EFFICACY OF SUPPLEMENT CONTAINING ARGININE AND ORNITHINE ON MALE SEXUAL FUNCTION IN HEALTHY JAPANESE
— A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY —

Masatomo NAJIMA[1], Mitsuhiko MUNEKATA[2] and Ryo KOHIYAMA[3]

1) JACTA (Japan Clinical Trial Association)
2) OZ Clinic
3) INSPIRE CO. LTD.

Abstract

Objectives: The objective of this research was to investigate the effectiveness of daily ingestion of a supplement, which contains arginine and ornithine on the male sexual function improvement.
Methods: In this randomized, placebo-controlled, double-blind trial, 31 subjects were randomized. To evaluate this objective, subjective reporting of sexual function (modified International Index of Erectile Function), blood flow velocity and Profile of Mood States were measured as the primary outcome.
Results: 7 subjects were withdrawn. 6 out of 7 were due to personal reasons (4; occupational conflict, 2; a cold) and 1 subject revealed to have extremely high score of potassium in the 6th week visit so that the physician in charge excluded. With 24 subjects, changes in values of modified IIEF were significant in the intergroup comparison after 12-week ingestion. Moreover, in the Vigor and Fatigue questionnaire, the magnitude of change from baseline was significantly different between the two groups after 12-week ingestion. 1 subject with extremely high score of potassium was examined as a sound individual overall by the physician in charge.
Conclusion: We found out that the ingestion of the supplement containing arginine and ornithine by healthy Japanese people for 12 weeks contributed to the improvement of the male sexual function. In addition, no safety-related matter occurred during 12-week test period.

Key words: arginine, ornithine, male sexual function, erectile dysfunction

1. INTRODUCTION

For men, male sexual function (erectile function) is not only an issue of sexual intercourse as the measure to preserve the species, but also an issue that relates to the self-confidence or the eagerness for life, the relationship between couples and ultimately the QOL (quality of life).

Erectile Dysfunction (ED) is defined as "a condition in which a man is unable to get or keep an erection firm enough for sexual intercourse"[1] (National Institute of Health (NIH), 1993), and the same definition is applied in our country. In an epidemiological survey conducted in Japan in 1998, about 8.7 million people were estimated to suffer from moderate ED (a condition in which a man can sometimes get or keep an erection firm enough for sexual intercourse), whereas about 2.6 million were estimated to be suffered from complete ED (a condition in which a man is always unable to get or keep an erection firm enough for sexual intercourse): those add up to about 11.3 million people, which means one in four men aged 25 years old or older were reported to have an erectile dysfunction problem[2], and the number is expected to increase due to the country's aging population.

It is commonly known that ED is brought by various factors such as aging, psychological factors, adult lifestyle-related diseases and/or a medicinal agent[3].

The current mainstream medical treatment for ED is the use of phos-phodiesterase5 inhibitor[4]. In Japan the use of Viagra became operational in 1999 and now three kinds of curative medicine are being used, all of which are regarded as a highly effective measure. However, it is also reported that their use may cause adverse events such as headache, variability in blood pressure or palpitation, and there are some medicines that should not be administered together with them[5]. In addition, it is relatively expensive to purchase these three medicines and they are only available on prescription. Therefore purchasing them is somewhat a hurdle for the male who does not have a symptom of ED but just desires to strengthen the male sexual function or the patients on the borderline. This fact accelerates the needs among men to strengthen their sexual function by taking the

foods they eat regularly or the supplements which both contain the ingredients effective for the revitalization of the male sexual function.

Based on these needs, many kinds of supplement products containing "arginine" are recently sold for men who want to strengthen their sexual function. Arginine is a kind of non-essential amino acid contained in chicken, soybean or Koya-dofu (freeze-dried bean curd), and it is reported to have functions such as increasing the production of growth hormones and improving blood flow by causing blood vessels to dilate[6)7)]. Another report illustrates the ingestion of arginine contributes to the improvement of the sexual function[8)].

Ornithine, on the other hand, is also a kind of non-essential amino acid contained in Shijimi (freshwater clam), cheese or flatfish, and is reported to be effective for fatigue recovery and growth promotion[9)]. However, although both arginine and ornithine have a great deal to do with intravital behavior of human, there are only a few reports about the relationship between the ingestion of ornithine and the sexual function, and as to the relationship between the use of both ingredients and the sexual function, we cannot find any reports scrutinizing it.

In this study, we examined the effect of the combination of arginine and ornithine for the male sexual function and the safeness of the combined use of them, excluding any deliberate effect of increasing vitality achieved by medicine. The test targets were healthy Japanese men, and the test method was a randomized, placebo-controlled, double-blind study using the supplement containing arginine and ornithine.

2. METHOD

2.1. Trial Design

A randomized, placebo-controlled, double-blind study was conducted with the aid of a fund from INSPIRE CO. LTD. (Tokyo) at two centers (OZ clinic, Tokyo and JACTA, Tokyo). The study period was 12 weeks, from July 18th to October 10th, 2015.

This study was conducted in accordance with the ethical principles of the declaration of Helsinki. The study protocol was approved by the Institutional Review Board of LLP. Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all subjects.

The allocation of the test product to the subjects was carried out by the person in charge of allocation. The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study.

2.2. Subjects

Healthy subjects participated in the present study. All of the subjects in this study were public volunteers who had enrolled in the monitor bank of CROee Inc. (Tokyo) and Rabbits-coco (Tokyo).

2.2.1. Inclusion Criteria

(1)Healthy males aged between 30 and 69 years;
(2)Males who have a sexual decline;
(3)Males who have a sexual partner.

2.2.2. Exclusion Criteria

(1)Subjects who are allergic to food related to the test material of this trial;
(2)Subjects who have previous medical history of the serious diseases (heart, liver, kidney, blood, digestive system, metabolism system);
(3)Subjects who are under treatment for hypertension, or who untreated hypertension (not less than level 2);
(4)Subjects who are under treatment for diabetes;
(5)Subjects who contract, or are under treatment for diseases of prostatic;
(6)Subjects who are judged as inappropriate for this study by the doctor in charge.

