



# Efficacy and Safety of 5-Aminolevulinic Acid for Patients with Symptoms of Late-Onset Hypogonadism: A Preliminary Study

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**Purpose:** As the concept of late-onset hypogonadism (LOH) has gained increased attention, the treatment of eugonadal patients with LOH symptom has become a clinical problem. Previous studies have shown the possible benefits of 5-aminolevulinic acid (5-ALA) on the somatic, psychological and sexual functions. We therefore conducted this randomized, double-blind, placebo-controlled study to confirm the efficacy and safety of 5-ALA for LOH symptoms.

**Materials and Methods:** Thirty-two eugonadal subjects with LOH symptoms were randomly divided into a 5-ALA group (n=15) and a placebo group (n=17). Treatment was continued for 8 weeks. The change of the Aging Males' Symptoms (AMS) scale score and several biochemical and endocrinological variables during treatment were compared between the groups.

**Results:** After treatment, the change in the total AMS in the 5-ALA group was significantly greater than that in the placebo group (-7.4±4.7 vs. -4.9±4.9, p=0.029). However, the differences between the groups in the change of the somatic, psychological, and sexual sub-scores of the AMS did not reach the statistical significance, although these changes in the 5-ALA group were greater than those in the placebo group. Furthermore, the change in the biochemical and endocrinological variables in the two groups did not differ to a statistically significant extent. During the 8-week treatment period, no patients discontinued 5-ALA due to treatment-emergent adverse events (TEAEs).

**Conclusions:** The intake of 5-ALA for 8 weeks was beneficial for eugonadal patients with symptoms of LOH and no severe TEAEs was experienced. 5-ALA should be considered as an option for those patients.

**Keywords:** Aminolevulinic acid; Efficacy; Hypogonadism; Safety; Testosterone

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## INTRODUCTION

Disability associated with the aging process has recently gained increased attention because the number of people of  $\geq 65$  years of age is increasing rapidly in

developing countries, including Japan. While aging may be an unavoidable process, from the viewpoint of public health and the national issue that the aging of society represents, it is important to extend healthy life expectancy. Due to its medical importance and interest

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raised by social media, testosterone has received attention as a key anti-aging molecule. It is well known that testosterone, which decreases with aging, is important in the physiology of various organs and tissues, such as the skin, muscle, liver, bone and bone marrow, brain, and sexual organs. The concept of late-onset hypogonadism (LOH), defined as “a clinical and biochemical syndrome associated with advancing age and characterized by typical symptoms and a deficiency in serum testosterone levels,” was suggested by the International Society of Andrology, International Society for the Study of the Aging Male, and European Association of Urology in 2005 [1]. The reported symptoms of LOH are easily recognized and involve the sexual function (*e.g.*, diminished sexual desire and erectile quality), psychological function (*e.g.*, depression, anxiety, anger, and changes in mood), and physiological function (*e.g.*, fatigue, decreased lean body mass with associated decreases in muscle volume and strength, increased visceral fat, decreased body hair, and decreased bone mineral density resulting in osteoporosis) [2-6]. The first-line treatment for LOH is testosterone replacement because the main cause of this symptomatic disorder is the age-related decrease in serum testosterone. Actually, testosterone replacement treatment has been reported to be beneficial for patients with symptoms of LOH, including symptoms associated with the sexual function, body composition, obesity, metabolic syndrome, diabetes type II, cardiovascular disease, and osteoporosis [7-9]. However, it is also apparent that the symptoms of LOH are not substantially related to any endocrinological parameters [10,11]. We also reported that 83.6% of 967 males with LOH symptoms showed normal endocrinological parameters, as assessed by the serum luteinizing hormone (LH) and testosterone concentrations [12]. Therefore, the current medical problem is how to treat eugonadal patients with LOH symptoms by means other than testosterone replacement.

