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Chronic Intake of a Meal Including Alaska Pollack Protein Increases Skeletal Muscle Mass and Strength in Healthy Older Women: A Double-Blind Randomized Controlled Trial

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ABSTRACT

Background: In animal studies, a meal containing Alaska pollack protein (APP) induces fast-twitch muscle hypertrophy. To our knowledge, no interventional studies have examined the benefits of APP intake on muscle mass and muscle weakness and the prevention of sarcopenia in older individuals.

Objectives: We evaluated the effects of APP intake on skeletal muscle mass, muscle strength, and physical performance among healthy community-dwelling older Japanese women.

Methods: In this double-blind randomized controlled trial, healthy women ≥ 65 y old were allocated to an APP or whey protein control (CON) group. Participants ingested test protein meals (5.0–5.1 g protein/serving) daily for 24 wk. Between-group differences in the change of skeletal muscle mass index (SMI) as the primary outcome and muscle strength as a secondary outcome were tested using multifrequency BIA and a handheld dynamometer, respectively, at baseline, and 4, 12, and 24 wk. The mean changes in the measured primary and secondary outcome variables from baseline to 4, 12, and 24 wk were compared using unpaired *t* tests.

Results: There were no between-group differences in nutritional status, food intake, or total energy and protein intakes at baseline, 12 wk, or 24 wk. The change in SMI was 0.12 kg/m^2 (95% CI: 0.01, 0.23 kg/m^2) and 0.11 kg/m^2 (95% CI: 0.03, 0.19 kg/m^2) greater in the APP group than in the CON group at 12 wk and 24 wk ($P \leq 0.03$) and knee extension strength was 0.07 Nm/kg BW (95% CI: 0.02, 0.12 Nm/kg BW) and 0.05 Nm/kg BW (95% CI: 0.00, 0.09 Nm/kg BW) higher in the APP group than in the CON group at these times ($P \leq 0.015$), respectively. The groups did not differ at 4 wk.

Conclusions: Daily intake of a meal containing APP compared with whey protein increases skeletal muscle mass and lower-extremity muscle strength in healthy older women, suggesting that an APP-containing meal may be useful in the prevention of sarcopenia in this group. This trial was registered at as UMIN000035718. *J Nutr* 2022;152:2761–2770.

Keywords: Alaska pollack protein, skeletal muscle mass, muscle strength, older adults, randomized controlled trial

Introduction

Sarcopenia is characterized by progressive and generalized skeletal muscle loss resulting in decreased muscle strength and/or physical function, as defined by the Asian Working Group for Sarcopenia (1). Factors such as aging, physical inactivity, malnutrition status, and chronic disease increase the risk of sarcopenia and associated adverse outcomes, including physical disability and a poor quality of life (QOL) (2). In the context of older Japanese individuals, the risk of falls is higher among women than among men, with decreased physical function being an additional risk factor for falls (3). Therefore, it is important to establish an intervention for preventing sarcopenia in older women, who are at high risk of fractures.

Awareness regarding the clinical importance of sarcopenia has increased in recent years; however, implementing preventative measures and therapeutic interventions remains a challenge (4). Protein-rich diets and resistance exercises are reportedly effective in regaining and maintaining muscle mass in older adults (5). However, the effect of consuming proteins from different food sources on the prevention and treatment of sarcopenia in older adults is unclear. Research suggests that protein from animal sources, such as milk (containing casein and whey), meat, fish, and eggs, can help prevent muscle atrophy and weakness more than plant-based protein sources (6, 7). Whey protein is a high-quality protein containing all the essential amino acids that are naturally found in dairy products. In particular, previous clinical trials have reported the effects of

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whey protein intake (≥22.5 g protein/d) on increase in muscle mass and strength in older adults (8).

Alaska pollack, a wild-caught whitefish, is commonly consumed worldwide owing to its quality, affordability, versatility, and nutritional content. Alaska pollack is a marine fish species of the cod family Gadidae. With a mild flavor and a delicate, flaky texture, Alaska pollack is low in fat and an efficient source of protein. Animal studies have previously showed the beneficial effect of Alaska pollack protein (APP) intake on fast-twitch muscle hypertrophy, compared with casein protein intake (9, 10). To our knowledge, no interventional studies have examined the benefits of APP intake, in the absence of resistance exercise, in enhancing muscle mass and reducing muscle weakness in older persons. Moreover, it is unclear whether APP intake contributes to preventing sarcopenia and increasing or maintaining skeletal muscle mass and strength in older adults.

Therefore, we aimed to evaluate the effects of APP meal intake on increasing skeletal muscle mass, muscle strength, and physical performance among healthy community-dwelling older Japanese women using a double-blind, randomized controlled design.

Methods

Participants

In total, 108 community-dwelling adult women were initially screened for this study. Participants were from the Kita-Harima community (in the cities of Kato, Miki, and Nishiwaki-yabu) in Hyogo Prefecture, Japan. Participants were recruited from March to April 2019, and the intervention study took place from May 2019 until March 2020. The eligibility criterion for the participants was healthy older women aged ≥ 65 y. The exclusion criteria were presence of sarcopenia; age < 65 y; presence of diabetes, chronic cardiac diseases, hepatic disease, respiratory diseases, and chronic kidney disease; performance of resistance exercise in the past 1 y [continuous use of weight training machines, body weight (BW) resistance exercises, and resistance band exercises for ≥1 wk]; and male sex. We confirmed that the patients did not have diabetes, chronic kidney disease, and other obvious diseases using a health check-up, clinical data, and medical interview. Using the Asian Working Group for Sarcopenia criteria (1), we excluded women with sarcopenia ($n = 3$), women aged < 65 y ($n = 9$), and those with diabetes ($n = 3$) and chronic kidney disease ($n = 1$).

After the initial assessments, a stratified randomization strategy was used to allocate the remaining 92 participants into 2 groups with a comparable age distribution between them: the APP meal intake intervention group (APP group; $n = 46$) and the whey protein control (CON) intake group (CON group; $n = 46$). This 2-arm, stratified randomization, placebo-controlled, double-blind, parallel-group trial was conducted over a period of 24 wk. The randomization list was generated by an independent statistician who was not involved in the study. According to the order on the randomization list, study staff randomly allocated the eligible participants (1:1, with 3 blocks of size 16) to the groups to receive APP or CON test meals during the intervention. All participants and study staff were blinded to the test meals.

