Synergic effect of *Aureobasidium Pullulans* produced beta-glucan and resistance exercise on muscle strength, biomarkers, and fitness profiles in adults with relative sarcopenia: a randomized, double-blinded, placebo-controlled trial

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SUPPLEMENTARY TABLE 1 Summary of comprehensive measurements by category

Category	Measurements
Muscle function (strength)	Peak torque (TQ) at 60°/s of knee extension/flexion, Handgrip strength
Lean body mass and fat%	Appendicular skeletal mass index, Skeletal muscle mass index, Body composition (Total fat%, Trucal fat%)
Training-induced fatigue	Lactate, Creatine kinase, Insulin growth factor-1, Growth hormone
Inflammatory status	High-sensitivity C-reactive protein
Quality of life	Euro-QoL-5D score
Safety monitoring	Blood pressure, Aspartate transaminase, Alanine transaminase, Creatinine, Glucose
Fitness profile	Sit-up, Sit and reach, Single leg stance, 6-min walk test, 400-meter walk test, 10-meter obstacle walk test, Short physical performance battery
Additional effects	Lipid profile, Insulin resistance, Free fatty acid

SUPPLEMENTARY TABLE 2 Resistance exercise program

Time	50 min per session. The three groups were arranged at 9:00–9:50 am, 10:00–10:50 am, and 11:00–11:50 am, respectively.
Frequency	Three times a week (Mon, Wed, and Fri)
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 times), and the number of sets (1-2 sets) were gradually increased. 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 were estimated to be 55–60% of 1RM.
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.

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SUPPLEMENTARY TABLE 3 Secondary outcome measure of the two groups (PP analysis)[1]

	APβG group (n = 30)		Placebo group (n = 30)		Adjusted difference of APβG vs placebo	P[2]
	Baseline	12 wk	Baseline	12 wk		
Handgrip (right), kg	26.1 ± 6.8	28.7 ± 7.4**	28.1 ± 9.2	29.5 ± 9.8**	1.40 (0.19, 2.61)	0.024
Handgrip (left), kg	25.8 ± 6.9	27.8 ± 7.1**	27.6 ± 9.2	28.3 ± 9.0	1.33 (0.01, 2.65)	0.048
ASM/height^2, kg/m^2	5.58 ± 1.05	5.73 ± 1.02**	5.95 ± 1.23	6.11 ± 1.20**	-0.03 (-0.19, 0.12)	0.679
ASM/weight x 100	23.8 ± 3.8	24.3 ± 3.3*	24.7 ± 4.0	25.1 ± 3.6	0.09 (-0.50, 0.67)	0.773
Total fat percent, %	37.8 ± 5.4	35.4 ± 5.4**	35.0 ± 6.8	33.6 ± 6.4**	0.07 (-0.79, 0.93)	0.874
Truncal fat percent, %	37.8 ± 6.0	36.8 ± 5.8**	36.8 ± 7.3	35.8 ± 6.6**	-0.21 (-1.30, 0.89)	0.708
Hs-CRP, mg/dl	0.40 [0.40-0.70]	0.50 [0.30-0.98]	0.60 [0.40-0.80]	0.50 [0.25-0.80]	-0.38 (-1.72, 0.96)	0.458
Lactate, mg/dL	10.6 ± 4.1	10.6 ± 4.2	10.7 ± 5.4	11.1 ± 4.9	1.00 (-1.15, 3.15)	0.356
CK, IU/L	114.0 [86.5-154.8]	95.0 [80.0-119.3]	93.0 [78.0-127.5]	96.5 [72.5-146.5]	-13.50 (-38.33, 11.34)	0.105
IGF-1, ng/mL	142.5 ± 38.3	163.7 ± 42.4**	146.1 ± 37.7	168.9 ± 42.8**	1.00 (-13.52, 15.53)	0.890
GH, ng/mL	0.91 [0.34-2.17]	0.82 [0.41-2.30]	0.68 [0.19-1.28]	1.18 [0.13-2.94]*	-0.02 (-2.04, 2.00)	0.661
EQ-5D-3L	0.86 ± 0.04	0.86 ± 0.04	0.85 ± 0.07	0.86 ± 0.04	0.01 (-0.01, 0.03)	0.170
EQ-VAS	80.6 ± 10.9	78.7 ± 12.5	79.7 ± 17.1	80.0 ± 13.1	1.91 (-5.21, 9.02)	0.594

[1]Values are mean ± SD, median [IQR] or mean (95% CI) unless otherwise indicated. PP, per-protocol; 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

[2]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.

