1.2	(1.5)	-0.5	(1.4)	**0.020**
	(kg)	12	1.2	(1.6)	-0.1	(2.1)	0.136
30-second chair stand test	(time)	6	2.9	(4.1)	0.7	(2.0)	0.151
	(time)	12	3.4	(5.4)	1.6	(2.1)	0.341
Timed Up and Go test	(Second)	6	0.1	(0.8)	0.4	(1.1)	0.510
	(Second)	12	0.1	(1.2)	0.4	(0.6)	0.493
Right lower limb extensor strength	(kg)	6	-2.1	(6.3)	0.3	(2.7)	0.281
	(kg)	12	-2.0	(6.5)	-0.8	(5.7)	0.630
Left lower limb extensor strength	(kg)	6	-0.7	(5.1)	1.4	(4.3)	0.336
	(kg)	12	-1.0	(6.8)	-1.5	(4.3)	0.843
Right lower limb circumference	(cm)	6	0.6	(1.3)	-1.1	(1.3)	**0.008**
	(cm)	12	0.5	(2.4)	-1.0	(1.3)	0.098
Left lower limb circumference	(cm)	6	-0.7	(3.2)	-1.0	(1.5)	0.729
	(cm)	12	0.8	(2.4)	-0.9	(1.3)	0.061
Welch's t-test; Mean (SD). Bold: A significant difference.							

Table 4: Changes from baseline to week 12 (all participants).

6.3 Subgroup analysis (pre-frail/frail women, frailty score ≥ 4)

Among pre-frail or frail women (n = 9), left-hand grip strength significantly improved from baseline in the AGO group at week 6 (Δ = +1.8 ± 1.8 kg) compared with placebo (Δ = −0.7 ± 1.3 kg; p = 0.049). In addition, left lower-limb circumference tended to increase (Δ = +0.4 ± 1.3 cm vs −0.9 ± 0.9 cm; p = 0.128), although not reaching statistical significance.

By week 12, between-group differences were attenuated and no longer significant (left grip Δ = +1.3 ± 1.8 kg vs +0.4 ± 1.7 kg; p = 0.482; left limb circumference Δ = +1.5 ± 2.9 cm vs −1.8 ± 1.3 cm; p = 0.067). Nevertheless, the AGO group consistently maintained higher numerical values for muscle strength and limb circumference throughout the study (Figure 3A–B; Table 5).

		Week of measurement	Group A (n = 5)		Group P (n = 4)		p-value
10-meter walking speed	(m/s)	6	0.9	(0.7)	1.2	(1.7)	0.809
	(m/s)	12	0.7	(0.6)	1.8	(1.0)	0.100
Right grip strength	(kg)	6	-0.3	(1.5)	-0.8	(1.8)	0.713
	(kg)	12	-0.2	(0.9)	-0.9	(0.6)	0.205
Left grip strength	(kg)	6	1.8	(1.8)	-0.7	(1.3)	**0.049**
	(kg)	12	1.3	(1.8)	0.4	(1.7)	0.482
30-second chair stand test	(time)	6	2.9	(2.8)	0.8	(1.0)	0.170

	(time)	12	3.7	(2.7)	0.6	(1.7)	0.076
Timed Up and Go test	(m/s)	6	0.1	(0.7)	0.0	(1.6)	0.914
	(m/s)	12	0.3	(1.1)	0.4	(0.5)	0.835
Right lower limb extensor strength	(kg)	6	-1.1	(5.4)	1.3	(3.0)	0.446
	(kg)	12	1.3	(7.7)	1.1	(3.6)	0.953
Left lower limb extensor strength	(kg)	6	0.9	(4.1)	3.2	(3.9)	0.302
	(kg)	12	2.7	(7.0)	0.8	(2.2)	0.597
Right lower limb circumference	(cm)	6	0.3	(1.0)	-0.6	(0.8)	0.152
	(cm)	12	0.8	(2.6)	-0.8	(0.5)	0.255
Left lower limb circumference	(cm)	6	0.4	(1.3)	-0.9	(0.9)	0.128
	(cm)	12	1.5	(2.9)	-1.8	(1.3)	0.067
Welch's t-test; Mean (SD). Bold: A significant difference.							

