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Efficacy of Black Currant, Red Perilla, and Myo–inositol in the Prevention of Late–onset Hypogonadism in Healthy Men Non–randomized Clinical Trial

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ABSTRACT

Background Late-onset hypogonadism (LOH) occurs in men from middle to older age. LOH presents with a wide range of symptoms, which are caused by a decrease in blood testosterone. We previously reported that components of black currant (*Ribes nigrum*), red perilla *(Perilla frutescens*), and sweet tea (*Rubus suavissimus*) promote testosterone production in testicular Leydig cells and that oral intake of the mixture of these food components increases serum testosterone levels in male mice. In this study, a clinical trial was conducted on healthy adult men to verify the effects of the mixture (Sterone Power Mix® or SPM) in humans.

Methods Healthy adult male volunteers (n=25) assigned 200 mg/day or 600 mg/day of SPM for eight weeks. Salivary testosterone levels were measured at 0, 4, and 8 weeks. Aging Males' Symptoms (AMS) score and Erection Hardness Score (EHS) were assessed at 0, 4, and 8 weeks and follow-up 4week after stop taking supplementation.

Results Daily intake of SPM tended to increase salivary testosterone levels and significantly improved the AMS score. No adverse events were observed in this study.

Conclusions SPM intake alleviated LOH symptoms and may serve as an effective dietary supplement for men suffering from LOH.

(UMIN-CTR: UMIN000054558)

KEY WORDS Testosterone; Late-onset hypogonadism; Aging Males' Symptoms score

INTRODUCTION

Testosterone is a steroid hormone that is mainly synthesized in the Leydig cells of the testes and secreted into the bloodstream in men. Testosterone plays a crucial role in the development of male reproductive organs, skeletal and muscular growth, as well as brain function and psychological well-being. A decline in testosterone levels causes a range of systemic disorders.

Late-onset hypogonadism (LOH) is a condition caused by age-related reductions in testosterone levels. Symptoms of LOH include decreased libido, depression, memory impairment, poor concentration and motivation, fatigue, hot flashes, sleep disturbances, and loss of muscle mass. Additionally, low testosterone levels are associated with increased risks of metabolic syndrome, cardiovascular disease, diabetes, respiratory disease, and more severe outcomes in COVID-19 infection, all of which impact life expectancy [1)].

LOH is prevalent among middle-aged and older men. It affects 12% of men in their fifties, 20% in their sixties, 30% in their seventies, and 50% in their eighties [2)]. Notably, LOH has also been reported in younger men, likely due to lifestyle and other factors [3)].

Historically, LOH has been considered a "disorder due to aging" and is sometimes misdiagnosed as "depression" because of its overlapping neuropsychological symptoms. However, recent research has revealed that LOH is a complex, multi-organ dysfunction that significantly reduces quality of life and has highlighted the renewed recognition of the role of testosterone during aging [4, 5)].

Testosterone replacement therapy (TRT) is the standard treatment for LOH. However, exogenous testosterone administration may cause testicular dysfunction [6)], and oral methyltestosterone treatment has been associated with hepatotoxicity in some cases [7)].

Given these concerns, dietary components that regulate endogenous production and secretion of testosterone have gained attention as alternative approaches [8-11)]. Previously, we identified black currant *(Ribes nigrum)*, red perilla *(Perilla frutescens*), and sweet tea (*Rubus suavissimus*) as food

sources that promote testosterone secretion in testicular Leydig cells. Blackcurrants are known as "cassis" in Japan, and their fruits are consumed as processed products such as jelly, jam, dried fruit, and juice. Red perilla is known as "Aka-shiso" in Japan and is consumed as processed foods such as shiso juice, as well as to produce umeboshi (pickled plums).

We also identified the bioactive components and underlying mechanisms. In that study, we demonstrated that a mixture containing black currant extract, red perilla extract, and myo-inositol (MI, a bioactive component of sweet tea) significantly elevated blood testosterone levels in male mice [12].

In the current study, we conducted a clinical trial to investigate whether supplementation with this mixture of black currant extract, red perilla extract, and MI, named "Sterone Power Mix® (SPM)," can increase testosterone levels in adult men and alleviate symptoms associated with LOH.

MATERIALS AND METHODS

1 Composition of the tested food supplement

Sterone Power Mix® (SPM, Japan Pharma Co., Ltd., Tokyo, Japan), a mixture of black currant extract, red perilla extract, and MI, was used as the food supplement (Table 1). Excipients were added to 200 mg of SPM, which was then encapsulated in hydroxypropyl methylcellulose capsules.