This study was approved by the ethics committee of Tokushima University (approval number: 3417), and the trial was registered at the University Hospital Medical Information Network (UMIN000035718). Informed consent was obtained from all study participants, and written informed consent was obtained from them to publish this article. Data from a previous investigation were utilized for sample size estimation. The calculation was based on an effect size of 0.692 [absolute difference of 0.18 kg/m^2 skeletal muscle mass index (SMI) with an SD of 0.26 kg/m^2] (11), α level of 0.05, and a power $(1 - \beta)$ of 80%; we identified as necessary 34 individuals for each study group. We aimed to enroll ≥43 individuals/group to be conservative and to allow for potential dropouts and a missing data rate of 20%.

Intervention design

Participants in both the APP and CON groups ingested a test meal (Nippon Suisan Kaisha, Ltd.) daily for 24 wk during the intervention period. Each test meal was ingested at breakfast (consisting of either APP or CON powder dissolved in 120 mL hot water). **Supplemental Table 1** shows the nutritional values of the APP powder (5.1 g; 4.5 g protein from Alaska pollack and 0.6 g protein from other sources) and CON powder (5.0 g; 4.5 g protein from whey and 0.5 g protein from other sources). The mean ± SD amount of APP or whey protein supplementation related to the body mass of the participants in this study was 0.09 ± 0.01 g · BW kg^{-1} · d^{-1} for APP and CON. Each amino acid was analyzed directly from the amount of amino acids (Japan Food Research Laboratories). The amount of tryptophan was analyzed directly using HPLC (Japan Food Research Laboratories). The amounts of other amino acid were analyzed directly using the amino acid analyzer method. The proteins were analyzed from the amount of nitrogen. Therefore, the difference in amount between amino acid and protein levels was observed. Both before and during the intervention, no participant correctly postulated which group they were part of.

APP supplementation was calculated based on results from a previous clinical study (12) and from the amount that could potentially be consumed in daily life in addition to normal meals. However, the amount of whey protein supplementation used in this study was relatively low compared with that used in previous studies (≥22 g/d of whey protein) (8). We assumed that 5 g/d of whey protein ingestion could be used in the CON groups because this amount is insufficient for stimulating intrinsic functions, such as skeletal muscle mass and muscle strength. In addition, the APP and CON test meals contained 448 mg and 460 mg leucine, respectively. During the intervention period, the test meal was provided by a researcher who was not involved in the study. All participants were instructed to record the day a test meal was consumed in a record book. Food and nutrition management was overseen by a registered dietitian. Participants underwent a course on food and nutrition management before and during the intervention, and the course was based on the Dietary Reference Intakes for Japanese, 2015 (13).

Outcome measure

The following outcomes were measured by blinded research staff during designated visits in the 1-mo preintervention period, during the intervention at 4 and 12 wk, and during a postintervention period at 24 wk. The change in SMI was the primary outcome parameter (1). Grip strength, knee extension strength, gait speed, the chair stand test, and health-related QOL were the secondary outcome parameters.

Body composition and skeletal muscle mass

BW and skeletal muscle mass (total muscle mass, upper and lower limb muscle mass) were measured with a body composition analyzer (In Body Bioelectrical Impedance Analyzer; In Body Japan) using direct segmental multifrequency BIA. BMI was calculated using the standard formula: BW (kg)/height squared (m^2). An underweight person was

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Supplemental Tables 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: Akt, protein kinase B; APP, Alaska pollack protein; BW, body weight; CON, whey protein control; ICC, intraclass correlation coefficient; MNA-SF, Mini Nutritional Assessment-Short Form; mTOR, mammalian target of rapamycin; QOL, quality of life; SMI, skeletal muscle mass index; TEI, total energy intake.

defined as someone with a BMI < 18.5. SMI was calculated by dividing the upper and lower limb skeletal muscle mass by the height squared: total upper and lower limb muscle mass (kg)/height squared (m^2) (1, 2). Body composition and skeletal muscle mass data were collected at baseline as well as at 4, 12, and 24 wk.

In accordance with the manufacturer's guidelines, participants wiped the bottom of their feet with a proprietary electrolyte tissue before standing on the electrodes embedded in the scale platform of the respective analyzer (14). The participants were instructed to stand upright and grasp the handles of the analyzer, thereby providing contact with a total of 8 electrodes (2 for each foot and hand). BIA was performed after urination and defecation, fasting for 6 h after a meal, and 5–10 min of rest. Before the BIA measurement, any exercise or activity that would cause sweating was prohibited. Excellent agreements were observed between DXA and direct segmental multifrequency BIA techniques in whole muscle mass quantification [intraclass correlation coefficient (ICC) female = 0.95, ICC male = 0.96]. In segmental muscle mass quantification, excellent agreements between the methods were demonstrated for the upper limbs (ICC female ≥ 0.91, ICC male ≥ 0.87) and lower limbs (ICC female ≥ 0.83, ICC male ≥ 0.85), with good agreements shown for the trunk measurements (ICC female = 0.73, ICC male = 0.70) (15).

Muscle strength

Grip strength and knee extension strength were used to evaluate muscle strength at baseline and at 4, 12, and 24 wk. Upper-extremity muscle strength was measured for each hand using the maximum isometric grip strength value while standing. Participants measured the Smedley hand dynamometer (GRIP-D TKK5401; Takei) in an upright position with the second joint of the index finger adjusted to 90 degrees. A handheld dynamometer (μTas F-1; ANIMA) was utilized to evaluate the maximum isometric knee extension strength, which represents the lower-extremity muscle strength. Participants sat on a bench with a force sensor fixed firmly to the distal end of the tibia and the tibia fixed to a rigid bar using a belt. We defined the maximal isometric knee extension strength as the highest value achieved by the participant. To estimate the knee extension strength, the knee extension value was divided by BW [Newton meter (Nm) force/kg BW]. A previous validation study showed that interrater reliability of isometric knee extension strength measurements was highly correlated with data measured by the handheld dynamometer (ICC male and female = 0.99, respectively) (16).

