



Effects and Long Term Safety of Fermented Hop Extract Supplementation During Physical Exercise Intervention in Healthy Middle-Aged and Older Adults: A Randomized, Double Blind, Placebo Controlled Trial

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● Abstract

Objective: 8-prenylnaringenin (8-PN), obtained by fermenting hop extract, is known as a functional polyphenol that has effects such as alleviating menopausal symptoms, maintaining bone mineral density, and reducing body fat. Recent studies have reported that muscle atrophy could be suppressed and muscle mass could be restored by 8-PN ingestion in animal experiments, however the effects in humans have not been clarified. In this study, we conducted a randomized controlled trial to investigate the effects of fermented hop extract supplementation, the principal component is 8-PN, in combination with physical exercise on body composition and muscle strength in middle-aged and elderly Japanese with a tendency toward obesity.

Methods: Seventy-eight participants, who aged 40-74 years and body mass index (BMI) was 23.0 kg/m² or over, agreed to participate in this trial. The participants were randomly divided into hop group (n=39) and placebo group (n=39). The hop group consumed one capsule per day, which contains 100 µg of 8-PN, for 12 weeks, on the other hand the placebo group consumed one capsule per day, not contained 8-PN, for 12 weeks. Each group were instructed to complete eight kinds of physical exercises and resistance tube training three times in a week through this trial. Body composition and muscle strength were assessed at baseline, after 8 weeks, and after 12 weeks. In addition, participants completed the Profile of Mood States 2 (POMS2) questionnaire, and blood and urine tests were performed at each assessment session.

Results: Percent changes from baseline to 12 weeks showed significant increases in appendicular muscle mass and skeletal muscle index (SMI) in the hop group compared with the placebo group ($p = 0.03$ for each). In addition, the increase in interleukin-6 (IL-6), a pro-inflammatory cytokine marker, was significantly attenuated in the hop group compared with the placebo group. Regarding safety, no serious adverse effects were observed in either group, and there were no differences in the frequency of adverse events between the groups.

Conclusions: 8-PN supplementation may effectively promote increases in appendicular muscle mass when combined with physical exercise. In addition, 8-PN intake may attenuate the exercise-induced increase in interleukin-6 (IL-6). Long-term safety of 8-PN intake was also demonstrated.

Keywords: 8-prenylnaringenin; hop extracts; physical exercise; skeletal muscle index

1. INTRODUCTION

Hops (*Humulus lupulus*) have been used not only as a key ingredient in brewing but also as a traditional medicinal plant in Europe and Asia, where they have traditionally been used for their sedative and digestive properties^{1)~3)}. Among the bioactive compounds in hops, 8-prenylnaringenin (8-PN) is a functional polyphenol obtained by fermenting hop extracts⁴⁾⁵⁾. Hop extracts has been reported to have effects such as alleviating menopausal symptoms⁶⁾, maintaining bone mineral density⁷⁾, and reducing body fat^{8)~9)}. Recently, the effects of 8-PN on muscle mass have also been reported, and animal studies have shown that 8-PN intake inhibits muscle atrophy and restores muscle mass^{10)~12)}. Hops also contain xanthohumol (XH) and isoxanthohumol (IX), and 8-PN is synthesized from IX by the intestinal microflora¹³⁾. However, it has been reported that approximately 60% of humans are unable to synthesize 8-PN in the intestine due to the lack of specific gut flora, making it difficult for such people to synthesize 8-PN¹⁴⁾.

Developed countries are facing a super-aging society, in which the decline in muscle strength among older adults has become a major public health issue. The decline in muscle strength and muscle mass leads to frailty in the elderly. Frailty increases the risk of falls, femoral fractures, and head injuries, which in turn increases the risk of needing nursing care¹⁵⁾. Consequently, the increase in nursing care costs associated with the super-aging population has become a social problem in Japan¹⁶⁾. Preventing falls is a critical factor of extending healthy life expectancy and reducing nursing care costs. Therefore, individuals are required to maintain their muscle mass, strength, and functional capacity through daily exercise. As the risk of falling increases with age, daily exercise is recommended for older adults and for middle-aged individuals to reduce falling risk.

In this study, a combined intervention of 8-PN supplementation and a home based exercise program was implemented in healthy middle-aged and older adults with a tendency toward obesity. The daily dosage of 8-PN was set at 100 µg, and a randomized, placebo-controlled trial design was employed. This study aimed to evaluate whether 8-PN intake enhances the effects of physical exercise on muscle mass and muscle strength, and to clarify its long-term safety, thereby providing important insights for developing strategies in a super aging society.

