



Preventing effects of exopolymers purified from *Aureobasidium pullulans* (EAP) supplementation and resistance exercise on muscle aging and loss in the Korean elderly: a randomized controlled trial

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Abstract

Objective In this study, an analysis was performed to observe the effect of resistance exercise and exopolymers purified from *Aureobasidium pullulans* (EAP) intake for 12 weeks on muscle reduction and improvement of muscle function in the elderly.

Methods A total of 45 elderly individuals (age distribution: 60–78 years; average age: 67.24 years) were randomized into 15 subjects in the high-dosage (1 g/d) intake and exercise group, 15 subjects in the low-dosage (0.5 g/d) intake and exercise group, and 15 subjects in the placebo intake and exercise group. Body composition, physical strength, isokinetic muscle function, blood lipids and blood pressure, and muscle metabolism-related hormones were measured in all three groups before and after the same 12-week exercise–nutrition program.

Results Resistance exercise for 12 weeks and 1 g/day intake of EAP improved physical strength, isokinetic muscle function, blood lipids, blood pressure, and some parameters related to muscle metabolism. In the hormones related to muscle metabolism, the catabolic hormones CK and LDH significantly decreased ($p < 0.05$), and otherwise, the anabolic hormone GH significantly increased ($p < 0.05$).

Conclusion It was confirmed that resistance exercise and EAP intake for 12 weeks can have positive and synergistic effects on muscle reduction and muscle improvement in the elderly.

Keywords Exercise · Exopolymers · *Aureobasidium pullulans* · Muscle aging and loss · A randomized controlled trial

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Introduction

According to the Geriatrics Fact Sheet, 2018, it is predicted that by 2029, elderly people aged 65 years or older will account for 25% of the population, and Korea will enter an ultra-aged society. As of 2018, the life expectancy is 79.7 years for men and 85.7 years for women, with an average age of 82.7 years old [1]. Among them, the average healthy life expectancy is 64.4 years old as of 2018. This is a slight decrease from the past, meaning that men in South Korea suffer for 15 years on average, and women about 20 years on average [1, 2].

Recently, it has been confirmed that it is very important to manage sarcopenia and muscle frailty so that the elderly can live a healthy daily life regardless of disease. Sarcopenia is a phenomenon in which the physical function decreases along with muscle strength due to a decrease in the skeletal muscle mass with aging [3, 4]. In addition, fat fills the place where the muscle is left, and the weight is maintained. Therefore, it is easy to know the state of muscle loss [5].

According to the Korea National Health and Nutrition Examination Survey [2], 10–28% of the population over the age of 65 years is reported to have sarcopenia. Their muscle mass in their 70 s was 30% lesser than in their 30 s and 40 s. The muscle strength is also observed to decline. It is known that the average grip strength of men in their 30 s in Korea is approximately 47 kg; however, the average grip strength of men in their 70 s is only approximately 34 kg [2].

Sarcopenia is a disease that was given a diagnostic code (M62. 84) in the US International Classification of Diseases (ICD)-10-CM in April 2016 [6]. The whole world paid attention to the decision of the USA in that there were many disease codes similar to sarcopenia, such as muscle atrophy and muscle loss. Subsequently, the World Health Organization (WHO) included sarcopenia in the 11th edition of the International Classification of Diseases [7]. In line with this trend, the Korean government is also planning to include a diagnostic code for sarcopenia in the 8th revision of the Korean Standard Classification of Diseases (KCD) in 2021 [8].

Recently, various drugs for treating sarcopenia have been developed. Eli Lilly and Company is developing LY2495655, an antibody to myostatin, which is a cytokine that inhibits muscle growth, differentiation, and neogenesis. Acceleron Pharma, Inc. is developing ACE-031 (a soluble form of activin receptor type-2B), the decoy receptor for an activin receptor type-2B (ACVR2B). Novartis International AG previously conducted clinical trials while developing bimagrumab, an antibody of ACVR2B; however, development was finally stopped in 2018. Thus, a

therapeutic agent for treating sarcopenia has not yet been developed [9, 10]. As an alternative to this, resistance exercise, protein supplementation, and supplementation with vitamin D have been established as the basic treatment for sarcopenia [10].

Several studies have confirmed the effects of preventing sarcopenia and increasing muscle mass and strength in the elderly by performing exercise along with ingesting functional materials, such as collagen peptides [11] and tea catechins [12], which can help to prevent muscle loss [3, 4]. β -glucans and β -glucan-rich extracts are known to have various physiological activities [13]. Exopolymers purified from *Aureobasidium pullulans* SM-2001 (EAP) contain 13% β -1,3/1,6-glucan [14]. It has been reported that EAP have antiosteoporotic effects in rats [15, 16] and in healthy women [17], and muscle atrophy-inhibiting effects in mice [18]. However, studies on the effects of exercise and EAP intake on muscle function improvement have not yet been conducted.

Therefore, in this study, to evaluate the effect of preventing sarcopenia, 45 elderly individuals were randomly divided into 15 individuals in the placebo intake and exercise group, 15 individuals in the low-dosage intake and exercise group (0.5 g of EAP with 0.5 g of crystalline cellulose), and 15 individuals in the high-dosage intake and exercise group (1 g of EAP). All three groups performed the same resistance exercises for 12 weeks, and measurements of body composition, physical strength, isokinetic muscle function, blood lipids and blood pressure, and hormones related to muscle metabolism were measured before and after the exercise–nutrition program.

Results

Information on the subjects who participated

If power (1—type 2 error [β]) is 0.80, the significance level (α) is 0.05, and the effect size (f) is 0.5 (the difference between groups is medium effect size), the result of calculating the number of subjects, 42 subjects are theoretically required for this study, using G*Power 3.0 program [19]. Considering the dropout rate (about 10%), a total of 45 subjects were planned as the number of subjects to be recruited. In this experiment, 50 elderly individuals were recruited as subjects, of which five individuals were excluded (those with acute infection or a history of malignant tumors and a history of cerebral infarction, myocardial infarction, peripheral vascular disease, or dementia in the last 6 months were excluded). The age distribution and average age of the 45 elderly subjects were 60–78 years and 67.24 years,

Table 1 The Gym-stick exercise program used in this study

Exercise	Contents	Period (weeks)	Intensity	Time (min)
Warm-up	Stretching & walking			10
Main (Resistance exercise)	Gym-stick ① high knee ② good morning exercise ③ biceps curl ④ triceps extension ⑤ bentover low ⑥ hip extension ⑦ curl up ⑧ scapular retraction ⑨ shoulder press ⑩ squat	1 ~ 4 (green tube) 5 ~ 12 (blue tube)	RPE 11–12 (8 ~ 12 times × 1 ~ 2 set) RPE 13–14 (8 ~ 15times × 2 ~ 3 set)	30
Cool down	Stretching & breathing			10
Total				50

