

Original Article

Modulation of Neural and Muscular Adaptation Processes During Resistance Training by Fish Protein Ingestions in Older Adults

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Abstract

Assessments of both neural and muscular adaptations during interventions would provide valuable information for developing countermeasures to age-related muscle dysfunctions. We investigated the effect of fish protein ingestion on training-induced neural and muscular adaptations in older adults. Twenty older adults participated 8 weeks of isometric knee extension training intervention. The participants were divided into two groups who took fish protein ($n = 10$, Alaska pollack protein, APP) or casein ($n = 10$, CAS). Maximal muscle strength during knee extension, lower extremity muscle mass (body impedance method), and motor unit firing pattern of knee extensor muscle (high-density surface electromyography) were measured before, during, and after the intervention. Muscle strength were significantly increased in both CAS ($124.7 \pm 5.8\%$) and APP ($117.1 \pm 4.4\%$) after intervention ($p < .05$), but no significant differences between the groups were observed ($p > .05$). Significant increases in lower extremity muscle mass from 0 to 8 weeks were demonstrated only for APP ($102.0 \pm 3.2, p < .05$). Greater changes in motor unit firing pattern following intervention were represented in CAS more than in APP. These results suggest that nutritional supplementations could modulate neural and muscular adaptations following resistance training and fish protein ingestion preferentially induces muscular adaptation without the detectable neural adaptation in older adults.

Keywords: Aging, Alaska pollack protein, Nutritional supplementation, Multichannel surface electromyography, Motor unit identification

It is well known that resistance exercise preferentially improves neural factors during early periods of resistance training before adaptations in muscular factors such as muscle hypertrophy (1). Although muscular adaptations following resistance training had been well reported as muscle hypertrophy in the previous studies (2,3), neural adaptation, that is, alterations in motor unit firing properties, and interaction between neural and muscular factors in training adaptation have not been fully understood. Recently, it has been pointed out that age-related muscle dysfunction cannot be explained by only muscle atrophy such as sarcopenia (4,5). Regarding the training intervention

to older adults, adaptations in neural factors highly contribute to improvement in muscle strength by resistance training in older adults (1) and meta-analysis by Borde and colleagues (6) suggested that resistance exercise for older adults improves muscle strength but had small effect on muscle morphology. These findings mean that plasticity during resistance training could be higher in neural factor than that in muscular factors in older adults. Our studies also demonstrated that in older adults, the maximal muscle strength are positively correlated with motor unit firing rate (7) and both muscle thickness and surface electromyography (EMG) amplitudes (8). Therefore, assessment of

neural factors would be important information to understand adaptation processes during resistance training or other interventions in older adults as well as muscular factor.

Protein supplementation improves muscle protein synthesis after resistance training session even in older adults (9). There has been growing recognition that increases in muscle mass and strength following resistance training is elicited by protein supplementation in older adults (10,11). Because protein supplementation mainly induces muscular adaptations such as muscle hypertrophy, contributions of neural and muscular factors to training-induced adaptation would not be coincidence between resistance training interventions with and without protein supplementation. In other words, combination of interventions of resistance training and protein supplementation may be useful research designs to determine or quantify the adaptations in neural and muscular factors separately.

In most of the previous studies, casein, whey, and/or milk protein have been used as protein supplementations to test the effect of its combination with resistance training (10,11), and these are widely developed as commercial protein supplements. In daily diets, meat is also recommended as protein source for older adults because larger quantity of essential amino acids and richer beneficial biological compounds such as creatine, carnitine, iron, and/or cobalamin are contained in meat (12). On the other hand, fish is other protein source that can be frequently consumed in daily meals and is recognized to reduce mortality and risks of cardiovascular diseases in human due to fish oil such as n-3-PUFA (13). Recently, effect of fish protein intake on muscle morphology has been investigated in animal studies. Mizushige and colleagues (14), Kawabata and colleagues (15), and Morisasa and colleagues (16) showed that Alaska pollack protein (APP) ingestion induced more marked muscle hypertrophy comparing with casein (CAS) in rat muscle (14–16). Moreover, we recently conducted the intervention research to investigate the effect of fish protein intake and resistance training and its combination on motor unit firing pattern, muscle volume, and physical performance in older adults (17). Although we could not detect any changes in muscle strength and muscle morphology in knee extensor muscles, improvement in motor function of lower extremity such as chair stand test and changes in motor unit firing pattern in knee extensor muscles were greater in the group with APP and resistance training than other groups (17). Because we applied leg-press exercise to train all lower extremity muscles, improvement in muscle strength on a muscle group, that is, knee extensors, could not be observed. To understand detailed mechanisms of training adaptation, increase in muscle strength for the interested muscles should be observed.

