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

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Abstract

Objectives: The objective of this research was to investigate the effectiveness of daily ingestion of a supplement, which contains arginine and citrulline on the male sexual function improvement.
Methods: In this randomized, placebo-controlled, double-blind trial, 49 subjects were enrolled. 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Results: 5 subjects were withdrawn. The withdrawal was due to personal reasons (4; occupational conflict, 1; a cold) .With 44 subjects, changes in scores of modified IIEF were significant in 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.
Conclusion: We found out that the ingestion of the supplement containing arginine and citrulline 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, citrulline, male sexual function, erectile dysfunction

1. INTRODUCTION

Currently in Japan, there are several countermeasures to the falling birth rate. Although it is difficult to indicate a specific cause of the falling birth rate since it involves a mix of factors, the infertility attributable to the decline in male sexual function is considered as one of the reasons. Now in Japan one out of six couples undergo some sort of treatment for infertility [1], and the survey conducted by WHO says that a half of reasons of the infertility relate to the male sexual function. The major reasons of the male infertility are such as Varicocele or Obstructive azoospermia: at the same time, Erectile Dysfunction (ED) is also considered as one of main reasons [2], and according to the statistics its frequency is reported as 15 to 30% which is definitely not low. 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" [3] (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 that one in four men aged 25 years old or older were reported to have an erectile dysfunction problem [4], and the number is expected to increase due to the country's aging population. It is commonly known that ED is brought on by various factors such as aging, psychological factors, adult lifestyle-related diseases and/or a medicinal agent [5].

In Japan the use of Viagra became operational in 1999 and now three kinds of phos-phodiesterase 5 inhibitor (PDE5I) are widely being used [6]. However, it is 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 [7]. In addition, since they are only available on prescription, purchasing them is somewhat a hurdle for the male who does not suffer from a serious symptom of ED. This fact accelerates the needs among men to improve their sexual function by taking the foods they eat regularly or the supplements. Based on these needs, many kinds of supplement are recently sold for men who want to improve their sexual function, and for these supplements, amino acids and/or herbs are

Table 1　Schedule for the study

Item \ Term	Screening	Pretrial 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

commonly contained as their main ingredients. Especially, amino acids have functions different from those of PDE5I, and they are popularly used since they are contained in the natural foods and therefore tend to be regarded as "safe". The types of amino acids widely used for these supplements are arginine, ornithine and/or citrulline. Arginine is a kind of non-essential amino acid contained in chicken, soybean or Koya-dofu (freeze-dried bean curd)[8)9)], 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[10)]. Ornithine is reported to be effective for fatigue recovery and growth promotion[11)]. Citrulline, on the other hand, is the free amino acid discovered from watermelon, and it is reported to have functions such as improving the function of blood vessels and recovering from fatigue[12)13)].

We previously studied the functions of arginine and ornithine to the male sexual function by using the supplement containing these two amino acids[14)]. On the other hand, although there are some reports that citrulline, which is said to have functionalities more similar to those of arginine compared to ornithine, is associated with the treatment of ED, there are only a few reports that scrutinize the relationship between the ingestion of citrulline and/or arginine, and its effects on the sexual function of Japanese male.

In this study, we examined the effect of the combination of arginine and citrulline 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 citrulline.

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 October 31st, 2015 to January 23rd, 2016.

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 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 20 and 69 years;
(2) Males who have a sexual decline;
(3) Males who have a sexual partner.
(4) Males who suitable for the sexual function questionnaire (**Appendix 1**).

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;