Physical performance

Gait speed test and the chair stand test were used to evaluate physical performance at baseline and at 4, 12, and 24 wk. The chair stand test is used to record the maximum number of repetitions completed in 30 s (17). Participants were instructed to sit on a standard chair with their arms crossed over their chest and then asked to stand in a fully extended standing position as many times as possible. For usual gait speed, participants were instructed to walk for 10 m at their normal speed, and the time required to travel 4 m (3–7 m) was measured (1, 2).

Health-related QOL

The health-related QOL of the surveyed participants was estimated using the Short-Form 36 Health Survey, a questionnaire with 36 questions measuring physical health and mental health (18). Health-related QOL data were collected at baseline and 24 wk.

Food, energy, and macronutrient intake

The food, energy, and macronutrient intake of the surveyed participants was estimated using an FFQ based on food groups (Eiyou-kun version 5.0, Kenpakusha Co., Ltd.). Data on the total energy intake (TEI) and total protein intake were adjusted according to BW. The TEI, TEI/kg BW, protein energy ratio, total protein, total protein intake/kg BW, and intake of specific food items (cereals, potatoes, fish and shellfish, meats, eggs, milk and dairy products, bean and soybean products, vegetables, fruits, fat and oil, and sugar and confectioneries) were collected at baseline and at 12 and 24 wk.

Nutritional status and physical activity

Nutritional status was determined using the Mini Nutritional Assessment-Short Form (MNA-SF) (19). MNA-SF scores < 8 indicated malnutrition. Participants wore triaxial accelerometers (EZ-063 CALORIZM; Taniata Co., Ltd.) during the intervention period for 12 wk except when sleeping or bathing, and the total daily number of steps and total energy expenditure were determined for each participant. Nutritional status data were collected at baseline and at 12 and 24 wk. Physical activity data were collected at 12 wk.

Statistical analyses

Statistical analysis was performed after excluding participants with missing data and those who withdrew at 4, 12, or 24 wk. Two-factor repeated-measures ANOVA was used to evaluate the effects of the intervention on the primary and secondary outcome measures between the groups. The mean changes (Δ) in the measured primary and secondary outcomes from baseline to 4 wk (baseline–4 wk = Δ4 wk), 12 wk (baseline–12 wk = Δ12 wk), and 24 wk (baseline–24 wk = Δ24 wk) were compared using the unpaired t test or χ^2 test. To clarify the efficacy of the APP or CON, the Δ SMI from baseline to 4, 12, and 24 wk was compared between the 2 groups using unpaired t tests.

Baseline between-group differences in the physical characteristics; primary and secondary outcomes; and total energy, protein, and food intake were evaluated using an independent group nonpaired t test or χ^2 test analysis. All statistical analyses were performed using IBM SPSS software (version 25.0; IBM). Primary and secondary outcome data were checked for normality using the Shapiro–Wilk test. The relation between Δ SMI and Δ grip strength at 12 and 24 wk of intervention was analyzed using Pearson's correlation. All statistical analyses were performed using IBM SPSS software (version 25.0; IBM) with a significance level of $\alpha = 0.05$. All statistical tests were 2-tailed with the level of significance set at $P < 0.05$.

Results

Eight participants were excluded from the analysis at 4 wk [missing data due to inability to collect outcome data (APP, $n = 4$; CON, $n = 4$)], 12 participants at 12 wk [missing data due to inability to collect outcome data (APP, $n = 3$; CON, $n = 7$), withdrawal due to difficulty in ingesting the test meal (APP, $n = 1$; CON, $n = 1$)], and 9 participants at 24 wk [missing data due to inability to collect outcome data (APP, $n = 3$), withdrawal due to difficulty in ingesting the test meal (APP, $n = 2$; CON, $n = 1$), withdrawal due to lack of motivation (APP, $n = 2$; CON, $n = 1$)]. Participants without outcome data were not withdrawn from this study. At 24 wk, the APP and CON groups comprised 38 and 43 participants, respectively (**Figure 1**).

Table 1 presents the baseline clinical characteristics of the participants. There were no differences in clinical characteristics or in primary/secondary outcomes between the groups at baseline. There were no between-group differences in the MNA-SF score at baseline. **Supplemental Table 2** shows data on test meal intake and physical activity during the intervention at 4, 12, and 24 wk. There was no difference in the intake rate of the test meals between the groups at 4, 12, and 24 wk and no difference in the total number of steps or total energy expenditure during the intervention at 12 wk. **Table 2** shows data on food, energy, and macronutrient intake at baseline and during the intervention at 12 and 24 wk. There were no between-group differences in the food, energy [TEI (kcal or kcal/kg BW)], and macronutrient [total protein intake (g or g/kg BW), protein energy ratio] intake at baseline and at 12 and 24 wk.

