

PAPER



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Combined effect of polycan, a β -glucan from *Aureobasidium pullulans*, and regular resistance exercise on muscle strength, biomarkers, and fitness profiles in adults with relatively low skeletal muscle mass: a randomised controlled trial†

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Background: Previous studies suggest that when combined with exercise, *Aureobasidium pullulans*-derived β -glucan (AP β G) may enhance muscle health and fitness profiles because of its ability to improve exercise-induced fatigue and preserve muscle mass. **Objectives:** The objective was to examine the combined effects and safety of AP β G consumption and regular resistance exercise for 12 weeks on muscle strength, biomarkers, and fitness profiles in adults with relatively low skeletal muscle mass. **Methods:** We conducted a randomized, double-blinded, placebo-controlled trial in adults aged ≥ 50 years with $<110\%$ of the standard lean mass. Eighty participants randomly received either 1000 mg of AP β G per day or a placebo for 12 weeks. All participants underwent resistance training three times per week. At baseline and 12 weeks after treatment, we assessed their knee extension/flexion strength, handgrip strength, body composition, and biomarkers. We also evaluated Euro-QoL-5D (EQ-5D) questionnaire, food intake, and physical activity at baseline and 12 weeks after treatment. **Results:** The combination of AP β G and regular resistance exercise over 12 weeks resulted in a higher right knee flexion strength by 4.49 Nm (95% CI: -0.12 – 8.86 Nm; $P = 0.044$) than the placebo according to the intention-to-treat analysis. The combination intervention also led to a higher right knee flexion strength of 5.60 Nm (0.18–11.02 Nm; $P = 0.043$) and left knee flexion strength of 7.25 Nm (0.22–14.28 Nm; $P = 0.043$) than the placebo according to the per-protocol (PP) analysis. In addition, compared to the placebo, the combined intervention enhanced right-hand grip strength by 1.40 kg (0.19–2.61 kg; $P = 0.024$) and left-hand grip strength by 1.33 kg (0.01–2.65 kg; $P = 0.048$) according to PP analysis. The combined intervention also resulted in a more significant reduction in the time required for the 400 m walk test than the placebo. None of the participants experienced adverse events. **Conclusion:** AP β G, in addition to regular resistance exercise, may enhance skeletal muscle strength and fitness in adults with relatively low skeletal muscle mass.

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Introduction

As aging progresses, muscle regeneration efficiency decreases because of intrinsic changes, such as increased senescence, epigenetic modifications, metabolic and autophagic defects, and apoptosis; extrinsic changes, such as inflammation; and alterations in the extracellular matrix and growth factors.¹ To rebuild muscles that have been gradually lost with age, nutrient intake, including essential amino acids and vitamin D, along with exercise is important.² Pharmacological interventions for improving sarcopenia have recently been preclinically and clinically tested on pathways, such as myostatin signaling, renin-angiotensin system, androgen receptor, and activated protein kinase signaling.^{3,4} However, the available evidence

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still lacks clarity. Further, side effects of sarcopenia-related pharmacotherapy, such as selective androgen receptor modulation, hepatotoxicity, unpredictable post-dose prognosis, peripheral edema, hyperglycemia, musculoskeletal pain, and cancer, have been reported.^{3–6} Therefore, studies on the potential use of various nutraceuticals and functional foods, such as curcumin, resveratrol, catechin, soy protein, ginseng, and *Schisandra chinensis* Baillon extracts with limited side effects as alternatives to ameliorate the loss of muscle mass, muscle strength, and even physical function for adults with low muscle mass are being conducted.^{7–9}

In addition to β -glucan, the black yeast, *Aureobasidium pullulans*, also considered safe for consumption as food use in both the United States (GRAS notice no. GRN 000099) and Europe (EFSA-Q-2003-138), produces abundant soluble β -1,3-1,6-glucan.^{10,11} *A. pullulans*-derived β -glucan (AP β G) has been shown to have intestinal immunostimulatory, anti-tumor, anti-metastatic, anti-microbial, anti-inflammatory, anti-diabetic, anti-lipemic, anti-allergic, anti-stress, and anti-osteoporotic activities in animal models and clinical studies.^{12,13} A recent study found that a water-soluble β -glucan ameliorated exercise-induced fatigue and injury-related biochemical biomarkers and improved exercise-induced oxidative stress in mice.¹⁴ Furthermore, exopolymers purified from *A. pullulans* SM-2001 (Polican) exhibited dose-dependent muscle-preserving effects on dexamethasone-induced catabolic muscle atrophy in mice.¹⁵ In addition, a human pilot study suggested the possibility of an additive effect of AP β G on muscle function when combined with resistance exercise.¹⁶ Exercise maintains and regenerates muscles: for instance, resistance exercise improves muscle strength and increases functional performance, such as balance, endurance, and walking speed in middle-aged and older adults.^{17,18} Therefore, we hypothesized that a combination of AP β G and regular exercise could improve muscle and fitness profiles in adults with relatively low skeletal muscle mass. In this randomized, double-blinded, placebo-controlled trial, we aimed to investigate the combined effect of AP β G administration and regular resistance exercise for 12 weeks on muscle strength, biomarkers, and fitness profiles in adults with relatively low skeletal muscle mass and test its safety.

Methods

Ethics approval

Written informed consent was obtained from all study participants recruited through advertisements at Kyungnam University, Changwon, South Korea. This study was approved by the Institutional Review Board at Kyungnam University (2021-029-HR), and registered on Clinicaltrials.gov (registration number NCT05106686). The study was conducted between June 2021 and January 2022 in accordance with the principles of the Declaration of Helsinki and the Korean Good Clinical Products guidelines.

Study design and outcomes

This was a randomised, placebo-controlled, double-blinded trial. The primary outcome measure was change in the peak torque (TQ) at 60° s⁻¹ of knee extension/flexion, compared to the baseline, representing muscle function at 12 weeks of AP β G or placebo use. Secondary outcome measures were changes in the appendicular skeletal mass index (ASMI), skeletal muscle mass index (SMI), body composition, handgrip strength, biomarkers [lactate, creatine kinase (CK), insulin growth factor (IGF)-1, growth hormone (GH), and high-sensitivity C-reactive protein (hs-CRP) concentrations], and Euro-QoL-5D (EQ-5D) score, which is representative of the quality of life,¹⁹ within the 12-week treatment period. Lactate, CK, IGF-1, and GH concentrations were measured to assess exercise-induced fatigue²⁰ and hs-CRP concentrations were measured to determine the inflammatory status (ESI Table 1†).²¹ The study was conducted at the Department of Basic Science Research Institute and the Department of Physical Education of Kyungnam University, Changwon, South Korea.