Table 1. Composition of SPM.

Ingredient	% w/w	Bioactive component
Black currant extract	27.8	Anthocyanins 30%
Red perilla extract	5.8	Rosmarinic acid 15%
Myo-inositol	66.3	97%

2 Subjects

Subjects were recruited from May 29 to June 3, 2024. After the study contents were fully explained, 25 healthy adult male volunteers who met the inclusion criteria, did not violate the exclusion criteria, and were able to provide written informed consent were selected.

The inclusion criteria were as follows:

1) Men aged 20 to 80 years at the time of obtaining consent to participate in the study.

2) Healthy individuals not currently receiving medical treatment or medication.

3) Individuals who could collect samples on the specified date and submit them as instructed.

4) Individuals who provided written consent after fully understanding the purpose and procedure of the study.

The exclusion criteria were as follows:

1) Individuals currently receiving medical treatment for any illness.

2) Individuals regularly using health foods containing the test ingredients (black currant extract, red perilla extract, or MI).

3) Individuals regularly using health foods or medication related to male menopause or sexual function (e.g., maca: *Lepidium meyenii*; krachai Dam: *Kaempferia parviflora*; fenugreek: *Trigonella foenum-graecum*; testosterone-containing drugs; erectile dysfunction [ED] drugs; androgenetic alopecia [AGA] drugs).

4) Individuals with known allergies to the food supplement.

5) Individuals with a history of or current condition related to heart and/or renal failure or hepatitis.

6) Individuals who consume alcohol excessively.

7) Participants in other clinical trials.

8) Individuals planning significant lifestyle changes during the study period.

Twenty-five subjects were assigned subject numbers by a person not directly involved in the study. To prevent allocation bias, the person in charge of group assignment, also independent of the experiment and data analysis, used age and BMI to assign subjects to either the 200 mg/day group (1 capsule/day) or the 600 mg/day group (3 capsules/day). Allocation details were not disclosed to the

analysts until the data collection was complete. Black currant, red perilla, and MI are traditionally consumed foods and have been evaluated in previous clinical trials [13-15]. Based on these studies, the intake levels used in this study were considered safe.

3 Clinical study protocol

The clinical trial was conducted in accordance with the Declaration of Helsinki (revised at the Fortaleza General Assembly in 2013) and the "Ethical Guidelines for Medical Research Involving Human Subjects" (promulgated by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare) and was reviewed and approved by the Morishita Jintan Ethics Review Committee (approval number: A0018; approval date: 05/15/2024) to ensure the human rights and safety of the subjects and the reliability of the test data. The test protocol was registered in advance with the University Hospital Medical Information Network (UMIN-CTR) (UMIN Study ID: UMIN000054558). The purpose and methods of the study were fully explained to the subjects, and written informed consent was obtained from the subjects before the study was conducted.

The trial was a before-and-after comparative study evaluating two dosage groups (200 mg/day and 600 mg/day) over an 8-week period. Salivary testosterone was measured at three points: before intake (0W), 4 weeks after the start of the dosage regimen (4W), and 8 weeks after the start of the dosage regimen (8W). The Aging Males' Symptoms (AMS) score and Erection Hardness Score (EHS), which were based on self-assessment questionnaires, were measured at 0W, 4W, 8W, and after a 4-week follow-up period (follow-up). All test samples and questionnaires were labeled by subject number. Saliva samples were collected at a facility equipped with a freezer and immediately frozen. The self-administered questionnaires (the AMS and EHS) were completed by subjects at their homes and mailed to the analyst.

4 Salivary testosterone

Saliva was collected at 0W, 4W, and 8W to assess the effect of SPM. Saliva samples were collected using the Saliva Bio Oral Swab Device and Swab Storage Tube (Salimetrics, LLC, Carlsbad, CA) in accordance with the manufacturer's protocol. Briefly, participants refrained from consuming alcohol, tobacco, and caffeine after 9:00 p.m. on the day before saliva collection. On the day of collection, the participants rinsed their mouths three times with water, then waited 10 min before collection. Samples were collected between 9:00 and 11:00 a.m., avoiding the hour after eating and 45 min after toothbrushing. The swab was held under the tongue for 2 min, placed in a swab storage tube, and immediately frozen. Saliva samples were thawed and centrifuged at 1500×g for 15 min at 4°C, and the supernatant was analyzed to quantify salivary testosterone using a Salivary Testosterone Enzyme ImmunoAssay (EIA) Kit (Salimetrics, LLC).