In this study, we investigated the effect of fish protein ingestion on resistance training-induced neural and muscular adaptations in older adults. We applied isometric knee extension resistance exercise to improve muscle strength on the target muscles. Because the previous studies in rat muscle showed that APP ingestion induces greater increase in muscle mass when compared with CAS (14–16), we hypothesized that the group with APP would demonstrate greater increase in muscle mass following resistance training. Also, it was hypothesized that neural adaptation processes in APP are not coincidence with CAS supplementation because APP could mainly act on muscular factors.

Materials and Methods

Participants

Twenty healthy older male and female adults (mean age \pm SD: 73.4 \pm 5.4 years, range: 69–84 years) participated in this study. The participants gave written informed consent for the study after receiving a detailed explanation of the purposes, potential benefits, and risks

associated with participation. All procedures used in this study were approved by the Research Ethics Committee of Chukyo University (2015-002, 2016–057) and conducted in accordance with the Declaration of Helsinki.

Experimental Design

All participants trained their knee extensor muscles for 8 weeks. We applied isometric knee extension resistance training twice a week. Participants performed three sets of 10 isometric contractions during unilateral knee extension for both right and left legs with a rest interval of 60–120 seconds. Contraction intensity was set at greater than 80% of their maximal voluntary contraction (MVC) torque, and the performed and target torques were shown on the monitor as visual feedback. One contraction was constructed by 10 seconds of relaxation and resting phase, 1 second of increasing phase to the target torque, and 4 seconds of sustained phase at or over the target torque. During contractions, the experimenter requested the participants to count aloud 5 seconds to keep breathing. Target torque was updated when MVC increased after the measurements at every 2 weeks. Dynamometer system and posture of the participants for training were the same as in MVC measurements (see below).

During this training intervention, the participants were divided into two groups who intook the supplementations including APP or CAS in addition to normal daily diets. The participants took them every day, and ingestion timing were freely chosen from breakfast, lunch, or dinner by each participant. The supplementation for a day included 5 g of APP or CAS and amount of protein supplementations related to body mass of the participants in this study was 0.09 ± 0.01 g/kg/d for APP and 0.08 ± 0.02 g/kg/d for CAS. This amount of APP supplementation was calculated from the results of previous studies using rat (333 mg/kg/d) (14,15) and from the amount which has the potential to eat in daily life in addition to normal meal for human. On the other hand, amount of CAS supplementation used in this study is relatively low in comparison with those used in the previous studies (10–63 g/d or 0.3 g/kg/d) (10,11). We assumed that 5 g and 0.08 ± 0.02 g/kg/d of CAS ingestion can be used as a control condition because this amount of CAS could not be enough to act intrinsic functions as protein supplementation on muscle strength and mass. A randomized, double-blind, placebo-controlled treatment was conducted for supplementations. A dietary survey was performed by the nutritionist with the license from Ministry of Health, Labour and Welfare in Japan, and they used a brief-type self-administered diet history questionnaire (18) to quantify total energy, carbohydrate, protein, and fat in daily diets and its normalized values by individual body mass during the intervention. The result of brief-type self-administered diet history questionnaire reflected the daily diet from 3–4 to 7–8 weeks after the start of intervention.

Measurements of muscle strength, motor unit firing pattern, and anthropometry tests were conducted every 2 weeks during training intervention: (i) on the first day before the intervention commenced (0 week), (ii) at 2 weeks, (iii) 4 weeks, (iv) 6 weeks, and (v) 8 weeks after beginning the intervention study. Before intervention, the participants came to the laboratory to familiarize themselves with the instrumentation and motor tasks.

Muscle strength and anthropometry tests

MVC during isometric knee extension for right leg was measured at 0, 2, 4, 6, and 8 weeks by a custom-made dynamometer (Takei Scientific Instruments Co., Ltd., Niigata, Japan) with a force transducer (LU-100KSE; Kyowa Electronic Instruments, Tokyo, Japan). The participants sat in the dynamometer with both hip and knee

joint angles set to 90° (180° corresponds to full extension) and their distal part of shank in right leg fixed to the force transducer. MVC was determined according to our previously reported procedures (7,17,19). The MVC trial included a gradual increase in knee extension force to maximum effort in 2–3 seconds, and a plateau phase at maximum effort was maintained for 2–3 seconds with a verbal count given at 1-second intervals. The participants performed at least two trials with a ≥ 2 minutes rest interval between them. The highest MVC force was chosen from MVC trials and used in further analysis and as a reference to set a target force in resistance exercise. MVC torque was calculated as the product of the knee extension force and distance between the estimated rotation axes for the knee joint in the sagittal plane.