2.3. Randomization

Recruited subjects were 150 persons. Subjects who fulfilled eligibility criteria were 31 persons. The inclusion criteria were judged by the principle investigator. All subjects were sequentially assigned based on a random number table to one of the masked products and randomized to group T (Test sample: 16) and group P (Placebo: 15). The allocation was pre-assigned on the basis of randomized numbers.

2.4. Description of test foods and blinding

The test food, "Force" ("FO") and placebo were prepared by INSPIRE CO. LTD. The amount of daily intake is 9 tablets (1 tablet contains 400mg, therefore 9 tablets contain 3600mg). The compositions of FO were arginine, ornithine, etc., while Placebo was mainly consisted of cellulose. Both tablets were indistinguishable in shape, color or taste. Tablets were managed by the identification symbol. All involved were blinded.

2.5. Experimental Procedures

2.5.1. Experimental protocol

Subjects consumed 9 tablets of the supplement with hot or cold water every day for 12 weeks. Subjects were instructed as follows: to take the assigned foods as indicated; to maintain their usual lifestyles and habits; to avoid excessive amounts of food, drink or alcohol; to maintain a daily record of lifestyle factors during the test period; and to send the diary to the study coordinator every Friday by mobile email.

2.5.2. Outcome

The objective of this study is to verify the male sexual function improvement of ingesting food containing arginine with ornithine. To evaluate this objective, subjective reporting of sexual function was observed as the primary outcome. The questionnaire is based on International Index of Erectile Function (IIEF)[10)]. Some questions are eliminated or modified because they are the criteria of Erectile Dysfunction (ED). This study is intended to exclude ED patients. The details are illustrated in **Appendix 1**.

Furthermore the blood flow velocity and Profile of Mood States ('POMS')[11)] were measured as the

Table 1　Schedule for the study.

Item \ Term	Screening	Pre Trial Test	Test period (12 w)	
			Week 6	Week 12
Informed consent	●			
Questionnaire on the treatment of sexual function	●			
Selection and/or allocation	●			
Modified IIEF		●	●	●
Blood flow velocity		●	●	●
POMS		●	●	●
Biochemical analysis of the blood		●	●	●
Urine analysis		●	●	●
Ingestion of test foods			↔	↔
Log			↔	↔

● : Implementation
↔ : Daily practice during the test period

secondary outcome. The blood flow velocity was tested by Bi-directional Doppler ES-100V3 (Hadeco, Inc., Tokyo). For POMS, Vigor and Fatigue were questioned. The details of questionnaire are listed in **Appendix 2**. Blood biochemical and urine parameters were recorded to evaluate the safety of the test foods as the secondary outcome.

These assessments were conducted upon entry into the study (pre-intervention), 6 weeks and 12 weeks (post-intervention). To evaluate the safety of the test foods, adverse events were collected by means of a written questionnaire during the study.

According to the schedule shown **Table 1**, we measured parameters on efficacy and safety.

2.6. Data Analysis

All analyses were performed on the on-treatment population in the study. The full analysis set principal was adopted in the present study and no sample size design was used.

Data were expressed as mean ± SD. For the modified IIEF and POMS, changes from baseline in the same group were assessed using the Wilcoxon signed-rank test. The Mann-Whitney U test was used for intergroup comparisons of changes from the baseline. For blood flow velocity and biochemical analyses of blood and urine, changes from the baseline in the same group were assessed using the paired t-test. Student's t-test was used for intergroup comparisons of changes from the baseline. Student's t-test was used to compare subject backgrounds between groups. Statistical analyses were performed using Statcel 3 (Yanai, 2011). The results were considered significant at the <5% level in the two-sided test.

3. RESULT

3.1. Participant Demographics

From all of 150 applicants, 119 were eliminated according to questionnaire, **Appendix 3**.

The 31 subjects were randomly assigned to intervention groups and made a start with ingestion. 7 dropped out. Out of 7, 6 subjects were eliminated due to personal reasons (4; occupational conflict, 2; a cold). 1 subject revealed to be extremely high score of potassium in 6-week visit so that the physician in charge of this trial excluded this subject. Thus, data obtained with 24 subjects was used for the analysis of efficacy (**Fig l**).

There was no significant difference in the mean age between the groups (**Table 2**).

3.2. Questionnaire of sexual function

Data obtained with respect to the scores of the modified IIEF are summarized in **Table 3**.

Changes in values of the questionnaire were detected as follows: The intake of FO resulted in a significant increase in the score of #2, #3, #4, #5, #6, #7, #8, #9 and #10 after 12-week ingestion. For intergroup comparison, the score of #2, #5, #6, #7, #8 and #10 showed significant increase after 6-week ingestion, also all 10 items represented significant increase after 12-week ingestion.

3.3. Blood flow velocity

Table 4 depicts changes in the blood flow velocity. Significant upward changes were seen in the FO group compared to the Placebo group after 6-week and 12-week ingestion.

3.4. Vigor and Fatigue

Changes of values were also evaluated in Vigor and Fatigue. The significant difference was found in the intragroup comparison with the FO group of 10 items after 6-week ingestion, also 14 items after 12-week ingestion (**Table 5**). For the intergroup comparison, 1 item after 6-week ingestion and 8 items after 12-week ingestion revealed the significant difference (**Table 6**).