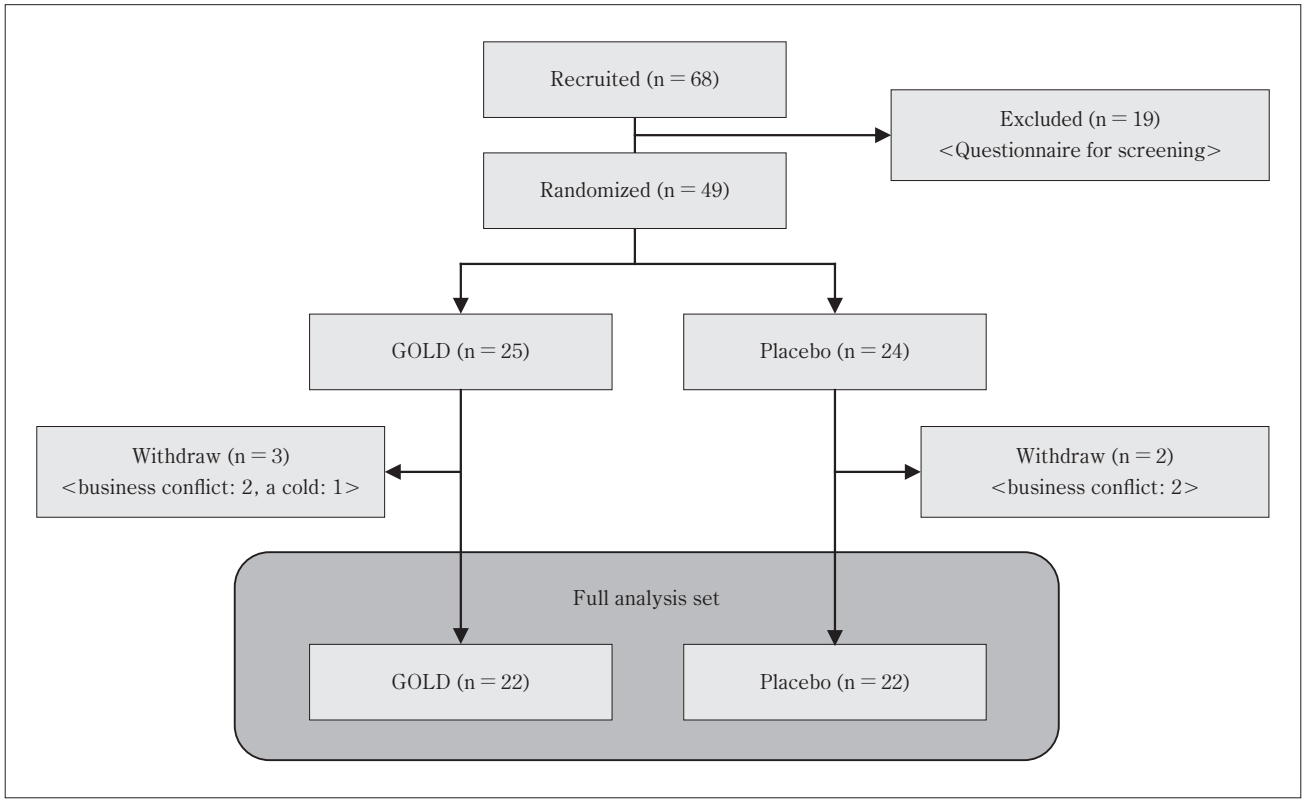

Fig. 1 Flow diagram of subject disposition

(5) Subjects who contract, or are under treatment for diseases of prostatic;
(6) Subjects who are judged as inappropriate for this study by the principle investigator.

2.3. Randomization

Recruited subjects were 68 persons. Subjects who fulfilled eligibility criteria were 49 persons. The inclusion criteria were judged by the principle investigator. All subjects were sequentially allocated to group T (Test sample: 25) and group P (Placebo: 24) using a randomized number table. In the process of subject assignment, background factors such as age were taken into consideration to avoid biased distribution.

2.4. Description of test foods and blinding

The test food, "The Gold Premium" ("GOLD") and placebo were prepared by INSPIRE CO. LTD. The amount of daily intake is 12 tablets (1 tablet weighs 330 mg, therefore 12 tablets weigh 3,960 mg). The compositions of GOLD were arginine, citrulline, 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 12 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 such as all the food items or number of steps they took for the day during the test period.

Table 2 Subject demographics

Item	Unit	GOLD	Placebo
Subjects	numbers	22	22
Age*	years	44.5 ± 9.1	44.5 ± 9.1

mean ± SD

* No significant difference

2.5.2. Outcome

The objective of this study is to verify the male sexual function improvement of ingesting food containing arginine and citrulline. To evaluate this objective, subjective reporting of sexual function was observed as the primary outcome. The questionnaire is based up on International Index of Erectile Function (IIEF) [15]. 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 2**.