Muscle mass of whole body and lower limb was estimated from body impedance measurements (InBody270, InBodyJapan Inc., Tokyo, Japan) at 0, 4, and 8 weeks. We also measured body mass and fat mass in these measurements.

Local muscle morphology was assessed from thicknesses of the knee extensor muscles of right leg by ultrasonographic imaging (FAZONE CB, FUJI FILM, Tokyo, Japan). Longitudinal B-mode images were taken at 50% of the distance between the head of the greater trochanter and inferior lateral edge of the patella, at the location of the center of the electrode grid for surface EMG recording. The vertical distances between superficial edge of the vastus lateralis (VL) muscle and superficial edge of the femur were measured as muscle thickness with image analysis software (Image J, National Institutes of Health, Baltimore, MD). This muscle thickness includes the VL muscle and vastus intermedius muscle. A single operator blinded to information on the participant groups and test periods carried out this analysis. These measurements had also been used in our previous studies (7,17,19,20).

Multichannel Surface EMG Recording and Motor Unit Decomposition

To identify motor unit firing patterns, submaximal isometric knee extension at two different force levels were performed by the participants at 0, 2, 4, 6, and 8 weeks. The MVC torque measured at 0 week was used to calculate the target force. Therefore, the participants performed the motor tasks with the same absolute force levels at various measurement periods. After the MVC trials, isometric knee extension ramp contractions from 0% to 30% of MVC (Ramp30) and 0% to 90% of MVC (Ramp90) were tested for right leg. Two or three trials were performed for each task with a >2-minute rest interval among them. Ramp30 consisted of 15 seconds of increasing phase from baseline to 30% of MVC force level with 2% MVC/s rate of force increase and 15 seconds of sustained phase at 30% of MVC force level. Ramp90 consisted of 18 seconds of increasing phase from baseline to 90% of MVC force level with 5% MVC/s rate of force increase. Out of two or three trials, the trial with the smaller error between the target and actual forces was selected for analysis for Ramp30 and Ramp90, respectively. The ramp contractions were applied to identify motor unit recruitment threshold and to calculate motor unit firing rate at various force levels.

Multichannel surface EMG signals were recorded from the VL muscle of the right leg during the submaximal ramp contractions. We used a semidisposable adhesive grid of 64 electrodes with 1 mm diameter and 8-mm interelectrode distance (ELSCH064R3S, OT Bioelectronica, Torino, Italy). The electrodes were organized in 13 rows and 5 columns of electrodes with one missing electrode at the upper left corner. The method used for determining the

electrode location was as previously described (7,17,20). Briefly, the center of the electrode grid was located at the midpoint of the longitudinal axis of the VL muscle, that is, the line between the head of the greater trochanter and inferior lateral edge of the patella, and the columns were aligned along the VL longitudinal axis. A reference electrode (WS1, OT Bioelectronica, Torino, Italy) was placed at right knee. Monopolar surface EMG signals were recorded and amplified by a factor of 500, sampled at 2,048 Hz, and converted to digital form by a 16-bit analog-to-digital converter (Quattrocento, OT Bioelectronica, Torino, Italy), together with the force transducer signal. Recorded monopolar surface EMG signals were transferred to analysis software (MATLAB R2018a, MathWorks GK, Tokyo, Japan), filtered by a band-pass filter (10–450 Hz), and differentiated between neighboring electrodes along the columns. Fifty-nine bipolar surface EMG signals were used for further analysis.

From multichannel surface EMG signals, individual motor unit firing patterns were decomposed by the Convolution Kernel Compensation technique (21–24). We followed the decomposition procedure that was previously extensively validated on signals from various skeletal muscles (7,17,21,25–28). The pulse-to-noise ratio, introduced by (29), was used as an indicator of the motor unit identification accuracy (29) and only motor units with pulse-to-noise ratio > 30 dB (corresponding to an accuracy of motor unit firing identification > 90%) were used for further analysis, whereas all other motor units were discarded (29). After decomposition, discharge patterns of individual motor units were independently inspected and correlated by two experienced investigators. Discharge times for individual motor units were used for calculation of instantaneous motor unit firing rates. We excluded the discharges with interdischarge intervals < 33.3 or > 250 ms because firing rates that calculated from this range of interdischarge intervals are unusually high (>30 Hz) or low (<4 Hz) for the VL muscle (7,20,21,30,31).