5 Aging Males' Symptoms (AMS) score

The AMS score is a 17-item self-administered questionnaire used internationally for screening and diagnosing LOH [16]. Each question was rated by the participants on a 5-point scale: "none" (1 point), "mild" (2 points), "moderate" (3 points), "severe" (4 points), and "extremely severe" (5 points). Each subject performed the test at 0W, 4W, 8W, and follow-up. The overall AMS score along with subdomains were analyzed: somatic subscore (questions 1–5, 9, 10), psychological subscore (questions 6–8, 11, 13), and sexual subscore (questions 12, 14–17).

6 Erection hardness score (EHS)

The EHS is a self-reported questionnaire to evaluate ED. Participants rated their own ED on a 5-point scale at 0W, 4W, 8W, and follow-up.

7 Statistical analysis

Results are presented as mean ± standard error of mean (SEM). Statistical analyses were performed using GraphPad Prism software (ver. 10, GraphPad Software, San Diego, CA, USA). The specific

statistical analyses methods are Two-way ANOVA, Dunnett's multiple comparison test. Differences were considered statistically significant when the p-value was less than 0.05.

RESULTS

1 Group allocation

Since SPM is a dietary supplement, healthy male subjects were recruited for this study to evaluate its preventive effect on LOH (Figure 1). Subjects were divided into two groups to ensure no significant differences in age and BMI, 13 participants were assigned to the 200 mg/day group and 12 participants to the 600 mg/day group. There were no significant differences in age and BMI between the two groups (Table 2). Each group consumed the food supplement for 8 weeks. No adverse events occurred during the trial. A daily intake of one capsule (200 mg/day group) is expected to provide 16.7 mg of black currant anthocyanins (BCAs), 1.7 mg of rosmarinic acid (RA), and 128.6 mg of MI. The doses of 200 mg/day and 600 mg/day were selected based on a previous animal study [12] and a preliminary examination conducted prior to the current study.

Table 2. Group allocation and baseline characteristics of study subjects.

	200 mg/day group	600 mg/day group	p-value
Age*	44.9±3.2	44.3±0.9	0.902
Body mass index*	24.6±0.9	24.0±0.7	0.650
Number of subjects	13	12	-

*No significant differences between the two groups (Student's t-test).

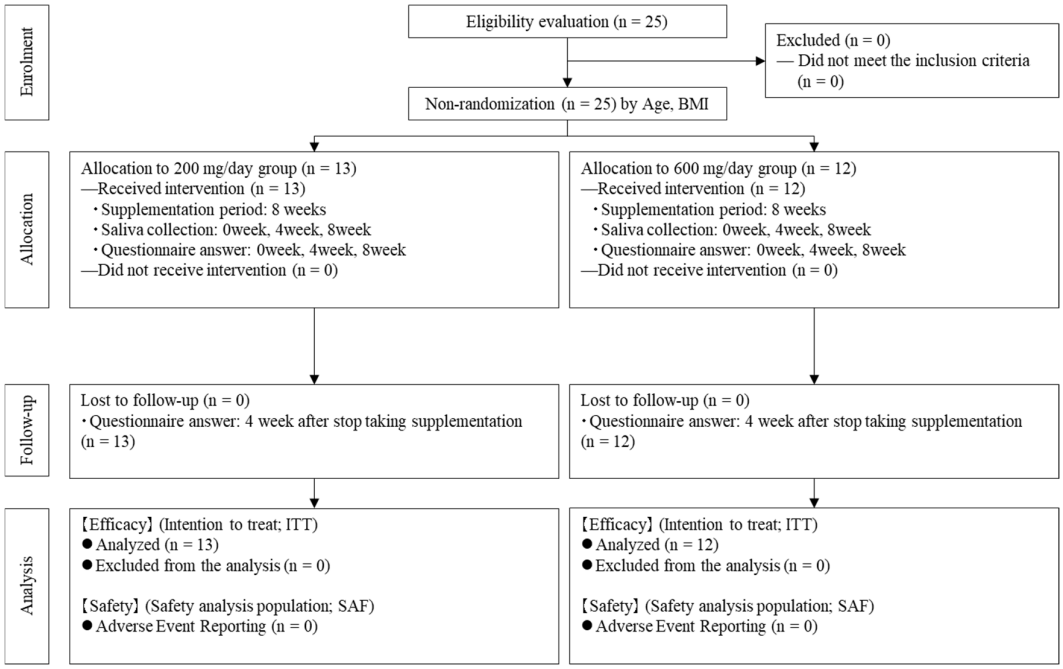

Fig. 1. Participant flowchart.