Furthermore the blood flow velocity, testosterone level and Profile of Mood States ('POMS') [16] were measured as the secondary outcome. The blood flow velocity was

Table 3　Modified IIEF (questionnaire of sexual function)

	Questionnaire	Time points	Scores		P-value [2]
			GOLD (n = 22) [1]	Placebo (n=22) [1]	
1	Over the past 6 weeks, how many times have you attempted sexual intercourse?	Baseline	1.6 ± 1.0	1.8 ± 1.0	
		Week 6	2.1 ± 1.0 **	1.4 ± 0.9	
		⊿ 0–6 w	0.5 ± 0.6	− 0.4 ± 1.0	0.001 ##
		Week 12	2.4 ± 0.7 **	1.5 ± 1.0	
		⊿ 0–12 w	0.8 ± 0.8	− 0.3 ± 1.1	< 0.001 ##
2	Over the past 6 weeks, when you attempted sexual intercourse, how often was it satisfactory for you?	Baseline	2.3 ± 1.0	3.1 ± 1.0	
		Week 6	2.7 ± 0.8 **	3.0 ± 1.2	
		⊿ 0–6 w	0.5 ± 0.7	− 0.1 ± 1.0	0.030 #
		Week 12	3.2 ± 0.8 **	2.7 ± 1.4	
		⊿ 0–12 w	1.0 ± 1.0	− 0.4 ± 1.4	< 0.001 ##
3	Over the past 6 weeks, how much have you enjoyed sexual intercourse?	Baseline	2.3 ± 0.7	2.6 ± 0.9	
		Week 6	2.3 ± 1.0	2.6 ± 0.7	
		⊿ 0–6 w	0.0 ± 1.4	0.0 ± 0.8	0.892
		Week 12	2.9 ± 0.6 **	2.6 ± 1.1	
		⊿ 0–12 w	0.6 ± 0.7	0.0 ± 1.0	0.028 #
4	Over the past 6 weeks, when you had sexual stimulation or intercourse how often did you ejaculate?	Baseline	3.4 ± 1.5	4.0 ± 1.0	
		Week 6	3.9 ± 1.3 †	4.0 ± 1.2	
		⊿ 0–6 w	0.5 ± 1.5	0.0 ± 0.8	0.167
		Week 12	4.2 ± 0.9 **	3.7 ± 1.5	
		⊿ 0–12 w	0.8 ± 1.0	− 0.2 ± 1.5	0.011 #
5	Over the past 6 weeks, when you had sexual stimulation or intercourse how often did you have the feeling of orgasm or climax?	Baseline	2.9 ± 1.3	2.9 ± 1.1	
		Week 6	3.3 ± 0.9 *	3.1 ± 1.1	
		⊿ 0–6 w	0.4 ± 0.7	0.2 ± 0.9	0.575
		Week 12	3.5 ± 0.9 **	2.9 ± 1.2	
		⊿ 0–12 w	0.6 ± 0.7	0.0 ± 1.4	0.067 ‡
6	Over the past 6 weeks, how often have you felt sexual desire?	Baseline	2.7 ± 1.0	2.9 ± 1.0	
		Week 6	3.0 ± 0.9	3.1 ± 0.8	
		⊿ 0–6 w	0.2 ± 0.8	0.2 ± 0.8	1.000
		Week 12	3.1 ± 0.8 †	3.3 ± 1.0	
		⊿ 0–12 w	0.4 ± 0.8	0.4 ± 1.0	1.000
7	Over the past 6 weeks, how would you rate your level of sexual desire?	Baseline	2.6 ± 0.5	2.6 ± 0.7	
		Week 6	2.8 ± 0.7	2.9 ± 0.5 †	
		⊿ 0–6 w	0.1 ± 0.8	0.3 ± 0.7	0.561
		Week 12	2.9 ± 0.5 †	2.9 ± 0.7 †	
		⊿ 0–12 w	0.3 ± 0.7	0.2 ± 0.6	0.820
8	Over the past 6 weeks, how satisfied have you been with you overall sex life?	Baseline	2.6 ± 0.7	2.7 ± 0.8	
		Week 6	3.0 ± 0.7*	3.0 ± 0.9	
		⊿ 0–6 w	0.3 ± 0.6	0.3 ± 0.8	0.833
		Week 12	3.2 ± 0.8 **	3.1 ± 0.7 *	
		⊿ 0–12 w	0.6 ± 0.8	0.4 ± 0.9	0.484
9	Over the past 6 weeks, how satisfied have you been with your sexual relationship with your partner?	Baseline	3.0 ± 0.6	2.7 ± 0.8	
		Week 6	2.9 ± 0.7	3.0 ± 0.8 †	
		⊿ 0–6 w	− 0.1 ± 0.9	0.4 ± 0.9	0.071 ‡
		Week 12	3.4 ± 0.7 †	3.2 ± 0.7 *	
		⊿ 0–12 w	0.4 ± 1.0	0.5 ± 0.9	0.742
10	Over the past 6 weeks how often have you experienced nocturnal penile tumescence?	Baseline	2.2 ± 0.7	2.3 ± 0.8	
		Week 6	2.4 ± 0.6	2.3 ± 0.8	
		⊿ 0–6 w	0.2 ± 0.8	0.0 ± 0.8	0.584
		Week 12	2.7 ± 0.5 *	2.5 ± 0.9 †	
		⊿ 0–12 w	0.5 ± 0.9	0.3 ± 0.6	0.323