Table 2 The diagnostic kits used in this study

	Diagnostic kit	Manufacturer
Total cholesterol	CHOL2	Roche Holding AG. ¹
Triglyceride	TRIGL	Roche Holding AG
HDL-C	HDL-HOLESTEROLGEN.4(HDLC4)RX	Roche Holding AG
LDH	LDHI2	Roche Holding AG
Glucose	Glucose HK Gen.3	Roche Holding AG
Creatine	CREJ2	Roche Holding AG
CK	Creatine Kinase	Roche Holding AG
IGF-1	LIAISON IGF-1	DiaSorin Inc. ²
GHRH	Immolute 2000 hGH	Siemens ³
Insulin	Elecsys Insulin	Roche Holding AG

HDL-C high-density lipoprotein cholesterol; *LDH* lactate dehydrogenase; *CK* creatine kinase; *IGF-1* insulin-like growth factor 1; *GHRH* growth hormone-releasing hormone

¹Roche Holding AG. (Basel, Switzerland)

²DiaSorin Inc. (Saluggia, Italy)

³S (München, Germany)

respectively (Table 1). The average age, average height, average weight, and average body mass index (BMI) of the subjects belonging to the three groups are shown in Table 2.

Body composition and waist and hip circumference

When the body composition, such as the body weight, BMI, body fat percentage, fat mass, bone mineral mass, and waist and hip circumferences, were measured before and after the 12-week exercise–nutrition program, the placebo intake and exercise group, the low-dosage intake and exercise group, and the high-dosage intake and exercise groups showed a tendency to decrease in the intra-group

Table 3 The age distribution and average age of the subjects in this study

	No	Age		
		Minimum	Maximum	Mean ± SD
Man	7	60	78	69.14 ± 5.98
Woman	38	60	78	66.89 ± 4.63
Total	45	60	78	67.24 ± 4.85

comparison. Otherwise, when comparing between groups, there was no significant change (Table S1).

Physical strength

To evaluate the change in physical strength, tests were conducted for left forward flexion, left grip strength, right grip strength, sit-up, unipedal stand, 10 m obstacle walking, and 6-min walking, and the results are shown in Table 3.

Sit-up, unilateral standing, and 6-min walking tests

When checking the change in the sit-up, unilateral standing, and 6-min walking tests, positive changes were found in all of the intra-group changes in the placebo group, the low-dosage intake and exercise group, and the high-dosage intake and exercise group; however, no statistically significant intergroup changes were found (Table 3).

Sit and reach (as flexibility)

The comparative analysis of sit and reach (as flexibility) within the group confirmed significant positive changes in all of the intra-group changes in the placebo group, the low-dosage intake and exercise group, and the high-dosage intake and exercise group ($p < 0.05$). When checking the changes between groups, the changes in the high-dosage intake and exercise group were higher than those in the low-dosage intake and exercise group and the placebo groups ($F = 4.114$, $p = 0.023$) (Table 3, Fig. 1).

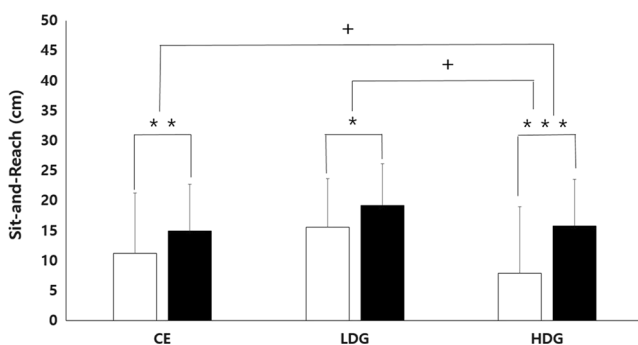


Fig. 1 The change of flexibility (sit and reach) in both intra- and intergroup comparisons of CG, LDG, and HDG group. CG: Control (placebo) intake and exercise group; LDG: Low-dosage (EAP 0.5 g) intake and exercise group; HDG: High-dosage (EAP 1.0 g) intake and exercise group. Before (□) and after (■) the 12-week exercise–nutrition program. *Significant different between group by paired t test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, +LSD test, $+p < 0.05$

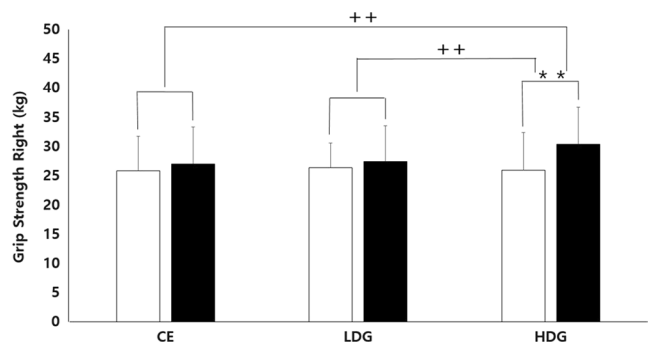


Fig. 2 The change of right-handed grip strength in both intra- and intergroup comparisons of CG, LDG, and HDG group. CG: Control (placebo) intake and exercise group; LDG: Low-dosage (EAP 0.5 g) intake and exercise group; HDG: High-dosage (EAP 1.0 g) intake and exercise group. Before (□) and after (■) the 12-week exercise–nutrition program. *Significant different between group by paired t test, $**p < 0.01$, +: LSD test, $+p < 0.05$, $++p < 0.01$

Left-handed grip strength

For left-handed grip strength, the intra-group comparative analysis showed significant differences between the high-dosage intake and exercise group and the placebo groups ($p < 0.05$). When the change between the groups was confirmed, it was observed that the changes in the high-dosage intake and exercise group were more than those in the low-dosage intake and exercise group ($F = 4.190$, $p = 0.022$) (Table 3).