Study participants

Participants ≥ 50 years of age, with body mass index (BMI) ranging from 18.5 to 30.0 kg m⁻² and with relatively low skeletal muscle mass ($<110\%$ of the skeletal muscle mass measured using the body composition analyzer InBody 720, Biospace Co. Ltd, Seoul, Republic of Korea)⁹ were eligible for the study. Participants with any central fracture within 1 year of enrollment, with abnormal liver or renal function (aspartate aminotransferase or alanine aminotransferase level ≥ 60 IU L⁻¹, creatinine level ≥ 1.2 mg dL⁻¹), uncontrolled diabetes (fasting glucose level ≥ 160 mg dL⁻¹), uncontrolled hypertension (blood pressure (BP) $\geq 160/100$ mmHg), uncontrolled thyroid disorder, or any severe cardio-cerebrovascular disease or cancer (diagnosed within the preceding 3 months), with severe gastrointestinal symptoms, known allergies, medication use for psychiatric illness (such as severe mood disorder, schizophrenia, and substance use), alcohol abuse, pregnancy or lactation, severe lower extremity musculoskeletal disorders or injuries, inability to communicate or walk, fragility of hip fractures, use of drugs or supplements that affect muscles within 2 weeks, or those who had participated in other clinical trials within the preceding 3 months, were excluded. Furthermore, participants with the following criteria were discontinued, dropped out of the trial: withdrawal of consent by the participant, occurrence of serious adverse reactions, protocol violations, not taking the test product or placebo for 5 days consecutively, failure to attend follow-up assessments, and poor compliance. Compliance was assessed by counting the number of capsules remaining at each visit. The participants were requested to log the time of taking the supplement in a diary, which was submitted along with the bottle, to the researcher at every visit. Adherence rates of $\geq 80\%$ were required for optimal therapeutic efficacy.

using a sphygmomanometer (BP-203 RV II; Colin Corp., Aichi, Japan). After a 12 h overnight fast, blood samples were collected at baseline and at 12 weeks after randomisation to evaluate the biomarkers of muscle metabolism and monitor the potential adverse effects of APβG. Furthermore, during the second and third visits, we measured knee extension strength, handgrip strength, body composition analysis, and physical performance profiles, such as sit-ups for muscular endurance, sit and reach (SR) for flexibility, single leg stance (SLS) for balance, 6-minute walk (6 MWT) for cardiorespiratory endurance, 400-meter walking (400 MWT) for mobility limitation, 10-meter obstacle walking (10 MWT) for coordination, and short physical performance battery protocol (SPPB) for lower extremity function and mobility. The details of the measurements performed have been described in our previous study.¹⁶ Additionally, the participants were counseled to maintain their usual physical activity and diet during the 12 weeks of the study. At baseline and after 12 weeks of the trial, the participants were asked to answer a questionnaire on dietary intake and physical activities that may influence the change in skeletal muscle status. Moreover, food intake was assessed using the CAN-Pro version 5.0 (The Korean Nutrition Society, Seoul, Republic of Korea) based on the recorded meals during each week at the start and end of the study.⁹ The number of physical activities was represented as the average daily walking calories (kcal per week), calculated using the walking log of the preceding 7 days and a 3-axis wrist-worn accelerometer (Mi Smart Band 6, Xiaomi, Beijing, China). Reports of any other adverse events or unpredictable allergic reactions were collected throughout the study.

Laboratory measurements

All analyses were conducted in a laboratory. For outcome assessment, lactate concentrations (ref. 03183700) were measured using a colorimetric assay on the Cobas c501 module (Roche Diagnostics, Mannheim, Germany), which had an inter- and intra-assay CV of 1.4% and 0.6%, respectively. CK concentrations (ref. 05168546) were analyzed by UV testing and hs-CRP (ref. 05950864) concentrations were measured by latex particle-enhanced immunoturbidimetric assay using the Cobas c701 module (Roche Diagnostics, Mannheim, Germany). The inter-CV and intra-CV were 4.8% and 1.2% for hs-CRP and 1.4% and 0.5% for CK, respectively. IGF-I concentrations were measured using a one-step sandwich chemiluminescence immunoassay (Liaison XL, DiaSorin S.p.A., Saluggia, Italy), and GH concentrations were measured using a solid-phase, two-site chemiluminescent immunometric assay on an IMMULITE® 2000 system (Siemens, Los Angeles, CA, USA). The inter-CV and intra-CV were 7.9% and 2.6% for IGF-I and 5.7% and 1.9% for GH, respectively. For monitoring safely, fasting serum glucose concentrations were measured using a hexokinase-based test (Glucose HK Gen.3, Roche Diagnostics, Mannheim, Germany), and liver enzyme concentrations were measured according to the recommendations of the IFCC on the Cobas c701 module (Roche Diagnostics, Mannheim, Germany). Serum creatinine (Roche,

ref. 05168589) concentrations were measured using an enzymatic method. The inter-CV and intra-CV were 1.2% and 0.7% for creatinine, respectively.