Recently, a randomized, double-blind, placebo-controlled study showed that 5-aminolevulinic acid (5-ALA), which is a precursor of heme (an essential component of hemoglobin, myoglobin, cytochromes, and drug metabolism enzymes), is beneficial for patients with symptoms of fatigue [13]. It was hypothesized that the intake of 5-ALA could reduce fatigue via the mitochondrial electron transport system, the activation of cytochrome c oxidase, and enhancement of adenosine triphosphate production, because heme proteins are

very important for energy production. Another study, based on the evaluation of questionnaires about symptoms, revealed that the intake of 5-ALA is effective for improving the mood and coping ability of prediabetic middle-aged and older adults, probably because brain serotonin levels are increased by 5-ALA [14]. Interestingly, the influence of 5-ALA on the increased levels of neuroactive substances, such as tryptophan, was also suggested as a possible underlying mechanism of the improved mood [14]. Furthermore, it is expected that this increase in tryptophan by the intake of 5-ALA has the potential to improve sexual dysfunction because the serum tryptophan concentration has been reported to be closely associated with the sexual function [15]. These findings regarding the beneficial effect of 5-ALA on physiological and psychological symptoms (fatigue and mood, respectively) and the possible improvement of the sexual function encouraged us to administer 5-ALA to patients with symptoms of LOH.

In the present study, we investigated the efficacy and safety of treatment by the oral administration of 5-ALA to eugonadal patients with moderate LOH symptoms, in comparison to placebo.

## MATERIALS AND METHODS

### 1. Patients

This study included 32 males who visited our hospital between January 2017 and October 2018, due to at least one of the following symptoms of LOH: lethargy, general fatigue, malaise, depression, insomnia, frustration, reduced concentration, sweating, hot flashes, coldness, tinnitus, headache, numbness, dizziness, stiff shoulder, night sweats, sexual dysfunction, and decreased libido. The inclusion criteria in this study were age  $\geq 40$  years, serum concentration of total testosterone  $>3.0$  ng/mL [16] and Aging Males' Symptoms (AMS) scale score (as questionnaire specific to LOH) 27–49, which reflects moderate complaints. The exclusion criteria were as follows: past history of treatment for malignant disease within 3 years, history of major depression, high prostate specific antigen (PSA) level ( $>4.0$  ng/mL), treatment refusal, heart failure, renal failure, hepatitis B, hepatitis C, liver dysfunction with values  $>1.5$  times the facility standard values, and severe allergic symptoms related to food or medicine. Our subjects were randomized, in a double-blind manner, into the 5-ALA group ( $n=15$ ) and the placebo group ( $n=17$ ).

Randomization was performed by a simple randomization method using a computed random number table. The 5-ALA group received three tablets of 5-ALA phosphate (10 mg/tablet per day, administered orally), for 8 weeks while the placebo group was given three placebo tablets per day for 8 weeks. Each tablet contained 1.2 mg of iron, 5 mg of zinc yeast, 3.3 mg of niacin, and 10 mg of cystine. The tablets used for this study were provided by SBI Pharmaceuticals Co. Ltd. (Tokyo, Japan). Both the 5-ALA and placebo tablets were the same size and color to make them indistinguishable.

## 2. Methods

Symptoms of LOH were assessed by the AMS score. Endocrinological variables included total testosterone (3.00–8.84 ng/mL), LH (1.7–8.6 mIU/mL), follicle-stimulating hormone (1.6–11.0 mIU/mL), prolactin (4.0–14.0 ng/mL), and cortisol (6.2–22.7 µg/dL). Biochemical data included liver enzymes (*e.g.*, aspartate aminotransferase [5–37 U/L] and alanine aminotransferase [6–43 U/L]), and metabolic and lifestyle factors (*e.g.*, hemoglobin A1c [4.6%–6.2%], fasting blood sugar [65–109 mg/dL], total cholesterol [150–219 mg/dL], high-density lipoprotein cholesterol [40–70 mg/dL], low-density lipoprotein cholesterol [70–139 mg/dL], triglyceride [30–149 mg/dL] and uric acid [3.5–6.9 mg/dL]). The serum concentrations of PSA (0.000–4.000 ng/dL) and zinc (65–110 µg/dL) were also assessed. All blood samples were collected between 09:00 and 11:00 to monitor biochemical and endocrinological variables. The AMS score was assessed before treatment, and after 4 and 8 weeks of treatment. All blood parameters were evaluated before treatment and after 8 weeks of treatment.