Right-handed grip strength

In the comparative analysis of the right-handed grip strength within the group, only the high-dosage intake and exercise

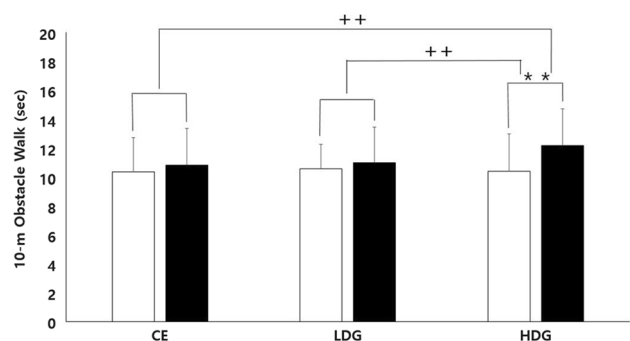


Fig. 3 The change of walking time in both intra- and intergroup comparisons of CG, LDG and HDG group in the 10-m obstacle walking test. CG: Control (placebo) intake and exercise group; LDG: Low-dosage (EAP 0.5 g) intake and exercise group; HDG: High-dosage (EAP 1.0 g) intake and exercise group. Before (□) and after (■) the 12-week exercise–nutrition program. *Significant different between group by paired t test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ +LSD test, $+p < 0.05$, $++p < 0.01$

group showed a significant increase ($p < 0.05$), and when the changes between the groups were compared, the strength of changes in the high-dosage intake and exercise group had higher than those in the low-dosage intake and exercise group and the placebo intake and exercise groups ($F = 5.301$, $p = 0.009$) (Table 3, Fig. 2).

10-m obstacle walking test

In the 10-m obstacle walking test, the intra-group comparative analysis confirmed significant positive changes in all of the intra-group changes in the placebo group, the low-dosage intake and exercise group, and the high-dosage intake and exercise group ($p < 0.05$). When the change between groups was compared, the number of changes in the high-dosage intake and exercise group were higher than those in the low-dosage intake and exercise group and the placebo intake and exercise groups ($F = 4.097$, $p = 0.024$) ($p < 0.05$) (Table 3, Fig. 3).

Isokinetic muscle function

The peak torque values of extension and flexion at 60° of the left and right knee joints and the peak torque values of extension and flexion at 180° of the left and right knee joints were measured before and after the 12-week exercise–nutrition program, and the analysis results are as follows (Table 4).

60° left extension, 60° right extension, 180° left extension, 180° right extension, and 180° right flexion

When checking the change in 60° left extension, 60° right extension, 180° left extension, 180° right extension, and 180° right flexion, there were no significant differences in either the intra-group or intergroup comparisons (Table 4).

60° left flexion

In the intra-group comparative analysis at 60° left flexion, only the high-dosage intake and exercise group showed a significant difference. The results of the comparative

analysis between groups showed that the high-dosage intake and exercise group was higher than the low-dosage intake and exercise group ($p < 0.05$), and the placebo group was higher than the low-dosage intake and exercise group ($p < 0.5$) (Table 4).

60° right flexion

As a result of intra-group comparative analysis at 60° right flexion, no significant difference was found. In the case of the comparative analysis between groups, the high-dosage intake and exercise group showed higher values than the low-dosage intake and exercise group ($p < 0.01$) (Table 4).

180° left flexion

In 180° left flexion, a significant difference was found in the high-dosage intake and exercise group ($p < 0.05$) and the low-dosage intake and exercise group ($p < 0.05$) in intra-group comparative analysis. As a result of the comparison between groups, the high-dosage intake and exercise group showed higher values than the low-dosage intake and exercise group ($p < 0.01$), and the placebo group showed higher values than the low-dosage intake and exercise group ($p < 0.05$) (Table 4).

Blood lipids and blood pressure

When evaluating changes in TC, TG, glucose, HDL-C, systolic blood pressure (SBP), and diastolic blood pressure (DBP), there were no significant differences in both intra- and intergroup comparisons (Table 5).

Hormones related to muscle metabolism

In this study, catabolism hormones [creatine kinase (CK), and lactate dehydrogenase (LDH)] and anabolic hormones [insulin-like growth factor (IGF-1), growth hormone (GH), and insulin] in the hormones related to muscle metabolism were measured and analyzed (Table 6).

Table 4 The average age, average height, average weight, and average BMI of the subjects belonging to the three groups

Group	Variables			
	Ages (yrs)	Height (cm)	Weight (kg)	BMI (kg/m ²)
CG (n = 15)	68.40 ± 4.97	156.34 ± 4.65	59.51 ± 4.25	24.37 ± 1.76
LDG (n = 15)	66.87 ± 4.12	156.55 ± 5.89	61.75 ± 7.66	25.13 ± 1.89
HDG (n = 15)	66.47 ± 5.49	157.78 ± 7.88	58.51 ± 7.28	23.49 ± 2.27

Values are means ± SD

CG, control intake and exercise group, LDG, low-dosage (0.5 g) EAP intake and exercise group; HDG, high-dosage (1.0 g) EAP intake and exercise group; BMI, body mass index

Table 5 The change in physical strength in the three groups (n = 15 per each group)

Variable	Group	Pre-exe	Post-exe	<i>t</i>	Δ	<i>F</i>	LSD
Sit-and-reach (cm)	CG (A)	11.16 ± 10.07	14.91 ± 7.80	− 4.374**	3.75 ± 3.32	4.114 ^{##}	C > A,B
	LDG (B)	15.57 ± 8.01	19.20 ± 6.95	− 2.902*	3.63 ± 4.84		
	HDG (C)	7.83 ± 11.09	15.77 ± 7.78	− 5.486***	7.94 ± 5.61		
Grip strength left (kg)	CG (A)	23.02 ± 6.21	26.19 ± 6.07	− 2.206*	3.17 ± 5.56	4.190 ^{##}	C > B
	LDG (B)	26.23 ± 4.65	26.61 ± 6.54	− 0.442	0.38 ± 3.33		
	HDG (C)	23.99 ± 5.09	28.98 ± 6.71	− 4.844***	4.99 ± 3.99		
Grip strength right (kg)	CG (A)	25.83 ± 5.91	27.01 ± 6.31	− 1.980	1.17 ± 2.29	5.301 ^{##}	C > A,B
	LDG (B)	26.38 ± 4.26	27.48 ± 6.13	− 1.804	1.10 ± 2.36		
	HDG (C)	25.97 ± 6.48	30.39 ± 6.33	− 3.865**	4.41 ± 4.42		
Sit-up (n/30 sec)	CG (A)	4.00 ± 4.49	6.47 ± 6.71	− 3.653**	2.47 ± 2.61	1.087	NS
	LDG (B)	3.40 ± 4.21	5.07 ± 6.02	− 2.860*	1.67 ± 2.26		
	HDG (C)	2.93 ± 3.33	6.00 ± 5.78	− 4.075**	3.07 ± 2.91		
Single-HDG standing (sec)	CG (A)	26.71 ± 21.36	54.07 ± 40.55	− 3.446**	27.35 ± 30.75	0.040	NS
	LDG (B)	28.29 ± 16.08	54.27 ± 39.02	− 3.292**	25.97 ± 30.56		
	HDG (C)	39.62 ± 24.90	68.60 ± 34.28	− 4.357**	28.98 ± 25.76		
10-m obstacle walk (sec)	CG (A)	10.30 ± 1.36	9.01 ± 1.26	2.751*	− 1.29 ± 1.82	4.097 ^{##}	C > A,B
	LDG (B)	10.47 ± 1.93	9.35 ± 0.90	2.560*	− 1.12 ± 1.69		
	HDG (C)	10.97 ± 1.48	8.16 ± 2.15	5.946***	− 2.81 ± 1.83		
6-min walk (m)	CG (A)	497.33 ± 39.32	574.33 ± 42.71	− 9.485***	77.00 ± 31.44	0.120	NS
	LDG (B)	498.33 ± 64.38	574.33 ± 51.96	− 4.483**	76.00 ± 65.66		
	HDG (C)	543.00 ± 41.82	612.33 ± 43.71	− 7.629***	69.33 ± 35.20		