3.5. Blood and Urine Test

Table 6 and **7** shows the blood biochemical and urine

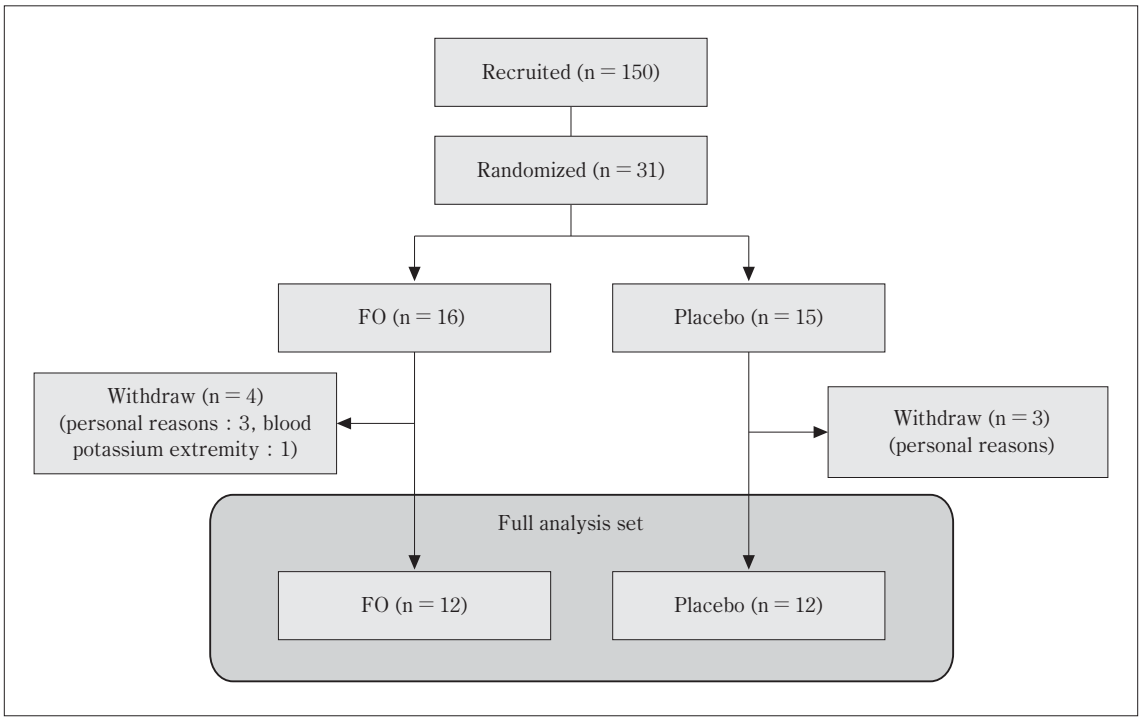

Fig. 1 Flow diagram of subject disposition

Table 2 Subject demographics

Item	Unit	FO	Placebo
Subjects*	—	12	12
Age	Years	48.4 ± 11.3	48.8 ± 11.4

* Number of subjects
mean ± SD

parameters. A significant difference was observed in the changes of Chloride, Potassium and Blood Sugar (Serum) of the FO group after 12 weeks of ingestion. The same difference was observed with urine pH. However, the investigator judged it as the range of physiological variation (or clinically safe).

3.6. Adverse Event

The subject whose blood potassium was extremely high was examined as a sound individual overall by the physician in charge of this study. Any other adverse event was not depicted during this trial.

4. DISCUSSIONS

We conducted a randomized, placebo-controlled, double-blind study for examining the efficacy of a supplement (FO) containing arginine and ornithine on the male sexual function improvement. As the primary outcome, the study showed the significant differences in the scores of modified International Index of Erectile Function (IIEF) on the questionnaire, which is designed to collect the subjective evaluations against the FO among the study subjects. In addition, the significant differences were observed in the blood flow velocity. Furthermore, several categories on the questionnaire of Profile of Mood States (POMS) showed the significant differences, and this result indicates the improvement of QOL. At the same time, as the secondary outcome of the observation of clinical findings such as medical interview, the blood and urine test revealed no abnormal change had been triggered by the ingestion of test products.

Main Findings

In this study we examined the male sexual function by the questionnaire, designed based upon IIEF which consists of ten items (the questions which presuppose ED were excluded by the doctor handling this investigation). Most items out of ten showed the significant differences, indicating the improvement of male sexual function; the intake of FO resulted in the significant increase in the score of #2, #3, #4, #5, #6, #7, #8, #9 and #10, and for intergroup comparison, on the other hand, the score of #2, #5, #6, #7, #8 and #10 showed significant differences. These results indicate that at least one out of ten items, consisting of “intercourse satisfaction” (#1, #2, #3), “orgasmic function” (#4, #5), “sexual desire” (#6, #7), “overall satisfaction” (#8, #9) and “erectile function” (#10), showed the significant difference of

Table 3　modified IIEF

	Questionnaire	Time points	Scores		Between-group difference (P-value)
			FO group (n = 12)	Placebo group (n = 12)	
1	Over the past 4 weeks, how many times have you attempted sexual Intercourse?	Baseline	1.6±1.0	2.1±1.4	
		Week 6	1.9±0.7	2.2±1.3	
		Change	0.3±1.3	0.1±0.7	0.237
		Week 12	2.1±0.5	1.7±0.9	
		Change	0.5±1.1	−0.4±1.1	0.026 #
2	Over the past 4 weeks, when you attempted sexual intercourse, how often was it satisfactory for you?	Baseline	1.8±0.6	3.3±1.2	
		Week 6	3.2±1.2 *	2.9±1.3 †	
		Change	1.4±1.4	−0.4±0.7	0.001 ##
		Week 12	2.9±1.4 *	2.8±0.9 *	
		Change	1.2±1.4	−0.5±0.7	0.003 ##
3	Over the past 4 weeks, how much have you enjoyed sexual intercourse?	Baseline	2.2±0.6	2.8±1.0	
		Week 6	2.9±0.5 *	3.1±0.7	
		Change	0.8±0.8	0.3±1.0	0.326
		Week 12	3.0±0.4 *	2.6±0.7	
		Change	0.8±0.7	−0.3±1.0	0.013 #
4	Over the past 4 weeks, when you had sexual stimulation or intercourse how often did you ejaculate?	Baseline	2.5±1.2	3.6±1.4	
		Week 6	4.0±1.2 *	4.3±1.0 †	
		Change	1.5±1.3	0.7±1.2	0.166
		Week 12	4.0±1.0 *	2.9±0.9 †	
		Change	1.5±1.6	−0.7±1.1	0.001 ##
5	Over the past 4 weeks, when you had sexual stimulation or intercourse how often did you have the feeling of orgasm or climax?	Baseline	2.4±0.9	3.7±1.2	
		Week 6	3.7±1.0 *	3.0±0.9 †	
		Change	1.3±1.1	−0.7±1.0	0.001 ##
		Week 12	3.9±0.8 **	2.7±0.8 **	
		Change	1.5±1.1	−1.0±0.7	< 0.001 ##
6	Over the past 4 weeks, how often have you felt sexual desire?	Baseline	3.2±0.9	3.4±1.2	
		Week 6	3.8±0.9	2.4±0.8 *	
		Change	0.6±1.0	−1.0±1.1	0.004 ##
		Week 12	3.9±0.5 *	2.3±0.5 **	
		Change	0.8±0.9	−1.2±0.9	< 0.001 ##
7	Over the past 4 weeks, how would you rate your level of sexual desire?	Baseline	3.0±0.6	3.9±0.8	
		Week 6	3.8±0.5 **	3.2±0.4 *	
		Change	0.8±0.5	−0.8±1.0	< 0.001 ##
		Week 12	3.8±0.6 *	2.7±0.7 **	
		Change	0.8±0.9	−1.3±0.8	< 0.001 ##
8	Over the past 4 weeks, how satisfied have you been with you overall sex life?	Baseline	2.3±0.6	3.3±1.3	
		Week 6	3.2±0.6 **	3.0±0.9	
		Change	0.9±0.7	0.3±1.1	0.007 ##
		Week 12	3.4±0.7 **	2.6±0.5 *	
		Change	1.2±0.9	−0.8±1.1	< 0.001 ##
9	Over the past 4 weeks, how satisfied have you been with your sexual relationship with your partner?	Baseline	2.5±0.7	3.1±1.0	
		Week 6	3.2±0.6 *	3.1±0.9	
		Change	0.7±0.7	0.0±1.1	0.126
		Week 12	3.3±0.7 *	2.9±0.7	
		Change	0.8±0.7	−0.2±0.8	0.011 #
10	Over the past 4 weeks how often have you experienced nocturnal penile tumescence?	Baseline	2.3±0.6	2.4±0.5	
		Week 6	3.2±0.7 **	2.3±0.8	
		Change	0.9±0.7	−0.2±1.0	0.017 #
		Week 12	3.2±0.4 **	2.0±0.6	
		Change	0.9±0.5	−0.4±0.8	< 0.001 ##