SUPPLEMENTARY TABLE 4 Additional benefits profile[1]

	APβG group		Placebo group		Adjusted difference of APβG vs. placebo	P[2]
	Baseline	12 wk	Baseline	12 wk		
Intention to treat (*n*=80)						
WC, cm	84.1 ± 7.8	81.6 ± 7.3**	86.8 ± 7.5	85.2 ± 7.4	-1.91 (-4.61, 0.80)	0.164
HOMA-IR	1.38 [0.82-2.05]	1.51 [1.03-2.11]	1.71 [1.04-2.45]	1.74 [1.02-2.63]	0.92 (-1.08, 2.91)	0.938
FFA. mg/dL	439.5 [311.8-583.0]	395.9 [302.5-526.8]*	455.0 [337.8-627.5]	370.0 [289.5-448.0]*	-64.32 (-5.03, 133.66)	0.648
TC, mg/dL	196.7 ± 39.8	191.6 ± 35.0	192.7 ± 38.1	185.7 ± 39.9	2.34 (-10.77, 15.44)	0.724
Triglyceride, mg/dL	101.5 [71.3-128.3]	100.5 [76.5-132.0]	115.0 [73.0-183.8]	126.3 [75.0-157.0]	-34.15 (-15.70, 84.00)	0.582
HDL-C, mg/dL	56.5 ± 11.1	55.1 ± 12.4	54.2 ± 15.9	55.9 ± 15.3	-1.78 (-6.53, 2.98)	0.458
LDL-C, mg/dL	118.3 ± 37.5	114.7 ± 36.7	110.8 ± 40.9	106.9 ± 38.2	0.47 (-12.34, 13.29)	0.941
Per protocol (*n*=60)						
WC, cm	82.9 ± 7.2	79.9 ± 7.6**	85.8 ± 6.9	85.0 ± 8.1	-2.56 (-5.58, 0.47)	0.096
HOMA-IR	1.38 [0.77-2.06]	1.44 [0.93-2.02]	1.49 [1.02-2.43]	1.45 [0.96-2.60]	-1.58 (-1.14, 4.30)	0.982
FFA. mg/dL	443.0 [302.3-641.8]	359.0 [295.0-566.0]*	453.5 [342.0-631.0]	316.5 [264.5-453.0]*	88.60 (-6.73, 183.93)	0.696
TC, mg/dL	199.9 ± 39.4	194.7 ± 38.4	192.6 ± 40.6	185.3 ± 44.1	-2.78 (-18.09, 12.52)	0.717
Triglyceride, mg/dL	107.0 [71.3-125.8]	91.0 [75.3-120.3]	97.0 [71.5-160.0]	101.0 [73.5-157.0]	53.80 (-14.52, 122.82)	0.478
HDL-C, mg/dL	56.1 ± 10.6	55.2 ± 12.3	56.0 ± 16.5	56.9 ± 16.7	-1.69 (-7.08, 3.69)	0.531
LDL-C, mg/dL	121.6 ± 37.6	118.5 ± 39.5	110.4 ± 43.2	105.8 ± 42.1	-4.62 (-18.62, 9.39)	0.511

[1]Values are mean ± SD or mean (95% CI) unless otherwise indicated. APβG, *Aureobasidium pullulans*-produced β-glucan; WC; waist circumference; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; FFA, free fatty acid; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol

[2]ANCOVA or Quade's rank ANCOVA adjusted for sex, protein intake %, and each baseline value as covariates over the 12-wk period. *P<0.05, **P<0.005 by paired *t*-test or Wilcoxon signed-rank test within each group.