Values are means ± SD

LSD: Post hoc analysis technique to determine in which group the mean difference between groups occurred

CG, control intake and exercise group; LDG, low-dosage (0.5 g) EAP intake and exercise group; HDG, high-dosage (1.0 g) EAP intake and exercise group; BMI, body mass index; LBM, lean body mass

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ^{##} $p < 0.01$

*Significant different between groups by paired *t* test

[#]Significant different in the amount of change (Δ) by one-way ANOVA

Creatinine, insulin-like growth factor (IGF-1), and insulin

When checking changes in the values of creatinine, IGF-1, and insulin, there were no significant differences in both the intra- and intergroup comparisons (Table 6).

Creatine kinase (CK)

CK significantly decreased in the high-dosage intake and exercise group and significantly increased in the low-dosage intake and exercise group due to intra-group comparative analyses ($p < 0.05$). As a result of the comparison between groups, the high-dosage intake and exercise group was significantly higher than the low-dosage intake and exercise group and the placebo group ($p < 0.05$) (Table 6, Fig. 4).

Lactate dehydrogenase (LDH)

As a result of an intra-group comparative analysis of LDH, significant differences were found in the high-dosage intake

and exercise group and the low-dosage intake and exercise group. As a result of comparison between groups, the high-dosage intake and exercise group showed a decrease in values compared to the control group ($p < 0.5$) (Table 6).

Growth hormone (GH)

In GH, the intra-group comparative analysis showed a significant difference only with the high-dosage intake and exercise group ($p < 0.05$), and a significant difference in the amount of change between groups ($F = 6.033$, $p = 0.005$). A post hoc analysis showed that the values of the high-dosage intake and exercise group were significantly higher than the low-dosage intake and exercise group and the placebo group ($p < 0.01$) (Table 6, Fig. 5).

Table 6 The change in isokinetic muscle function in the three groups (n = 15 in each group)

Variable	Group	Pre-exe	Post-exe	<i>t</i>	Δ	<i>F</i>	LSD
60° Left Extension (Nm)	CG (A)	80.54 ± 18.91	81.33 ± 16.96	−0.211	0.79 ± 14.53	1.630	NS
	LDG (B)	71.47 ± 27.68	69.45 ± 21.27	0.503	−2.03 ± 15.62		
	HDG (C)	82.15 ± 20.19	89.47 ± 25.10	−2.115	7.32 ± 13.40		
60° Left Flexion (Nm)	CG (A)	41.98 ± 13.55	45.03 ± 12.07	−1.633	3.05 ± 7.22	3.915 [#]	C > B, A > B
	LDG (B)	41.78 ± 13.27	38.40 ± 9.81	1.168	−3.38 ± 11.21		
	HDG (C)	44.23 ± 13.79	49.21 ± 14.21	−2.962*	4.98 ± 6.51		
60° Right Extension (Nm)	CG (A)	77.94 ± 21.10	77.90 ± 21.19	0.017	−0.04 ± 8.89	0.306	NS
	LDG (B)	74.72 ± 21.85	75.44 ± 24.92	−0.350	0.72 ± 7.96		
	HDG (C)	86.17 ± 18.61	88.76 ± 17.28	−0.888	2.59 ± 11.31		
60° Right Flexion (Nm)	CG (A)	44.03 ± 14.74	44.41 ± 13.11	−0.309	0.39 ± 4.84	3.952 [#]	C > B
	LDG (B)	42.72 ± 13.23	39.77 ± 10.09	1.622	−2.95 ± 7.04		
	HDG (C)	44.37 ± 17.84	49.19 ± 14.40	−1.867	4.83 ± 10.01		
180° Left Extension (Nm)	CG (A)	48.16 ± 12.18	48.33 ± 12.27	−0.093	0.17 ± 7.24	0.605	NS
	LDG (B)	43.76 ± 13.65	44.51 ± 10.95	−0.484	0.75 ± 5.97		
	HDG (C)	50.67 ± 12.09	53.36 ± 14.29	−1.617	2.69 ± 6.45		
180° Left Flexion (Nm)	CG (A)	32.01 ± 9.85	32.99 ± 9.24	−0.639	0.98 ± 5.94	7.256 ^{##}	C > B, A > B
	LDG (B)	33.18 ± 6.23	28.14 ± 6.43	2.568*	−5.04 ± 7.60		
	HDG (C)	31.97 ± 9.89	36.53 ± 9.40	−2.428*	4.56 ± 7.27		
180° Right Extension (Nm)	CG (A)	47.41 ± 12.73	46.45 ± 8.67	0.517	−0.95 ± 7.14	0.956	NS
	LDG (B)	45.47 ± 11.36	47.05 ± 13.10	−1.257	1.59 ± 4.89		
	HDG (C)	52.23 ± 11.09	53.58 ± 12.28	−1.239	4.83 ± 10.01		
180° Right Flexion (Nm)	CG (A)	33.81 ± 11.35	31.90 ± 11.72	1.308	−1.91 ± 5.65	3.135	NS
	LDG (B)	31.15 ± 7.54	28.97 ± 6.72	1.539	−2.18 ± 5.49		
	HDG (C)	32.87 ± 13.05	35.95 ± 10.34	−1.492	3.08 ± 7.99		

Values are means ± SD

LSD: Post hoc analysis technique to determine in which group the mean difference between groups occurred

CG, control intake and exercise group; LDG, low-dosage (0.5 g) EAP intake and exercise group; HDG, high-dosage (1.0 g) EAP intake and exercise group; BMI, body mass index; LBM, lean body mass

* $p < 0.05$, [#] $p < 0.05$, ^{##} $p < 0.01$

*Significant different between groups by paired *t* test

[#]Significant different in the amount of change (Δ) by one-way ANOVA

Discussion

The present study was aiming to evaluate the effects of combined treatment of resistance exercise and EAP intake on preventing sarcopenia among elderly individuals.