Mean firing rates of individual motor units were calculated from instantaneous firing rates during each 5% of MVC for Ramp30 and 10% of MVC for Ramp90, for example, the mean firing rate at 15% of MVC for Ramp30 was calculated from the instantaneous firing rates on the interval from 12.5% to 17.5% of MVC and the mean firing rate at 50% of MVC for Ramp90 was calculated from instantaneous firing rates on 45%–55% of MVC interval. Mean firing rates with >30% coefficient of variation were excluded for further analysis (32). Detected motor units were divided into four and three groups by the recruitment force: motor units recruited at <10%, 10%–15%, 15%–20%, and 20%–25% of MVC for Ramp30, and 0%–20%, 20%–40%, and 40%–60% of MVC for Ramp90. These four and three motor unit groups for Ramp30 and Ramp90 were used for further analyses, respectively. All motor units from the participants of the same group were merged into a motor unit group, and averaged values were calculated for each motor unit group with different recruitment thresholds at different periods. These procedures for calculating motor unit firing rates had been previously used in our studies (7,17,20).

Statistics

The results are reported as the mean \pm SD. Before the statistical analysis processes, we performed the Shapiro–Wilk test to assess the normal distribution of data for further analysis. Because our results included non-normal distributed data, the present study used nonparametric statistical tests. The Mann–Whitney test was used to compare age, height, body mass, muscle mass, fat mass

estimated by InBody, muscle tissue thicknesses measured by ultrasonography, and MVC between the groups at 0 week. The body mass, body fat, and whole-body and lower limb muscle mass estimated from InBody, muscle tissue thicknesses measured by ultrasonography, and MVC at 2, 4, 6, and 8 weeks were normalized by the values at 0 week to test the effect of intervention and to compare the data between the groups. Percentages of timing of taking the given nutritional supplementations were also compared between the groups by the Mann–Whitney test. To test the effect of intervention, the Friedmann test was applied to the normalized values of body mass, body fat, whole-body and lower limb muscle mass, muscle tissue thicknesses, and MVC of 0, 2, 4, 6, and 8 weeks for each participant group. If significant effects of intervention were detected, the Bonferroni–Dunn test was used to compare the values at 2, 4, 6, and 8 weeks with the value at 0 week (33). Normalized values of body mass, body fat, whole-body and lower limb muscle mass, muscle tissue thicknesses, MVC, and nutritional parameters were compared between the groups at each period by the Mann–Whitney test.

Effect of intervention on motor unit firing rate for each force level and motor unit group was tested by the Kruskal–Wallis test. When a significant effect of the intervention was identified by the Kruskal–Wallis test, firing rates were compared among 0, 2, 4, 6, and 8 weeks by the Dunn's test (34). Motor unit firing rate at each force level was also compared among the motor unit groups with different recruitment forces by the Kruskal–Wallis test. If the Kruskal–Wallis test detected a significant effect of the motor unit group, firing rates at a force level were compared among the motor unit groups with different recruitment forces by the Dunn's test (34). Statistical analysis was performed using SPSS (version 21.0, SPSS, Tokyo, Japan) and MATLAB (R2018a, MathWorks GK).

Results

Muscle Strength and Anthropometry Tests

There were no significant differences between CAS and APP in age, height, body mass, muscle mass, fat mass estimated by InBody, muscle tissue thicknesses measured by ultrasonography, and MVC at 0 week ($p > .05$; Table 1) and nutritional parameters during intervention ($p > .05$; Supplementary Table 1). Percentages of timing of taking the given nutritional supplementations for CAS and APP were $34.5 \pm 44.3\%$ and $65.0 \pm 40.1\%$ with breakfast, $16.4 \pm 18.4\%$ and $15.8 \pm 22.3\%$ with lunch, and $49.1 \pm 39.3\%$ and $19.2 \pm 29.1\%$ with dinner. There were no significant differences in timing of taking the nutritional supplementations between CAS and APP ($p > .05$).

Body mass and muscle thickness were not changed during intervention in both CAS and APP ($p > .05$; Table 2 and Supplementary Table 3). For whole-body muscle mass, lower limb muscle mass, and fat mass, significant effects of intervention were found in APP ($p < .05$), whereas there were no significant effects of intervention in CAS ($p > .05$; Table 2 and Supplementary Table 3). Whole-body muscle mass and lower limb muscle mass in APP at 8 weeks were significantly greater than those at 0 week ($p < .05$; Table 2 and Supplementary Table 3). Fat mass in APP significantly decreased at 8 weeks when compared with 0 week ($p < .05$; Table 2 and Supplementary Table 3). MVC was significantly influenced by intervention in both CAS and APP ($p < .05$; Figure 1, Table 2, and Supplementary Table 3). MVC at 6 and 8 weeks were significantly greater than that at 0 week in CAS and MVC at 4, 6, and 8 weeks were significantly greater than that at 0 week in APP ($p < .05$; Figure 1, Table 2, and Supplementary Table 3).