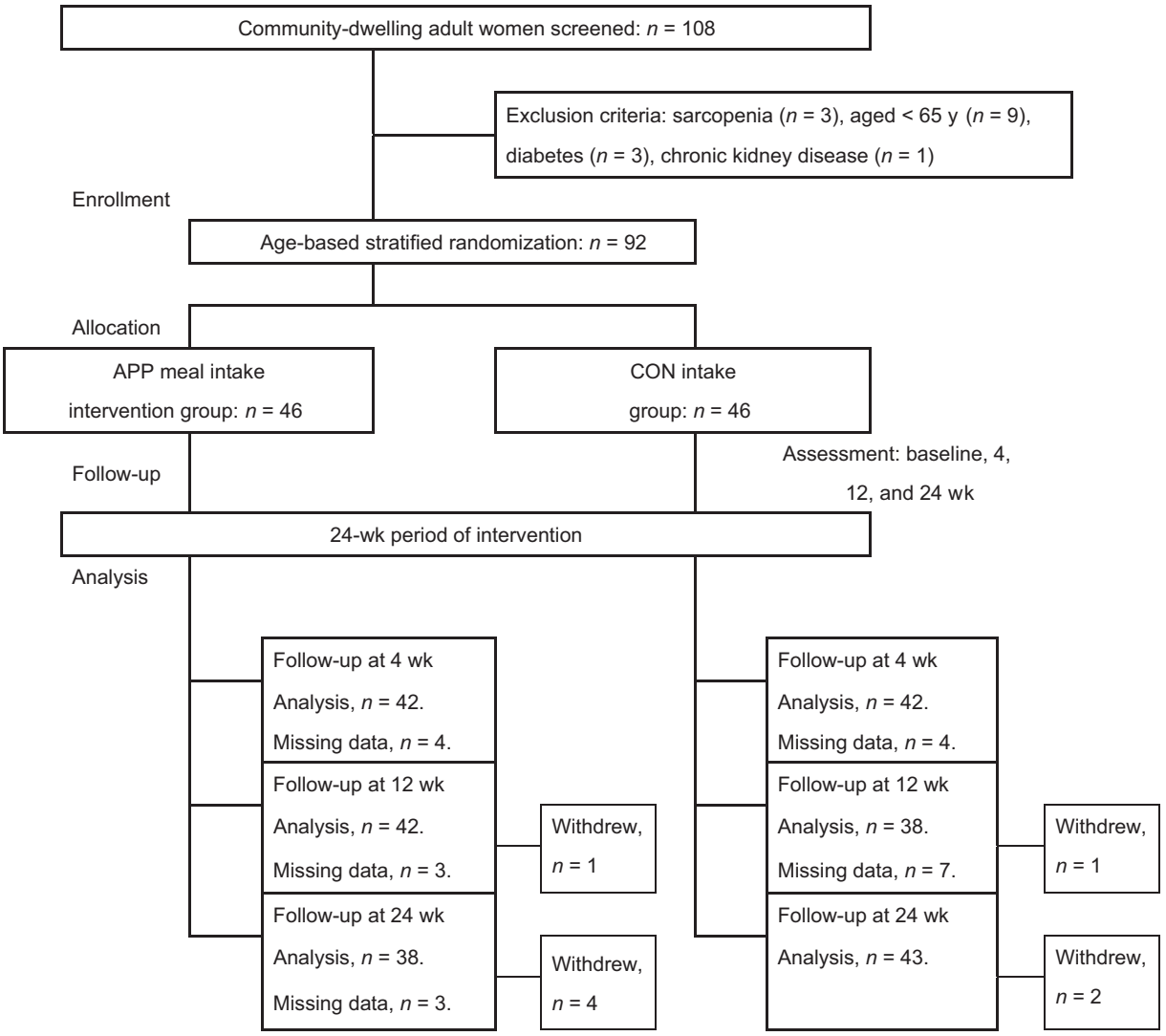

FIGURE 1 Flowchart showing the study participants. APP, Alaska pollack protein; CON, whey protein control.

Table 3 and **Supplemental Table 3** show the 4-, 12-, and 24-wk intervention period changes in the primary and secondary outcomes. A significant group-by-time interaction was identified for SMI (12 wk, $P = 0.029$; 24 wk, $P = 0.013$), skeletal muscle mass (12 wk, $P = 0.015$; 24 wk, $P = 0.015$), grip strength (12 wk, $P = 0.041$), and knee extension strength (12 wk, $P = 0.015$; 24 wk, $P = 0.013$) at 12 and 24 wk, respectively. Figure 2 shows the mean changes (Δ) from baseline to the postintervention period for SMI, grip strength, and knee extension strength. From baseline to 12 and 24 wk of intervention, Δ SMI, Δ skeletal muscle mass, Δ grip strength, and Δ knee extension strength were significantly higher in the APP group than in the CON group (Δ SMI: 12 wk, $P = 0.030$; 24 wk, $P = 0.011$; Δ skeletal muscle mass: 12 wk, $P = 0.015$; 24 wk, $P = 0.013$; Δ grip strength: 12 wk, $P = 0.041$; Δ knee extension strength: 12 wk, $P = 0.010$; 24 wk, $P = 0.015$). From baseline to 12 and 24 wk of intervention, Δ gait speed, Δ chair stand test score, and Δ physical and Δ mental QOL were not significantly different in the APP group than in the CON group (Δ gait speed: 12 wk, $P = 0.301$; 24 wk, $P = 0.225$; Δ chair stand test: 12 wk, $P = 0.201$; 24 wk, $P = 0.129$; Δ physical QOL: 24 wk, $P = 0.549$; Δ mental QOL: 24 wk, $P = 0.771$).

In the APP group, the Δ grip strength was positively correlated with the Δ SMI at 12 and 24 wk of intervention (12 wk, $r = 0.340$, $P = 0.027$; 24 wk, $r = 0.422$, $P = 0.008$).

Discussion

We reported the effectiveness of APP meal intake compared with whey protein in increasing skeletal muscle mass and knee extension strength in healthy community-dwelling older Japanese women. In our study, APP meal intake resulted in greater increase in SMI, skeletal muscle mass, and knee extension strength during the intervention at 12 and 24 wk, than did whey protein intake.

A short-term period of APP intake (~12 wk) compared with whey protein was suggested to increase skeletal muscle mass. This study indicated that APP as a protein source may help prevent the loss of skeletal muscle mass and strength, compared with whey protein in older adults. APP is commonly consumed worldwide owing to its quality, affordability, versatility, and nutritional content. During the intervention period, adherence to APP test meal intake was

TABLE 1 Baseline characteristics of healthy older women in the APP and CON groups[1]

	APP ($n = 46$)	CON ($n = 46$)	*P* value[2] APP vs. CON
Age, y	70.4 ± 4.6	70.2 ± 5.3	0.883
Body height, cm	154.9 ± 6.0	155.3 ± 5.6	0.172
BW, kg	55.8 ± 7.0	53.4 ± 6.9	0.169
BMI, kg/m^2	23.3 ± 3.5	22.9 ± 2.8	0.505
BMI < 18.5 kg/m^2, *n*	1	1	0.557
MNA-SF, score	12.9 ± 1.3	12.7 ± 1.2	0.412
Malnutrition, %	0	0	—
SMI, kg/m^2	6.24 ± 0.63	6.25 ± 0.62	0.916
Skeletal muscle mass, kg	20.3 ± 2.4	20.3 ± 2.3	0.894
Grip strength, kg	25.3 ± 4.2	25.0 ± 3.9	0.682
Gait speed, m/s	1.32 ± 0.17	1.31 ± 0.14	0.679
Knee extension strength, Nm/kg BW	1.41 ± 0.23	1.39 ± 0.22	0.642
Chair stand, rep	25.4 ± 4.6	24.1 ± 5.0	0.262
Physical QOL, score	45.3 ± 10.7	46.7 ± 8.0	0.489
Mental QOL, score	54.5 ± 8.9	53.3 ± 8.5	0.519