Results revealed that there was no statistically significant difference in the body composition between the high-dosage intake and exercise group, the low-dosage intake and exercise group, and the placebo group after 12 weeks of exercise and EAP intake. However, upon checking the average amount of change, the largest intake group showed the greatest decrease in weight, body mass index, body fat percentage, and waist circumference, which are related to obesity. The increase in lean mass and bone mineral mass, which are related to the muscle and bone, was found to be the largest (Table S1). In particular, the ingestion of EAP along with exercise rather than exercise alone showed a

tendency to improve obesity-related factors. In addition, a 1 g intake showed a positive tendency to improve obesity more effectively than 0.5 mg (Table S1). EAP intake has been found to have a positive effect on the improvement of obesity caused by exercise.

When checking the change in the physical strength after 12 weeks of exercise and EAP intake group, it was found that the exercise and the high-dosage intake and exercise group showed better sitting trunk flexion performance, left grip strength, right grip strength, and 10-m obstacle walking compared to the other groups (Table 3). When these changes were evaluated, the amount of change in the EAP 1,000 mg group (the high-dosage intake and exercise group) was more positive than the EAP 500 mg group (the low-dosage intake and exercise group) and the control (placebo) group who only exercised (Table 3). These results indicate that the intake of EAP increased myogenesis by increasing the

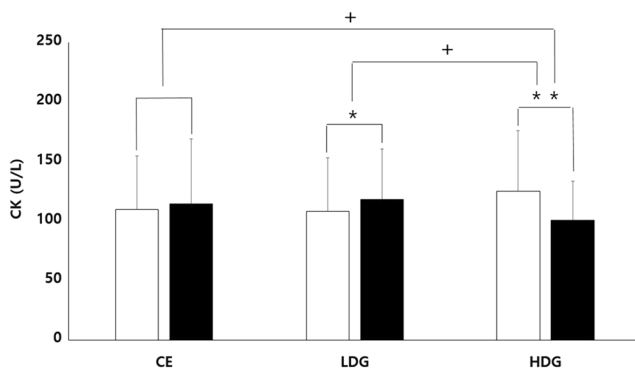


Fig. 4 The change of creatine kinase (CK) activity in both intra- and intergroup comparisons of CG, LDG, and HDG group. CG: Control (placebo) intake and exercise group; LDG: Low-dosage (EAP 0.5 g) intake and exercise group; HDG: High-dosage (EAP 1.0 g) intake and exercise group. Before (□) and after (■) the 12-week exercise–nutrition program. *Significant different between group by paired *t* test, **p* < 0.05, ***p* < 0.01, ****p* < 0.001 + LSD test, + *p* < 0.05, ++ *p* < 0.01.

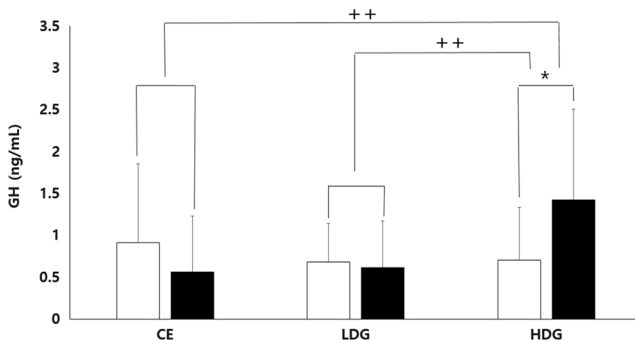


Fig. 5 The change of growth hormone (GH) activity in both intra- and intergroup comparisons of CG, LDG, and HDG group. CG: Control (placebo) intake and exercise group; LDG: Low-dosage (EAP 0.5 g) intake and exercise group; HDG: High-dosage (EAP 1.0 g) intake and exercise group. Before (□) and after (■) the 12-week exercise–nutrition program. *Significant different between group by paired *t* test, **p* < 0.05, ***p* < 0.01, ****p* < 0.001 + LSD test, + *p* < 0.05, ++ *p* < 0.01.

expression levels of genes and proteins of pAMPK/AMPK, pACC/ACC, Sirt 1, and PGC-1 α in skeletal muscle cells [18], and it is believed that the production of muscles and nerves through exercise caused an increase in the physical strength [20]. These results confirmed that EAP intake had a positive effect on the physical fitness improvement according to exercise.

The change in isokinetic muscle function by combination treatment with EAP 1 g intake and resistance exercise for 12 weeks was increased in the 60°, 180° left flexor, and 60° right flexor group than in the low-dosage intake and exercise group and placebo group (Table 4). The ingestion of EAP along with exercise has been shown to improve the

isokinetic muscle function. It was also found that 1 g of intake was more effective than 0.5 g. These results suggest that EAP intake with exercise has a positive effect on the improvement of flexor muscles. In the results of Kim [21], as a result of intensive training for strengthening lower limb muscles using isokinetic exercise equipment, there was a significant effect on strengthening the flexor muscles than the extensors. In addition, considering that the higher the age, the greater the rate of flexor defects in the quadriceps femoris and hamstring muscles [22], the increase in flexor muscle strength is considered to be a very positive effect in the elderly over the age of 60 years, with a high flexor defect rate. The isokinetic muscle function improvement in this study is believed to be caused by the muscle protection effect of EAP administration on muscle atrophy; additionally, the expression of genes and proteins of the mitochondrial biosynthesis regulators PGC-1 α , NRF-1, and Tfam in skeletal muscle cells increased, thereby increasing the myogenesis in mice [18] and increased the isokinetic muscle strength of flexors weakened by aging due to the nutritional and exercise synergies by the generation of muscles and nerves by resistance exercise [20]. Therefore, it was confirmed that the intake of EAP had a positive effect on the improvement of flexor muscles according to exercise.

There were no significant differences in the values of blood lipids and blood pressure in all groups according to the combined treatment of EAP intake and resistance exercise for 12 weeks (Table 5). It was found that the exercise group, exercise, and EAP intake group continued to maintain the normal range because the subjects were not obese, and their blood lipids and blood pressure were normal.