Scores are expressed as the mean ± SD.

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

2) ‡ $p < 0.1$, # $p < 0.05$, ## $p < 0.01$ between-group differences in change from baseline.

Table 4 Blood flow velocity

Item	Time points	Values		P-value [2]
		GOLD (n = 22) [1]	Placebo (n = 22) [1]	
Blood flow velocity (cm/s, KHz)	Baseline	4.9 ± 3.0	6.8 ± 3.6	
	Week 6	7.2 ± 3.9 **	5.8 ± 3.1	
	⊿ 0–6 w	2.3 ± 2.2	− 1.0 ± 2.8	< 0.001 ##
	Week 12	7.4 ± 3.8 **	4.5 ± 3.6 *	
	⊿ 0–12 w	2.5 ± 3.1	− 2.2 ± 3.9	< 0.001 ##
Testosterone (ng/mL)	Baseline	4.94 ± 1.56	4.89 ± 1.52	
	Week 6	5.01 ± 1.48	4.97 ± 1.50	
	⊿ 0–6 w	0.07 ± 1.21	0.08 ± 1.23	0.976
	Week 12	4.78 ± 1.67	4.70 ± 1.68	
	⊿ 0–12 w	− 0.16 ± 0.95	− 0.19 ± 1.29	0.926

Values are expressed as the mean ± SD.

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

2) ‡ $p < 0.1$, # $p < 0.05$, ## $p < 0.01$ between-group differences in change from baseline.

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 3**. 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

A full analysis set was adopted in the present study and no sample size design was used. All statistics were expressed as mean ± standard deviation (SD). For the modified IIEF, blood flow velocity, testosterone, the POMS, 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. Multiplicity according to the occasions was not adjusted. Any subjects with missing values were eliminated from the analysis. Statistical analyses were performed using Statcel 3 (Yanai, 2011). The results were considered significant at the < 5% level in the two-sided test.

3. RESULTS

3.1. Participant demographics

The 49 subjects were randomly assigned to intervention groups and made a start with ingestion. 5 were withdrawn due to personal reasons (4; occupational conflict, 1; a cold). Thus, data obtained with 44 subjects (GOLD; 22, Placebo; 22) 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. Modified IIEF (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 GOLD resulted in a significant increase in the score of #1, #2, #3, #4, #5, #8, and #10 after 12-week ingestion. For intergroup comparison, the score of #1 and #2 showed significant increase after 6-week ingestion, and the score of #19, #2, #3 and #4 showed significant increase after 12-week ingestion.

3.3. Blood flow velocity and Testosterone

Table 4 depicts changes in the blood flow velocity, testosterone level. In the blood flow velocity, significant upward changes were seen in the GOLD compared to the Placebo after 6-week and 12-week ingestion. There was no significant difference in testosterone.