Scores are expressed as the mean ± SD.
† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.
# $p < 0.05$, ## $p < 0.01$ between-group differences in change from baseline.

Table 4 Blood flow velocity

unit	Time points	Values		Between-group difference (P-value)
		FO group (n = 12)	Placebo group (n = 12)	
cm/s, KHz	Baseline	5.3±2.5	7.5±4.7	
	Week 6	7.0±3.4 *	6.0±3.2	
	Change	1.8±2.1	−1.5±3.8	0.017 #
	Week 12	7.5±3.1 **	5.9±3.8 †	
	Change	2.3±2.2	−1.7±3.0	0.001 ##

Values are expressed as the mean ± SD.

† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.

# $p < 0.05$, ## $p < 0.01$ between-group differences in change from baseline.

improvement. Although items #1, #3 and #9 did not achieve the satisfactory level of significant differences, these greatly relate to the relationship between the partners, and therefore the improvement may not be accomplished only by the improvement of subject's sexual function.

Based on the findings illustrated above, it can be said that from a standpoint of IIEF score, the ingestion of FO has improved all aspects of male sexual function. Although IIEF was originally developed to examine a state change associated with treatment of ED, it has been confirmed that the use of IIEF is available for the examination of male sexual function of healthy people[12)13)]. Therefore it should be appropriate as an evaluation criterion for the male sexual function of this study.

The main factor for evaluating the male sexual function is an erection. It is considered that an erection is achieved based on the following mechanism[14)15)]; firstly, sexual stimulation causes agitation on the sexual center of the cerebrum, and the agitation passes through the pelvic nerve, pelvic plexus and cavernous nerves of the penis. Then Nitrogen monoxide (NO) is secreted from nerve terminals and it increases the level of cGMP in the unstriated muscle of the corpus cavernosum penis. Then the unstriated muscle relaxes, and this relaxation lets the blood flow into the cavernous body. The flow extends the tunica albuginea corporum cavernosorum, then the draining vein is closed and finally an erection is achieved.

Arginine contained in FO produces NO, and it is reported that when the NO reaches the vascular smooth muscle, it increases the level of cGMP and causes dilatation of blood vessels[16)]. There is also a report that has shown a significant subjective improvement in sexual function in men with organic ED who have the decreased production of NO, as a result of the 5g/day ingestion of L-arginine[8)].

On the other hand, ornithine reportedly has a function of mitigating tiredness from having worked out[9)], and this function possibly contributes to reducing the fatigue of men during sexual intercourse. In addition, ornithine converts into arginine through the urea cycle[17)], therefore the concurrent ingestion of arginine and ornithine is expected to have a mutual influence. There is also a report that has shown the muscle strengthening function of men as a result of the concurrent ingestion of both ingredients[18)] and this finding may affect the function of unstriated muscle when an erection occurs. These functions are different from those of curative medicine for ED (PDE5I) which has a function of inhibiting biodegradation of cGMP. Therefore, FO containing both arginine and ornithine is expected to have an improvement effect of male sexual function, while excluding any deliberate effect of increasing vitality achieved by medicine such as PDE5I

Secondary Findings

In this study we observed a significant difference of the blood flow velocity in the FO group compared to the Placebo group, after 6-week and 12-week ingestion. As illustrated above, this result can be explained as the function of improving the blood flow the amino acids such as arginine or ornithine (contained in FO) reportedly have[16)]. The blood flow of the subjects who ingested FO has improved and its velocity has also increased. The variation was within the standard level, and did not deviate from the usual range.