2. MATERIALS AND METHODS

2.1. Participants

Inclusion criteria for the participants were: (1) healthy adults aged 40 to 74 years at the time of consent, (2) body mass index (BMI) of 23.0 kg/m² or higher, (3) capable of daily exercise, and (4) consent to participate after

thoroughly understanding the purpose of the study. Exclusion criteria were: (1) hop allergy, (2) treatment for hyperlipidemia, (3) taking visceral fat-reducing medication, (4) performing high-intensity strength training at least three days a week, (5) participation in other clinical trials within three months prior to consent, (6) pregnancy or breastfeeding, (7) judged by the collaborating doctor of ineligibility based on baseline blood tests, or (8) determination by the principal investigator of ineligibility for other reasons. Based on a previous study, the number of participants was calculated based on the previous study¹⁷⁾, which evaluated the effects of combination of plant-derived supplement intake and physical exercise. We hypothesized that the increase in muscle mass would be 0.6 kg in the hop group and 0.2 kg in the placebo group, with a standard deviation of 0.62 kg. The statistical power ($1-\beta$) was set at 0.80, and the significance level (α) was set at 0.05. Consequently, the sample size was calculated to be 37.7 participants per group. Assuming a 15% dropout rate, a total of 90 participants was deemed appropriate for the present study. Recruit of the participants were conducted from 11/12/2024 to 19/4/2025. Informed consent was obtained from all subjects involved in the study. The measurement sessions were conducted in a rented facility in Kobe, Japan.

2.2. Test foods

The test food capsules were prepared by Daicel Corporation (Tokyo, Japan). The capsule of hop group contained fermented hop extract, mainly 100 µg of 8-PN, in a capsule (ASTROHOP™), conversely the capsule of placebo group contained dextrin instead of hop extracts. The two test capsules were visually identical. ASTROHOP is a registered trademark of Daicel Corporation in Japan and a trademark of Daicel Corporation in the United States and Canada.

2.3. Study design and interventions

This study employed a randomized, double-blind, placebo-controlled trial design. After baseline assessment session, the participants were divided into hop group or placebo group randomly in a 1:1 allocation ratio, with sex, age, and whole-body muscle mass at baseline as allocation factors using the SAS program's "PROC SURVEYSELECT" procedure. Randomization was performed by Koutatsu Maruyama (last author), who was not involved in participant recruitment or assessment sessions, and he solely managed the group assignments. All other investigators remained blinded to the group allocations until all assessments were completed and the database was locked. The participants consumed a capsule of each group after morning meal every day for 12 weeks, and were instructed not to change their lifestyles except for the interventions of this trial. Throughout this trial, the participants were required to complete following 8-items

of physical training: (1) lower body squats; (2) thigh exercise; (3) back muscle exercise; (4) back and buttocks exercise; (5) upper body abdominal exercise; (6) lower body abdominal exercise; (7) arm exercise; (8) calf muscle training. In addition, for upper body training, resistance training was performed using rubber tubes. We instructed the participants to perform each exercise thrice per week during the study, performing approximately 15 repetitions at their 15RM per set.

2.4. Diary records

To ensure compliance, participants were required to keep a health diary of daily capsule intake adherence, the items of physical training done, changes in their health condition, and lifestyle changes. After the end of the study, adverse events were identified based on this diary.

2.5. Outcomes

In this study, three assessments were conducted: at baseline, at 12 weeks after the intervention, and additionally at 8 weeks for safety monitoring. The measurement items are as follows.

Body composition was assessed at baseline, after 8 weeks, and after 12 weeks using a bioelectrical impedance analyzer (MC-780A; Tanita, Tokyo, Japan). Height was measured only at baseline. The parameters were as follows: body weight, body mass index (BMI), body fat percentage, trunk body fat percentage, visceral fat level, whole-body muscle mass (primary endpoint), trunk muscle mass, appendicular muscle mass, skeletal muscle index (SMI).

Physical performance was assessed at baseline, after 8 weeks, and after 12 weeks. The indices included grip strength, isometric knee extension strength, hip adductor strength and hip abductor strength, and one-leg standing time with eyes open. Grip strength was measured using the digital dynamometer (T.K.K.5401; Takei Scientific Instruments Co., Ltd. Niigata, Japan). Isometric knee extension strength was measured using the digital dynamometer (T.K.K.5715; Takei Scientific Instruments Co., Ltd.). Hip adductor strength and hip abductor strength were measured using (T.K.K.3367B; Takei Scientific Instruments Co., Ltd.). To measure one-leg standing time, the participants were instructed to stand on their self-selected leg with their arms at rest alongside their body and facing a mark at eye level. Standing time was measured up to 120 seconds. Each measurement was taken twice, and the higher value was used.

Blood samples were collected at baseline, after 8 weeks, and after 12 weeks. Routine biochemical parameters were measured to evaluate the long-term safety of 8-PN, including total protein, albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyltransferase (γ -GT), uric acid, blood urea nitrogen (BUN), creatinine, sodium, calcium, potassium, magnesium, high-density lipoprotein cholesterol (HDL-C),

total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), amylase, glucose, estimated glomerular filtration rate (eGFR), complete blood count, and HbA1c. In addition, the following specialized biomarkers were measured to explore the effects of 8-PN: interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) and malondialdehyde-modified LDL (MDA-LDL).

Urinalysis was performed at baseline, after 8 weeks, and after 12 weeks to evaluate the long-term safety of 8-PN supplementation. The measured parameters included routine urinary components, urinary creatinine, and urinary uric acid.

To assess subjective mood conditions, the Profile of Mood States Second Edition (POMS2) questionnaire was administered three times: at baseline, at week 8, and at week 12. The Japanese version of the POMS2¹⁸⁾, which is based on the original POMS2 developed by Heuchert and McNair¹⁹⁾, is a self-administered questionnaire consisting of 35 items. The Total Mood Disturbance (TMD) score was used as the overall indicator, and seven subscales were evaluated: Anger-Hostility (AH), Confusion-Bewilderment (CB), Depression-Dejection (DD), Fatigue-Inertia (FI), Tension-Anxiety (TA), Vigor-Activity (VA), and Friendliness (F). The Friendliness (F) subscale is not included in the TMD score. Except for VA and F, higher scores indicate a worse mood state.