3.4. POMS (vigor and fatigue)

Changes of scores were also evaluated in POMS (subscales of vigor and fatigue). The significant difference was found in the intragroup comparison with the GOLD of 1 item after 6-week ingestion, also 9 items after 12-week ingestion (**Table 5**). For the intergroup comparison, 1 item after 6-week ingestion and 3 items after 12-week ingestion revealed the significant difference.

3.5. Blood and urine test

Table 6 and **7** show the blood biochemical and urine parameters. A significant difference was observed in the changes of AST and LD (LDH) of the GOLD after 6 weeks of ingestion. There was no significant difference of the GOLD after 12 weeks of ingestion. In addition, no significant change or difference was shown in urine test. However, the investigator judged it as the range of

Table 5　POMS (vigor and fatigue)

Questionnaire		Time points	Scores		P-value [2]
			GOLD (n = 22) [1]	Placebo (n=22) [1]	
1	Vigor Lively	Baseline	2.1 ± 0.9	1.5 ± 0.9	
		Week 6	2.2 ± 0.9	2.0 ± 0.7 *	
		⊿ 0–6 w	0.1 ± 0.8	0.5 ± 0.9	0.204
		Week 12	2.0 ± 0.8	2.0 ± 0.7 *	
		⊿ 0–12 w	0.0 ± 0.7	0.5 ± 0.9	0.046 #
2	Active	Baseline	1.9 ± 0.8	2.4 ± 0.5	
		Week 6	2.1 ± 0.9	2.1 ± 0.8	
		⊿ 0–6 w	0.2 ± 0.9	− 0.3 ± 0.9	0.053 ‡
		Week 12	2.6 ± 0.7 **	2.0 ± 0.8 *	
		⊿ 0–12 w	0.7 ± 0.6	− 0.5 ± 0.9	< 0.001 ##
3	Energetic	Baseline	1.5 ± 0.5	1.8 ± 0.7	
		Week 6	1.8 ± 0.7 †	1.5 ± 0.7	
		⊿ 0–6 w	0.3 ± 0.7	− 0.3 ± 0.9	0.023 #
		Week 12	1.9 ± 0.6 **	1.8 ± 0.7	
		⊿ 0–12 w	0.5 ± 0.7	0.0 ± 0.8	0.082 ‡
4	Cheerful	Baseline	2.1 ± 0.9	1.6 ± 0.9	
		Week 6	2.3 ± 0.8	2.0 ± 0.7 *	
		⊿ 0–6 w	0.2 ± 0.8	0.5 ± 0.8	0.337
		Week 12	2.2 ± 0.8	2.2 ± 0.9 *	
		⊿ 0–12 w	0.1 ± 0.7	0.6 ± 1.1	0.077 ‡
5	Alert	Baseline	2.0 ± 0.7	1.2 ± 0.8	
		Week 6	2.3 ± 0.8	1.7 ± 0.8 **	
		⊿ 0–6 w	0.3 ± 0.8	0.5 ± 0.6	0.390
		Week 12	2.3 ± 0.8 †	2.0 ± 0.8 **	
		⊿ 0–12 w	0.3 ± 0.8	0.7 ± 0.8	0.087 ‡
6	Full of pep	Baseline	2.0 ± 0.8	1.9 ± 0.7	
		Week 6	2.2 ± 0.9	1.9 ± 0.6	
		⊿ 0–6 w	0.2 ± 0.8	0.0 ± 0.9	0.489
		Week 12	2.4 ± 0.9 **	1.8 ± 0.6	
		⊿ 0–12 w	0.4 ± 0.7	− 0.1 ± 0.9	0.045 #
7	Carefree	Baseline	1.6 ± 0.8	1.4 ± 0.8	