At the same time, for evaluating the QOL of the subjects the doctor conducted a questionnaire using POMS. For POMS, vigor and fatigue were questioned. After 12-week ingestion of FO, 14 items (of the questionnaire) showed the significant differences and the comparison between the Placebo group indicated the significant differences on 8 items (Vigor Lively, Active, Alert, Carefree, Fatigue worn out, Listless, Fatigued and Exhausted). These include both items about the fatigue and the vigor, and although for the items of "Vigor Lively" and "Active", FO group answered as they felt lively, at the same time they answered as they felt tired on the items such as "Fatigue worn out". It is possible that the fatigue is the result of intensity of their sexual intercourse after gaining some confidence in their sexual ability, but only this result is not sufficient to conclude the improvement of QOL has been achieved by the

Table 5　POMS

Questionnaire		Time points	Scores		Between-group difference (P-value)
			FO group (n = 12)	Placebo group (n = 12)	
1	Vigor Lively	Baseline	1.6 ± 0.7	1.8 ± 1.0	
		Week 6	2.0 ± 0.6	2.3 ± 1.1	
		Change	0.4 ± 0.8	0.4 ± 1.1	0.729
		Week 12	2.6 ± 0.7 **	1.8 ± 0.8	
		Change	1.0 ± 0.6	− 0.1 ± 0.7	0.002 ##
2	Active	Baseline	1.4 ± 0.7	2.1 ± 0.9	
		Week 6	2.3 ± 0.5 *	2.3 ± 1.1	
		Change	0.9 ± 0.8	0.3 ± 1.1	0.106
		Week 12	2.6 ± 0.7 **	2.0 ± 0.7	
		Change	1.2 ± 1.0	− 0.1 ± 0.8	0.007 ##
3	Energetic	Baseline	1.2 ± 0.4	1.6 ± 1.1	
		Week 6	2.3 ± 0.9 **	1.7 ± 0.5	
		Change	1.1 ± 0.7	0.1 ± 0.9	0.011 #
		Week 12	2.4 ± 0.5 **	2.6 ± 0.7	
		Change	1.3 ± 0.5	1.0 ± 1.0	0.665
4	Cheerful	Baseline	1.4 ± 0.8	2.1 ± 1.1	
		Week 6	2.1 ± 0.7 *	2.3 ± 1.1	
		Change	0.7 ± 0.7	0.3 ± 1.4	0.248
		Week 12	2.3 ± 0.5 *	3.1 ± 1.0 *	
		Change	0.8 ± 0.7	1.0 ± 1.3	0.862
5	Alert	Baseline	1.1 ± 0.9	1.9 ± 0.9	
		Week 6	2.2 ± 0.9 *	2.3 ± 0.8	
		Change	1.1 ± 1.2	0.3 ± 0.8	0.106
		Week 12	2.3 ± 0.7 *	1.7 ± 0.7	
		Change	1.3 ± 1.1	− 0.3 ± 0.5	0.002 ##
6	Full of pep	Baseline	1.1 ± 0.7	1.5 ± 1.0	
		Week 6	2.0 ± 0.7 *	1.8 ± 1.2	
		Change	0.9 ± 0.9	0.3 ± 1.7	0.260
		Week 12	1.9 ± 0.8 *	1.5 ± 0.8	
		Change	0.8 ± 0.8	0.0 ± 1.5	0.0999 ‡
7	Carefree	Baseline	1.2 ± 0.6	1.8 ± 0.9	
		Week 6	2.0 ± 0.7 *	1.8 ± 1.6	
		Change	0.8 ± 0.9	− 0.1 ± 1.9	0.157
		Week 12	2.1 ± 0.5 *	1.5 ± 0.7	
		Change	0.9 ± 0.8	− 0.3 ± 1.2	0.0102 #
8	Vigorous	Baseline	1.2 ± 0.7	1.6 ± 1.0	
		Week 6	1.9 ± 0.8 *	2.3 ± 1.1	
		Change	0.8 ± 0.6	0.7 ± 1.4	0.795
		Week 12	2.2 ± 0.7 **	2.6 ± 0.9 *	
		Change	1.0 ± 0.7	1.0 ± 1.4	1.000
9	Fatigue worn out	Baseline	1.8 ± 1.1	1.9 ± 1.1	
		Week 6	2.0 ± 1.0	1.6 ± 1.4	
		Change	0.3 ± 1.3	− 0.3 ± 1.7	0.525
		Week 12	2.5 ± 0.8 *	1.2 ± 0.9 †	
		Change	0.8 ± 1.1	− 0.8 ± 1.3	0.006 ##
10	Listless	Baseline	1.5 ± 0.9	1.5 ± 1.1	
		Week 6	2.3 ± 0.6 *	1.3 ± 1.0	
		Change	0.8 ± 0.9	− 0.2 ± 1.5	0.126
		Week 12	2.5 ± 0.7 *	1.3 ± 1.3	
		Change	1.0 ± 0.9	− 0.3 ± 1.9	0.028 #
11	Fatigued	Baseline	1.8 ± 0.9	2.1 ± 1.0	
		Week 6	2.7 ± 0.8 *	2.0 ± 1.0	
		Change	0.8 ± 0.8	− 0.1 ± 1.4	0.073 ‡
		Week 12	2.7 ± 0.7 *	1.8 ± 0.6	
		Change	0.8 ± 0.9	− 0.3 ± 1.2	0.026 #
12	Exhausted	Baseline	1.1 ± 0.8	1.3 ± 1.3	
		Week 6	2.2 ± 0.7 **	1.5 ± 0.9	
		Change	1.1 ± 0.7	0.2 ± 1.3	0.069 ‡
		Week 12	2.3 ± 0.5 **	0.8 ± 0.8	
		Change	1.2 ± 0.6	− 0.5 ± 1.2	0.001 ##
13	Sluggish	Baseline	1.4 ± 0.7	1.2 ± 1.2	
		Week 6	1.9 ± 1.0	1.8 ± 1.1 †	
		Change	0.5 ± 1.1	0.6 ± 1.1	0.751
		Week 12	2.2 ± 0.9 *	1.2 ± 0.9	
		Change	0.8 ± 1.1	0.0 ± 1.4	0.194
14	Weary	Baseline	1.2 ± 0.8	0.8 ± 1.0	
		Week 6	1.6 ± 0.8	1.8 ± 1.0 *	
		Change	0.4 ± 1.2	1.1 ± 1.2	0.299
		Week 12	1.8 ± 0.8 †	1.1 ± 0.9	
		Change	0.6 ± 1.1	0.3 ± 1.3	0.583
15	Bushed	Baseline	1.5 ± 0.9	1.0 ± 1.3	
		Week 6	1.9 ± 0.9	1.3 ± 0.9	
		Change	0.4 ± 1.2	0.3 ± 1.8	0.817
		Week 12	2.3 ± 0.9 *	1.1 ± 0.9	
		Change	0.8 ± 0.9	0.1 ± 1.4	0.166

Scores are expressed as the mean ± SD.
† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.
‡ $p < 0.1$, # $p < 0.05$, ## $p < 0.01$ between-group differences in change from baseline.