The primary endpoint of this study was the comparison of the change in the total AMS score ( $\Delta$  total AMS) between the 5-ALA group and the placebo group during treatment. The secondary endpoints were the comparisons of the change in the somatic, psychological and sexual sub-scores of the AMS ( $\Delta$  somatic AMS,  $\Delta$  psychological AMS, and  $\Delta$  sexual AMS) between the groups during the treatment. Furthermore, the total AMS score and each sub-score of the AMS before treatment and after 8 weeks of treatment were compared in both the 5-ALA group and the placebo group. The change of each of the endocrinological and biochemical variables was also compared between the groups during treatment. Finally, treatment-emergent adverse events (TEAEs) were assessed according to the patients'

complaints during the study period.

## 3. Statistical analysis

The comparison of all factors, including age and body mass index (BMI; body weight [kg]/height<sup>2</sup> [m<sup>2</sup>]) between the 5-ALA group and the placebo group before treatment was performed by the Mann–Whitney U-test. The comparison of the change in the total AMS score after 4 and 8 weeks of treatment was compared between the 5-ALA group and the placebo group by a one-way analysis of variance (ANOVA). In each group, the total score and sub-scores of the AMS after 8 weeks of treatment were compared to the respective score pre-treatment scores using a paired Student's t-test. During the treatment period, the degree of change in other parameters was also analyzed by a one-way ANOVA. A p-value of <0.05 was considered to indicate statistical significance. All statistical analyses were conducted with the JMP<sup>®</sup> 13 software program (SAS Institute Inc., Cary, NC, USA).

## 4. Ethics statement

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and written informed consent was obtained from all patients. The procedures were approved by Institutional Review Board of the Juntendo University (approval number: 16-108, UMIN–Clinical Trials Registry: UMIN 000025353).

## RESULTS

### 1. Patient population

Table 1 shows the patient's characteristics at the start of the trial. The mean age was 52.3±8.8 years and the mean BMI was 24.4±4.4 kg/m<sup>2</sup>. The mean total AMS score was 38.7±5.7, and the mean sub-scores of the AMS were as follows: somatic function, 16.2±2.3; psychological function, 8.8±2.5; and sexual function, 13.7±3.6. The mean total testosterone level was 5.1±1.2 ng/mL. No significant differences were found between the 5-ALA and placebo groups in age, BMI, AMS score, or in the endocrinological and biochemical variables (Table 1).

### 2. Primary endpoint

The  $\Delta$  total AMS value of the 5-ALA group was significantly greater than that of the placebo group after