Table 1. Characteristics of the Participants

	CAS	APP
<i>n</i>	10	10
<i>n</i> of male	8	8
Age (y)	73.1 \pm 1.5	73.6 \pm 2.0
Height (cm)	161.6 \pm 2.8	161.3 \pm 2.3
Body mass (kg)	62.23 \pm 3.8	59.86 \pm 2.6
Muscle mass (kg)	24.99 \pm 5.6	24.29 \pm 3.6
Fat mass (kg)	15.93 \pm 4.6	15.35 \pm 4.1
Muscle thickness (cm)	3.75 \pm 0.59	3.68 \pm 0.66
Subcutaneous tissue thickness (cm)	0.71 \pm 0.19	0.78 \pm 0.28
MVC (N)	342.7 \pm 45.2	375.6 \pm 39.3

Notes: APP = Alaska pollack protein; CAS = casein; MVC = maximal voluntary contraction.

Motor Unit Firing Patterns

In total, 2,154 motor units were identified: 774 in CAS and 633 in APP for Ramp30 and 408 in CAS and 339 in APP for Ramp90 (Supplementary Table 2).

For Ramp30, a significant effect of intervention was observed in motor units recruited at 15%–20% of MVC for CAS ($p < .05$). At 25% of MVC, their motor unit firing rates at 4 weeks were significantly lower than at 0 and 8 weeks ($p < .05$; Figure 2). For Ramp90, a significant effect of intervention was observed in motor units recruited at 20%–40% of MVC for CAS ($p < .05$). At 80% of MVC, their motor unit firing rates at 4 weeks were significantly greater than at 0 week ($p < .05$; Figure 3).

For both CAS and APP, there were significant differences in firing rates among motor unit groups with different recruitment thresholds. The number of these significant differences at different force levels increased consistently with the time course of intervention in CAS, but not in APP (Supplementary Figures 1 and 2). In particular, for Ramp30, the number of groups with significant differences increased from 0 to 2 weeks for CAS ($\Delta 4$), but not for APP ($\Delta 0$), from 0 to 4 weeks ($\Delta 7$ and $\Delta 1$ for CAS and APP), from 0 to 6 weeks ($\Delta 5$ and $\Delta 3$ for CAS and APP), and from 0 to 8 weeks for CAS ($\Delta 6$), but not for APP ($\Delta 0$; Supplementary Figure 1). Thus, the number of significant differences was greater in CAS than in APP. These results indicate that relationships in firing rates among motor units with different recruitment thresholds are changed during intervention and this change is greater in CAS than in APP. This tendency was also found in Ramp90, that is, CAS showed greater changes in firing rates among motor unit groups with different recruitment thresholds than APP (Supplementary Figure 2).

Discussion

We investigated the effect of fish protein supplementation on neural and muscular adaptations following 8 weeks of isometric resistance training in older adults. Two groups with different nutritional supplementations, that is, CAS and APP, showed the resistance training-induced strength gain following the intervention. Although there were no significant differences in MVC between the groups at all periods, significant increases in MVC were observed 4 weeks onwards in APP and from 6 weeks onwards in CAS, respectively (Figure 1 and Table 1). These results suggest that ingestion of APP induces earlier improvement in muscle strength when compared with ingestion of CAS. Similar trend of results was reported in our previous study (17). During 6 weeks of resistance training in leg-press exercise,

Table 2. Results of Anthropometric Parameters and Motor Function Tests (Relative to 0 Week)

	Weeks	CAS		APP	
Body mass	0	100.0 ± 0.0		100.0 ± 0.0	
	4	100.1 ± 0.5		100.1 ± 1.3	
	8	100.3 ± 1.7		100.2 ± 1.2	
Whole-body muscle mass	0	100.0 ± 0.0		100.0 ± 0.0	
	4	101.3 ± 1.8		101.6 ± 2.4	
	8	101.6 ± 2.3		102.1 ± 1.9	*.039
Lower limb muscle mass	0	100.0 ± 0.0		100.0 ± 0.0	
	4	101.1 ± 2.1		100.4 ± 1.4	
	8	101.3 ± 3.2		102.0 ± 3.2	*.006
Fat mass	0	100.0 ± 0.0		100.0 ± 0.0	
	4	96.9 ± 4.1		96.8 ± 5.0	
	8	96.7 ± 3.5		94.4 ± 6.6	*.039
Muscle thickness	0	100.0 ± 0.0		100.0 ± 0.0	
	2	98.7 ± 5.6		97.8 ± 4.2	
	4	101.4 ± 6.0		97.5 ± 4.2	
	6	100.1 ± 6.2		99.0 ± 6.0	
	8	100.2 ± 6.9		102.2 ± 6.1	
MVC	0	100.0 ± 0.0		100.0 ± 0.0	
	2	102.8 ± 4.0		105.6 ± 2.4	
	4	114.2 ± 4.1		110.2 ± 3.5	*.012
	6	119.0 ± 5.7	*.004	113.4 ± 3.7	*.004
	8	124.7 ± 5.8	*.004	117.1 ± 4.4	*.004

Notes: APP = Alaska pollack protein; CAS = casein; MVC = maximal voluntary contraction.
 *Significant difference versus 0 wk.