2 Salivary testosterone

Salivary testosterone can be collected without surgical intervention and is used in various clinical studies because it correlates with free testosterone in the blood [17, 18]. The baseline salivary testosterone levels at 0W were 204±16 pg/mL in the 200 mg/day group and 202±20 pg/mL in the 600 mg/day group, with no significant difference between the two groups. Compared with the testosterone level at 0W, in the 200 mg/day group, testosterone levels changed by +22±14 pg/mL at 4W (95%CI [−16, 59], p=0.31) and −4±12 pg/mL at 8W (95%CI [−35, 27], p=0.94). In the 600 mg/day group, changes were +34±16 pg/mL at 4W (95%CI [−7, 76], p=0.11) and −12±22 pg/mL at 8W (95%CI [−70, 47], p=0.86) compared with that at 0W (Figure 2a). No significant difference in salivary testosterone levels was observed between the 200 mg/day group and the 600 mg/day group at any measurement points.

3 AMS score and EHS grade

A decrease in AMS scores indicates improvement in LOH symptoms. At 0W, the 200 mg/day group had a slightly higher score (32.5±3.3) than the 600 mg/day group (26.7±2.8), but the difference was not statistically significant (mean diff. 5.9, 95%CI [−3.0, 14.7], p=0.18). The 200 mg/day group showed a decrease in AMS score throughout the trial period, but a significant decrease was observed only at follow-up (mean diff. −7.8, 95%CI [−14.7, −0.9], p=0.027), while the 600 mg/day group showed significant decreases at 8W (mean diff. −5.0, 95%CI [−9.4, −0.6], p=0.026) and follow-up (mean diff. −4.8, 95%CI [−9.0, 0.7], p=0.023) (Figure 2b).

Analysis of the AMS score was also performed for the three subscores: somatic subscore, psychological subscore, and sexual subscore (Table 3). In the 200 mg/day group, the somatic subscore significantly improved at 4W (mean diff. −2.9, 95%CI [−5.7, −0.2], p=0.039), 8W (mean diff. −4.0, 95%CI [−7.3, −0.7], p=0.018), and follow-up (mean diff. −4.0, 95%CI [−7.5, −0.5], p=0.024) compared with that at 0W. The psychological or sexual subscores showed non-significant trends of improvement from 4W to follow-up. In the 600 mg/day group, in comparison to 0W, the somatic subscores significantly improved at 8W (mean diff. −2.3, 95%CI [−4.2, −0.3], p=0.027) and follow-up (mean diff. −2.2, 95%CI [−3.5, −0.8], p=0.0027). The psychological and sexual subscores showed non-significant improvement trends.

Table 3. AMS subscores across study timepoints.

Subscore	Group	Test period				Comparison (0W − 8W)		
		0W	4W	8W	Follow-up	Mean diff.	95%CI	p-value
Somatic	200 mg/day	14.6±1.3	11.7±1.4*	10.6±1.2*	10.6±1.3*	−4.0	(−7.3, −0.7)	0.018
	600 mg/day	11.4±1.0	10.4±0.9	9.2±0.6*	9.3±0.9**	−2.3	(−4.2, −0.3)	0.027
Psychological	200 mg/day	7.6±1.1	6.7±1.0	6.8±0.9	6.7±0.8	−0.85	(−3.2, 1.5)	0.670
	600 mg/day	6.7±0.9	6.0±0.6	5.9±0.4	5.7±0.6	−0.75	(−2.2, 0.7)	0.403
Sexual	200 mg/day	10.3±1.2	8.2±1.4	8.3±1.3	7.5±1.3	−2.0	(−5.2, 1.2)	0.267
	600 mg/day	8.6±1.2	8.2±1.1	6.6±0.6	6.9±0.7	−2.0	(−4.4, 0.4)	0.106

*p<0.05, **p<0.01 vs. 0W for each group (two-way ANOVA, Dunnett's multiple comparisons test).