Table 1. Patient characteristics

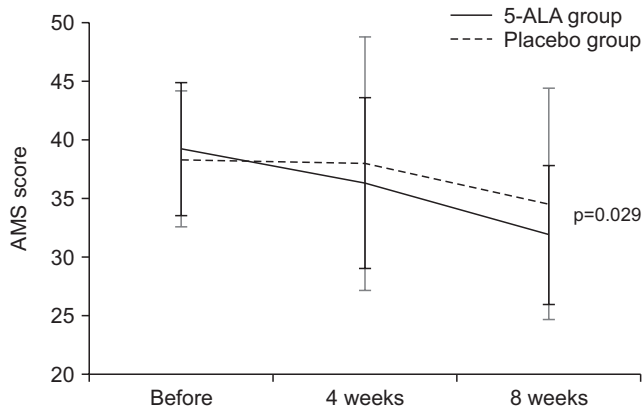
Variable	Total (n=32)			5-ALA (n=15)			Placebo (n=17)		
	Mean±standard deviation	Median	95% confidence interval	Mean±standard deviation	Median	95% confidence interval	Mean±standard deviation	Median	95% confidence interval
Age (y)	52.3±8.8	52	49.1–55.5	51.5±8.2	50	46.9–56.0	53.4±9.9	53	48.3–58.5
BMI (kg/m <sup>2</sup> )	24.4±4.4	24.4	22.8–26.0	23.1±5.5	22.2	20.1–26.2	25.5±3.0	25	24.0–27.0
AMS score	38.7±5.7	39	36.7–40.8	39.3±5.7	40	36.1–42.4	38.4±5.8	36	35.4–41.3
Somatic	16.2±2.3	17	15.4–17.0	16.4±2.0	17	15.3–17.5	15.9±2.5	16	14.6–17.3
Psychological	8.8±2.5	8	7.9–9.8	8.5±2.3	8	7.2–9.7	9.3±2.5	8	8.0–10.6
Sexual	13.7±3.6	13.5	12.4–15.0	14.4±4.5	15	11.9–16.9	13.1±2.5	13	11.8–14.4
Total testosterone (ng/mL)	5.1±1.2	5.2	4.7–5.6	5.1±1.4	5.2	4.4–5.9	5.1±1.2	5.3	4.5–5.7
LH (mIU/mL)	6.2±3.6	5.2	4.9–7.5	7.1±4.2	5.9	4.8–9.4	5.4±2.9	4.4	3.9–6.9
FSH (mIU/mL)	8.0±4.5	6.8	6.3–9.6	9.6±5.6	6.8	6.5–12.7	6.6±2.7	5.8	5.2–8.0
PRL (ng/mL)	11.2±12.5	7.5	6.7–15.7	15.2±17.4	9.2	5.6–24.9	8.2±4.1	7.1	6.1–10.3
Cortisol (µg/dL)	9.4±2.8	9.2	8.4–10.4	9.6±3.3	9.4	7.8–11.4	9.0±2.5	8.9	7.7–10.3
AST (U/L)	25.7±9.5	24.5	22.3–29.1	26.8±11.7	21	20.3–33.3	24.5±7.2	24	20.8–28.3
ALT (U/L)	27.6±15.3	25	22.1–33.1	29.1±19.7	20	18.2–20.0	26.6±10.6	27	21.2–32.0
HbA1c (%)	5.9±0.7	5.7	5.6–6.1	5.8±0.9	5.7	5.3–6.3	5.9±0.6	5.8	5.6–6.2
FBS (mg/dL)	105.4±22.7	97.5	97.2–113.6	107.8±30.5	95	90.9–124.7	102.1±12.8	98	95.6–108.7
T-cholesterol (mg/dL)	203.2±36.7	208	189.8–216.7	213.1±45.0	219	187.1–239.1	194.6±26.3	185	181.1–208.2
HDL-cholesterol (mg/dL)	55.8±12.8	57	51.1–60.5	55.1±11.2	58	48.6–61.5	55.1±11.7	56	49.1–61.2
LDL-cholesterol (mg/dL)	111.7±24.7	110	102.7–120.8	116.6±29.3	117.5	99.7–133.5	108.5±20.6	110	97.9–119.1
TG (mg/dL)	193.1±297.5	139	84.0–302.2	249.4±429.5	155.5	101.7–169.4	149.5±104.7	116	95.7–203.3
UA (mg/dL)	6.0±1.6	6.5	5.4–6.6	5.7±1.7	6.1	4.6–6.7	6.2±1.4	6.1	5.5–6.9
PSA (ng/mL)	0.9±0.6	0.8	0.7–1.1	0.9±0.7	0.8	0.5–1.3	0.9±0.5	0.7	0.6–1.2
Zn (µg/dL)	77.1±17.9	79	73.9–83.4	73.5±21.0	79	70.5–83.6	80.5±14.3	76	72.6–88.4

5-ALA: 5-aminolevulinic acid, BMI: body mass index, AMS: Aging Males' Symptoms, LH: luteinizing hormone, FSH: follicle-stimulating hormone, PRL: prolactin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HbA1c: hemoglobin A1c, FBS: fasting blood sugar, T: total, HDL: high-density lipoprotein, LDL: low-density lipoprotein, TG: triglyceride, UA: uric acid, PSA: prostate specific antigen, Zn: zinc.