[1] Values are mean ± SD unless indicated otherwise. APP, Alaska pollack protein; BW, body weight; CON, whey protein control; MNA-SF, Mini Nutritional Assessment-Short Form; Nm, Newton meter; QOL, quality of life; rep, repetition; SMI, skeletal muscle mass index.
[2] Unpaired *t* test or χ^2 test.

high (87.4%–92.1%), suggesting that APP intake is acceptable and sustainable for older adults. Kim et al. (20) reported that the mean adherence rate to a resistance exercise program, over a 3-mo intervention period, was 80.5% in the resistance exercise only group in older women. Niemeijer et al. (21) showed by meta-analysis and systematic review that exercise intervention increased the RR of adverse events. However, in this study, there were no reported adverse events, such as incidental fall, hip fracture, death, or hospital admission. Therefore, an APP-containing meal may be proposed as a new dietary intervention to prevent sarcopenia in older adults.

There were no differences in nutritional status, total energy, protein, or food intake between the groups during the intervention at 12 and 24 wk. The study did not include participants undergoing protein restriction with a total protein intake < 0.8 g · kg BW^{-1} · d^{-1}. Participants consumed adequate protein from daily meals, not the APP and CON test meals. Therefore, the energy and protein, fat, and carbohydrate amounts contained in the APP or whey protein test meal were not included in the nutrition and food intake survey.

Consequently, the effects of APP meal intake on skeletal muscle mass and strength could be clearly demonstrated. We included healthy older adults without sarcopenia. Thus, daily

TABLE 2 Food, energy, and macronutrient intakes in healthy older women who consumed APP or CON meals daily for 24 wk[1]

	Baseline[2]		12 wk[2]		24 wk[2]	
	APP ($n = 46$)	CON ($n = 46$)	APP ($n = 42$)	CON ($n = 38$)	APP ($n = 38$)	CON ($n = 43$)
TEI,[3] kcal/d	1741 ± 252	1732 ± 239	1703 ± 144	1709 ± 201	1764 ± 237	1723 ± 216
TEI/BW,[3] kcal · kg BW^{-1} · d^{-1}	31.6 ± 5.5	32.8 ± 6.8	31.2 ± 4.5	32.8 ± 5.4	31.8 ± 5.5	32.5 ± 5.5
Total protein energy ratio,[3] g/kcal	13.8 ± 2.2	13.5 ± 2.1	13.9 ± 1.7	13.6 ± 2.0	13.4 ± 2.1	13.6 ± 2.0
Total protein intake,[3] g/d	60.4 ± 16.0	60.0 ± 16.0	59.3 ± 8.9	58.6 ± 13.5	59.4 ± 12.8	58.5 ± 11.8
Total protein intake/BW,[3] g · kg BW^{-1} · d^{-1}	1.09 ± 0.30	1.12 ± 0.35	1.07 ± 0.20	1.12 ± 0.27	1.07 ± 0.28	1.09 ± 0.22
Cereals, g/d	358 ± 77	342 ± 81	359 ± 80	357 ± 75	362 ± 105	358 ± 83
Potatoes, g/d	61 ± 24	56 ± 19	58 ± 19	55 ± 13	63 ± 26	62 ± 31
Fish and shellfish, g/d	71 ± 31	68 ± 30	70 ± 23	70 ± 28	70 ± 27	70 ± 28
Meats, g/d	56 ± 33	58 ± 30	56 ± 27	58 ± 30	60 ± 22	55 ± 25
Eggs, g/d	36 ± 14	38 ± 17	40 ± 14	40 ± 18	38 ± 14	38 ± 14
Milk and dairy product, g/d	166 ± 86	168 ± 49	167 ± 59	174 ± 55	168 ± 39	172 ± 44
Bean and soybean product, g/d	67 ± 40	63 ± 40	67 ± 40	64 ± 40	65 ± 49	70 ± 46
Vegetables, g/d	292 ± 95	276 ± 87	282 ± 91	266 ± 82	290 ± 86	279 ± 93
Fruits, g/d	110 ± 53	115 ± 47	114 ± 52	116 ± 43	104 ± 65	99 ± 55
Fat and oil, g/d	10 ± 6	10 ± 5	10 ± 3	11 ± 5	12 ± 5	11 ± 5
Sugar and confectioneries, g/d	10 ± 4	11 ± 6	9 ± 5	10 ± 6	11 ± 7	11 ± 7

[1] Values are mean ± SD. APP, Alaska pollack protein; BW, body weight; CON, whey protein control; TEI, total energy intake.
[2] Unpaired *t* test; there were no between-group differences in the food, energy, and macronutrient intakes.
[3] Participants consumed adequate protein from daily meals, not the APP and CON test meals. Therefore, the energy and protein, fat, and carbohydrate amounts contained in the APP or whey protein test meals were not included in the nutrition and food intake survey.