When the changes in muscle metabolism-related hormones were confirmed according to the combined treatment of 12 weeks of EAP intake and resistance exercise, the indices of muscle damage, CK, and LDH decreased the most in the maximal intake group, and GH, an anabolic hormone, increased the most in the maximal intake group (Table 6). In particular, it has been shown to improve the muscle metabolism-related hormones when taking EAP along with exercise rather than just exercising. In addition, a 1 g intake was more effective than 0.5 g intake and showed an increasing trend toward muscle metabolism-related hormones. The results of this study confirmed that the combination of EAP intake and exercise had a positive effect on the muscle metabolism hormone.

Muscle atrophy caused by various causes, such as nerve damage and aging, causes muscle weakness and increased muscle fatigue due to a decrease in the diameter of muscle fibers by a decrease in protein in muscle cells [23]. The content of creatine and creatine kinase in the body is always kept constant, and maintains a constant equilibrium with the activity and amount of skeletal muscle [24, 25]. Therefore, the content of creatine in blood is used as an important blood

biochemical indicator to measure the activity and amount of skeletal muscle, and an increase in the creatine content means destruction of the skeletal muscle fibers [26, 27]. Additionally, CK is an important enzyme responsible for supplying ATP through the breakdown of creatine in various cells, especially muscle cells, and LDH is an enzyme responsible for lactic acid degradation and is intensively present in red blood cells and heart muscle cells. Therefore, when the cells in which they exist are released into the blood, they can be used as an indicator of cell damage, such as muscle damage. In particular, the simultaneous elevation of CK and LDH in blood has been used as a biochemical indicator of muscle damage [27–29], and a remarkable increase in blood CK and LDH is also caused during muscle atrophy [30]. The decrease in CK in this experiment is thought to be a result of the muscle protection effect of EAP and the adaptation effect of the human body to a 12-week moderate-intensity resistance exercise training [20].

On the other hand, the decrease in GH observed in the elderly is associated with a decrease in muscle and bone density and an increase in the visceral fat. In addition, GH is a hormone that increases the uptake of amino acids in cell membranes and promotes protein synthesis by acting on transcription and translation processes in the cell nucleus and ribosomes, respectively. EAP mainly comprise carbohydrate, sugar 3.7%, and crude protein 4.2% [14]. It is thought that the ingestion of EAP increases GH via certain physiological metabolism(s), which induces protein synthesis [31], and increases metabolic sensitivity through resistance exercise for 12 weeks, which is thought to have the potential to promote muscle protein synthesis [20]. According to the results of this study, it was confirmed that EAP intake had a positive effect on muscle metabolism hormones in addition to exercise.

The limitations of this study are as follows. In the results of this study, there was no significant difference between groups in some variables, such as body composition, blood lipids, and muscle-related hormones, which is considered a problem as all groups were treated with exercise. In addition, as can be seen from the positive effects on body composition change in the previous study by Song [32] that ingesting 6 g of EAP, it is necessary to increase the intake through further research and to experiment only with the EAP intake group and the placebo group without exercise. In addition, considering that the subjects of this study showed a positive trend in improving obesity-related factors, even though they were not obese, it is estimated that the exercise synergistic effect of EAP could be confirmed if an experiment was conducted on obese people in further research.

Materials and methods

Test material

The experimental and placebo products used in this clinical research study were packaged inside boxes provided by Glucan Co. Ltd. (Jinju-si, Republic of Korea). The experimental products contained 1 g of EAP for the high-dosage intake and exercise group and 0.5 g of EAP with 0.5 g of crystalline cellulose for the lower dosage group, respectively. The placebo product contained 950 mg of crystalline cellulose and 50 mg of gardenia red dye. The test food was manufactured as a powder in stick packing. Some EAP samples were placed in the herbarium of Silla University (Busan, Korea; encoded as Polycan2020Choi01). A total of 40 sticks were provided to each subject, taking into consideration a 12-week dosage [36 days (3 days/week), 36 sticks], a visitation window of 2 days (2 sticks), and storage products (2 sticks).

Study Design

A total of 45 elderly participants were classified into groups of 15 individuals, namely the placebo and exercise group, the low-dosage intake and exercise group (0.5 g of EAP), the high-dosage intake and exercise group (1 g of EAP), and subsequently, the same resistance exercise was performed for 12 weeks. The changes in body composition, physical fitness, isokinetic muscle function, and blood lipids and muscle metabolism-related hormones in the elderly were analyzed before and after the exercise–nutrition program. The three groups were as follows:

- Control intake and exercise group (CG): placebo (0.95 g of crystalline cellulose and 0.05 g of gardenia red dye) + Gym-stick exercise.
- Low-dosage intake and exercise group (LDG): EAP (0.5 g) and crystalline cellulose (0.5 g) + Gym-stick exercise.
- High-dosage intake and exercise group (HDG): EAP (1 g) + Gym-stick Exercise.

The study was conducted from June 5, 2020, to August 28, 2020, at the Health Anti-Aging Center in Kyungnam University (Changwon-si, Gyeongsangnam-do, Republic of Korea). The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of Kyungnam University (Approval no. 1040460-A-2020-018).

Recruitment of subjects

Among men and women in their 60 s and 70 s residing in Changwon-si, those who agreed to participate in the study after explanation of the purpose and program of the study before the start of the study were selected as subjects. Among them (50 individuals), those with acute infections or a history of malignant tumors in the last 1 month, a history of cerebral infarction and myocardial infarction in the last 6 months, a history of peripheral vascular disease, and a history of dementia were excluded (5 individuals). Therefore, a total of 45 subjects were divided into 15 subjects in the placebo group, 15 subjects in the low-dosage intake and exercise group, and 15 subjects in the high-dosage intake and exercise group by a simple random sampling method using G*Power 3.1 program [19].

Intake of EAP

EAP (0.5 g/d or 1 g/d) and placebo were administered at 8:00 am with enough water.

Exercise program

The exercise program of this study was a medium-intensity muscular endurance exercise program using a Gym-stick (Gymstick International Oy, Ratavartijankatu 11, 15,170 Lahti, Finland) for 50 min a day, three times a week for 12 weeks. The exercise time, frequency, and intensity were configured with reference to the American College of Sports Medicine [33] (Table 7).

Exercise type

Gym-stick exercises using an elastic band as muscle endurance exercise were performed in this study.

Frequency

Three times a week (Mon, Wed, and Fri) were performed.