For assessment of additional effects of APβG, free fatty acids (FFA) concentrations were determined using an enzymatic colorimetric assay (NEFA-HR2, ACS-ACOD; Wako Chemicals, Neuss, Germany) on a Hitachi LABOSPECT 008 (Hitachi High-Tech Co., Tokyo, Japan). Insulin (ref. 07027559190) was measured using an enzyme immunoassay based on the sandwich principle of the Cobas e801 module (Roche Diagnostics, Mannheim, Germany). The inter-CV and intra-CV were 1.2% and 0.6% for FFA, and 2.4% and 1.7% for insulin, respectively. Insulin resistance was assessed by calculating the homeostatic model assessment for insulin resistance (HOMA-IR) using the following formula: (fasting glucose × fasting insulin)/405.²² Plasma total cholesterol (TC), triglyceride, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) concentrations were measured using an enzymatic colorimetric assay on the Cobas c701 module (Roche Diagnostics, Mannheim, Germany). The inter-CV and intra-CV were 1.5% and 0.6% for TC, 1.9% and 0.8% for triglyceride, 1.5% and 0.5% for HDL-C, and 1.2% and 1.0% for LDL-C, respectively. More detailed information is provided in ESI Table 1.†

Muscle strength

Knee extension strength and handgrip strength were measured using the Biodex System 3 Pro isokinetic dynamometer (Biodex, Inc., Shirley, NY, USA) and a Jamar hydraulic hand dynamometer (Model 5030 J1, Sammons Preston Rolyan, Bolingbrook, IL, USA), respectively. The detailed method of measurement has been described in our previous study.^{9,16}

Body composition analysis

Body composition analysis was performed using whole-body dual-energy X-ray absorptiometry (DXA, Hologic Horizon W, Software: Apex Version 5.6.0.5, Hologic Inc., Marlborough, MA, USA) in the supine position according to the manufacturer's guidelines. Appendicular skeletal mass (ASM) was defined as the total lean soft-tissue mass in the four limbs. In this study, the ASMI and SMI were estimated using the following formulas: $ASM \text{ (kg)}/height \text{ (m)}^2$ and $ASM/weight \text{ (kg)} \times 100$, respectively.^{23,24}

Resistance exercise program

All participants completed an exercise program for 12 weeks at The Reflex Balance Thera Fitness Center from a certified professional fitness instructor. It included three weekly sessions (50 min each) with a total of 36 sessions; it was a medium-intensity muscular endurance exercise program using Gymstick (Gymstick International Oy, Ratavirtijankatu 11, 15170 Lahti, Finland), as implemented in our previous study.¹⁶ and in ESI Table 2 and Fig. 1A–C.† Attendance rates of ≥80% were required for measuring optimal thera-

peutic efficacy, which was calculated using the program attendance log.

Statistical analysis

We used MedCalc version 20.109 (MedCalc Software Ltd, Ostend, Belgium) to calculate the sample size, based on a previous similar study,¹⁶ which tested the effects of exercise on isokinetic function in older adult women. The estimated sample size was 30 participants per group with 80% power to detect a mean difference of 9.4 Nm in the knee flexion peak TQ at an angular velocity of 60° s^{-1} , assuming a standard deviation of 12.7 Nm in the primary outcome variable and an alpha error of 5%. The sample size was adjusted to 40 participants per group to allow for a 25% dropout rate. Intention-to-treat (ITT) analysis ($N = 80$) was used to compare outcomes between the AP β G and placebo groups, with multiple imputation of missing data. The percentage of missing values at the 12-week follow-up was 25.0% for all variables. Five imputed datasets were created, and the results of the analyses from the different imputed datasets were pooled according to Rubin's rules using R software version 3.6.2 (R Foundation for Statistical Computing). Multivariate imputation by the chained equations algorithm was used with the predictive mean matching method. A per-protocol (PP) analysis was also performed ($N = 60$) to assess the effectiveness of the supplementation. The Shapiro–Wilk test was used to test the normality assumption. Intergroup comparisons of baseline characteristics were performed using the 2-sample *t*-test for continuous variables (or Mann–Whitney's *U* test for nonparametric continuous variables) and the chi-square test for categorical variables (or Fisher's exact test for nonparametric categorical variables). ANCOVA or rank ANCOVA was used for the main analysis, with adjustments for sex, protein intake percent, and each baseline variable, all of which were calculated as a delta, *i.e.*, 12-week minus baseline as covariates. The model assumptions were checked using histograms, normal probability plots, and

residual scatter plots. Safety analyses included all randomised patients who were exposed to at least one dose of the study intervention. Statistical significance was set at $P < 0.05$. Data were analyzed using IBM SPSS Statistics (version 22.0; IBM Inc., Armonk, NY, USA) and R software version 3.6.2 (<https://www.r-project.org/>).

Results

Baseline characteristics of the participants

A total of 82 participants were enrolled in this study, with 80 (mean age 62.1 ± 7.4 years) of them randomly allocated to the AP β G or placebo group. Twenty-four (30.0%) participants were male. Overall, 60 participants (75.0%) completed the trial. The characteristics of the participants who dropped out ($n = 20$) were similar to those of others who completed the study (Fig. 2). Randomisation was successful as most variables were comparable between the two groups, and no significant differences were observed in the baseline demographic and anthropometric characteristics, comorbid conditions, calorie intake, and walking activity between the groups (Table 1). There were no significant changes in calorie intake and walking activity during the study period among the participants, reflecting no additional effects that might have influenced muscle status or function besides from the intervention (Table 2). During the entire study period, a double-blinded requirement was well-maintained. In both groups, supplement adherence exceeded 90%, on average, during the 12-week study period in the PP population.

Primary outcome

At 12 weeks, in the ITT analysis, the AP β G group showed significant improvement in concentric knee flexion strength (peak TQ) at 60° s^{-1} of the right leg by 4.49 Nm (95% confidence interval: -0.12 – 8.86 Nm; $P = 0.044$) compared to the placebo group (Fig. 3). The PP analysis also revealed that the

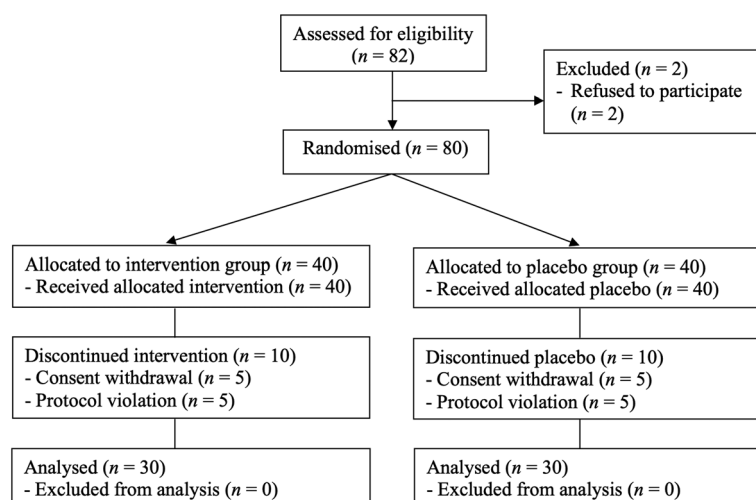


Fig. 2 Flow diagram of the study participants.