Table 5: Changes from baseline to week 12 (pre-frail subset).

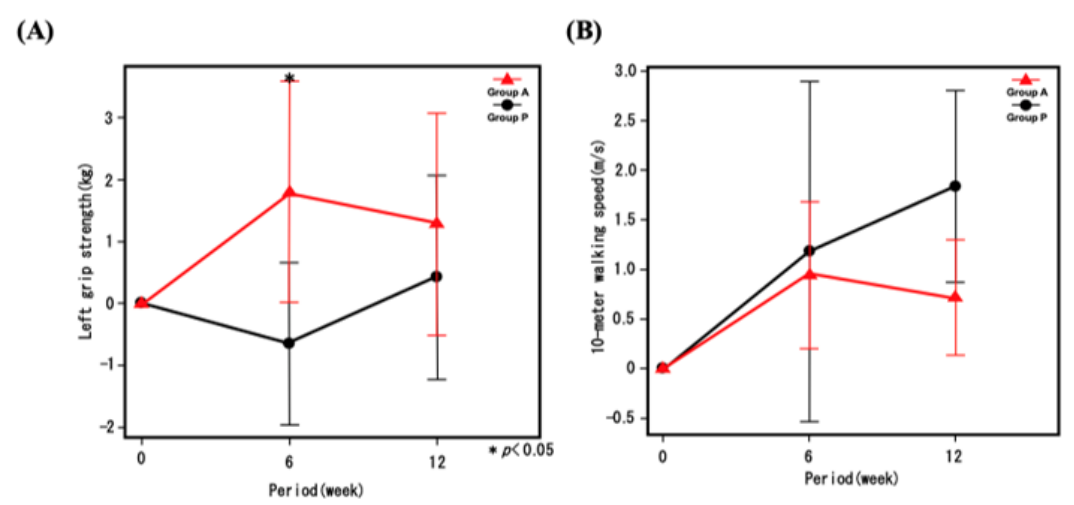

Figure 3. Pre-frail subgroup (frailty score ≥4).

(A) Left-hand grip strength (kg) and (B) 10-meter walking speed (m/s) measured at baseline, week 6, and week 12. Participants classified as pre-frail/frail at baseline (frailty score ≥4) received agaro-oligosaccharides (AGO; 500 mg/day; n = 5) or placebo (n = 4) for 12 weeks. Data are presented as mean ± SD. Between-group comparisons were performed on change from baseline (Δ) using Welch's t-test (two-sided α = 0.05). Asterisks indicate significant differences versus placebo at the same time point (*p < 0.05). Measurement procedures were identical to those described for Figure 2.

Abbreviations: AGO, agaro-oligosaccharides; Δ, change from baseline; SD, standard deviation.

6.4 Safety

No serious adverse events occurred in either group. AGO was well tolerated.

Discussion

In this randomized, double-blind controlled trial, daily intake of agaro-oligosaccharides (AGO, 500 mg) for 12 weeks resulted in early improvements in muscle strength and limb circumference among community-dwelling older women. Specifically, the AGO group exhibited significantly greater gains in left-hand grip strength and right calf circumference after 6 weeks compared with placebo, with more pronounced effects observed in women categorized as pre-frail. Although these between-group differences diminished by week 12, the direction of change remained favorable, suggesting transient but biologically meaningful benefits of AGO intake on skeletal muscle maintenance.

Physiological and clinical interpretation

Grip strength is a validated surrogate marker of global muscle function and a predictor of disability, hospitalization, and mortality [4,8]. The 6-week enhancement in non-dominant (left-hand) grip strength implies a systemic rather than localized response, since non-dominant strength is less influenced by daily activity [12]. The concurrent increase in calf circumference suggests that AGO may help preserve peripheral muscle mass, a key determinant of mobility and frailty prevention. These findings support the potential of AGO as a nutritional intervention for early-stage frailty, where targeted modulation of muscle function can delay progression to functional impairment.