Individual AMS questions with significant differences are presented in Table 4. The 200 mg/day group showed significant improvements in sleep quality [Q4 on sleep problems: 4W (mean diff. −1.1, 95%CI [−1.8, −0.3], p=0.0074), 8W (mean diff. −1.0, 95%CI [−1.8, −0.2], p=0.015), follow-up (mean diff. −1.2, 95%CI [−2.0, −0.4], p=0.0057)], fatigue [Q5 on increased need for sleep, often feeling tired: 4W (mean diff. −0.7, 95%CI [−1.3, −0.1], p=0.016), 8W (mean diff. −1.0, 95%CI [−1.7, −0.3], p=0.0095), follow-up (mean diff. −0.8, 95%CI [−1.6, −0.1], p=0.024)], and muscle strength [Q10 on decrease in muscular strength: 4W (mean diff. −0.5, 95%CI [−0.8, −0.1], p=0.020), 8W (mean diff. −0.8, 95%CI [−1.5, −0.2], p=0.014), follow-up (mean diff. −0.8, 95%CI [−1.5, −0.2], p=0.014)]. The 600 mg/day group showed significant improvements in overall well-being [Q1 on decline in feeling of general well-being: 8W (mean diff. −0.4, 95%CI [−0.8, −0.01], p=0.043), follow-up (mean diff. −0.4, 95%CI [−0.8, −0.01], p=0.043)] and libido [question17 on decrease in sexual desire/libido: 8W (mean diff. −0.6, 95%CI [−1.1, −0.1], p=0.030)].

EHS scores did not change significantly during the trial period, likely because participants did not exhibit ED at baseline (Table 5).

Table 4. AMS individual questions with statistically significant differences.

Questionnaire	Group	Test period				Comparison (0W − 8W)		
		0W	4W	8W	WO	Mean diff.	95%CI	p-value
Q1	600 mg/day	2.4±0.3	1.3±0.2**	1.4±0.1*	1.2±0.1**	−0.4	(−0.8, −0.01)	0.043
Q4	200 mg/day	2.6±0.3	1.9±0.3*	1.6±0.2**	1.8±0.2*	−1.0	(−1.8, −0.2)	0.015
Q5	200 mg/day	2.5±0.2	2.0±0.3*	1.6±0.2*	1.6±0.3*	−1.0	(−1.7, −0.3)	0.010
Q10	200 mg/day	1.6±0.2	1.4±0.2	1.2±0.1*	1.2±0.1*	−0.8	(−1.5, −0.2)	0.014
Q17	600 mg/day	1.8±0.2	1.7±0.3	1.3±0.1*	1.3±0.1	−0.6	(−1.1, −0.1)	0.030

*p<0.05, **p<0.01 vs. 0W for each group (two-way ANOVA, Dunnett's multiple comparisons test).

Table 5. EHS grade* during the trial period.

	Test period				Comparison (0W − 8W)		
	0W	4W	8W	Follow-up	Mean diff.	95%CI	*p*-value
200 mg/day group	3.4±0.2	3.5±0.1	3.4±0.1	3.5±0.1	0.0	(−0.4, 0.4)	>0.99
600 mg/day group	3.3±0.2	3.4±0.2	3.4±0.2	3.3±0.2	0.2	(−0.1, 0.5)	0.357

*Grade 0: Penis does not enlarge; 1 Penis is larger, but not hard; 2 Penis is hard, but not hard enough for penetration; 3 Penis is hard enough for penetration, but not completely hard; 4 Penis is completely hard and fully rigid

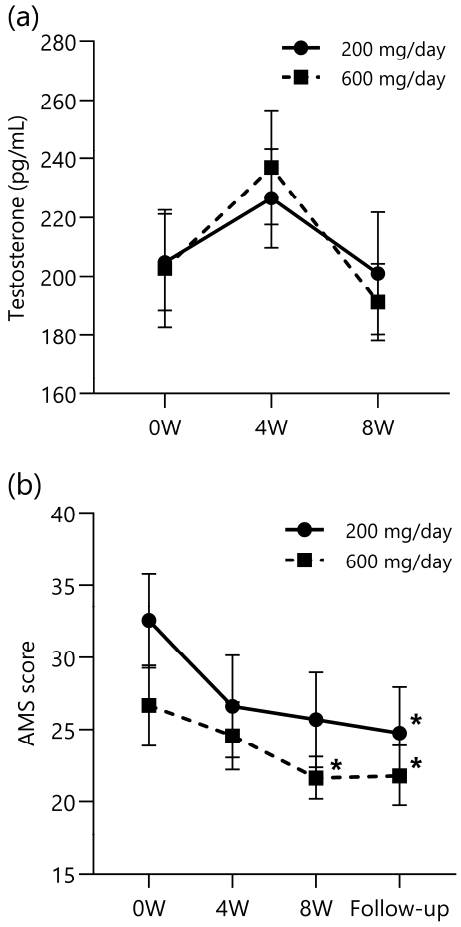

Fig. 2. Salivary testosterone concentration and AMS score. (a) Salivary testosterone concentration before the intake (0W), 4 weeks after intake (4W), and 8 weeks after intake (8W) of the tested food supplement. (b) AMS score at 0W, 4W, 8W and follow-up 4 weeks after stop taking supplementation (Follow-up). *p<0.05 vs. 0W for each group (two-way ANOVA, Dunnett's multiple comparisons test).