Table 1 Baseline characteristics of the study group^a

Variables	Intention-to-treat population			Per-protocol population		
	APβG group (n = 40)	Placebo group (n = 40)	P ^b	APβG group (n = 30)	Placebo group (n = 30)	P ^b
Age, years	63.0 ± 7.4	61.3 ± 7.4	0.329	62.1 ± 7.2	61.9 ± 7.6	0.889
Male, %	10 (25.0)	14 (35.0)	0.329	6 (20.0)	11 (36.7)	0.152
Height, cm	159.9 ± 8.9	161.4 ± 8.4	0.455	159.7 ± 6.4	161.1 ± 8.3	0.477
Weight, kg	60.8 ± 9.5	63.5 ± 10.1	0.224	59.8 ± 7.5	62.7 ± 10.4	0.222
Body mass index, kg m ⁻²	23.7 ± 2.4	24.3 ± 2.7	0.303	23.4 ± 2.3	24.1 ± 2.9	0.332
Body fat percent, %	36.5 ± 6.3	35.6 ± 6.6	0.516	36.7 ± 6.8	35.0 ± 6.0	0.307
Truncal fat percent, %	37.6 ± 6.4	37.4 ± 7.0	0.867	37.8 ± 6.0	36.8 ± 7.3	0.560
Waist circumference, cm	84.1 ± 7.8	86.8 ± 7.5	0.111	82.9 ± 7.2	85.8 ± 6.9	0.123
Systolic BP, mmHg	137.0 ± 20.8	134.8 ± 18.2	0.615	136.1 ± 21.5	135.3 ± 16.5	0.877
Diastolic BP, mmHg	85.4 ± 11.7	85.6 ± 10.0	0.935	86.0 ± 11.6	86.0 ± 10.4	1.000
Co-morbidities			0.707			0.598
Hypertension	13 (32.5)	9 (22.5)		8 (29.0)	5 (15.6)	
Dyslipidemia	11 (27.5)	14 (35.0)		9 (29.0)	13 (40.6)	
Diabetes mellitus	4 (10.0)	5 (12.5)		2 (6.5)	4 (12.5)	
Others	8 (20.0)	9 (22.5)		7 (22.6)	7 (21.9)	
Total energy intake, kcal d ⁻¹	1936.8 ± 407.8	1868.3 ± 405.0	0.453	1917.4 ± 410.2	1889.1 ± 408.0	0.792
Protein, %	13.7 ± 1.6	14.4 ± 2.1	0.077	13.6 ± 1.7	14.6 ± 2.3	0.059
Fat, %	19.9 ± 6.4	20.2 ± 6.7	0.843	20.0 ± 6.5	21.1 ± 7.1	0.549
Carbohydrate, %	65.3 ± 7.3	64.2 ± 8.0	0.498	65.3 ± 7.6	62.9 ± 8.3	0.248
Waking, kcal per week	621.9 [365.2–882.2]	549.7 [267.1–909.6]	0.476	529.6 [335.7–893.0]	476.6 [221.3–937.4]	0.433
Adherence, %	87.7 ± 12.4	88.0 ± 9.9	0.929	92.5 ± 6.2	91.6 ± 5.5	0.570

^a Values are mean ± SD or n (%), except for waking, which is median [IQR]. APβG, *Aureobasidium pullulans*-produced β-glucan; BP, blood pressure.

^b P value by 2-sample *t* test or Mann–Whitney's *U* test for continuous variables and chi-square test for categorical variables.

Table 2 Energy intake and physical activity between the two groups for 12 weeks^a

	APβG group		Placebo group		Adjusted difference of APβG vs. placebo	<i>P</i> ^b
	Baseline	12 weeks	Baseline	12 weeks		
Intention-to-treat (<i>n</i> = 80)						
Energy intake, kcal d ^{−1}	1936.8 ± 407.8	2000.0 ± 290.4	1868.3 ± 405.0	1993.6 ± 325.1*	−114.07 (−247.83, 19.70)	0.093
Protein, %	13.7 ± 1.6	15.1 ± 1.5**	14.4 ± 2.1	14.8 ± 2.4	−0.57 (−0.29, 1.43)	0.193
Fat, %	19.9 ± 6.4	21.7 ± 7.5	20.2 ± 6.7	19.0 ± 5.2	2.56 (−0.15, 5.27)	0.063
Carbohydrate, %	65.3 ± 7.3	62.4 ± 8.3*	64.2 ± 8.0	64.8 ± 6.3	−2.57 (−5.68, −0.54)	0.104
Walking, kcal per week	621.9 [361.0–886.2]	603.1 [413.5–906.2]	549.7 [249.9–918.7]	568.8 [335.6–861.1]	43.24 (−44.92, 131.39)	0.428
Per-protocol (<i>n</i> = 63)						
Energy intake, kcal d ^{−1}	1917.4 ± 410.2	1992.1 ± 300.8	1889.1 ± 408.0	2004.2 ± 305.8	−136.12 (−286.00, 13.76)	0.074
Protein, %	13.6 ± 1.7	15.1 ± 1.6**	14.6 ± 2.3	15.0 ± 2.6	0.48 (−0.60, 1.56)	0.377
Fat, %	20.0 ± 6.5	21.6 ± 7.8	21.1 ± 7.1	19.1 ± 5.5*	2.64 (−0.49, 5.78)	0.097
Carbohydrate, %	65.3 ± 7.6	62.8 ± 8.7	62.9 ± 8.3	64.5 ± 6.6	−2.42 (−5.82, 1.34)	0.215
Walking, kcal per week	529.6 [335.7–893.0]	559.9 [395.9–902.9]	476.6 [221.3–937.4]	460.1 [290.9–883.9]	75.86 (−34.55, 186.26)	0.976

^a Values are mean ± SD or median [IQR] or mean (95% CI). APβG, *Aureobasidium pullulans*-produced β-glucan. ^b MANCOVA adjusted for baseline total energy intake, and each macronutrient % or rank ANCOVA adjusted for baseline walking calories as covariates over the 12-week period. **P* < 0.05, ***P* < 0.005 by paired *t*-test or Wilcoxon signed-rank test within each group.