TABLE 3 Muscle mass and strength outcome variables in healthy older women who consumed APP or CON meals daily for 24 wk[1]

Outcome	*n*	Mean ± SD	Mean change (95% CI) from baseline	Mean change (95% CI), estimated between-group	*P*-interaction[2]
Change after 4 wk					
SMI, kg/(m^2 · 4 wk)				0.00 (−0.07, 0.08)	0.905
APP	42	6.25 ± 0.63	0.05 (0.00, 0.10)		
CON	42	6.32 ± 0.65	0.05 (−0.01, 0.10)		
Skeletal muscle mass, kg/4 wk				0.08 (−0.13, 0.29)	0.420
APP	42	20.5 ± 2.3	0.21 (0.08, 0.33)		
CON	42	20.4 ± 2.4	0.13 (−0.04, 0.30)		
Grip strength, kg/4 wk				0.46 (−0.40, 1.33)	0.290
APP	42	25.7 ± 4.2	0.38 (−0.34, 1.10)		
CON	42	25.0 ± 4.0	−0.08 (−0.59, 0.42)		
Gait speed, m/(s · 4 wk)				0.01 (−0.02, 0.04)	0.471
APP	42	1.34 ± 0.13	0.00 (−0.03, 0.02)		
CON	42	1.31 ± 0.13	0.01 (−0.01, 0.02)		
Knee extension strength, Nm/(kg BW · 4 wk)				0.03 (−0.02, 0.09)	0.269
APP	42	1.45 ± 0.26	0.01 (−0.03, 0.06)		
CON	42	1.39 ± 0.24	−0.02 (−0.05, 0.02)		
Chair stand test, rep/4 wk				0.45 (−0.67, 1.57)	0.423
APP	42	25.2 ± 4.3	−0.31 (−1.16, 0.54)		
CON	42	23.8 ± 5.2	−0.76 (−1.52, −0.01)		
Change after 12 wk					
SMI, kg/(m^2 · 12 wk)				0.12 (0.01, 0.23)	0.029
APP	42	6.36 ± 0.64	0.08 (0.02, 0.15)*		
CON	38	6.15 ± 0.57	−0.04 (−0.12, 0.05)		
Skeletal muscle mass, kg/12 wk				0.33 (0.06, 0.60)	0.015
APP	42	20.7 ± 2.4	0.19 (0.05, 0.33)*		
CON	38	20.0 ± 2.3	−0.14 (−0.37, 0.09)		
Grip strength, kg/12 wk				1.03 (0.04, 2.02)	0.041
APP	42	26.4 ± 4.2	0.80 (0.03, 1.58)*		
CON	38	24.5 ± 3.5	−0.23 (−0.86, 0.40)		
Gait speed, m/(s · 12 wk)				0.04 (−0.03, 0.10)	0.686
APP	42	1.33 ± 0.15	−0.03 (−0.09, 0.04)		
CON	38	1.31 ± 0.11	0.01 (−0.01, 0.03)		
Knee extension strength, Nm/(kg BW · 12 wk)				0.07 (0.02, 0.12)	0.012
APP	42	1.48 ± 0.27	0.04 (0.00, 0.08)*		
CON	38	1.38 ± 0.22	−0.03 (−0.06, 0.00)		
Chair stand test, rep/12 wk				0.82 (−0.45, 2.09)	0.201
APP	42	26.0 ± 5.1	0.43 (−0.48, 1.34)		
CON	38	23.9 ± 5.1	−0.39 (−1.30, 0.51)		
Change after 24 wk					
SMI, kg/(m^2 · 24 wk)				0.11 (0.03, 0.19)	0.013
APP	38	6.31 ± 0.65	0.04 (0.00, 0.09)*		
CON	43	6.19 ± 0.66	−0.07 (−0.14, 0.00)		
Skeletal muscle mass, kg/24 wk				0.29 (0.06, 0.51)	0.015
APP	38	20.5 ± 2.5	0.13 (0.00, 0.27)*		
CON	43	20.1 ± 2.3	−0.15 (−0.33, 0.03)		
Grip strength, kg/24 wk				1.04 (−0.02, 2.09)	0.053
APP	38	26.4 ± 4.3	0.93 (0.06, 1.80)		
CON	43	24.8 ± 3.4	−0.11 (−0.76, 0.54)		
Gait speed, m/(s · 24 wk)				0.02 (−0.01, 0.05)	0.209
APP	38	1.31 ± 0.15	0.00 (−0.02, 0.03)		
CON	43	1.28 ± 0.13	−0.02 (−0.04, 0.00)		
Knee extension strength, Nm/(kg BW · 24 wk)				0.05 (0.00, 0.09)	0.021
APP	38	1.43 ± 0.25	0.04 (0.00, 0.07)*		
CON	43	1.37 ± 0.23	−0.02 (−0.04, 0.01)		
Chair stand test, rep/24 wk				0.81 (−0.24, 1.85)	0.129
APP	38	26.3 ± 5.2	0.74 (0.06, 1.41)		
CON	43	24.0 ± 4.7	−0.07 (−0.87, 0.73)		
Physical QOL, score/24 wk				1.31 (−3.41, 6.04)	0.544
APP	38	48.2 ± 7.5	2.12 (−2.06, 6.29)		

(*Continued*)

TABLE 3 (*Continued*)

Outcome	*n*	Mean ± SD	Mean change (95% CI) from baseline	Mean change (95% CI), estimated between-group	*P*-interaction[2]
CON	43	47.7 ± 7.0	0.80 (−1.52, 3.13)		
Mental QOL, score/24 wk				0.25 (−3.33, 3.83)	0.722
APP	38	52.7 ± 6.7	−2.17 (−4.67, 0.33)		
CON	43	50.7 ± 5.8	−2.68 (−5.01, −0.34)		

[1] Between-group differences were evaluated using unpaired *t* tests; *different from CON, $P < 0.05$. APP, Alaska pollack protein; BW, body weight; CON, whey protein control; Nm, Newton meter; QOL, quality of life; rep, repetition; SMI, skeletal muscle mass index.
[2] Main effect × interaction; 2-factor repeated ANOVA.

intake of APP meal can be considered effective in the prevention of sarcopenia among healthy older adults. The effects of APP meal intake on knee extension strength were clearly observed. Weakness of the lower-extremity muscle strength is an important indicator of the risk of falls in older adults (22, 23). Thus, the daily intake of APP meal can be considered an effective method for the prevention of falls among healthy older adults.

Currently, standard treatment to increase skeletal muscle mass and strength in older adults involves high-intensity resistance exercise. However, this type of exercise may decrease motivation and increase withdrawal rates in older adults. In this study, APP meal intake increased skeletal muscle mass and muscle strength without resistance exercise in older adults. Therefore, APP intake is proposed for the prevention of sarcopenia in older adults who face difficulty with resistance-based exercises.