DISCUSSION

The current initial human clinical trial was conducted to verify whether the oral intake of SPM, a mixture of black currant extract, red perilla extract, and MI, could support testosterone production and improve LOH symptoms in adult men.

To avoid the need for blood sampling, salivary testosterone, which has been shown to correlate with serum free testosterone [18], was measured in this study. Because testosterone levels show diurnal variation, high from dawn until mid-morning and gradually decreasing from the afternoon onward [19], saliva was collected between 9:00 and 11:00 am to minimize variability.

After 4 weeks of oral SPM intake, salivary testosterone levels increased in both groups (the 200 mg/day and 600 mg/day groups), with a non-significant trend toward a dose-dependent effect (Figure 2a). The increase in testosterone is consistent with the results of a mouse experiment [12] and indicates that the intake of the mixture of black currant extract, red perilla extract, and MI tends to increase testosterone levels in human subjects as well. Although statistical significance was not reached, the combined data from both groups (n=25) showed a significant increase in testosterone levels at 4W compared with 0W (mean diff. 27.8, 95%CI [2.0, 53.5], p=0.033), suggesting that a larger sample size may better capture the effect of SPM on testosterone levels.

Although elevated at 4W of SPM administration, by 8W testosterone levels declined to near-baseline level (as at 0W) in both groups (Figure 2a). This pattern may be explained by the transient increase in testosterone at 4W resulting in negative feedback that lowers testosterone levels. In support of this hypothesis, intramuscular injections of testosterone enanthate, which transiently increases testosterone levels, have been reported to gradually decrease luteinizing hormone (LH) levels from day 5 onward after injection [20]. LH is an endogenous hormone that stimulates testosterone biosynthesis in the testes. Therefore, this LH-mediated negative feedback may explain the decrease in testosterone levels at 8W in this study. Although LH was not measured in this study, future trials should

include this to assess potential feedback inhibition.

While it is possible that diurnal variations in testosterone levels could have influenced the results, we collected saliva samples between 9:00 and 11:00 am, a time window during which testosterone levels are known to fluctuate minimally (approximately 5%) [19]. In contrast, the changes in testosterone levels observed in this study were notably larger: a 12% increase from 0W to 4W, followed by an 11% decrease from 4W to 8W. Therefore, the observed changes are unlikely to be solely due to natural fluctuations and are considered to reflect the effect of SPM supplementation.

The AMS score, an indicator of LOH symptoms, improved over time after the SPM intake in both the 200 mg/day and 600 mg/day groups (Figure 2b). We initially expected that the score would return to the baseline value after discontinuation of SPM supplementation. However, the actual score was improved even after the 4-week washout period. This finding suggests that the effects of SPM persist for at least a month after intake ceases. However, because testosterone levels were not measured at the end of the follow-up period, it remains unclear whether this sustained improvement is accompanied by hormonal changes. Future trials should include post-SPM intervention testosterone measurements to clarify the duration of the effects of SPM.

Among the three AMS subscores, the consumption of SPM significantly improved somatic factors (Table 3). While this observation may reflect that SPM primarily affects physiological function, it can also be explained by the simple fact that the initial score for somatic factors was relatively high compared with the other two factors. The fact that the psychological and sexual subscores showed a downward trend (Table 3) and a significant reduction was observed specifically in sexual desire/libido in the 600 mg/day group (Table 4) may support the latter assumption.

The food supplement (SPM) used in this study contains black currant and red perilla extract, both of which have previously been shown to enhance testosterone biosynthesis in mouse testicular Leydig cell model I-10 cells. Their key bioactive components are BCAs and RA, both of which promote

testosterone production by increasing the expression of steroidogenic acute regulatory protein, one of the rate-limiting proteins in the steroid hormone biosynthesis pathway, in the testes [12]. The third component, MI, a bioactive component found in sweet tea, also supports testosterone secretion in I-10 cells. MI acts by reducing the expression of the aromatase gene (*Cyp19a1*), which converts testosterone to estradiol [12]. Based on these previous observations, the observed tendency toward increased testosterone levels at 4W in the current clinical trial may be attributed to the combined action of BCAs, RA, and MI.