APβG group showed significant improvements in concentric knee flexion strength (peak TQ) at 60° s⁻¹ in the right and left legs of 5.60 Nm (0.18–11.02 Nm; *P* = 0.043) and of 7.25 Nm (0.22–14.28 Nm; *P* = 0.043), respectively, compared with the placebo group (Fig. 4). The placebo group showed significant improvement in concentric knee extension strength (peak TQ) at 60° s⁻¹ of the left leg by 9.21 Nm (0.94–17.49 Nm; *P* = 0.03) compared to the APβG group in the PP analysis.

Secondary outcome

As Table 3 shows, the ITT analysis revealed that changes in secondary outcomes did not differ between the two groups after 12 weeks. However, after 12 weeks, the PP analysis revealed that the APβG group showed a significant increase in right handgrip strength by 1.40 kg (0.19–2.61 kg; *P* = 0.024) and left handgrip strength by 1.33 kg (0.01–2.65 kg; *P* = 0.048) com-

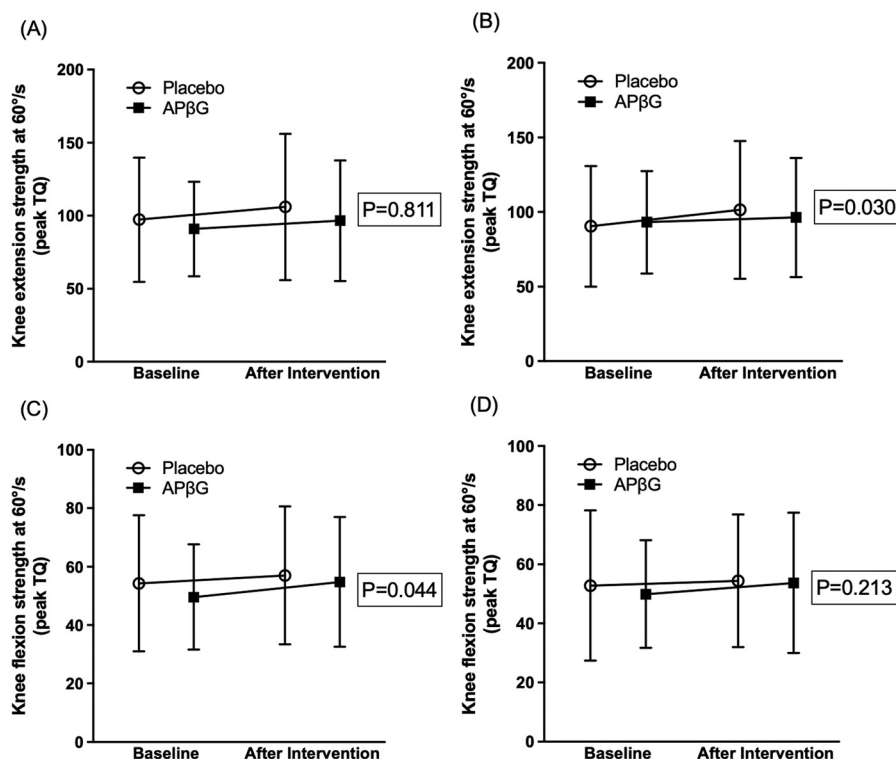


Fig. 3 Primary outcome measure of the two groups (intention-to-treat analysis). (A) Right, (B) left, (C) right, (D) left. Values are mean $\pm$ SD or mean (95% CI) unless otherwise indicated. APβG, *Aureobasidium pullulans*-produced β -glucan; TQ, torque. P value by ANCOVA adjusted for sex, protein intake %, and each baseline value as covariates over the 12-week period.

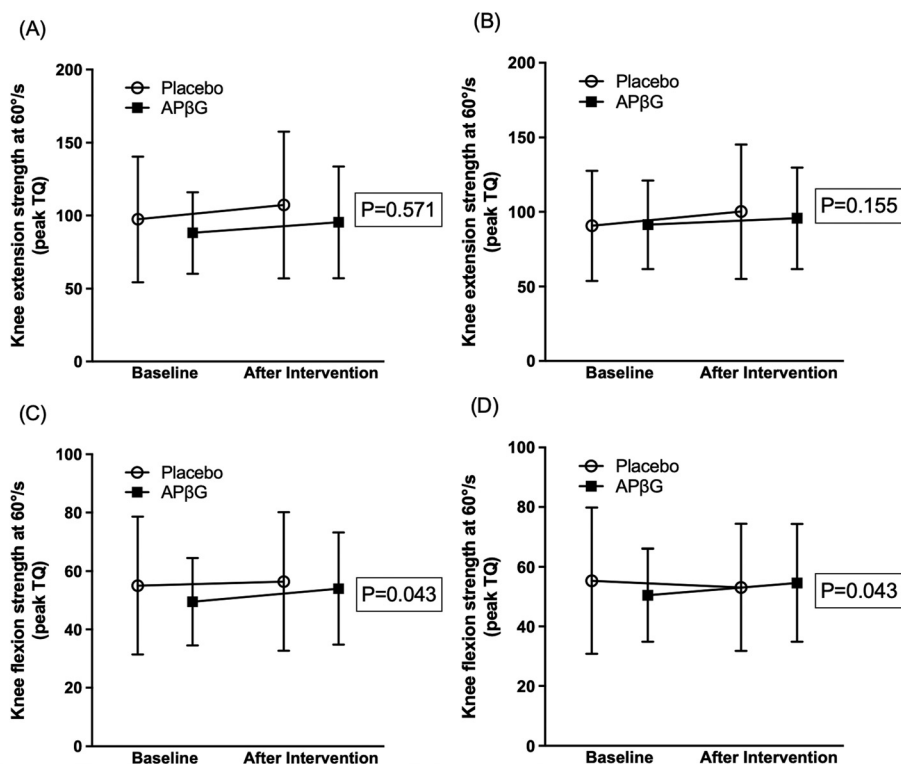


Fig. 4 Primary outcome measure of the two groups (per-protocol analysis). (A) Right, (B) left, (C) right, (D) left. Values are mean $\pm$ SD or mean (95% CI) unless otherwise indicated. APβG, *Aureobasidium pullulans*-produced β -glucan; TQ, torque. P value by ANCOVA adjusted for sex, protein intake %, and each baseline value as covariates over the 12-week period.

pared with the placebo group. Nevertheless, the other secondary outcomes did not differ between the two groups throughout the study period (ESI Table 3†).