8 weeks of treatment ( $-7.4 \pm 4.7$  vs.  $-4.9 \pm 4.9$ ,  $p=0.029$ ; Fig. 1); however, no significant difference was observed after 4 weeks of treatment ( $p=0.177$ ).

### 3. Secondary endpoints

The total AMS score and the sub-scores of AMS at



**Fig. 1.** The comparison of the change in the total AMS score between the 5-ALA group and the placebo group during treatment. The change in the 5-ALA group was significantly greater than that in the placebo group after 8 weeks of treatment ( $-7.4 \pm 4.7$  vs.  $-4.9 \pm 4.9$ ,  $p=0.029$ ). AMS: Aging Males' Symptoms, 5-ALA: 5-aminolevulinic acid.

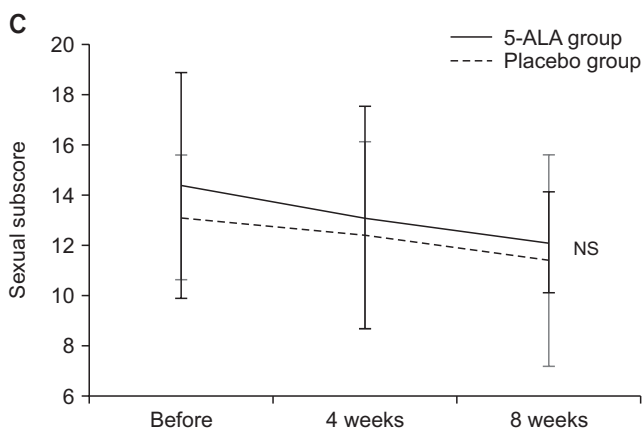
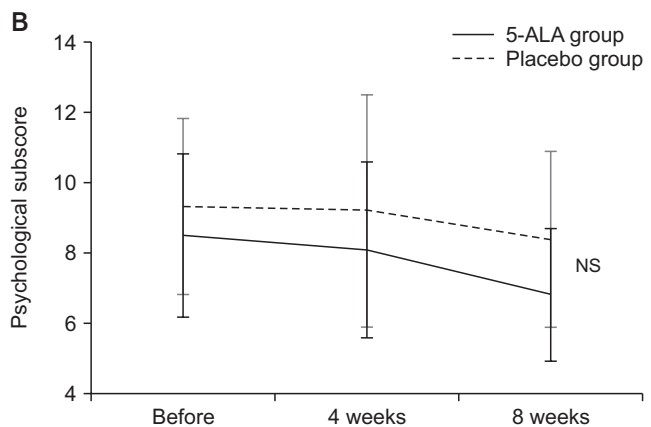
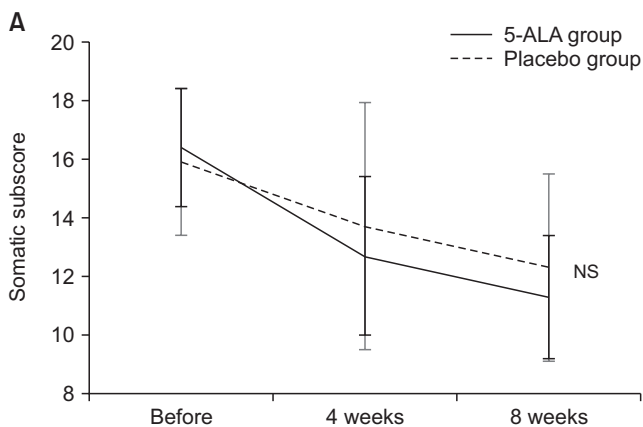
the point after 8 weeks was significantly lower than those before the treatment in the 5-ALA group. However, these tendencies were also found in the placebo group (Table 2). Thus, although the  $\Delta$  somatic AMS (A),  $\Delta$  psychological AMS (B) and  $\Delta$  sexual AMS (C) values were greater in the 5-ALA group than those in the placebo group (somatic,  $-5.1 \pm 1.6$  vs.  $-3.6 \pm 2.8$ ; psychological,  $-1.7 \pm 2.4$  vs.  $-0.9 \pm 1.5$ ; sexual,  $-2.2 \pm 2.5$  vs.  $-1.7 \pm 2.5$ ) after 8 weeks of treatment, none of the differences reached