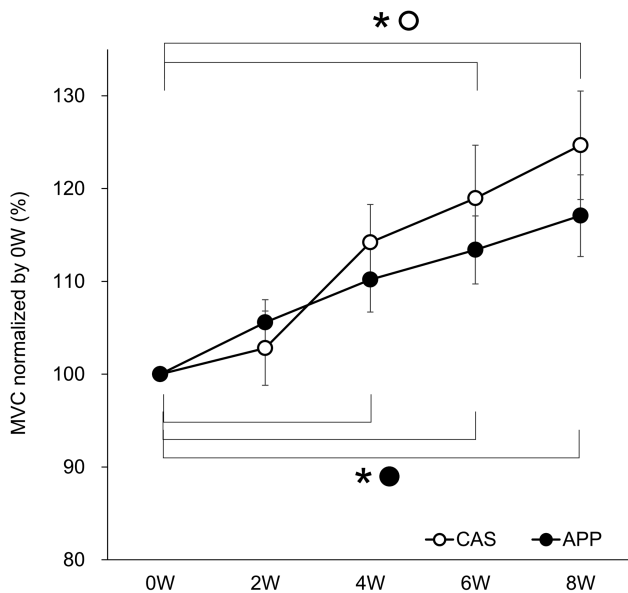


Figure 1. Maximal voluntary contraction (MVC) during isometric knee extension for the groups with ingestions of Alaska pollack protein (APP) and casein (CAS). *Significant differences ($p < .05$) among the periods.

improvements in chair stand test were found at 3 weeks after intervention in the older adults who ingested APP, whereas they were found at 6 weeks after intervention in the older adults who ingested placebo food (17). Generally, strength gain in 4 or 6 weeks of resistance training have been explained by neural adaptation (35,36). In the present study, greater changes in motor unit firing pattern were mainly found in CAS, but not in APP, during training periods including early phases such as 4 and 6 weeks (Figures 2 and 3 and Supplementary

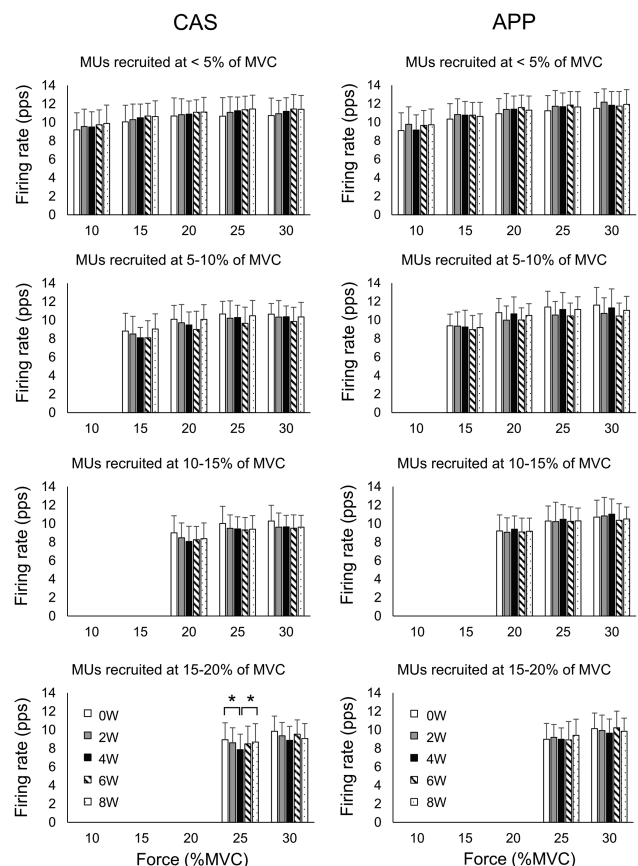


Figure 2. Motor unit firing rates during Ramp30 for the groups with ingestions of Alaska pollack protein (APP) and casein (CAS). MU = motor units. *Significant differences ($p < .05$) among the periods.