Previous animal studies reported that dietary APP meal intake significantly increased gastrocnemius and extensor digitorum longus muscle weight after 6 or 8 wk of feeding in rats (9). Moreover, dietary APP meal increased gastrocnemius skeletal muscle mass after only 2 d of feeding, regardless of the dietary fat content (24). In addition, APP intake significantly increased gastrocnemius skeletal muscle mass after 56 wk of feeding (24). Thus, APP induces acute and sustainable skeletal muscle hypertrophy in rats.

More previous animal studies have also demonstrated that dietary APP intake, in comparison with dietary casein, can suppress muscle atrophy, and it increased muscle hypertrophy in rats (24–26). Uchida et al. (24) demonstrated that, compared with dietary casein, dietary APP causes suppression of the gene expression of negative muscle mass regulators, such as *Fbxo32*/atrophy gene-1, *Mstn*/Myostatin, and *Trim63*/muscle-specific RING finger protein 1, in rats. Therefore, it was speculated that muscle hypertrophy induced by dietary APP could be attributed to the suppression of protein degradation by the ubiquitin–proteasome pathway.

In addition, the concentrations of muscle protein synthesis factors, such as phospho-protein kinase B (p-Akt)/Akt, p-70S6K/70S6K, and mammalian target of rapamycin (mTOR), increased significantly in the dietary APP group compared with those in the dietary casein group in rats (26). Therefore, compared with dietary casein, APP promoted skeletal muscle hypertrophy by activating the Akt/mTOR signaling pathway to enhance protein synthesis in rats (26). Intake of protein with a high content of leucine (≥2500 mg) enhances muscle protein synthesis in older adults (5). In this study, 448 mg leucine was a comparatively low dose. The APP group had increased SMI and skeletal muscle mass despite a lack of change in baseline total energy and protein intake. In addition, there was no difference in the amount of leucine between the APP and CON test meals in this study. It is possible that active peptides from the digestion of fish proteins (such as myosin, actin, troponin, and tropomyosin), and not the leucine in APP meal, accounted for skeletal muscle hypertrophy (24), because protein and leucine were at low doses in this study. On the other hand, daily APP meal intake could promote protein synthesis by activating the Akt/mTOR signaling pathway, which is also promoted by exercise (26). The active peptides derived by the hydrolyzing APP proteins could be responsible for causing muscle fiber hypertrophy by activating the Akt/mTOR signaling pathway and promoting protein synthesis, which is also promoted by exercise. Daily intake of a meal containing APP would be expected to have a muscle hypertrophic effect as a specific food function that is different from other protein sources. Our future research will provide that the specific food function of APP and active peptides of hydrolyzing APP are effective on promoting muscle hypertrophy.

Although previous research reported that 5 g of daily intake of APP meal paired with resistance exercise could increase skeletal muscle mass and knee extension strength after 8 wk in older adults (12), this study showed that an intervention with 5 g of daily APP meal intake without resistance exercise also increased SMI and knee extension strength after 12 wk. The whey protein dosage along with resistance exercise were reported to increase muscle mass and muscle strength in older adults (12, 27). However, a previous study demonstrated that whey protein intake without resistance exercise was not proven to increase skeletal muscle mass and strength in older adult women (27). Thus, although whey protein or APP intake accompanied with resistance exercise may increase skeletal muscle mass and muscle strength in healthy older adults, the effects of APP meal intake alone may be different from those of whey protein intake. APP meal intake might be more effective than whey protein intake in the prevention of sarcopenia. Thus, these findings emphasize the need for older adults to consume APP meal daily to maintain muscle mass and strength.

In this study, smaller increases in SMI (mean ± SD Δ SMI: +1.38% ± 3.41%) and knee extensor strength (Mean ± SD Δ knee extension strength: +2.69% ± 8.93%) after 12 wk of APP meal intake were observed than those in a previous study (12), in which APP meal and resistance exercise intervention were combined (Mean ± SD Δ skeletal muscle mass: +2.0% ± 3.2%; Mean ± SD Δ knee extension strength: +17.1% ± 4.4%). A previous study showed that the average rate of loss of skeletal muscle mass in people aged >70 y was in the range of 0.5%–1.0%/y (28). Therefore, daily intake of APP meal may have a clinically important antiaging effect of increasing SMI by 1.38% ± 3.41% in older women aged >70 y. On the other hand, Bai et al. (29) reported that the effect of BCAA-enriched supplementation was a 1.0 ± 0.8 kg increase in grip strength. Bo et al. (11) reported that 6 mo of nutritional supplementation