Intensity

Depending on the degree of the adaptation of the subject and the degree of improvement in the muscle strength, the length of the elastic band, the number of repetitions (8–12

Table 7 The changes in blood lipids, glucose, and blood pressure in the three groups (n = 15 in each group)

Variable	Group	Pre-exe	Post-exe	<i>t</i>	Δ	<i>F</i>	LSD
TC (mg/dl)	CG (A)	181.20 ± 50.05	185.80 ± 43.54	−0.601	4.60 ± 29.66	1.724	NS
	LDG (B)	187.47 ± 34.95	173.67 ± 29.30	2.587*	−13.80 ± 20.66		
	HDG (C)	193.47 ± 33.71	185.40 ± 31.56	0.984	−8.07 ± 31.75		
TG (mg/dl)	CG (A)	130.73 ± 62.52	140.73 ± 41.36	−0.514	10.00 ± 75.38	0.293	NS
	LDG (B)	134.80 ± 64.54	132.87 ± 65.09	0.146	−1.93 ± 51.39		
	HDG (C)	103.33 ± 40.23	99.07 ± 45.32	0.644	−4.27 ± 25.65		
Glucose (mg/dl)	CG (A)	99.33 ± 14.41	96.33 ± 13.21	1.262	−3.00 ± 9.20	0.603	NS
	LDG (B)	102.87 ± 44.21	91.47 ± 11.73	1.262	−11.40 ± 34.98		
	HDG (C)	93.13 ± 12.78	88.40 ± 11.20	1.457	−4.73 ± 12.58		
HDL (mg/dl)	CG (A)	52.40 ± 17.30	50.33 ± 16.27	1.153	−2.07 ± 6.94	1.012	NS
	LDG (B)	55.87 ± 10.47	50.93 ± 9.71	1.813	−4.93 ± 10.54		
	HDG (C)	57.00 ± 12.51	56.40 ± 9.46	0.308	−0.60 ± 7.54		
Systolic blood pressure (mmHg)	CG (A)	137.20 ± 16.13	137.40 ± 15.26	−0.067	0.20 ± 11.62	1.451	NS
	LDG (B)	136.13 ± 18.07	130.13 ± 16.17	2.803*	−6.00 ± 8.29		
	HDG (C)	131.33 ± 19.23	126.67 ± 12.97	1.607	−4.67 ± 11.25		
Diastolic blood pressure (mmHg)	CG (A)	81.87 ± 8.20	82.07 ± 9.02	−0.100	0.20 ± 7.78	1.250	NS
	LDG (B)	84.53 ± 7.85	82.00 ± 7.15	2.465*	−2.53 ± 3.98		
	HDG (C)	82.20 ± 8.28	82.80 ± 6.29	−0.436	0.60 ± 5.33		

Values are means ± SD

LSD: Post hoc analysis technique to determine in which group the mean difference between groups occurred

CG, control intake and exercise group; LDG, low-dosage (0.5 g) EAP intake and exercise group; HDG, high-dosage (1.0 g) EAP intake and exercise group; BMI, body mass index; LBM, lean body mass; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol

* $p < 0.05$

*Significant different between groups by paired *t* test

times), and the number of sets (1–2 sets) were gradually increased (Table 7). Subjects exercised with a value of 11–12 rated perceived exertion (RPE) scale (light activity) using a green band for 1–4 weeks, and a blue band was used to set a value of 13–14 of RPE (somewhat hard activity) for 6–12 weeks. In the case of resistance exercise, it is dangerous to measure 1 repetition maximum (1RM) in the elderly; therefore, 8–12 repetitions of movements by Westcott & Baechle [34] were estimated to be 55–60% of 1RM.

Time

1 h 50 min per day. The three groups were arranged at 9:00–9:50 am, 10:00–10:50 am, and 11:00–11:50 am, respectively, and these timings were changed on the day of exercise in turn.

Place

The Reflex Balance Thera Fitness Center (Changwon-si, Gyeongsangnam-do, Republic of Korea) maintained temperature and humidity of 20–22 °C and 40–50%, respectively.

Body composition measurement

The height, weight, skeletal muscle mass, lean mass, BMI, body fat mass, body fat percentage, and abdominal fat percentage were measured using a bioelectrical impedance analysis (InBody720 Body Composition Analyzer, Biospace Co. Ltd., Seoul, Korea). All subjects were asked to stand upright on the Inbody 720's electrode plate while fasting for 10 h. Thereafter, holding the handles in both hands and making the electrode switch lightly pressed with the thumb with the armpit away from the body, the measurement was made using the skin electrical resistance of the left and right soles and fingers.

Physical fitness measurement

Muscular strength (grip strength)

Grip strength was measured using a grip dynamometer (TKK 5101 grip-D, Takei Scientific Instruments, Co. Ltd., Tokyo, Japan). The width was adjusted so that the second joint of the second finger was at a right angle and was carried out twice in an upright position. The maximum value was measured in kg.

Muscular endurance (sit-ups)

The subjects were asked to lay on a lift-up table (Casio HS-20; Casio Co. Ltd., Tokyo, Japan), raise their knees, hold both hands behind their neck, and use only their abdominal muscles to raise the upper body and bend it forward for 30 s. Subsequently, it was measured by the number of times.

Flexibility (sit and reach)

The flexibility was measured in cm units by performing two forward bends with the knee kept straight using a sit-to-sit forward bend meter (Helmas SH-9600G, O2Run Co. Ltd., Seoul, Korea).

Balance (one-legged stand)

The duration of one leg stand was measured (Casio HS-20); however, the maximum time was limited to 120 s. The recording was performed in seconds, and after two times, the record of the good side was recorded.

Cardiorespiratory endurance (6-min walk)

Subjects were asked to lightly extend both elbows along the straight line marked on the floor in the gym, maintain a good walking posture as much as possible, and walk for 6 min at their usual walking speed (Casio HS-20). Records were made in 5 m increments, and those less than 5 m were trimmed.

Coordination (10 m obstacle walking)

The coordination was measured using a measuring instrument (Takei Scientific Instruments Co. Ltd.). The subjects were asked to approach the center of the obstacle on the start line as close as possible and stand with both feet together. The subjects started walking according to the start signal and had six obstacles spread across the bridge. The time at which one leg touched the ground after crossing the obstacle at the 10 m point was measured.

Isokinetic muscle function

Using the Biodex System 3 (Biodex, Inc., New York, USA), an isokinetic muscle function, the extensor and flexor peak torque values of the knee joint were measured, and the % BW was calculated considering the weight.