For evaluation of safety of long-term 8-PN supplementation, the number of adverse events were counted during this trial by the participants daily records. When the adverse events were judged as a potential causation by the corroborator doctor, those were regarded as side effects. In this study, adverse events were divided into seventeen symptoms as follows: Cold (including COVID-19), Influenza, Other infectious diseases, Allergies, Systemic symptoms, Digestive symptoms, Respiratory symptoms, Bodily pain, Nervous system disorders, injured, Skin symptoms, Dental symptoms, Sleep/mental disorders, unexplained symptoms, Gynecologic symptoms, Abnormal blood test results, Abnormal urine test results. Regarding each event, the numbers of participants had once or more, were analyzed between the two groups.

2.6. Statistical analysis

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The effects of 8-PN supplementation were analyzed using non-parametric analysis by comparing the percent changes from baseline to 12 weeks between the two groups, as the percent changes were not assumed to follow a normal distribution. No covariates were included in these analyses. The frequency of adverse events were analyzed for all participants allocated to either group using χ squared test or Fisher's exact test. For each test, a significant difference was defined as $p < 0.05$.

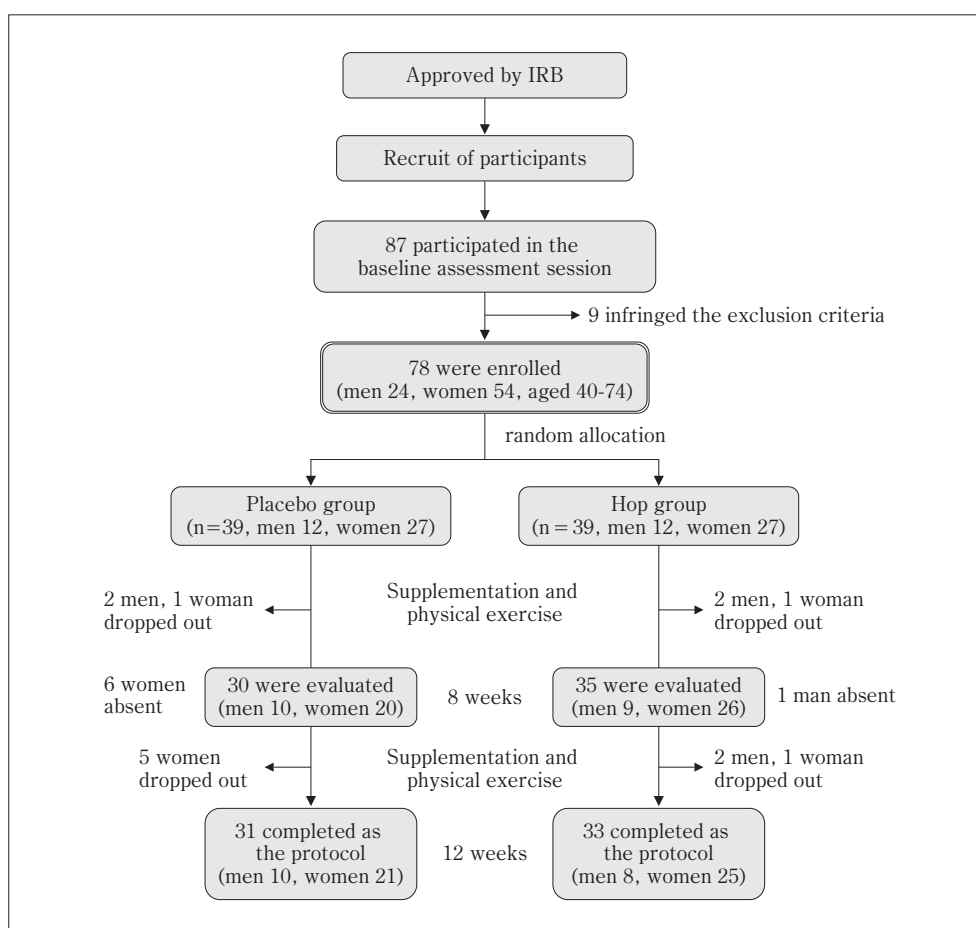


Fig. 1 The participant flow chart

2.7. Ethics statement UMIN

The study was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Life Science and Medical Research Involving Human Subjects in Japan. The protocol was approved by the Institutional Review Board of Ehime University Hospital (Toon, Japan). This trial was registered at the clinical trial registry of Japan (registration number: UMIN000056664) under the title “Effects on body composition of the combination of continuous intake of plant-derived polyphenols and physical exercise: a randomized, placebo-controlled, double-blind study” on 16 April 2025. The trial information is available at the following URL: https://center6.umin.ac.jp/cgi-bin/ctr/ctr_view_reg.cgi?recptno=R000064700.