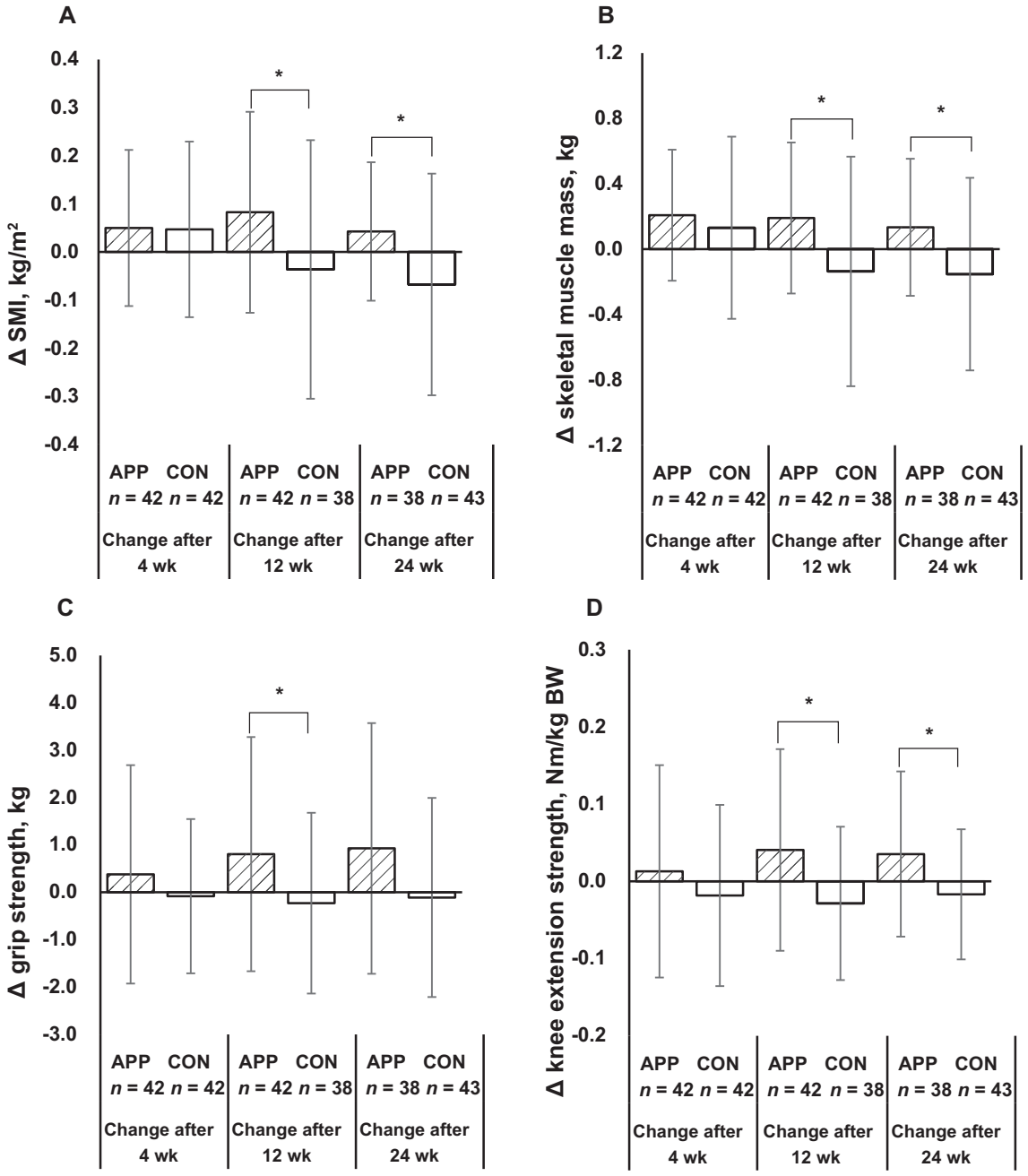

FIGURE 2 Mean changes in muscle mass and strength outcome variables in healthy older women who consumed APP or CON meals daily for 24 wk. Mean changes (Δ) in the (A) SMI, (B) skeletal muscle mass, (C) grip strength, and (D) knee extension strength from baseline to the 12-wk and 24-wk intervention periods in the 2 groups [APP meal intake intervention group (4 wk, $n = 42$; 12 wk, $n = 42$; 24 wk, $n = 38$) and CON meal intake group (4 wk, $n = 42$; 12 wk, $n = 38$; 24 wk, $n = 43$)] in healthy older women. Data are presented as mean change ± SD. Between-group differences were evaluated using unpaired *t* tests; *different from CON, $P < 0.05$. APP, Alaska pollack protein; BW, body weight; CON, whey protein control; Nm, Newton meter; SMI, skeletal muscle mass index.

(enriched with energy, protein, and vitamins D and E) increased skeletal muscle mass by 0.20 ± 1.1 kg in older adults. Kim et al. (20) reported that 3 mo of leucine-enriched essential amino acid increased skeletal muscle mass by 0.20 ± 2.4 kg and grip strength by 0.5 ± 5.2 kg in older women. Therefore, daily intake of APP meal increased skeletal muscle mass and strength by the same amount in this study as in previous studies by Bai et al. (29), Bo et al. (11), and Kim et al. (20). In the APP group, the Δ grip strength was positively correlated with the Δ SMI at 12 and 24 wk of intervention. At 24 wk, the APP group showed a small increase in skeletal muscle mass and muscle strength compared with the APP intake and resistance exercise observed in a previous study (12), but the moderate effect sizes for SMI and knee extension muscle strength in the CON group were 0.57 and 0.55, respectively (Supplemental Table 3). Therefore, daily intake of APP meal can be considered to have a physiologic effect on muscle strength and increase skeletal muscle mass among healthy older adults.

In addition, although APP meal intake increased skeletal muscle mass and strength in this study, it did not increase postintervention gait speed or physical QOL. A previous meta-analysis that included resistance exercise interventions for older

adults reported an improvement in physical and mental QOL (30). Another study showed that a 12-wk resistance exercise program with nutritional supplementation effectively increased the chair stand test and gait speed and also improved the physical QOL (31). Moreover, research has demonstrated that resistance exercise improves walking ability and psychological status in community-dwelling older adults (32, 33). Thus, the intervention of APP meal intake without resistance exercise is limited and may not affect walking ability or physical QOL. The effects of APP meal intake are limited to skeletal muscle mass and strength, and a combination of APP meal and resistance exercise may be necessary to improve physical performance and QOL in older adults.

This study has some limitations that should be addressed. First, our study was limited to women to eliminate known sex differences in age-related muscle atrophy. Second, we could not evaluate serum albumin concentration in our participants, which is known to be related to muscle hypertrophy (34). However, participants in this study were healthy older adults without malnutrition. Although we estimated muscle mass using direct segmental multifrequency BIA measurements, a strong positive correlation between DXA- and direct segmental multifrequency BIA–based measurements of muscle mass has been previously reported, confirming the validity of direct segmental multifrequency BIA measurements of muscle mass among older adults (15). Nevertheless, future intervention trials should consider using DXA to assess body composition precisely. Finally, because our intervention study reported a relatively small increase in muscle mass and strength at 24 wk, future studies are required to clarify the benefits of a prolonged intake of APP meal for an intervention period > 24 wk in increasing muscle mass and strength.

In conclusion, we demonstrated that daily intake of a meal containing APP compared with whey protein increases skeletal muscle mass and lower-extremity muscle strength in healthy older women, suggesting that an APP-containing meal may be useful in the prevention of sarcopenia in this group.

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Data Availability

All clinical data sets generated and analyzed during this study are not publicly available. However, clinical data sets are available from the corresponding author upon reasonable request.

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