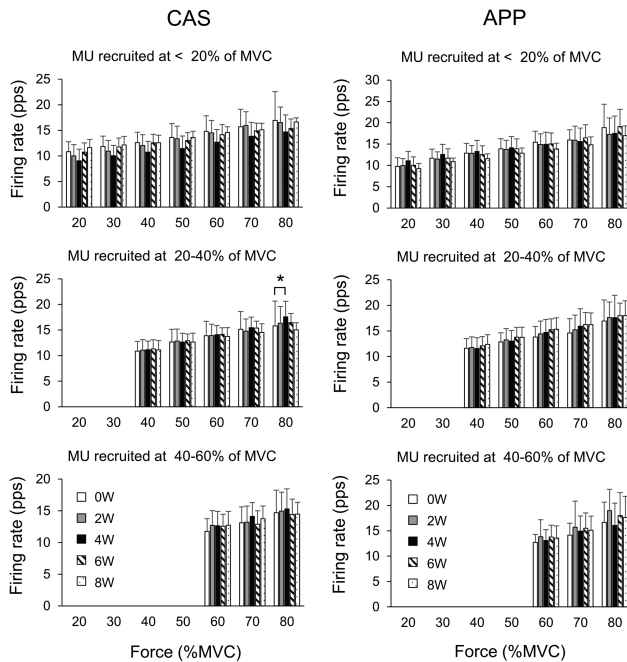


Figure 3. Motor unit firing rates during Ramp90 for the groups with ingestions of Alaska pollack protein (APP) and casein (CAS). MU = motor units. *Significant differences ($p < .05$) among the periods.

Figures 1 and 2), meaning that it could be difficult to explain the earlier improvement in muscle strength on APP by neural adaptation that was assessed by motor unit firing pattern in the present study. Also, detectable morphological changes were not observed in APP and CAS at 4 and 6 weeks in this study (Table 2). Therefore, the results of neural and muscular factors in our study could not explain different time course in resistance training-induced improvements of muscle strength between ingestions of APP and CAS. Because it is unlikely for ingestion of APP to act directly on the neural factors, we thus estimated that undetectable morphological changes might have occurred in muscle tissues during 4 or 6 weeks of the intervention in the present study (3). Seynnes and colleagues (3) reported an increase in quadriceps cross-sectional area measured by magnetic resonance imaging system even in 20 days of resistance training. Because we used ultrasonography and body impedance methods to assess muscular adaptation, potential muscular adaptation may not have been detected in the present study. We provide further discussion for muscular adaptation with ingestion of APP in the later parts.

In the present study, significant increases in whole-body and lower extremity muscle mass were found at 8 weeks only for APP ($p < .05$, Table 2). Because an increase in muscle mass was not observed for CAS ($p > .05$, Table 2), we can assume that APP ingestion can enhance an increase in muscle mass following resistance training in older adults. Also, we found a decrease in fat mass from 0 to 8 weeks for APP ($p < .05$, Table 2), but not for CAS ($p > .05$, Table 2). The previous study demonstrated that ingestions of APP for 6 or 8 weeks induce significant increases in the gastrocnemius muscle weight and fiber diameters when comparing with ingestion of CAS (14–16) and a significant increase of myosin heavy-chain gene expression in *Myh4* (14) in rats. Also Mizushige and colleagues (15) showed decrease in serum triglycerides and inhibition of visceral body fat accumulation in rats, and Morisasa and colleagues reported that lipid molecules were significantly changed in APP-fed rats than in CAS-fed rats (16). Moreover, Mizushige and colleagues (15) and

Kawabata and colleagues (14) suggested that muscle hypertrophy induced by APP ingestion leads the enhancement of basal energy expenditure. It may be difficult to directly apply these results in rats to human, but APP ingestion could act on muscle protein synthesis and on energy metabolisms inducing reduction of fat mass, which were conformed in animal studies.