Mechanistic considerations

Beyond canonical antioxidant and anti-inflammatory effects, AGO may exert human-relevant actions via the gut–muscle axis, including improvements in substrate availability, intestinal barrier/viscosity-mediated nutrient absorption, and neuromuscular performance. Because low-molecular-weight AGO can reach the circulation, a direct effect on muscle mitochondrial homeostasis is also plausible. These hypotheses warrant confirmation using biomarker panels (e.g., IL-10/TNF-α balance, circulating SCFAs, myokines) and microbiome profiling in future trials.

The beneficial effects of AGO likely arise from a combination of gut–muscle axis modulation, anti-inflammatory and antioxidant actions. In vitro and animal studies demonstrate that AGO suppresses NF-κB activation, reduces pro-inflammatory cytokines (TNF-α, IL-1β), and enhances intestinal barrier integrity [9,10,25,26]. Moreover, low-molecular-weight AGO can translocate across the intestinal epithelium and enter systemic circulation [21,22], providing a plausible route for direct effects on skeletal muscle. This may counteract chronic low-grade inflammation ("inflammaging"), which accelerates sarcopenic processes [2,7].

Furthermore, experimental data suggest that AGO can upregulate heme oxygenase-1 (HO-1) [9] and modulate oxidative stress responses, potentially improving mitochondrial homeostasis within muscle cells. Recent studies have also shown that marine-derived oligosaccharides promote myotube differentiation and contractile protein synthesis [13], aligning with our observed short-term increases in muscle performance.

AGO may additionally benefit joint and connective tissue function. It has been shown to inhibit collagenase activity and cartilage degradation in arthritic models [21], indicating possible mitigation of joint discomfort. Because musculoskeletal pain limits physical activity and exacerbates frailty [22], AGO's anti-arthritic and anti-inflammatory properties may indirectly sustain habitual movement and leg strength.

Our present results in older women complement a recent case-series study in prefrail older men, which demonstrated potential improvements in functional capacity following AGO intake [26]. Together, these studies suggest that AGO may confer benefits across both sexes, with differential magnitudes depending on baseline frailty status.

Interpretation of non-significant change in 10-meter walking speed

Although the primary outcome, 10-meter walking speed, showed a favorable trend in the AGO group, the between-group difference did not reach statistical significance. Walking speed is a multifactorial parameter influenced by strength, balance, coordination, and habitual gait pattern. The muscle strength gains observed over 6 weeks may not have been sufficient to yield measurable improvements in gait performance within the 12-week timeframe. Previous trials have shown that significant improvements in gait speed among older adults often require interventions longer than 16–24 weeks or combined exercise–nutrition programs rather than nutritional supplementation alone [18,19,23].

In addition, baseline gait speed in our participants was relatively high (mean ≥ 1.0 m/s), indicating good functional capacity and a possible ceiling effect that limited detectable improvement. The modest sample size (n = 23) and lack of control for habitual activity or environmental conditions may also have contributed to variability. Future studies should include frailer participants, longer intervention durations, and objective gait analysis to enhance sensitivity and reproducibility.

Temporal pattern and adaptation

The attenuation of effects between weeks 6 and 12 may reflect a plateau or adaptive response in systemic inflammatory and metabolic pathways. Feedback regulation within short-chain fatty acid (SCFA) signaling and GPR43-mediated immune modulation [1,3] could normalize host responses over time. Alternatively, the relatively small sample size and absence of dietary or exercise control may have limited statistical sensitivity to detect sustained differences. Future trials should incorporate biomarker analyses (e.g., IL-10/TNF-α ratios, circulating SCFAs, myokine profiles) and gut microbiome sequencing to clarify mechanistic links between AGO intake and musculoskeletal outcomes.

Public health implications

Frailty and sarcopenia impose significant social and economic burdens in super-aging societies such as Japan. AGO supplementation offers a safe, low-cost, and food-derived approach to mitigate early muscle decline, requiring minimal behavior modification. Its demonstrated tolerability and compatibility with routine diets suggest high scalability for community-based frailty prevention programs. Confirmation of these preliminary findings in larger, multi-center, and longer-duration studies will be essential to determine optimal dosage, durability of effect, and relevance to outcomes such as falls, hospitalization, and quality of life.