Physical performance profiles

In the ITT analysis, SLS was significantly increased by 9.64 s ($P = 0.017$) and 400 MWT was decreased by 8.60 s ($P = 0.048$) in the APβG group compared with that in the placebo group after 12 weeks of treatment. The PP analysis also revealed that sit-ups in the APβG group increased by 1.34 ($P = 0.007$) and 400 MWT decreased by 13.11 s ($P = 0.015$) after 12 weeks of treatment, compared with that in the placebo group. However, the other secondary outcomes did not differ between the two groups throughout the study period (Table 4).

Evaluation of safety and additional benefits

All participants completed the protocol without any adverse events or serious adverse events, and none of them had any complaints. Additionally, waist circumference and free fatty acid concentrations remained unchanged in both groups during the 12 weeks of the trial (ESI Table 4†). For safety, no significant changes in BP, concentrations of liver enzymes, fasting glucose concentrations, or creatinine concentrations were observed between the two groups during the 12 weeks of the trial (ESI Table 5†). After 12 weeks, none of the participants had elevated liver enzyme concentrations more than double the reference value or creatinine levels that were 1.5 times the reference value. In addition, after 12 weeks of the trial, there was no discernable change observed between the two groups of participants who had systolic BP ≥ 160 mmHg, diastolic BP ≥ 100 mmHg, and fasting glucose ≥ 160 mg dL⁻¹ (ESI Table 3†).

Discussion

In this trial of adult patients with relatively low skeletal muscle mass, administration of 1000 mg APβG as an adjunct to regular resistance exercise for 12 weeks effectively improved muscle strength and fitness. This study investigated the combined effect of exercise and APβG on muscle health, similar to a previous study conducted on older adults by Shin *et al.*, which was a pilot investigation with a limited sample size aimed at determining the appropriate daily dosage of APβG.¹⁶ This previous study reported partial effects of the administration of APβG in combination with resistance exercise on improving muscle function. However, the purpose of the present study was to identify and address the limitations of the study conducted by Shin *et al.*¹⁶ and to corroborate the results of previous studies on the anti-sarcopenia effects of APβG.^{15,16} Our study had several key differences from Shin *et al.*'s study.¹⁶ Firstly, this study included participants aged 50 years and above, thus including middle-aged individuals, unlike Shin *et al.*,¹⁶ whose research participants were restricted to individuals aged 60 years or older. In addition, the proportion of males in the previous study was 15%,¹⁶ which corresponded to half of that (30%) in the present study. Furthermore, the previous pilot study did not compare comorbidities, energy intake, and physical activity between the control, low-dosage, and high-dosage groups at the start of the study.¹⁶ Additionally, there was no within-group or between-group comparison of energy intake and physical activity before and after 12 weeks.¹⁶ Moreover, Shin *et al.*'s study¹⁶ did not assess whether participants had relatively low muscle mass, which meant that their participants could have had various muscle conditions. In contrast, our study only selected participants with relatively low muscle mass using the body composition

Table 3 Secondary outcome measure of the two groups (ITT analysis)^a

	APβG group ($n = 40$)		Placebo group ($n = 40$)		Adjusted difference of APβG vs. placebo	P^b
	Baseline	12 weeks	Baseline	12 weeks		
Handgrip (right), kg	26.5 $\pm$ 8.0	28.6 $\pm$ 7.4*	27.9 $\pm$ 10.2	28.5 $\pm$ 9.6	0.59 (−2.25, 3.42)	0.681
Handgrip (left), kg	25.8 $\pm$ 8.6	27.5 $\pm$ 7.3	27.0 $\pm$ 9.7	28.3 $\pm$ 8.3	−0.55 (−3.03, 1.93)	0.660
ASM/height ² , kg m ^{−2}	5.67 $\pm$ 1.02	5.78 $\pm$ 0.95	5.98 $\pm$ 1.15	6.05 $\pm$ 1.11	−0.12 (−0.41, 0.17)	0.413
ASM/weight $\times$ 100	24.0 $\pm$ 4.0	24.1 $\pm$ 3.4	24.6 $\pm$ 3.8	24.9 $\pm$ 3.5	−0.71 (−1.74, 0.33)	0.177
Total fat percent, %	36.5 $\pm$ 6.3	35.6 $\pm$ 5.1	35.6 $\pm$ 6.6	34.2 $\pm$ 5.9**	1.13 (−0.26, 2.52)	0.110
Truncal fat percent, %	37.6 $\pm$ 6.4	37.4 $\pm$ 7.0	37.4 $\pm$ 7.0	36.1 $\pm$ 6.0*	1.13 (−0.34, 2.59)	0.129
Hs-CRP, mg dL ^{−1}	0.50 [0.40–1.00]	0.55 [0.30–1.28]	0.60 [0.40–0.88]	0.56 [0.30–1.18]	−0.11 (−1.07, 0.85)	0.371
Lactate, mg dL ^{−1}	10.7 $\pm$ 4.1	10.7 $\pm$ 5.0	10.7 $\pm$ 5.0	11.1 $\pm$ 4.4	0.57 (−1.05, 2.19)	0.481
CK, IU L ^{−1}	108.5	101.0	93.0	103.9	−8.52 (−29.38, 12.35)	0.517
	[83.5–154.0]	[83.5–119.3]	[74.0–127.8]	[73.3–137.5]		
IGF-1, ng mL ^{−1}	141.1 $\pm$ 36.3	161.9 $\pm$ 38.2**	150.1 $\pm$ 39.4	169.4 $\pm$ 39.8**	−1.10 (−13.99, 11.79)	0.866
GH, ng mL ^{−1}	1.03 [0.32–2.34]	1.08 [0.47–2.75]	0.68 [0.16–1.40]	1.20 [0.20–2.89]*	0.03 (−1.57, 1.63)	0.699
EQ-5D-5L	0.86 $\pm$ 0.04	0.86 $\pm$ 0.05	0.85 $\pm$ 0.07	0.85 $\pm$ 0.04	0.00 (−0.14, 0.02)	0.662
EQ-VAS	80.9 $\pm$ 11.6	79.1 $\pm$ 11.6	77.9 $\pm$ 16.8	79.9 $\pm$ 11.9	−0.48 (−5.89, 4.93)	0.859