**Table 2.** Change in the AMS scores of the 5-ALA and placebo groups

AMS		Before	8 weeks	p-value
Total	5-ALA	39.3 $\pm$ 5.7	31.9 $\pm$ 5.9	<0.001
	Placebo	38.4 $\pm$ 5.8	34.6 $\pm$ 9.9	0.019
Somatic	5-ALA	16.4 $\pm$ 2.0	11.3 $\pm$ 2.1	<0.001
	Placebo	15.9 $\pm$ 2.5	12.3 $\pm$ 3.2	<0.001
Psychological	5-ALA	8.5 $\pm$ 2.3	6.8 $\pm$ 1.9	0.018
	Placebo	9.3 $\pm$ 2.5	8.4 $\pm$ 2.5	0.027
Sexual	5-ALA	14.4 $\pm$ 4.5	12.1 $\pm$ 4.4	0.003
	Placebo	13.1 $\pm$ 2.5	11.4 $\pm$ 4.2	0.013

Values are presented as mean $\pm$ standard deviation.

AMS: Aging Males' Symptoms, 5-ALA: 5-aminolevulinic acid.



**Fig. 2.** Comparison of the change in the (A) somatic, (B) psychological, and (C) sexual sub-scores of the AMS ( $\Delta$  somatic AMS,  $\Delta$  psychological AMS and  $\Delta$  sexual AMS) between the groups during treatment. Although each respective change in the 5-ALA group was greater than that in the placebo group after 8 weeks of treatment, none of the differences reached statistical significance. AMS: Aging Males' Symptoms, 5-ALA: 5-aminolevulinic acid, NS: not significant.

statistical significance (Fig. 2). The comparison of the change in the endocrinological and biochemical variables showed no significant differences between the groups in any of the endocrinological and biochemical variables (Table 3).

#### 4. Safety

During the 8-week treatment period, no patients discontinued 5-ALA due to TEAEs, although one patient developed diarrhea transiently without the cessation of 5-ALA. In addition, none of the endocrinological or

laboratory variables changed from within the normal range before treatment to out of normal range after 8 weeks of treatment.

## DISCUSSION

In recent years, symptoms of LOH have gained increased attention in developed countries, including Japan, as social problem in aging societies. Testosterone replacement treatment was the main option for patients with symptoms of LOH, according to the