Table 6 Changes in biochemical blood test ①

Item	Unit	Std. Value	Time points	Values	
				FO group (n = 12)	Placebo group (n = 12)
Total Bilirubin	mg/dL	0.2-1.2	Baseline	0.71±0.32	0.61±0.32
			Week 6	0.69±0.34	0.45±0.16 †
			Change	−0.02±0.24	−0.16±0.26
			Week 12	0.66±0.22	0.58±0.22
			Change	−0.05±0.22	−0.03±0.39
Total Protein	g/dL	6.5-8.3	Baseline	7.4±0.4	7.4±0.5
			Week 6	7.4±0.3	7.4±0.4
			Change	−0.0±0.3	0.0±0.3
			Week 12	7.7±0.3 †	7.5±0.4
			Change	0.2±0.4	0.1±0.4
Albumen	g/dl	3.8-5.3	Baseline	4.6±0.3	4.6±0.4
			Week 6	4.7±0.3 *	4.7±0.4
			Change	0.2±0.2	0.1±0.2
			Week 12	4.6±0.4	4.5±0.3 *
			Change	0.1±0.3	−0.1±0.2
AST (GOT)	U/L	8-38	Baseline	20.3±3.1	19.8±3.6
			Week 6	20.8±4.6	21.6±5.9
			Change	0.5±3.0	1.8±4.5
			Week 12	22.1±4.6 †	21.0±4.0 †
			Change	1.8±3.5	1.3±2.0
ALT (GPT)	U/L	4-43	Baseline	19.7±9.3	20.8±10.3
			Week 6	20.3±11.4	22.5±13.0
			Change	0.6±3.9	1.7±4.2
			Week 12	23.3±13.2 †	21.4±8.9
			Change	3.7±6.0	0.6±5.7
ALP	U/L	110-354	Baseline	231.3±61.2	231.2±69.6
			Week 6	223.2±54.4	219.8±53.1
			Change	−8.1±24.7	−11.4±38.1
			Week 12	234.0±42.1	212.5±56.1 †
			Change	2.8±45.9	−18.7±34.2
LD (LDH)	U/L	121-245	Baseline	180.2±17.9	187.3±19.3
			Week 6	168.0±19.0 *	181.0±17.7
			Change	−12.2±15.5	−6.3±17.2
			Week 12	176.9±22.5	173.3±18.3 **
			Change	−3.3±17.8	−14.1±10.1 ‡
γ-GT (γ GTP)	U/L	86 and under	Baseline	42.0±48.7	37.2±40.9
			Week 6	43.9±47.2	42.4±45.8 *
			Change	1.9±6.5	5.3±6.3
			Week 12	50.9±51.8 †	41.8±43.9
			Change	8.9±15.5	4.7±10.8
CK (CPK)	U/L	38-196	Baseline	132.5±34.1	148.7±74.8
			Week 6	138.3±54.1	107.8±27.6
			Change	5.8±39.6	−40.8±85.9
			Week 12	146.3±80.8	138.8±73.4
			Change	13.8±73.3	−9.8±114.6
Total Cholesterol	mg/dL	130-219	Baseline	210.7±29.0	205.3±29.4
			Week 6	201.7±26.3	217.3±38.9
			Change	−9.0±29.4	12.0±24.0 ‡
			Week 12	217.6±29.8	214.7±39.9
			Change	6.9±39.9	9.4±26.3

Values are expressed as the mean ± SD.
† $p < 0.1$, * $p < 0.05$ against baseline.
‡ $p < 0.1$ between-group differences in change from baseline.

Table 6　Changes in biochemical blood test ②

Item	Unit	Std. Value	Time points	Values	
				FO group (n = 12)	Placebo group (n = 12)
Neutral Fat (TG)	mg/dL	30-149	Baseline	161.3±75.8	152.7±105.2
			Week 6	178.8±136.0	235.8±213.0
			Change	17.4±112.5	83.2±177.4
			Week 12	179.2±101.8	149.4±63.1
			Change	17.8±91.2	−3.3±98.6
Sodium	mEq/L	135-150	Baseline	144.3±1.7	144.1±1.8
			Week 6	143.7±1.8	143.5±1.4
			Change	−0.6±1.7	−0.6±2.1
			Week 12	144.0±2.0	143.9±1.7
			Change	−0.3±2.2	−0.2±2.1
Chloride	mEq/L	98-110	Baseline	104.8±2.3	105.0±2.2
			Week 6	104.5±1.0	103.3±2.1 **
			Change	−0.3±2.3	−1.8±1.9 ‡
			Week 12	103.5±1.9 *	103.4±1.1 *
			Change	−1.3±1.9	−1.6±1.9
Potassium	mEq/L	3.5-5.3	Baseline	3.7±0.2	3.7±0.2
			Week 6	4.1±0.4 **	4.0±0.3 **
			Change	0.3±0.3	0.3±0.2
			Week 12	4.0±0.2 **	4.0±0.4 *
			Change	0.3±0.2	0.3±0.3
Calcium	mg/dL	8.4-10.2	Baseline	9.8±0.3	9.9±0.4
			Week 6	9.7±0.3	9.9±0.3
			Change	0.0±0.2	0.0±0.3
			Week 12	10.0±0.4	9.9±0.3
			Change	0.2±0.5	0.0±0.4
Inorganic Phosphorus	mg/dL	2.5-4.5	Baseline	3.6±0.8	3.7±0.5
			Week 6	3.1±0.5 *	3.3±0.4 *
			Change	−0.5±0.7	−0.4±0.5
			Week 12	3.3±0.4	3.6±0.4
			Change	−0.2±0.8	−0.1±0.5
Urea Nitrogen	mg/dL	8.0-22.0	Baseline	13.9±3.0	15.1±1.9
			Week 6	13.9±2.4	14.4±2.2
			Change	0.1±2.9	−0.7±1.9
			Week 12	14.8±3.5 †	14.7±2.1
			Change	0.9±1.7	−0.4±2.3
Creatinine	mg/dL	0.61-1.04	Baseline	0.90±0.13	0.89±0.15
			Week 6	0.83±0.13 **	0.82±0.13 **
			Change	−0.07±0.07	−0.07±0.07
			Week 12	0.87±0.13	0.87±0.14
			Change	−0.03±0.06	−0.02±0.06
Blood Sugar (Serum)	mg/dL	60-109	Baseline	61.5±7.7	71.3±24.4
			Week 6	58.3±13.1	64.0±11.9
			Change	−3.2±12.1	−7.3±15.1
			Week 12	73.0±19.7 *	66.5±14.2
			Change	11.5±17.9	−4.8±15.8 #

Values are expressed as the mean ± SD.
† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.
‡ $p < 0.1$, # $p < 0.05$ between-group differences in change from baseline.