^a Values are mean $\pm$ SD, median [IQR] or mean (95% CI) unless otherwise indicated. ITT, intention-to-treat; APβG, *Aureobasidium pullulans*-produced β -glucan; ASM, appendicular skeletal muscle; Hs-CRP, high-sensitive C-reactive protein; CK, creatinine kinase; IGF-1, insulin growth factor-1; GH, growth hormone; EQ-5D-5L, EuroQol-5 dimensions-5-levels; VAS, visual analogue scale. ^b ANCOVA or Quade's rank ANCOVA adjusted for sex, protein intake %, and each baseline value as covariates over the 12-week period. * $P < 0.05$, ** $P < 0.005$ by paired t -test or Wilcoxon signed-rank test within each group.

Table 4 Fitness profiles^a

	APβG group		Placebo group		Adjusted difference of APβG vs. placebo	<i>P</i> ^b
	Baseline	12 weeks	Baseline	12 weeks		
Intention to treat (<i>n</i> = 80)						
Sit-up	6.0 [0.0–13.3]	9.2 [5.0–12.2]**	10.0 [0.0–16.8]	11.6 [2.0–18.8]*	−0.63 (−1.51, 2.76)	0.123
SR, cm	15.0 [5.3–17.7]	13.2 [9.4–18.7]	12.3 [3.7–19.1]	13.7 [4.8–19.0]	3.37 (−10.82, 4.07)	0.776
SLS, s	70.0 [46.8–105.5]	120.0 [83.2–120.0]**	55.0 [36.0–120.0]	87.2 [46.1–120.0]**	9.64 (−5.14, 24.42)	0.017
6 MWT, meter	586.5 ± 59.3	622.9 ± 63.3*	585.0 ± 48.4	604.0 ± 65.3*	20.93 (−10.87, 52.72)	0.194
400 MWT, s	255.2 ± 30.5	235.3 ± 24.0**	255.7 ± 25.8	243.8 ± 23.2**	−8.60 (−17.13, 0.07)	0.048
10 MWT, s	17.1 ± 2.8	14.6 ± 2.3**	16.5 ± 3.6	14.2 ± 2.8**	−0.04 (−0.93, 0.86)	0.938
SPPB	11.0 [11.0–11.5]	12.0 [11.2–12.0]**	11.0 [11.0–11.0]	12.0 [11.0–12.0]**	0.09 (−0.16, 0.33)	0.188
Per protocol (<i>n</i> = 60)						
Sit-up	3.5 [0.0–11.0]	8.0 [3.0–15.2]**	10.0 [0.0–16.3]	10.0 [0.0–19.5]*	1.34 (−0.48, 3.17)	0.007
SR, cm	15.1 [5.7–17.9]	15.2 [9.5–20.3]	13.2 [3.9–19.5]	13.6 [4.2–19.1]	1.26 (−1.05, 3.56)	0.205
SLS, s	80.5 [39.4–120.0]	120.0 [88.3–120.0]**	48.1 [33.5–120.0]	80.5 [37.5–120.0]**	12.35 (−2.73, 27.42)	0.085
6 MWT, meter	587.3 ± 63.6	625.2 ± 71.5*	587.0 ± 48.4	598.3 ± 73.1	33.50 (−10.69, 77.69)	0.134
400 MWT, s	251.3 ± 19.5	231.8 ± 22.0**	255.6 ± 26.6	245.9 ± 26.1*	−13.11 (−23.60, −2.62)	0.015
10 MWT, s	17.0 ± 2.3	14.3 ± 2.0**	16.3 ± 3.6	14.5 ± 3.1**	−0.60 (−1.46, 0.26)	0.170
SPPB	11.0 [11.0–11.8]	12.0 [12.0–12.0]**	11.0 [11.0–11.0]	12.0 [11.0–12.0]**	0.18 (−0.12, 0.49)	0.164

^a Values are mean ± SD or median [IQR] or mean (95% CI) unless otherwise indicated. APβG, *Aureobasidium pullulans*-produced β-glucan; SR, sit and reach; SLS, single leg stance; 6 MWT, 6 min walk test; 400 MWT, 400-meter walk test; 10 mWT, 10-meter obstacle walk test, SPPB, short physical performance battery. ^b ANCOVA or Quade's rank ANCOVA adjusted for age, sex, bodyweight, baseline values, and the changes from baseline in dietary total caloric intake and protein intake as covariates over the 12-week period. *P < 0.05, **P ≤ 0.005 by paired t-test or Wilcoxon signed-rank test within each group.

analyzer InBody 720, ensuring that all participants had a similar condition. Regarding the statistical analysis method, in this study, considered the missing data from the ITT analysis, which was treated with multiple imputation, and the difference in results between the two groups before and after 12 weeks was confirmed by ANCOVA or rank ANCOVA considering covariate variables. Additionally, nutrition and physical activity, which can affect muscle function, were assessed both before and after the study to identify any differences between the two groups. Finally, in contrast to the pilot study conducted by Shin *et al.*,¹⁶ in which body composition was analysed using bioelectrical impedance analysis, this study utilized a more accurate and reliable method of whole-body DXA. Thus, body composition analysis variables such as ASMI, SMI, total fat percentage, and trunk fat percentage were included as secondary outcomes in this study. In addition, changes in quality of life were also added as secondary outcomes. Consequently, our study presents more extensive and evidence-based findings regarding the impact of APβG supplementation with resistance exercise on muscle function. Our study showed that a 1000 mg dose of APβG administered daily over 12 weeks, in combination with resistance exercise, increased the right and left peak TQ at 60° s^{−1} of knee flexion to 5.60 and 7.25 Nm, respectively, compared with that in the placebo group (PP analysis). However, it had no apparent favorable effects on muscle mass status. Additionally, our study revealed that the grip strength in both hands was positively affected in the intervention group as well (PP analysis). These results at 12 weeks were presumed to be caused by the additive

effect of APβG supplementation in combination with regular resistance exercise. Muscle strength gradually decreases with age, and in particular, the decline in knee flexor strength is more pronounced than the decline in knee extensor strength.^{25,26} This eventually causes an imbalance in the hamstrings-to-quadriceps (knee flexor-to-extensor) ratio, increasing the risk of muscle or ligament damage in the lower extremities. In the present study, the combined effect of APβG and exercise mainly improved knee flexor strength, suggesting that the hamstrings-to-quadriceps ratio was improved in this aspect.