**Table 3.** The biochemical and endocrinological variables of the 5-ALA and placebo groups

Group	Before	8 weeks	Δ	p-value	
Total testosterone (ng/mL)	5-ALA	5.1±1.4	4.7±1.7	-0.4±1.1	0.507
	Placebo	5.1±1.2	5.0±1.3	-0.1±1.2	
LH (mIU/mL)	5-ALA	7.1±4.2	6.2±4.8	-0.9±2.6	0.478
	Placebo	5.4±2.9	5.1±2.6	-0.3±2.5	
FSH (mIU/mL)	5-ALA	9.6±5.6	9.2±5.7	-0.4±0.9	0.677
	Placebo	6.6±2.7	6.5±3.2	-0.1±1.4	
PRL (ng/mL)	5-ALA	15.2±17.4	11.3±6.7	-3.9±13.5	0.110
	Placebo	8.2±4.1	11.3±9.2	3.1±5.9	
Cortisol (µg/dL)	5-ALA	9.6±3.3	11.3±4.9	1.7±4.2	0.681
	Placebo	9.0±2.5	10.2±2.5	1.3±3.4	
AST (U/L)	5-ALA	26.8±11.7	29.4±11.6	2.6±5.6	0.300
	Placebo	24.5±7.2	25.1±5.3	0.6±6.0	
ALT (U/L)	5-ALA	29.1±19.7	32.5±27.1	3.4±9.4	0.754
	Placebo	26.6±10.6	29.2±10.1	2.6±5.9	
HbA1c (%)	5-ALA	5.8±0.9	5.8±0.9	0.0±0.2	0.089
	Placebo	5.9±0.6	5.8±0.5	-0.1±0.2	
FBS (mg/dL)	5-ALA	107.8±30.5	112.9±45.3	5.1±27.6	0.837
	Placebo	102.1±12.8	107.3±14.1	5.2±12.2	
T-cholesterol (mg/dL)	5-ALA	213.1±45.0	208.0±45.8	-3.8±23.2	0.842
	Placebo	194.6±26.3	192.3±28.8	-2.3±18.2	
HDL-cholesterol (mg/dL)	5-ALA	55.1±11.2	50.9±9.7	-4.4±5.0	0.295
	Placebo	55.1±11.7	53.4±12.3	-1.8±6.4	
LDL-cholesterol (mg/dL)	5-ALA	116.6±29.3	115.7±23.4	0.0±14.6	0.623
	Placebo	108.5±20.6	111.3±22.2	2.8±14.9	
TG (mg/dL)	5-ALA	249.4±429.5	212.1±268.5	-26.6±172.9	0.364
	Placebo	149.5±104.7	163.5±104.4	14.0±56.2	
UA (mg/dL)	5-ALA	5.7±1.7	5.7±1.8	0.2±1.7	0.551
	Placebo	6.2±1.4	6.2±1.2	-0.1±0.7	
PSA (ng/mL)	5-ALA	0.9±0.7	1.2±1.3	0.2±1.0	0.282
	Placebo	0.9±0.5	0.8±0.5	-0.1±0.2	
Zn (µg/dL)	5-ALA	73.5±21.0	92.5±13.7	15.8±13.2	0.840
	Placebo	80.5±14.3	94.1±35.9	13.9±32.8	

Values are presented as mean±standard deviation.

5-ALA: 5-aminolevulinic acid, LH: luteinizing hormone, FSH: follicle-stimulating hormone, PRL: prolactin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HbA1c: hemoglobin A1c, FBS: fasting blood sugar, T: total, HDL: high-density lipoprotein, LDL: low-density lipoprotein, TG: triglyceride, UA: uric acid, PSA: prostate specific antigen, Zn: zinc.

definition of LOH. However, a cross-sectional study of 606 male over 50 years showed that 30.8% of male complained of symptoms of LOH regardless of whether their serum testosterone concentration was within the normal range [17]. Nevertheless, there have been few beneficial treatment options for eugonadal patients with symptoms of LOH. In Japan, several herbal medicines have been used in such cases. We already reported that one herbal medicine, “saikokaryuukotsuboreitou,” was effective for such patients, probably through its change of several cytokines [18,19]. 5-ALA has been expected to be another option for the treatment of the symptoms of LOH, which include physiological symptoms (*e.g.*, fatigue) psychological symptoms (*e.g.*, mood) and sexual dysfunction.

The actual mechanism underlying the improvement of LOH symptoms by the intake of 5-ALA is still unknown. Recently, a study with depressive patients that investigated the effects of exercise (interval walking training) showed that training days, training impulse, and time of fast walking significantly increased in the group that received 5-ALA and sodium ferrous citrate in comparison to a placebo group [20]. Interestingly, that study also showed that the specific questionnaire for depression scores only showed a significant decrease in the 5-ALA and sodium ferrous citrate group, although the dose of 5-ALA in that study was much greater than that in our study. It was hypothesized that 5-ALA and sodium ferrous citrate supplementation might lower the physical and psychological barriers to achieving moderate or higher intensity exercise training to decrease the score of the questionnaire for depression, because the required exercise intensity might have been too difficult for depressive patients. We also hypothesize that a similar mechanism may underlie the beneficial effects of 5-ALA on the symptoms of LOH, such as depression and physical inactivity. Regarding the somatic aspect, it was shown that body weight gain and fat accumulation might be reduced by 5-ALA treatments in a murine model of a diet-induced obesity, probably influenced by the liver lipid metabolism [21]. Furthermore, it was recently reported that the intake of 5-ALA increased muscle mass and improved the physical performance of 100-week-old mice with sarcopenia and chronic kidney disease via muscle mitochondrial activation [22]. These findings raise the expectation that 5-ALA will be effective for symptoms of LOH, because one of the typical symptoms of LOH is