3. RESULTS

3.1. Participant Flow and Baseline Characteristics

Seventy-eight participants met the eligibility criteria and were enrolled in the study. Participants were randomly assigned to the hop ($n = 39$) or placebo ($n = 39$) group. Fourteen participants (hop, 6 and placebo, 8) dropped out and sixty-four participants (hop, 33 and placebo, 31) completed the trial. The analysis for efficacy was

conducted by per-protocol set consisting of these 64 participants, whereas the analysis of adverse events was performed for all 78 participants who were allocated to either group (**Fig. 1**). Reasons for dropout mainly included personal circumstances or loss of follow up. None of the dropouts were related to the intervention, and their exclusion from analysis was not considered to introduce bias. Missing data were not imputed, and efficacy outcomes were analyzed only for participants who completed the 12 week intervention.

The mean ages were 55.4 ± 8.4 years in the placebo group and 55.9 ± 8.8 years in the hop group, with no significant differences between the groups (**Table 1**). Also, regarding body composition, physical performance, blood test and urine test, there were no significant difference between the groups. In VA score of POMS2, the hop group (10.5 ± 4.0) was higher than the placebo group (8.2 ± 3.9) significantly, whereas no significant difference was observed in other subscales.

3.2. Effects of Hop Intake on Body Composition

Table 2 shows the measurements at baseline, 8 weeks, and 12 weeks, and **Table 3** shows the percent changes in each group from baseline to 12 weeks. A significant increase in appendicular muscle mass and SMI was

Table 1 The baseline characteristics of participants

	Placebo group (n = 39)			Hop group (n = 39)			p value**
	mean	s.d.		mean	s.d.		
Age (years)	55.4	8.4		55.9	8.8		0.78
Appendicular muscle mass (kg)	20.3	4.8		20.0	4.8		0.74
SMI (kg/m ²)	7.72	1.07		7.65	1.20		0.79
Grip strength (right, kg) *	28.0	22.5	34.8	27.2	20.55	36.12	0.65
Grip strength (left, kg) *	26.6	21.5	32.9	26.1	19.75	34.58	0.77
Hip adductors strength (kg)	21.9	5.2		20.5	6.2		0.28
Hip abductors strength (kg)	23.9	6.2		23.1	8.5		0.64
Isometric knee extension strength (right, kg)	34.3	10.5		36.8	13.1		0.34
Isometric knee extension strength (left, kg)	34.2	10.5		37.0	12.9		0.30
One-leg standing time (s) *	54.4	19.0	155.8	59.6	23.93	148.62	0.68
Body weight (kg)	70.1	11.0		69.3	11.0		0.74
BMI (kg/m ²)	26.9	2.3		26.7	2.5		0.83
Body fat weight (kg)	24.1	5.1		23.5	6.0		0.62
Body fat percentage (%)	34.6	6.2		34.0	6.5		0.68
Whole-body muscle mass (kg) *	42.6	35.0	51.8	42.5	35.01	51.48	0.93
Leg muscle mass (right, kg) *	7.86	6.29	9.82	7.73	6.16	9.71	0.75
Leg muscle mass (left, kg) *	7.77	6.20	9.75	7.60	6.07	9.54	0.67
Arm muscle mass (right, kg) *	2.13	1.74	2.60	2.09	1.67	2.61	0.72
Arm muscle mass (left, kg) *	2.06	1.68	2.53	2.02	1.61	2.52	0.65
POMS2	46.0	9.6			45.83	9.323	0.93
AH*	3.3	1.7	6.4	3.0	1.35	6.47	0.49
CB	3.7	3.1		2.8	2.6		0.17
DD*	2.0	0.9	4.7	1.9	0.87	4.07	0.76
FI*	4.4	2.0	10.0	3.8	1.86	7.96	0.42
TA	5.5	3.8		4.2	3.1		0.09
VA	8.2	3.9		10.5	4.0		0.01
F	10.4	4.0		11.4	3.2		0.24
TMD	13.7	16.1		7.4	14.4		0.07
Blood test	93.0	11.2		96.4	9.8		0.16
MDA-LDL Cholesterol (U/L)	93.7	27.1		96.8	27.5		0.62
IL-6 (pg/mL) *	1.66	1.06	2.60	1.73	1.08	2.76	0.69
TNF- α (pg/mL) *	0.61	0.41	0.93	0.62	0.45	0.85	0.98

* Geometric mean (25-75% range), ** Unpaired t-test, Chi-squared test, Fisher's exact test.

observed in the hop group (+2.4%) than in the placebo group ($\pm 0\%$) ($p=0.03$). **Fig. 2** shows the percent change in SMI from baseline to 8 weeks, and 12 weeks in the hop and placebo groups. In the lower body muscle mass, that of left leg increased significantly in the hop group (+2.1%) compared with the placebo group ($\pm 0\%$) ($p=0.02$). On the other hand, body weight, BMI, body fat mass, and body fat percentage decreased significantly in the placebo group compared with the hop group ($p=0.02, 0.02, 0.03, 0.04$ respectively).

3.3. Effects of Hop Intake on Physical Performance

With respect to physical performance, no significant differences were detected between the two groups in any of the measured indices.

3.4. Effects of Hop Intake on Blood and Urinary Biomarkers

Regarding the blood test results, IL-6 secretion was significantly reduced in the hop group compared with the placebo group ($p=0.04$). In urine test, no significance differences were detected between the two groups (**Table 3**).

3.5. Safety Assessment

No significant differences were observed between the two groups for any item, indicating no safety concerns associated with long-term hop intake (**Table 4**).