SUPPLEMENTARY TABLE 5 Laboratory findings evaluating the adverse effects[1]

	APβG group		Placebo group		Adjusted difference of APβG vs. placebo over	P[2]
	Baseline	12 wk	Baseline	12 wk		
Intention to treat (*n*=80)						
Systolic BP, mmHg	137.0 ± 20.8	133.5 ± 14.9	134.8 ± 18.0	131.0 ± 16.6	1.22 (-4.92, 7.36)	0.694
Diastolic BP, mmHg	85.4 ± 11.7	84.1 ± 9.0	85.6 ± 10.0	83.5 ± 9.9	1.07 (-2.41, 4.54)	0.543
AST, IU/L	23.0 [21.0-28.8]	24.0 [20.0-27.8]	21.5 [18.3-26.0]	23.2 [19.3-26.0]	0.31 (-2.24, 2.86)	0.862
ALT, IU/L	18.0 [15.0-26.0]	19.7 [15.0-23.9]	18.5 [14.0-27.8]	19.0 [13.3-27.0]	4.24 (0.10 8.37)	0.236
Creatinine, mg/dL	0.77 ± 0.13	0.78 ± 0.16	0.83 ± 0.24	0.85 ± 0.23	-0.01 (-0.07, 0.04)	0.631
Glucose, mg/dL	92.0 ± 24.2	92.1 ± 16.0	95.1 ± 21.9	95.8 ± 20.6	-2.58 (-8.92, 3.76)	0.420
Per protocol (*n*=60)						
Systolic BP, mmHg	136.1 ± 21.4	133.3 ± 16.3	135.3 ± 16.5	132.7 ± 16.8	-1.75 (-9.50, 6.00)	0.653
Diastolic BP, mmHg	86.0 ± 11.6	84.9 ± 9.9	86.0 ± 10.8	84.6 ± 10.8	-0.27 (-4.54, 4.01)	0.901
AST, IU/L	26.5 [21.0-30.5]	24.0 [19.8-28.3]	22.5 [18.8-26.0]	22.0 [19.0-26.3]	-0.02 (-3.20, 3.16)	0.494
ALT, IU/L	18.5 [15.0-26.5]	17.5 [14.0-24.3]	17.0 [14.0-27.3]	17.5 [13.0-27.3]	4.62 (-0.54, 9.79)	0.953
Creatinine, mg/dL	0.77 ± 0.14	0.75 ± 0.15	0.84 ± 0.25	0.84 ± 0.26	0.23 (-0.07, 0.02)	0.226
Glucose, mg/dL	88.6 ± 20.3	91.6 ± 18.3*	96.6 ± 23.6	97.8 ± 23.1	-0.05 (-6.22, 6.13)	0.988

[1]Values are mean ± SD or median [IQR] or mean (95% CI) unless otherwise indicated. APβG, *Aureobasidium pullulans*-produced β-glucan; BP, blood pressure; AST, aspartate transaminase; ALT, alanine transaminase

[2]ANCOVA or Quade's rank ANCOVA adjusted for sex, protein intake %, and each baseline value as covariates over the 12-wk period. *P<0.05 by paired *t*-test or Wilcoxon signed-rank test within each group.

SUPPLEMENTARY TABLE 6 Laboratory findings evaluating adverse events at 12 weeks

Variables	Intention-to-treat population		Per-protocol population	
	APβG group (*n*=40)	Placebo (*n*=40)	APβG group (n=30)	Placebo (n=30)
High AST (≥2×ULN)	0	0	0	0
High ALT (≥2×ULN)	0	0	0	0
High Cr (≥1.5×ULN)	0	0	0	0
SBP (≥160 mmHg)	4	3	2	2
DBP (≥100 mmHg)	1	2	1	2
FBG (≥160 mg/dL)	1	1	1	1

APβG, *Aureobasidium pullulans*-produced β-glucan; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Cr, creatinine; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; ULM, upper limit of normal.

All P values > 0.05 by Fisher's exact test

SUPPLEMENTARY FIGURE 1A Resistance exercise

SUPPLEMENTARY FIGURE 1B Resistance exercise

SUPPLEMENTARY FIGURE 1C Resistance exercise