Conclusion

In this randomized, double-blind, placebo-controlled trial, daily intake of agaro-oligosaccharides (AGO, 500 mg) for 12 weeks was safe and well tolerated and produced early improvements in muscle strength and limb circumference among community-dwelling older women. These effects were more evident in pre-frail participants, suggesting that AGO supplementation may help maintain peripheral muscle mass and strength in individuals at risk for frailty.

Although the primary endpoint, 10-meter walking speed, did not show a statistically significant difference between groups, favorable trends were observed, implying that longer interventions or combined lifestyle strategies may be required for functional improvement. Mechanistically, the benefits of AGO appear to involve modulation of the gut–muscle axis through anti-inflammatory and antioxidant pathways, including suppression of NF-κB signaling and induction of heme oxygenase-1.

AGO represents a promising, food-derived, and feasible nutritional approach for the prevention of frailty and sarcopenia in older adults. Future large-scale and longer-duration randomized controlled trials integrating clinical, biochemical, and microbiome outcomes are warranted to confirm these findings and establish the mechanistic and translational significance of AGO supplementation. Future multicenter trials with longer follow-up, dose–response evaluation, and stratification by baseline frailty status are warranted to confirm generalizability and durability of effects.

Acknowledgements

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

We thank the participants and staff who assisted with recruitment, assessments, and data collection.

Conflict of Interest

- Koji Karasawa and Toshiyuki Ochi are employees of Ina Food Industry Co., Ltd., which manufactured and provided the agaro-oligosaccharide (AGO) and placebo tablets used in this study.
- The company had no role in participant recruitment, data collection, statistical analysis, or interpretation of the results.
- All other authors declare no competing financial or personal interests related to this study.
- This disclosure is consistent with our previous report on AGO supplementation in prefrail older men (Kurasawa., *et al*. Preventive Medicine Research, in press).

Author Contributions

Conceptualization: Ryoji Hirota; Methodology: Ryoji Hirota; Investigation: Rina Kurasawa; Formal analysis: Rina Kurasawa;

Writing – original draft: Rina Kurasawa; Writing – review and editing: all authors; Study tablets – preparation and provision: Koji Karasawa, Toshiyuki Ochi; Supervision: Hiroyuki Hasegawa, Suguru Kurokawa, Koji Karasawa, and Takahiro Kubota. All authors read and approved the final manuscript.

Bibliography

1. Ashall L., *et al*. "Pulsatile Stimulation Determines Timing and Specificity of Nf-Kappab-Dependent Transcription". *Science* 324.5924 (2009): 242-246.

2. Bano G., *et al*. "Inflammation and Sarcopenia: A Systematic Review and Meta-Analysis". *Maturitas* 96 (2017): 10-15.

3. Bjorkman L., *et al*. "The Neutrophil Response Induced by an Agonist for Free Fatty Acid Receptor 2 (Gpr43) Is Primed by Tumor Necrosis Factor Alpha and by Receptor Uncoupling from the Cytoskeleton but Attenuated by Tissue Recruitment". *Molecular and Cellular Biology* 36.20 (2016): 2583-2595.

4. Bohannon R W. "Grip Strength: An Indispensable Biomarker for Older Adults". *Clinical Interventions in Aging* 14 (2019): 1681-1691.

5. Li M., *et al*. Isolation and characterization of an agaro-oligosaccharide-hydrolyzing bacterium from the gut microflora of Chinese individuals. PLoS ONE 9; e91106: 2014. doi: 10.1371/journal.pone.0091106.

6. Chen X., *et al*. "Agar Oligosaccharides: A Review of Preparation, Structures, Bioactivities and Application". *Carbohydrate Polymer* 265 (2021): 118076.

7. Dalle S., *et al*. "The Role of Inflammation in Age-Related Sarcopenia". *Frontiers in Physiology* 8 (2017): 1045.

8. Dodds RM., *et al*. "Grip Strength across the Life Course: Normative Data from Twelve British Studies". *PLoS One* 9.12 (2014): e113637.

9. Higashimura Y., *et al*. "Oligosaccharides from Agar Inhibit Murine Intestinal Inflammation through the Induction of Heme Oxygenase-1 Expression". *Journal of Gastroenterology* 48.8 (2013): 897-909.