Table 2 All measurements from baseline to 12 weeks

	Placebo group (n = 31)						Hop group (n = 33)					
	baseline			8 weeks			baseline			8 weeks		
	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%
Appendicular muscle mass (kg)	18.3	17.1	23.8	18.5	17.5	24.5	18.2	17.2	24.7	17.7	16.3	21.7
SMI (kg/m ²)	7.54	6.97	8.37	7.52	7.15	8.47	7.46	7.04	8.47	7.35	6.63	8.25
Grip strength (right, kg)	29.3	24.1	33.2	28.4	23.5	34.9	29.0	23.2	33.8	24.9	24.0	28.1
Grip strength (left, kg)	26.5	22.0	32.2	27.1	22.8	30.3	27.0	23.0	31.2	25.1	21.2	29.1
Hip adductors strength (kg)	20.8	18.4	22.4	19.6	13.0	23.1	22.6	22.0	23.6	20.1	17.2	23.8
Hip abductors strength (kg)	22.9	20.6	26.0	20.1	13.0	28.6	24.0	21.5	27.3	20.3	17.5	29.2
Isometric knee extension strength (right, kg)	33.5	28.5	37.4	35.1	32.4	43.7	35.4	32.1	40.4	35.2	25.9	46.1
Isometric knee extension strength (left, kg)	33.2	25.1	40.1	36.8	30.4	41.1	36.2	31.7	42.6	35.8	23.8	44.4
One-leg standing time (s)	86.0	31.0	120	84.0	28.0	120	120.0	65.0	120	82.0	39.0	120
Body weight (kg)	70.2	61.8	76.8	67.7	61.6	75.0	67.8	60.7	74.3	65.9	61.5	75.4
BMI (kg/m ²)	26.50	25.40	28.90	26.20	25.20	28.60	25.80	24.50	28.40	26.20	24.80	27.80
Body fat weight (kg)	24.1	19.9	27.0	23.0	19.2	24.8	23.7	18.5	25.1	22.1	20.4	25.0
Body fat percentage (%)	34.6	30.7	39.6	33.6	29.4	38.5	33.8	29.3	37.7	35.4	32.0	38.0
Whole-body muscle mass (kg)	40.3	36.7	50.1	40.1	37.3	52.8	39.8	36.7	52.1	39.4	36.5	46.2
Leg muscle mass (right, kg)	7.2	6.8	9.4	7.4	6.9	9.5	7.3	6.7	9.8	7.1	6.4	8.7
Leg muscle mass (left, kg)	7.1	6.7	9.4	7.4	6.8	9.7	7.3	6.7	9.8	6.9	6.4	8.4
Arm muscle mass (right, kg)	2.0	1.8	2.6	2.1	1.9	2.7	2.1	1.8	2.6	2.0	1.8	2.4
Arm muscle mass (left, kg)	1.9	1.7	2.5	2.0	1.8	2.5	2.0	1.8	2.5	1.9	1.7	2.2
POMS2	42.9	38.9	52.8	42.6	39.6	55.7	42.3	38.9	55.0	41.9	38.7	49.3
AH	3.0	2.0	5.0	3.0	2.0	5.0	3.0	1.0	6.0	3.0	1.0	5.0
CB	3.0	1.0	5.0	2.0	1.0	5.0	3.0	1.0	5.0	2.0	1.0	4.0
DD	1.0	1.0	3.0	1.0	0.0	4.5	1.0	0.0	5.0	1.0	0.0	3.0
FI	5.0	2.0	8.0	4.0	2.0	7.5	5.0	2.0	10.0	4.0	2.0	6.0
TA	5.0	2.0	8.0	5.0	1.5	8.0	5.0	2.0	8.0	4.0	1.0	5.0
VA	8.0	6.0	10.0	7.5	5.0	12.0	8.0	5.0	10.0	11.0	8.0	13.0
F	10.0	8.0	11.0	9.5	8.0	13.0	9.0	7.0	11.0	11.0	10.0	13.0
TMD	11.0	2.0	19.0	9.0	-1.5	16.5	10.0	1.0	23.0	6.0	-1.0	9.0
Blood test	94.0	87.0	97.0	93.0	85.0	98.0	94.0	87.0	100.0	94.0	90.0	97.0
MDA-LDL Cholesterol (U/L)	92.0	79.0	108.0	96.5	80.0	111.0	96.0	78.0	121.0	91.0	73.0	118.0
IL-6 (pg/mL)	1.50	1.20	2.20	1.55	1.20	2.70	2.00	1.40	2.60	1.70	1.20	2.40
TNF- α (pg/mL)	0.58	0.46	0.69	0.54	0.39	0.60	0.57	0.44	0.78	0.61	0.49	0.73