Table 7 Transition of Urinalysis

Item	Unit	Std. Value	Time points	Value	
				FO group (n = 12)	Placebo group (n = 12)
Specific Gravity	mg/dL	1.010-1.025	Baseline	1.021±0.006	1.023±0.007
			Week 6	1.018±0.007 †	1.020±0.006
			Change	−0.003±0.005	−0.002±0.008
			Week 12	1.022±0.005	1.019±0.005 †
			Change	0.001±0.006	−0.003±0.006 ‡
pH	g/dL	4.5-8.0	Baseline	5.6±0.6	5.7±0.4
			Week 6	6.0±0.6 †	6.0±0.7
			Change	0.3±0.5	0.3±0.8
			Week 12	6.3±0.9 *	6.4±1.0 *
			Change	0.6±0.7	0.7±1.0

Values are expressed as the mean ± SD.

† $p < 0.1$, * $p < 0.05$ against baseline.

‡ $p < 0.1$ between-group differences in change from baseline.

ingestion of the FO.

And in this study, it was observed that based upon clinical findings such as blood test and urine test, no abnormal change was triggered by ingestion of the test product. In the blood test, significant difference was observed in the changes of Chloride, Potassium and Blood Sugar (Serum) of the FO group after 12-week ingestion. The same difference was observed with urine PH. In either case, since the difference was minor one, the investigator judged it as the range of physiological variation (or clinically safe).

During the test period seven subjects discontinued the test. Six of them stopped the test because of their personal reason such as impossibility of continuing the test due to their business. As for the remaining one subject, on the other hand, the measurement after 6-week ingestion showed an abnormal value of potassium, but after the medical interview the doctor determined the abnormity was due to the backache and ruled out any relationship with the test itself. Therefore, based upon the medical interview, blood test and urine test, we observed no harmful influence against biochemical and/or physiological matters of the subjects which seem to have a causal relationship with FO.

These results indicated the safety of the ingestion of the test product (FO) for the 12-week test period.

General Information

The male sexual function is a major factor of self-confidence and vigor since it relates to the sexual desire, which is one of three major desires. Therefore not only the serious ED patients but also those who are not suffering from ED desire to improve their sexual function. Also, there is an opinion that the loss of confidence toward their sexual function tends to decrease the frequency of sexual intercourse and this tendency is one of the causes of the decline in the birth rate[19]. If they are able to improve their sexual function by simple and convenient methods such as obtaining supplements instead of the prescription of medical drugs, they may regain their confidence, try to increase the frequency of sexual intercourse, and eventually build a good relationship with partners. The birth rate would also increase.

The FO we used in this study contains the amino acids, the foods such as chicken or Shijimi (freshwater clam) plentifully have. The use of this supplement enables to gain the amino acids such as arginine and ornithine without selectively having plenty of these foods, and it plays an important role in improving the quality of one's sex life.

Limitations

In order to evaluate male sexual function it is necessary to have both an erection (a condition) and sexual intercourse (an action), and for the evaluation of these condition and action the subjective questionnaire plays an important role since they are quite personal matters. Although this study used the questionnaire based on IIEF (which is internationally recognized), it is inevitable to have some errors.

Although we found an improvement effect of male sexual function attained by the ingestion of arginine and ornithine, there is also a report that points out arginine is not functional for the mixed-type impotence[20], and this indicates that depending on the person the targeted improvement by the supplement may not be achieved. In addition, we have not yet discovered the mutual influences among arginine, ornithine and the other ingredients contained in the FO, and this point should be further scrutinized.

Conclusion

We found out that the ingestion of the supplement FO containing arginine and ornithine by healthy Japanese

people for 12 weeks contributed to the improvement of male sexual function. In addition, no safety-related matter occurred during the 12-week test period.

CONFLICT OF INTEREST

Ryo Kohiyama is a principal of INSPIRE CO. LTD. All remaining authors have declared no conflicts of interest.