A human study by Matsumoto et al. reported that oral intake of 33 mg/kg BCAs (10.8% anthocyanin) led to peak plasma concentrations of 73.4 ± 35.0 nM for delphinidin-3-rutinoside and 46.3 ± 22.5 nM for cyanidin-3-rutinoside, the two most abundant anthocyanins in black currant [21]. In contrast, another study by Röhrig et al. reported that a 1.5 g oral dose of black currant extract (28.3 ± 1.4% anthocyanin) produced significantly lower maximum plasma concentrations (8.6 ± 5.8 nM for delphinidin-3-rutinoside and 9.8 ± 3.1 nM for cyanidin-3-rutinoside) [22]. While the discrepancy between these two studies is unclear, both confirm that BCAs are absorbed in intact form and could act directly in the human body to support testosterone production.

For RA, Noguchi-Shinohara et al. reported that the consumption of *Melissa officinalis* extract containing 500 mg of RA was associated with maximum plasma concentrations of 124.03 ± 39.13 nM [23]. This study also revealed some RA is absorbed in its intact form, suggesting it may exert direct physiological effects, including on testosterone production in the human body.

The doses of BCAs and RA consumed in the present study (16.7 mg BCAs, 1.7 mg RA in the 200 mg/day group, and 50.1 mg BCAs, 5.1 mg RA in the 600 mg/day group) are much lower than those used in bioavailability studies [21-23]. Furthermore, the effective concentrations observed in in vitro studies are considerably higher than what would be expected from these oral doses [12]. Given the low estimated bioavailability of BCAs (less than 1%) in the bioavailability studies, it is plausible that

the observed effects may involve indirect contributions of BCAs and RA, such as metabolite effects, rather than the parent compound alone. This issue requires further investigation.

A key limitation of this study lies in the nature of the study population. Healthy male participants were recruited to evaluate the potential effect of SPM on LOH rather than to assess its therapeutic effects on diagnosed patients. Our selection criteria did not include marital or relationship status, baseline testosterone levels, or AMS scores. Marital status has been shown to affect testosterone levels, with partnered men typically exhibiting lower testosterone levels than unpartnered men [24, 25]. Thus, variability in marital status and associated differences in baseline testosterone levels may have influenced the current results. Another limitation is the small sample size, as this study was an initial evaluation of the effectiveness of SPM. Due to the small number of participants, the trial was conducted without a placebo group, resulting in less reliable outcomes. Despite these limitations, we believe the present results justify the need for future double-blind, placebo-controlled trials.

CONCLUSION

This study suggested that the oral intake of SPM may increase testosterone levels and improve the symptoms of LOH, with no adverse events observed in this study, indicating acceptable safety of the tested food supplement. While further validation with larger sample sizes and double-blind, placebo-controlled studies is needed to statistically confirm the efficacy, SPM shows promise as a potential food supplement for preventing LOH.

Abbreviation

LOH: Late-onset hypogonadism, SPM: Sterone Power Mix®, AMS: Aging Males' Symptoms, EHS: Erection Hardness Score, BCAs: black currant anthocyanins, RA: rosmarinic acid

MI: myo-inositol

【Conflict of interest】

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REFERENCES

1) Lanser L, Burkert FR, Thommes L, Egger A, Hoermann G, Kaser S, et al. Testosterone Deficiency Is a Risk Factor for Severe COVID-19. Front Endocrinol (Lausanne). 2021;12:694083.

2) Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR, Baltimore Longitudinal Study of A. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. J Clin Endocrinol Metab. 2001;86:724–31.

3) Cohen J, Nassau DE, Patel P, Ramasamy R. Low Testosterone in Adolescents & Young Adults. Front Endocrinol (Lausanne). 2019;10:916.

4) Laughlin GA, Barrett-Connor E, Bergstrom J. Low serum testosterone and mortality in older men. J Clin Endocrinol Metab. 2008;93:68–75.

5) Novak A, Brod M, Elbers J. Andropause and quality of life: findings from patient focus groups and clinical experts. Maturitas. 2002;43:231–7.

6) Majzoub A, Sabanegh E, Jr. Testosterone replacement in the infertile man. Transl Androl Urol.

2016;5:859–65.

7) Westaby D, Paradinas FJ, Ogle SJ, Randell JB, Murray-Lyon I. Liver Damage from Long-Term Methyltestosterone. The Lancet. 1977;310:261–3.