Table 3 The percent changes from baseline to 12 weeks

	Placebo group (n = 31)				Hop group (n = 33)				p value*	
	△ 8 weeks		△ 12 weeks		△ 8 weeks		△ 12 weeks		△ 8 w	△ 12 w
	Median	25%	75%	Median	25%	75%	Median	25%	75%	
Appendicular muscle mass (kg)	0.56	- 1.42	3.35	0.00	- 1.66	3.47	1.20	- 0.63	3.97	0.61
SMI (kg/m ²)	0.56	- 1.42	3.35	0.00	- 1.66	3.47	1.20	- 0.63	3.97	0.60
Grip strength (right, kg)	1.29	- 5.35	6.54	0.00	- 4.35	8.50	2.78	- 3.73	5.91	0.74
Grip strength (left, kg)	2.74	- 6.36	5.91	1.85	- 5.04	7.57	2.51	- 3.88	5.86	0.98
Hip adductors strength (kg)	- 1.41	- 32.40	12.17	9.36	1.75	17.07	- 3.25	- 28.28	12.74	0.64
Hip abductors strength (kg)	- 7.96	- 44.23	7.12	4.32	- 2.88	15.43	- 0.63	- 21.21	7.69	0.29
Isometric knee extension strength (right, kg)	8.17	2.21	13.13	12.74	- 2.78	20.44	- 0.30	- 7.14	13.36	0.09
Isometric knee extension strength (left, kg)	8.16	- 1.55	25.34	12.11	3.01	21.45	2.58	- 6.03	17.65	0.99
One-leg standing time (s)	0.00	- 4.65	12.77	0.00	0.00	48.15	0.00	0.00	46.34	0.82
Body weight (kg)	- 0.39	- 2.34	0.34	- 1.04	- 3.34	0.14	0.17	- 0.81	1.08	0.51
BMI (kg/m ²)	- 0.36	- 2.34	0.37	- 1.09	- 3.11	0.00	0.28	- 0.75	1.13	0.02
Body fat weight (kg)	- 4.86	- 8.15	- 1.40	- 6.30	- 9.79	- 1.23	- 0.86	- 3.40	2.44	0.02
Body fat percentage (%)	- 4.42	- 6.83	- 1.17	- 4.80	- 6.53	- 1.28	- 0.98	- 2.67	1.50	0.03
Whole-body muscle mass (kg)	0.89	- 0.25	1.57	0.81	- 0.25	1.30	0.52	0.00	1.31	0.04
Leg muscle mass (right, kg)	0.00	- 1.41	2.94	0.00	- 2.67	4.23	1.09	- 1.35	3.28	0.51
Leg muscle mass (left, kg)	0.00	- 1.75	1.89	0.00	- 3.01	3.13	1.43	- 1.32	3.57	0.14
Arm muscle mass (right, kg)	3.65	0.00	5.56	0.00	0.00	5.26	0.00	0.00	5.00	0.02
Arm muscle mass (left, kg)	3.70	0.00	5.88	0.00	0.00	5.88	0.00	0.00	5.26	0.41
POMS2	0.91	- 0.28	1.48	0.76	- 0.23	1.36	0.49	0.00	1.42	0.14
AH	0.00	- 15.13	41.67	0.00	- 33.33	20.00	0.00	- 10.00	66.67	0.50
CB	0.00	- 33.33	20.00	0.00	- 33.33	40.00	0.00	- 28.57	50.00	0.07
DD	0.00	- 95.00	0.00	0.00	- 50.00	0.00	0.00	- 50.00	0.00	0.79
FI	0.00	- 16.67	16.67	0.00	- 14.29	33.33	0.00	0.00	50.00	0.94
TA	0.00	- 27.92	25.00	0.00	- 25.00	50.00	0.00	- 20.00	25.00	0.52
VA	0.00	- 11.81	41.43	0.00	- 25.00	42.86	0.00	- 18.18	25.00	0.77
F	0.00	- 11.11	16.23	- 9.09	- 20.00	12.50	8.33	- 10.53	18.18	0.64
TMD	- 14.14	- 58.33	24.04	- 11.59	- 52.63	50.00	- 17.39	- 66.67	21.43	0.26
Blood test	1.12	- 4.21	3.61	2.41	- 4.08	5.77	0.00	- 3.30	3.19	1.00
MDA-LDL Cholesterol (U/L)	4.27	- 11.69	22.78	10.00	- 18.07	34.85	3.30	- 8.64	20.18	0.66
IL-6 (pg/mL)	- 4.68	- 22.22	33.33	16.67	0.00	41.18	- 5.41	- 22.58	36.84	0.41
TNF-α (pg/mL)	- 18.58	- 37.04	- 7.69	- 5.56	- 24.14	21.62	- 17.24	- 27.40	- 8.70	0.84
										0.04
										0.53

*Mann-Whitney U-test

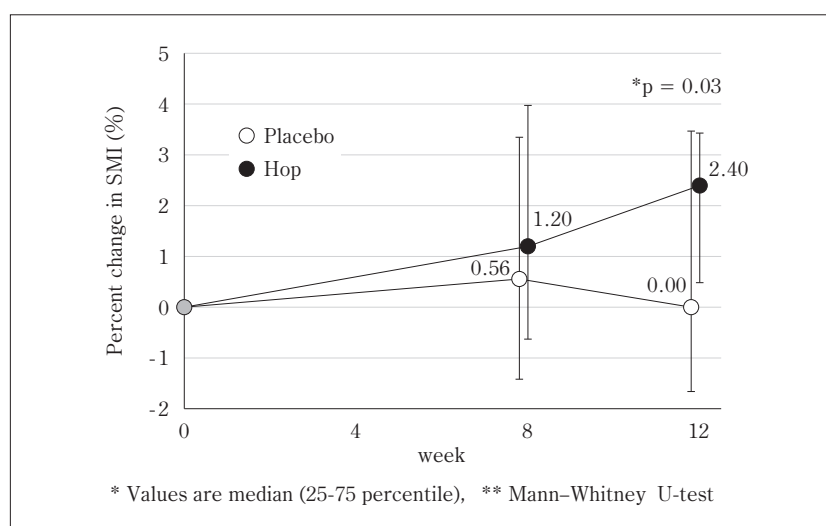


Fig. 2 Percent change in SMI from baseline to 12 weeks.