REFERENCES

1) NIH Consensus Development Panel on Impotence: Impotence. JAMA. 1993; **270**(1): 83-90.
2) Marui E. Epidemiology and risk factors for ED in Japan. J Clin Exp Med. 2002; **201**(6): 397-400.
3) Kamata N, Takahashi S. Epidemiology and risk factors for ED in Japan. J. Nihon Univ. Med. Ass. 2013; **72**(3): 142-5.
4) Shamloul R, Ghanem H. Erectile dysfunction. Lancet. 2013; **381**(9861): 153-65.
5) Chen L, Staubli SE, Schneider MP et al. Phosphodiesterase 5 inhibitors for the treatment of erectile dysfunction: a trade-off network meta-analysis. Eur Urol. 2015; **68**(4): 674-80.
6) Jabłecka A, Bogdański P, Balcer N et al. The effect of oral L-arginine supplementation on fasting glucose, HbA1c, nitric oxide and total antioxidant status in diabetic patients with atherosclerotic peripheral arterial disease of lower extremities. Eur Rev Med Pharmacol Sci. 2012; **16**(3): 342-50.
7) Dong JY, Qin LQ, Zhang Z et al. Effect of oral L-arginine supplementation on blood pressure: a meta-analysis of randomized, double-blind, placebo-controlled trials. Am Heart J. 2011; **162**(6): 959-65.
8) Chen J, Wollman Y, Chemichovsky T et al. Effect of oral administration of high-dose nitric oxide donor L-arginine in men with organic erectile dysfunction: results of a double-blind, randomized, placebo-controlled study. BJU Int. 1999; **83**(3): 269-73.
9) Sugino T, Shirai T, Kajimoto Y et al. L-ornithine supplementation attenuates physical fatigue in healthy volunteers by modulating lipid and amino acid metabolism. Nutr Res. 2008; **28**(11): 738-43.
10) Rosen RC, Riley A, Wagner G, et al. The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. Urology. 1997; **49**(6): 822-30.
11) McNair DM, Lorr M, Droppleman LF. EITS manual for profile of mood states. Educational and Industrial Testing Service. San Diego. 1971.
12) Momoi M, Sasaki F, Nibei F et al. Evaluation of Efficacy and Safety of the Mountain Ginseng on Male Sexual Function for 12-weeks Treatment. Jpn Pharmacol Ther. 2015; **43**(6): 859-67.
13) Marumo K, Nagatsuma K, Murai M. Effect of Aging and Diseases on Male Sexual Function Assessed by the Internationl Index of Erectile Function. Jpn J Urol. 1999; **90**(12): 911-9.
14) Nagano K. Cavernosal Anatomy and Mechanism of Erectile Function. J Smooth Muscle Res (Jpn.Sec). 2005; **9**: J37-J45.
15) Benet AE, Melman A. The epidemiology of erectile dysfunction. Urol Clin North Am. 1995; **22**(4): 699-709.
16) Huynh NT, Tayek JA. Oral arginine reduces systemic blood pressure in type 2 diabetes: its potential role in nitric oxide generation. J Am Coll Nutr. 2002; **21**(5): 422-7.
17) Shi HP, Fishel RS, Efron DT et al. Effect of supplemental ornithine on wound healing. J Surg Res. 2002; **106**(2): 299-302.
18) Elam RP, Hardin DH, Sutton RA, et al. Effects of arginine and ornithine on strength, lean body mass and urinary hydroxyproline in adult males. J Sports Med Phys Fitness. 1989; **29**(1): 52-6.
19) Momoi M,Sasaki F, Nibei F et al. Clinical Effects of the Mountain Ginseng on Male Sexual Function fo Short Term Treatment. Jpn Pharmacol Ther. 2015; **43**(6): 869-76.
20) Klotz T, Mathers MJ, Braun M, et al. Effectiveness of oral L-arginine in first-line treatment of erectile dysfunction in a controlled crossover study. Urol Int. 1999; **63**(4): 220-3.

Appendix 1. Questionnaire of sexual function

Question #1: Over the past 4 weeks, how many times have you attempted sexual Intercourse?
(0) No attempts
(1) 1-2 attempts
(2) 3-4 attempts
(3) 5-6 attempts
(4) 7-8 attempts
(5) 11 or more attempts
NOTE: similar to IIEF #6

Question #2: Over the past 4 weeks, when you attempted sexual intercourse, how often was it satisfactory for you?
(0) Did not attempt intercourse
(1) Almost never or never
(2) A few times (much less than half the time)
(3) Sometimes (about half the time)
(4) Most times (much more than half the time)
(5)Almost always or always
NOTE: similar to IIEF #7

Question #3: Over the past 4 weeks, how much have you enjoyed sexual intercourse?
(0) No intercourse
(1) Not enjoyable
(2) Not very enjoyable
(3) Fairly enjoyable
(4) Highly enjoyable
(5) Very highly enjoyable
NOTE: similar to IIEF #8

Question #4: Over the past 4 weeks, when you had sexual stimulation or intercourse how often did you ejaculate?
(0) Did not attempt intercourse
(1) Almost never or never
(2) A few times (much less than half the time)
(3) Sometimes (about half the time)
(4) Most times (more than half the time)
(5) Almost always or always
NOTE: similar to IIEF #9

Question #5: Over the past 4 weeks, when you had sexual stimulation or intercourse how often did you have the feeling of orgasm or climax?
(0) No sexual stimulation or intercourse
(1) Almost never or ever
(2) A few times (much less than half the time)
(3) Sometimes (about half the time)
(4) Most times (much more than half the time)
(5) Almost always or always
NOTE: similar to IIEF #10

Question #6: Over the past 4 weeks, how often have you felt sexual desire?
(0) Almost never or never
(1) A few times (much less than half the time)
(2) Sometimes (about half the time)
(3) Most times (much more than half the time)
(4) Almost always or always
※ Question #6 and #7 ask about sexual desire. Let's define sexual desire as a feeling that may include wanting to have a sexual experience (for example, masturbation or intercourse) thinking about having sex or feeling frustrated due to a lack of sex.
NOTE: similar to IIEF #11

Question #7: Over the past 4 weeks, how would you rate your level of sexual desire?
(0) Very low or none at all
(1) Low
(2) Moderate
(3) High
(4) Very high
NOTE: similar to IIEF #12

Question #8: Over the past 4weeks, how satisfied have you been with you overall sex life?
(0) Very dissatisfied
(1) Moderately dissatisfied
(2) About equally satisfied and dissatisfied
(3) Moderately satisfied
(4) Very satisfied
NOTE: similar to IIEF #13

Question #9: Over the past 4 weeks, how satisfied have you been with your sexual relationship with your partner?
(0) Very dissatisfied
(1) Moderately dissatisfied
(2) About equally satisfied and dissatisfied
(3) Moderately satisfied
(4) Very satisfied
NOTE: similar to IIEF #14

Question #10: Over the past 4 weeks how often have you experienced nocturnal penile tumescence?
(0) No attempts
(1) 1-2 attempts
(2) 3-4 attempts
(3) 5-6 attempts
(4) 7-8 attempts
(5) 11 or more attempts
NOTE: original

Appendix 2. POMS

Vigor
(1) Lively
(2) Active
(3) Energetic
(4) Cheerful
(5) Alert
(6) Full of pep
(7) Care free
(8) Vigorous

Fatigue
(1) Worn out
(2) Listless
(3) Fatigued
(4) Exhausted
(5) Sluggish
(6) Weary
(7) Bushed

The scores of answers are as follows:
Vigor
(0) Not at all
(1) A little
(2) Moderate
(3) Quite a bit
(4) Extremely

Fatigue
(0) Extremely
(1) Quite a bit
(2) Moderate
(3) A little
(4) Not at all

Appendix 3. Questionnaire for screening