visceral fat obesity. With regard to aging, a previous study using a rat model demonstrated that ALA synthetase activity significantly decreased during aging [23]. In humans, this aging process was also reported in a study using normal skin fibroblasts obtained from healthy volunteers of various ages. That study clearly showed that the mitochondrial complex IV (cytochrome c oxidase), including heme and cytochrome biosynthesized from ALA, decreased with age [24]. Because heme proteins are very important for energy production, this age-related decrease of ALA synthetase activity may have the potential to cause the somatic symptoms, psychological symptoms, and sexual dysfunction associated with LOH. In the present study, we clearly show that the  $\Delta$  total AMS values in the 5-ALA group were significantly greater than those in the placebo group after 8 weeks of treatment. This means that 5-ALA had a beneficial effect in patients with LOH symptoms, although the improvement of the sub-scores of the AMS did not reach statistical significance. However, it was apparent that the  $\Delta$  somatic AMS,  $\Delta$  psychological AMS, and  $\Delta$  sexual AMS values in the 5-ALA group were greater than those in the placebo group. These non-significant differences might be due to the relatively small study population. Although a little more dose may be advantageous, we decided to use 30 mg per day according to the previous study showing the safety of 5-ALA, because we worried seriously about adverse effects in this clinical trial. In addition, this present study showed that no endocrinological variables, including total testosterone, were altered by the intake of 5-ALA (Table 2). Thus, endocrinological improvement by 5-ALA was completely ruled out as the mechanism through which the symptoms of LOH were relieved. This finding seems to be in line with the findings of our previous study with a herbal medicine [18,19], although cytokines were not evaluated in the present study. Regarding safety, reported adverse effects of 5-ALA at high doses include hyper-photosensitivity and digestive symptoms (*e.g.*, abdominal fullness, stomachache, constipation, and diarrhea) [25]. Furthermore, in general, hypotension and alteration of liver functions have been reported as potential adverse effects of 5-ALA. In the present study, one patient experienced mild and transient diarrhea as a TEAE without the cessation of 5-ALA. Thus, 5-ALA supplementation at a dose of 30 mg per day should be considered sufficiently enough safe.

The present study was associated with some limitations. First, the study population was relatively small. It was difficult to obtain informed consent from the patients because the patients who visited our clinic were seeking better treatment for symptoms of LOH. Second, the 8-week observation period was short for observing clinical efficacy. Third, blood testing to measure the biochemical and endocrinological variables was only performed once. Blood testing should be repeated, especially for the assessment of the endocrinological profile, because it is known that several hormones are secreted seasonally and diurnally. Although these limitations cannot be ignored, we believe that our findings may be helpful for eugonadal patients with symptoms of LOH. Further long-term observation with a large number of subjects will be needed to resolve these problems.

## CONCLUSIONS

After 8 weeks of treatment of eugonadal patients with symptoms LOH, the total AMS score of the 5-ALA group was significantly improved in comparison the placebo group. Because no severe TEAEs were experienced, 5-ALA should be considered as an option for the treatment of eugonadal patients with symptoms of LOH.

## Conflict of Interest

The authors have nothing to disclose.

## Author Contribution

Conceptualization: HK, SH. Data curation: HK, KM. Formal analysis: HK, KM, AT. Investigation: HK. Methodology: HK, SH. Project administration: HK, SH. Supervision: KM, TA, SM, SI, TT, MN, AT. Validation: HK, SH. Visualization: HK, SH. Writing – original draft: HK, KM. Writing – review & editing: HK, AT.

## Data Sharing Statement

The data analyzed for this study have been deposited in HARVARD Dataverse and are available at <https://doi.org/10.7910/DVN/XZOMSZ>.

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