8) Terada N, Nagase T, Kayooka H, Adachi Y, Kato E. alpha-Tocotrienol in rice bran enhances steroidogenesis in mouse Leydig cell via increased gene expression of steroidogenic acute regulatory protein and induction of its mitochondrial translocation. Biosci Biotechnol Biochem. 2024;88:189–95.

9) Li W, Pandey AK, Yin X, Chen JJ, Stocco DM, Grammas P, et al. Effects of apigenin on steroidogenesis and steroidogenic acute regulatory gene expression in mouse Leydig cells. J Nutr Biochem. 2011;22:212–8.

10) Chen YC, Nagpal ML, Stocco DM, Lin T. Effects of genistein, resveratrol, and quercetin on steroidogenesis and proliferation of MA-10 mouse Leydig tumor cells. J Endocrinol. 2007;192:527–37.

11) Ito A, Shirakawa H, Takumi N, Minegishi Y, Ohashi A, Howlader ZH, et al. Menaquinone-4 enhances testosterone production in rats and testis-derived tumor cells. Lipids Health Dis. 2011;10:158.

12) Kato E, Inagaki Y, Kayooka H, Adachi Y, Nagase T, Terada N, et al. Bioactive Components in Black Currant Fruit, Red Perilla (Shiso) Leaf, and Chinese Sweet Tea That Enhance Testosterone Production in Leydig Cells and Their Combined Effect in Male Mice. ACS Food Sci Technol. 2024;4:1209–15.

13) Ohguro H, Ohguro I, Katai M, Tanaka S. Two-year randomized, placebo-controlled study of black currant anthocyanins on visual field in glaucoma. Ophthalmologica. 2012;228:26–35.

14) Takano H, Osakabe N, Sanbongi C, Yanagisawa R, Inoue K, Yasuda A, et al. Extract of Perilla frutescens enriched for rosmarinic acid, a polyphenolic phytochemical, inhibits seasonal allergic rhinoconjunctivitis in humans. Exp Biol Med (Maywood). 2004;229:247–54.

15) Carlomagno G, Unfer V. Inositol safety: clinical evidences. Eur Rev Med Pharmacol Sci. 2011;15:931–6.

16) Heinemann LAJ, Zimmermann T, Vermeulen A, Thiel C, Hummel W. A new ‘aging males’ symptoms' rating scale. The Aging Male. 2009;2:105–14.

17) Lood Y, Aardal-Eriksson E, Webe C, Ahlner J, Ekman B, Wahlberg J. Relationship between testosterone in serum, saliva and urine during treatment with intramuscular testosterone undecanoate in gender dysphoria and male hypogonadism. Andrology. 2018;6:86–93.

18) Arregger AL, Contreras LN, Tumilasci OR, Aquilano DR, Cardoso EM. Salivary testosterone: a reliable approach to the diagnosis of male hypogonadism. Clin Endocrinol (Oxf). 2007;67:656–62.

19) Brambilla DJ, Matsumoto AM, Araujo AB, McKinlay JB. The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. J Clin Endocrinol Metab. 2009;94:907–13.

20) Nakazawa R, Baba K, Nakano M, Katabami T, Saito N, Takahashi T, et al. Hormone profiles after intramuscular injection of testosterone enanthate in patients with hypogonadism. Endocr J. 2006;53:305–10.

21) Matsumoto H, Inaba H, Kishi M, Tominaga S, Hirayama M, Tsuda T. Orally administered delphinidin 3-rutinoside and cyanidin 3-rutinoside are directly absorbed in rats and humans and appear in the blood as the intact forms. J Agric Food Chem. 2001;49:1546–51.

22) Röhrig T, Kirsch V, Schipp D, Galan J, Richling E. Absorption of Anthocyanin Rutinosides after Consumption of a Blackcurrant (Ribes nigrum L.) Extract. J Agric Food Chem. 2019;67:6792–7.

23) Noguchi-Shinohara M, Ono K, Hamaguchi T, Iwasa K, Nagai T, Kobayashi S, et al. Pharmacokinetics, Safety and Tolerability of Melissa officinalis Extract which Contained Rosmarinic Acid in Healthy Individuals: A Randomized Controlled Trial. PLoS One. 2015;10:e0126422.

24) Holmboe SA, Priskorn L, Jorgensen N, Skakkebaek NE, Linneberg A, Juul A, et al. Influence of marital status on testosterone levels-A ten year follow-up of 1113 men. Psychoneuroendocrinology. 2017;80:155–61.

25) Babak R, Rasmus B, Saad E. Marital Status and Serum Level of Testosterone: In Relation to Serum Level of Cortisol. Archive of Urological Research. 2024;8:018–22.