Table 4 The incidents of adverse effects during the trial in both groups

	Placebo group		Hop group		p value*	
	n	rate (%)	n	rate (%)	Chi	Fisher
Cold (including COVID-19)	8	20.5	7	17.9	0.77	0.49
Influenza	0	0.0	2	5.1		
Other infectious diseases	2	5.1	0	0.0	0.77	0.49
Allergies	3	7.7	1	2.6		
Systemic symptoms	8	20.5	7	17.9	0.76	1.00
Digestive symptoms	7	17.9	6	15.4		
Respiratory symptoms	1	2.6	2	5.1	1.00	1.00
Bodily pain	14	35.9	14	35.9		
Nervous system disorders	1	2.6	1	2.6	0.78	1.00
Injured	2	5.1	2	5.1		
Skin symptoms	2	5.1	2	5.1	0.78	1.00
Dental symptoms	2	5.1	1	2.6		
Sleep/mental disorders	1	2.6	0	0.0	0.78	1.00
Unexplained symptoms	9	23.1	8	20.5		
Gynecologic symptoms	0	0.0	0	0.0	0.78	1.00
Abnormal blood test results						
AST $\geq$ 80 IU/L	0	0.0	0	0.0	1.00	1.00
ALT $\geq$ 80 IU/L	0	0.0	0	0.0		
γ -GT $\geq$ 100 IU/L	1	2.6	2	5.1	1.00	1.00
BUN $\geq$ 40 mg/dL	0	0.0	0	0.0		
Creatinine $\geq$ 1.6 mg/dL	0	0.0	0	0.0	1.00	1.00
HbA1c $\geq$ 8.0%	0	0.0	1	2.6		
Hemoglobin $<$ 8.0 g/dL	0	0.0	0	0.0	0.62	0.62
Elevated LDL Cholesterol	3	7.7	1	2.6		
Abnormal urine test results					1.00	1.00
Positive Urinary ketone body	1	2.6	0	0.0		
Urinary occult blood	1	2.6	2	5.1	1.00	1.00
Positive Urinary glucose	0	0.0	1	2.6		

*Chi-squared test, Fisher's exact test.

4. DISCUSSION

This study was conducted using a randomized, double-blind design to evaluate the effects and long-term safety of fermented hop extracts, containing 8-PN as its principal active component, combined with physical exercise on body composition and physical performance in middle-aged and older adults with a tendency toward obesity. As a result, the hop group showed significantly higher percent increases in appendicular muscle mass and SMI from baseline to 12 weeks than the placebo group. As shown in **Table 2** & **Table 3**, the significant decreases were observed in the body weight, BMI, body fat percentage in the placebo group compared with the hop group. These decreases were likely attributable to the substantial effects of the physical exercise intervention implemented in both groups, which may have overcome the detectable effects of 8-PN. For instance, the median body fat percentage decreased in both groups: from 34.6% at baseline to 33.8% at 12 weeks in the placebo group, and from 35.4% to 34.3% in the hop group. In this trial, it is considered that such significant reductions in the placebo group may have occurred by chance. Regarding the isometric knee extension strength, both groups showed significant increase, reflecting the effects of daily physical training. For instance, in the right isometric knee extension strength increased from 33.5 kg at baseline to 35.4 kg at 12 weeks in the placebo group, and 35.2 kg to 39.1 kg in the hop group. Similarly, the left isometric knee extension strength increased from 33.2 kg to 36.2 kg in the placebo group, 35.8 kg to 40.5 kg in the hop group (**Table 2**). In contrast, grip strength showed no significant changes in either group, with median percent changes of 0% in both groups (**Table 2** & **Table 3**). This suggests that increases in upper-body muscle mass and strength are more difficult to achieve than those in the lower body. Because the lower limbs routinely support body weight, they can be more easily overloaded through exercises such as squats. Conversely, applying sufficient load to the upper arms is difficult when relying solely on bodyweight exercises performed at home. Although participants were instructed to perform resistance tube training three times per week, the median percent changes in muscle mass of both arms remained 0% in each group after 12 weeks. The absence of change in grip strength further indicates that a greater training load would have been required to induce measurable increases in upper-arm muscle mass and strength. Therefore, the observed increases in appendicular muscle mass largely reflect increases in lower-limb muscle mass, suggesting that 8-PN supplementation enhanced increasing in lower body muscle mass in the present study.