10. Higashimura Y., *et al*. "Protective Effect of Agaro-Oligosaccharides on Gut Dysbiosis and Colon Tumorigenesis in High-Fat Diet-Fed Mice". *American Journal of Physiology-Gastrointestinal and Liver Physiology* 310.6 (2016): G367-375.

11. Higashimura, Y., *et al*. "Oligosaccharides from Agar Inhibit Pro-Inflammatory Mediator Release by Suppressing the Nuclear Factor-Kappa B Pathway in Macrophages". *Clinical and Experimental Immunology* 172.2 (2013): 230-239.

12. Hogrel J Y. "Grip Strength Measured by High Precision Dynamometry in Healthy Subjects from 5 to 80 Years". *BMC Musculoskeletal Disorder* 16 (2015): 139.

13. Huang Y., *et al*. "The Effects of Agaro-Oligosaccharides Produced by Marine Bacteria (Rheinheimera Sp. (Hy)) Possessing Agarose-Degrading Enzymes on Myotube Function". *Marine Drugs* 22.11 (2024).

14. Jiang C., *et al*. "Advances in Agaro-Oligosaccharides Preparation and Bioactivities for Revealing the Structure-Function Relationship". *Food Research International* 145 (2021): 110408.

15. Jo E., *et al*. "Odd-Numbered Agaro-Oligosaccharides Produced by Alpha-Neoagaro-Oligosaccharide Hydrolase Exert Antioxidant Activity in Human Dermal Fibroblasts". *Marine Drugs* 22.11 (2024).

16. Karasawa K., *et al*. "Improvement of Gait Speed with Agaro-Oligosaccharide Intake in Older Men". Japanese Patent No. 7236712 (registration date: 2023-03-02) (in Japanese) (2023)

17. Kurasawa R., *et al*. "Preliminary Evidence for the Feasibility and Potential Functional Benefits of Agaro-Oligosaccharides in Prefrail Older Men: A Case-Series with Random Allocation". *Preventive Medicine Research* (In press.)

18. Liu CK and RA Fielding. "Exercise as an Intervention for Frailty". *Clinics in Geriatric Medicine* 27.1 (2011): 101-110.

19. Pahor M., *et al*. "Effect of Structured Physical Activity on Prevention of Major Mobility Disability in Older Adults: The Life Study Randomized Clinical Trial". *JAMA* 311.23 (2014): 2387-2396.

20. Shen J., *et al*. "Eco-Friendly Production and Probiotic Purification of Agarose Degradation Products: Oligosaccharides and 3,6-Anhydro-L-Galactose". *International Journal of Biological Macromolecules* 281.2 (2024): 135682.

21. Shirai I., *et al*. "Orally Administered Low-Molecular Weight Agaro-Oligosaccharides Are Absorbed into the Plasma of Healthy Humans". *Frontiers in Nutrition* 10 (2023): 1273328.

22. Shirai I., *et al*. "Intestinal Permeability of Agaro-Oligosaccharides: Transport across Caco-2 Cell Monolayers and Pharmacokinetics in Rats". *Frontiers in Nutrition* 9 (2022): 996607.

23. Studenski S., *et al*. "Gait Speed and Survival in Older Adults". *JAMA* 305.1 (2011): 50-58.

24. Wang L., *et al*. "In Vitro and in Vivo Protective Effects of Agaro-Oligosaccharides against Hydrogen Peroxide-Stimulated Oxidative Stress". *Polymers (Basel)* 15.7 (2023).

25. Wang Q., *et al*. "Agaro-Oligosaccharides Mitigate Deoxynivalenol-Induced Intestinal Inflammation by Regulating Gut Microbiota and Enhancing Intestinal Barrier Function in Mice". *Food Function* 15.7 (2024): 3380-3394.

26. Yuan L., *et al*. "Multi-Omics Analysis Reveals That Agaro-Oligosaccharides with Different Degrees of Polymerization Alleviate Colitis in Mice by Regulating Intestinal Flora and Arginine Synthesis". *Food Function* 15.21 (2024): 10628-10643.