Table 8 The changes in hormones related to muscle metabolism in the three groups (n = 15 in each group)

Variable	Group	Pre-exe	Post – exe	<i>t</i>	Δ	<i>F</i>	LSD
Creatinine (mg/dl)	CG (A)	0.75 ± 0.15	0.73 ± 0.13	1.468	− 0.03 ± 0.07	1.694	NS
	LDG (B)	0.71 ± 0.14	0.72 ± 0.10	− 0.222	0.01 ± 0.12		
	HDG (C)	0.76 ± 0.16	0.71 ± 0.15	2.779*	− 0.05 ± 0.07		
CK (IU/ml)	CG (A)	109.33 ± 45.44	114.07 ± 55.02	− 0.745	4.73 ± 24.62	8.548 ^{##}	C < A,B
	LDG (B)	108.07 ± 44.95	117.93 ± 42.34	− 2.274*	9.87 ± 16.81		
	HDG (C)	124.73 ± 51.02	100.33 ± 33.34	3.133 ^{**}	− 24.40 ± 30.16		
LDH (IU/ml)	CG (A)	212.13 ± 30.75	208.53 ± 31.89	0.796	− 3.60 ± 17.51	2.722	C < A
	LDG (B)	228.20 ± 33.79	210.20 ± 25.97	2.841*	− 18.00 ± 24.54		
	HDG (C)	217.47 ± 26.07	198.53 ± 25.97	4.144 ^{**}	− 18.93 ± 17.69		
IGF-1 (ng/ml)	CG (A)	134.58 ± 34.58	132.85 ± 41.50	0.346	− 1.73 ± 19.41	1.123	NS
	LDG (B)	168.54 ± 66.03	158.85 ± 52.37	1.083	− 9.69 ± 34.68		
	HDG (C)	144.59 ± 36.76	148.88 ± 46.19	− 0.840	4.29 ± 19.76		
GH (ng/ml)	CG (A)	0.91 ± 0.94	0.56 ± 0.67	2.024	− 0.35 ± 0.68	6.033 ^{##}	C > A,B
	LDG (B)	0.68 ± 0.46	0.61 ± 0.56	0.334	− 0.07 ± 0.76		
	HDG (C)	0.70 ± 0.63	1.42 ± 1.09	− 2.481*	0.72 ± 1.12		
Insulin (IU/ml)	CG (A)	7.74 ± 4.56	9.95 ± 8.85	− 1.764	2.21 ± 4.86	1.547	NS
	LDG (B)	8.27 ± 3.08	8.06 ± 4.39	0.266	− 0.21 ± 3.01		
	HDG (C)	7.41 ± 4.57	7.04 ± 4.52	0.268	0.37 ± 5.31		

Values are means ± SD

LSD: Post hoc analysis technique to determine in which group the mean difference between groups occurred

CG, control intake and exercise group; LDG, low-dosage (0.5 g) EAP intake and exercise group; HDG, high-dosage (1.0 g) EAP intake and exercise group; BMI, body mass index; LBM, lean body mass; CK, creatine kinase; LDH, lactate dehydrogenase; IGF-1, insulin-like growth factor 1, GH, growth hormone

* $p < 0.05$, ** $p < 0.01$, ^{##} $p < 0.01$

*Significant different between groups by paired *t* test

[#]Significant different in the amount of change (Δ) by one-way ANOVA

Blood pressure, waist circumference, and hip circumference measurement

Blood pressure

After stabilizing for 10 min using an automatic sphygmomanometer (HEM-7122; OMRON Co, Ltd., Kyoto, Japan), the average value was calculated by measuring twice at 10-min intervals on the left upper arm.

Waist circumference

Cross the subject's hands to the thoracic cage so that he can stand comfortably and then breathe normally. After the subject has finished breathing, the circumference of the most indented part between the lowermost bone of the rib (10th) and the iliac crest is measured using a tape measure.

Hip circumference

Subject was made to stand upright. The subject's hip circumference was measured with a tape measure over the pubis and the most protruding part of the hip.

Blood lipid, catabolism and anabolic hormone

For blood collection, approximately 5 mL was collected from the cubital vein after the subject maintained an empty stomach for 10 h or more. The collected venous blood was analyzed after separating the serum by centrifugation (3,000 rpm × 10 min; Sorvall Legend XTR Centrifuge TX-1000, Thermo Fisher Scientific Inc., Waltham, MA, USA) at room temperature and stored at − 70 °C until further analysis. The total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and glucose in serum were analyzed using diagnostic kits (Table 8) and a Biochemistry analyzer (BioSystems Co. Ltd., Cumbria, UK).

The catabolic hormones, creatine kinase (CK), lactate dehydrogenase (LDH), anabolic hormone, insulin-like growth factor 1 (IGF-1), growth hormone-releasing hormone (GHRH), and insulin were analyzed using an enzyme immunoassay (Table 8) and UV/VIS spectrophotometer (Thermo Scientific Multiskan GO, Fisher Scientific Co., USA).

Statistical analysis

The mean value (M) and standard deviation (SD) of all measurement results were calculated and statistically analyzed using SPSS for Windows (ver. 20, SPSS Inc., Chicago, IL, USA). The comparative analysis between measurements before and after the exercise–nutrition program was performed by paired *t* test, the three groups were compared by one-way analysis of variance (ANOVA), and the post hoc test was performed using the Fisher's Least Significant Difference (LSD) test. The level of statistical significance was set at $p < 0.05$.

Conclusions

These results showed that resistance exercise for 12 weeks and ingestion of 1 g of EAP daily improved physical strength, isokinetic muscle function, blood lipids, blood pressure, and some variables related to muscle metabolism. In particular, among the hormones related to muscle metabolism, the catabolic hormones CK and LDH were significantly reduced, and the anabolic hormone GH was significantly increased. Based on these physiological activities, 12 weeks of resistance exercise and EAP intake could have a positive effect and synergistic effect on muscle reduction and muscle improvement in the elderly.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13530-021-00090-w>.

Declarations

Conflict of interest Hye-Su Park, Minseo Kang, Yong-Min Kim, Jong-Min Lim, Young Suk Kim, Hee-Taek Kim, and Jae-Suk Choi declare that they have no conflict of interest.

Consent for publications This study “Preventing effects of exopolymers purified from *Aureobasidium pullulans* (EAP) supplementation and resistance exercise on muscle aging and loss in the Korean elderly: a randomized controlled trial” has not been published or submitted for publication elsewhere, and all the listed authors have read and approved the submitted manuscript. All authors have contributed significantly and that all authors are in agreement with the content of the manuscript and agree to the conditions outlined in the copyright assignment form. In addition, this manuscript revised by an English-speaking consultant.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This clinical research was approved by the Institutional Review Board of Semyung University Korean Medicine Hospital (Approval No. SMJOH 2015-10-07).

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