Although increase of in muscle mass was found only in APP, similar degrees of muscle strength gain in CAS and APP groups were observed in the present study. We therefore assume that the other factors than muscle morphology, such as neural factor, contribute to improvement in muscle strength in CAS. We demonstrated difference in effect of resistance training on motor unit firing patterns between APP and CAS ingestions (Figures 2 and 3; Supplementary Figures 1 and 2). In addition to our previous study (17), the present study also confirmed that the food ingestion can modulate motor unit firing pattern following resistance training. In comparisons among the different periods, significant changes in motor unit firing rate were shown slightly only for CAS, but not for APP (Figures 2 and 3). For Ramp30, motor units recruited at 15%–20% of MVC at 4 weeks indicate significant lower firing rate at 25% of MVC when comparing with those at 0 and 8 weeks ($p < .05$; Figure 2). This change of motor unit firing rate is similar to motor units recruited at <20% of MVC during Ramp90 for CAS (Figure 3). On the other hand, motor units recruited at 20%–40% of MVC during Ramp90 exhibited significantly greater firing rates at 4 weeks than at 0 week for CAS ($p < .05$; Figure 3). Therefore, we found different trends of motor unit firing rate changes during 8 weeks of resistance training among the motor units with different recruitment thresholds for CAS. Also, training-induced changes in firing rate of motor units recruited at 15%–20% of MVC for Ramp30 and motor units recruited at 20%–40% of MVC for Ramp90 were observed at 4 weeks. At 8 weeks, these values returned to baseline. The results in the previous studies have varied as to whether resistance training increases, decreases, or changes motor unit firing rate. Therefore, changes in motor unit firing pattern following resistance training seemed to be strongly influenced by the training regimen. For example, the previous study that is similar to our research design showed that increase in motor unit firing rate during submaximal contraction in young adults (37) and no change in motor unit firing rate during maximal and submaximal contraction in older adults (38). It could be difficult to explain the physiological mechanisms of increase/decrease in motor unit firing rate in this study. On the other hand, we also found that changes of relationship in firing rate among motor unit groups following resistance training were different between the CAS and APP (Supplementary Figures 1 and 2). To quantify the relationship in firing rate among motor unit groups, we counted the numbers of significant differences in firing rate between different motor unit groups for each period and participant group. As the results demonstrate (Supplementary Figures 1 and 2), greater increases in the number of significant differences in firing rate between motor unit groups with different recruitment thresholds during training intervention (from 0 to 8 weeks) were shown in CAS when compared with APP for Ramp30 and 90. Significant differences in firing rate among the motor units with different recruitment thresholds are normally presented in young adults (7) and this is known as onion skin phenomenon (39,40). However, this structure is changed in older adults where firing rates among the motor units with different recruitment thresholds start to overlap (7). This age-related change in relationship in firing rate among different motor unit groups may be muscle specific and can be explained by decrease in the firing rate of low-recruitment threshold motor units in older adults (7). Our previous study demonstrated that resistance training intervention induces

an increase in firing rate in low-recruitment threshold motor units and changes relationship in firing rate among motor unit groups with different recruitment thresholds in older adults (17). Therefore, changes in firing rate relationship among motor unit groups with different recruitment thresholds during the CAS intervention can be interpreted as one of the neural adaptations following resistance training. Observed larger alterations in motor unit firing rates in CAS than in APP at least partially explain why the muscle strength increased without muscle hypertrophy in CAS and with muscle hypertrophy in APP intervention.

In this study, we assessed neuromuscular activation only in agonist muscle during the resistance training interventions. Previous studies suggested that increases in maximal strength following resistance training may have accomplished by a reduction in coactivation in older adults (19). We should note that the intervention of resistance training in this study may alter neuromuscular activations not only in agonist knee extensor muscles, but also in antagonist knee flexor muscles.

Conclusion

We investigated the effect of fish protein supplementations on resistance training-induced neural and muscular adaptations in older adults. For this purpose, we compared muscle strength, muscle mass, and motor unit firing patterns following 8 weeks of isometric knee extension resistance training between the older adults with ingestions of APP and CAS. Muscle strength of knee extensor was significantly increased in both groups at 8 weeks ($124.7 \pm 5.8\%$ for CAS and $117.1 \pm 4.4\%$ for APP, $p < .05$), and degrees of strength gains were not significantly different between CAS and APP at each period ($p > .05$). Significant increases in lower extremity muscle mass from 0 to 8 weeks were demonstrated for APP (102.0 ± 3.2 , $p < .05$), but not for CAS (101.3 ± 3.2 , $p > .05$). Greater changes in relationship in firing rates among motor unit groups with different recruitment thresholds following resistance training intervention were represented in CAS than in APP. These results suggest that nutritional supplementations could modulate neural and muscular adaptations following resistance training. Fish protein ingestion preferentially induces muscular adaptation without the detectable neural adaptation in older adults.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

Supplementary Figure 1. Relationships in firing rates among motor units with different recruitment thresholds during Ramp30 for the groups with ingestions of Alaska pollack protein (APP) and casein (CAS). MU: motor units. The symbols indicate significant differences ($p < .05$) between the motor units with different recruitment thresholds, respectively.

Supplementary Figure 2. Relationships in firing rates among motor units with different recruitment thresholds during Ramp90 for the groups with ingestions of Alaska pollack protein (APP) and casein (CAS). MU: motor units. The symbols indicate significant differences ($p < .05$) between the motor units with different recruitment thresholds, respectively.

Supplementary Table 1. Results of diet survey using a brief-type self-administered diet history questionnaire (BDHQ).

Supplemental Table 2. Number of motor units detected and considered for analysis for each group.

Supplementary Table 3. Results of anthropometric parameters and motor function tests.

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Conflict of Interest

None reported.

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