Regarding the POMS2 scores, no significant effects were observed. Previous studies have reported that intake of

hop extracts alleviates menopausal symptoms in women⁶⁾²⁰⁾. However, the participants in the present study were not limited to middle-aged women experiencing menopausal symptoms, and therefore the study population was not optimized to detect the effects related to menopausal health. In addition, the baseline TMD scores were unbalanced between the two groups, with scores of 13.7 in the placebo group and 7.4 in the hop group, respectively (**Table 1**). These factors suggest that the effects of 8-PN may have been underestimated in this trial. Furthermore, numerous studies have consistently reported beneficial effects of physical exercise on psychological conditions²¹⁾. Thus, to appropriately evaluate the effects of fermented hop extracts on psychiatric health, a study design such as the following would be more suitable: enrolling women aged 40-50 years around menopause and avoiding interventions involving resistance training, or limiting physical activity to light aerobic exercise such as walking.

In the blood test, IL-6 secretion was significantly reduced in the hop group compared with the placebo group. This result suggests that the anti-inflammatory effect of 8-PN attenuated the increase in serum IL-6 induced by high-intensity exercise. To our knowledge, this is the first study to demonstrate an anti-inflammatory effect in humans following high-intensity exercise. These findings indicate that 8-PN intake may help regulate excessive inflammatory responses, including cytokine secretion, triggered by physical exercise.

The main finding of this study is that 8-PN intake significantly enhances the increase in appendicular muscle mass when combined with physical training. Since several studies have reported a significant association between appendicular muscle mass and fall risk²²⁾²³⁾, the findings of this study suggest that the consumption of 8-PN may contribute to the prevention of falls and reduced care needs in middle-aged and older adults. In the Asian Working Group for Sarcopenia 2025 (AWGS 2025) criteria, the SMI, which is calculated from appendicular muscle mass, is used instead of whole-body muscle mass for the diagnosis of sarcopenia²⁴⁾. The reason is that the decrease in appendicular muscle mass has a strong association with physical performance; furthermore, independence in daily living depends primarily on appendicular muscles rather than trunk muscles²⁵⁾²⁶⁾. Additionally, the present study is significant in that it evaluates not only the effects of the supplement alone but also its combined effects with physical training. In recent years, numerous supplements and health foods have appeared on the market; however, there is a concern that people tend to underestimate the importance of exercise²⁷⁾²⁸⁾. Numerous studies have reported beneficial effects of physical exercise not only on increasing physical strength but also on cognitive performance, sleep quality, and mental health²¹⁾. Thus, the design of the present study, which combined

supplement intake with physical exercise, may be appropriate for health promotion in a super aging society¹⁷⁾²⁹⁾. Furthermore, the finding regarding the effects of IL-6 secretion suppression is the strength of this study. The effect of hop-extracts was elucidated for the first time among human, though have reported in animal study³⁰⁾. Notably, this study also demonstrated the safety of 8-PN supplementation, routine biochemical parameters. In addition, the incidence of adverse events did not differ from those observed in the placebo group. Most previous studies on hop extracts have focused on female participants⁶⁾⁷⁾²⁰⁾; however, the present study provides evidence supporting the safety of 8-PN in both men and women.

The mechanism underlying the enhancement in muscle mass increase by 8-PN supplementation may result from its estrogen-like activity⁶⁾, along with 8-PN's ability to prevent muscle atrophy, promote recovery, and accumulate in tissues with prolonged retention^{10)~12)31)32)}. In addition, the regulation of IL-6 secretion observed in this study suggests that 8-PN also exerts anti-inflammatory effects, which may further contribute to creating a physiological environment that favors muscle hypertrophy.

The present study has several limitations. First, the number of participants did not reach the expected sample size of 90. Although the minimum required number of 76 was initially exceeded, participant dropout occurred, resulting in 31 individuals in the placebo group and 33 in the hop group completed this trial. Second, the effects of 8-PN were observed only in lower body muscle mass, with no significant changes in upper body muscle mass or upper body muscle strength. An increase in lower body muscle mass is considered important for fall prevention, whereas maintaining or improving grip strength has been recognized as a key factor in reducing the risk of dementia³⁶⁾³⁷⁾. To detect of the effects of 8-PN on increasing upper-body muscle mass and strength, it may be necessary to increase either the frequency or the load of the resistance-tube training.

5. CONCLUSIONS

The combination of 8-PN supplementation and physical exercise could enhance the increase in appendicular muscle mass. In addition, 8-PN intake could attenuate the elevation of IL-6 induced by high-intensity training. Furthermore, the long-term safety of 8-PN intake was confirmed based on subjective symptoms and routine biochemical parameters. Further research is required to clarify the potential effects of 8-PN supplementation on upper body muscle mass and grip strength.

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original draft preparation, T.Ki.; writing—review and editing, A.K., N.Y. and K.M.; supervision, T.Ki.; project administration, A.K. All authors have read and agreed to the published version of the manuscript.”

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ABBREVIATIONS

The following abbreviations are used in this manuscript:

8-PN	8-prenylnaringenin
AH	Anger–Hostility
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CB	Confusion–Bewilderment
DD	Depression–Dejection
F	Friendliness
FI	Fatigue–Inertia
HbA1c	Hemoglobin A1c
HDL	High-Density Lipoprotein Cholesterol
IL-6	Interleukin-6
IX	Isoxanthohumol
LDL	Low-Density Lipoprotein Cholesterol
POMS2	Profile of Mood States 2
SMI	Skeletal Muscle Index
TA	Tension–Anxiety
TMD	Total Mood Disturbance
TNF-α	Tumor Necrosis Factor- α
VA	Vigor–Activity
XH	Xanthohumol
γ-GT	Gamma-glutamyltransferase

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