β-Glucans are a group of physiologically active compounds composed of D-glucose monomers linked by 1,3-, 1,4-, or 1,6-β-glycosidic bonds from natural sources with proven bioactive properties. Traditionally, β-glucan, abundant in mushroom cell walls, is the main functional component of mushrooms.²⁷ Furthermore, β-glucans are enzymatically extracted from the cell walls of fungi, yeasts, and some plants. *Aureobasidium pullulans*, popularly known as black yeast, is one of the most widespread saprophytic fungi associated with a wide range of terrestrial organisms. Moreover, APβG, an exopolysaccharide biosynthesized in a culture medium that does not require purification, is water-soluble and easy to process. In addition, it is almost tasteless and odorless, does not impair the flavor of food, and exhibits excellent water retention and thickening properties. Notably, it is widely used as a substitute for mushroom-derived β-glucan because of its high stability.¹³ In this study, we used a β-1,3/1,6-glucan (β-1,3 linkage 68:β-1,6 linkage 32) with an average molecular weight of 2.6 × 10⁵ Da fermented from *A. pullulans* SM-2001.¹⁵

Research on the biomedical efficacy of AP β G is limited to laboratory studies and animal experiments.¹³ The mechanism underlying the effects of β -glucan on muscle health has been reported in *in vitro* and animal models.^{15,27–29} Recently, Hwang *et al.*³⁰ reported that AP β G induced the suppression of C2C12 myotube atrophy by activating the phosphoinositide 3-kinase/protein kinase B pathway in *in vitro* models. In addition, AP β G was observed to exhibit a potential for skeletal muscle-preserving therapy in dexamethasone-induced catabolic muscle atrophy by exhibiting the anti-inflammatory and antioxidant effects of AP β G, which were mediated by upregulating the expression of genes related to muscle protein synthesis (Akt1, PI3K, A1R, and TRPV4) and inhibiting the expression of genes related to muscle protein degradation (atrogin-1, MuRF1, myostatin, and SIRT1) in mice.¹⁵ The researchers further confirmed that AP β G activated myogenic transcription (mRNA expression levels of Myf5, MyoD, myogenin, and MEF2) and inhibited muscle proteolytic factors (mRNA expression levels of FoxO3 α , MuRF1, and atrogin-1), resulting in the improvement and protection of muscle atrophy in mdx (C57BL/10ScSn-Dmdmdx/J) mice models for Duchenne-type muscular dystrophy.²⁹ A previous pilot study¹⁶ reported that the group administered a combination of high-dosage intake of AP β G and resistance exercise group became more flexible than the control group. In addition, the 10 m obstacle walking time in the high-dose intake and resistance exercise group was shorter than that of the control group, and the grip strength of both hands in the group was higher than that of the control group at week 12. Moreover, concentric knee flexion strength (peak TQ) at 60° s⁻¹ in the right leg in the high-dosage and resistance exercise group increased more than that in the low-dosage and resistance exercise group at week 12. However, there were a few limitations in that study pertaining to the small sample size and the statistical analysis method in which one-way ANOVA was used without adjusted ANCOVA. In addition, this study did not test the normality assumption of the measured variables.¹⁶ In another pilot study, β -glucan administration (2 g per day) for 4 weeks showed no difference in lower extremity muscle strength; however, it improved grip strength in professional table tennis players.³¹

As an extension of previous experimental and human pilot studies,^{15,16,29,30} the present human study showed that AP β G does not affect CK, IGF-1, and GH concentrations. Such differences compared to those reported in previous animal studies could be partly explained by the fact that the participants in this study were adults with relatively low skeletal muscle mass. In animal studies, the dexamethasone-induced muscle atrophy in the mdx (C57BL/10ScSn-Dmdmdx/J) mice model is probably equivalent to severe sarcopenia in humans. Severe sarcopenia is identified when all three criteria of the definition (low muscle mass, muscle strength, and physical performance) are met.³² Therefore, further studies are needed to verify the effects of AP β G on muscle biomarkers in adults with sarcopenia.

This study has some limitations, including the lack of biological confirmation to determine the mechanism of action of

AP β G on muscle health and fitness. This study focused on participants with relatively low skeletal muscle mass; therefore, the effect of AP β G in healthy people or patients with definite or severe sarcopenia remains unknown. Our study participants were physically active, apparently healthy, older adults aged ≥ 50 years; hence, they do not represent the entire population. Furthermore, although the dropout rate was within the protocol's expectations, it was relatively high. Despite these limitations, this study can still be considered to have significant value because of several strengths. To our knowledge, this is the first well-designed clinical study to examine the combined efficacy and tolerability of AP β G supplementation and resistance exercise in adults with relatively low skeletal muscle mass. Another strength of this study is the use of a 3-axis wrist-worn accelerometer to evaluate the participants' physical activity and a valid self-report instrument to evaluate the participants' dietary intake.

Conclusion

AP β G supplementation, in addition to regular resistance exercise, may enhance skeletal muscle strength and fitness in adults with relatively low skeletal muscle mass without any toxicity or severe adverse effects. However, further clinical studies are needed to reveal the biomedical mechanisms underlying the combination effect of AP β G supplementation and exercise in adults with sarcopenia.

Data availability

The data described in the manuscript, code book, and analytic code will be made available upon request, pending application and approval.

Author contributions

Conceptualisation and study design – S. Y. L.; patient recruitment – H. J. K., J. S. S., W. G. K.; analysis and interpretation of data – S. Y. L., J. S. S.; drafting of manuscript – S. Y. L.; critical revision of the manuscript – H. J. K., J. S. S.; W. G. K.; study supervision – S. Y. L., H. J. K. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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