		Week 6	1.9 ± 1.1	1.9 ± 1.0 †	
		⊿ 0–6 w	0.3 ± 0.8	0.5 ± 1.1	0.517
		Week 12	1.9 ± 0.7 †	1.9 ± 0.9 *	
		⊿ 0–12 w	0.2 ± 0.5	0.5 ± 1.0	0.251
8	Vigorous	Baseline	1.9 ± 0.8	1.7 ± 0.8	
		Week 6	2.1 ± 0.9 †	1.8 ± 0.6	
		⊿ 0–6 w	0.2 ± 0.6	0.1 ± 0.7	0.490
		Week 12	2.1 ± 0.8 †	1.9 ± 0.8	
		⊿ 0–12 w	0.3 ± 0.6	0.2 ± 1.0	0.855
9	Fatigue worn out	Baseline	2.1 ± 1.0	1.8 ± 1.1	
		Week 6	1.9 ± 0.8 †	1.5 ± 1.1	
		⊿ 0–6 w	− 0.2 ± 0.8	− 0.3 ± 0.9	0.860
		Week 12	1.5 ± 1.1 **	1.4 ± 1.0	
		⊿ 0–12 w	− 0.6 ± 0.8	− 0.4 ± 1.2	0.383
10	Listless	Baseline	1.5 ± 1.1	1.6 ± 0.9	
		Week 6	1.4 ± 0.9	1.3 ± 0.9 †	
		⊿ 0–6 w	− 0.1 ± 0.7	− 0.4 ± 0.8	0.247
		Week 12	1.3 ± 0.9	1.3 ± 1.1 †	
		⊿ 0–12 w	− 0.1 ± 0.8	− 0.4 ± 0.8	0.358
11	Fatigued	Baseline	2.4 ± 1.0	2.2 ± 1.0	
		Week 6	2.0 ± 0.8 †	2.0 ± 0.8	
		⊿ 0–6 w	− 0.3 ± 0.9	− 0.2 ± 1.1	0.771
		Week 12	1.7 ± 0.9 **	1.8 ± 1.1 †	
		⊿ 0–12 w	− 0.7 ± 1.0	− 0.4 ± 1.0	0.285
12	Exhausted	Baseline	1.7 ± 1.2	1.5 ± 1.2	
		Week 6	1.5 ± 1.0 †	1.4 ± 0.9	
		⊿ 0–6 w	− 0.3 ± 0.8	− 0.1 ± 1.1	0.641
		Week 12	1.1 ± 0.9 **	1.2 ± 1.1	
		⊿ 0–12 w	− 0.6 ± 0.8	− 0.4 ± 1.0	0.410
13	Sluggish	Baseline	2.0 ± 1.0	1.8 ± 1.1	
		Week 6	1.5 ± 1.0 *	1.6 ± 0.9	
		⊿ 0–6 w	− 0.5 ± 0.9	− 0.2 ± 1.0	0.339
		Week 12	1.2 ± 0.9 **	1.3 ± 1.1 †	
		⊿ 0–12 w	− 0.7 ± 0.9	− 0.5 ± 1.1	0.381
14	Weary	Baseline	1.5 ± 1.2	1.5 ± 1.2	
		Week 6	1.1 ± 1.0 †	1.3 ± 0.8	
		⊿ 0–6 w	− 0.3 ± 1.0	− 0.3 ± 1.0	0.880
		Week 12	1.0 ± 1.0 *	1.1 ± 1.0 †	
		⊿ 0–12 w	− 0.4 ± 1.1	− 0.5 ± 1.1	0.889
15	Bushed	Baseline	1.6 ± 1.2	1.5 ± 1.2	
		Week 6	1.3 ± 1.1	1.1 ± 1.1	
		⊿ 0–6 w	− 0.3 ± 0.9	− 0.4 ± 1.2	0.673
		Week 12	1.2 ± 1.0 *	1.0 ± 1.1 †	
		⊿ 0–12 w	− 0.4 ± 1.1	− 0.5 ± 1.2	0.789

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

Table 6 Changes in biochemical blood test (1)

Item	Unit	Std. Value	Time points	Values	
				GOLD (n = 22)	Placebo (n=22)
Total Bilirubin	mg/dL	0.2-1.2	Baseline	0.65 ± 0.28	0.66 ± 0.25
			Week 6	0.72 ± 0.29	0.65 ± 0.21
			⊿ 0–6 w	0.07 ± 0.27	0.00 ± 0.22
			Week 12	0.69 ± 0.31	0.67 ± 0.27
			⊿ 0–12 w	0.04 ± 0.18	0.01 ± 0.23
Total Protein	g/dL	6.5-8.3	Baseline	7.5 ± 0.3	7.3 ± 0.4
			Week 6	7.4 ± 0.2	7.3 ± 0.4
			⊿ 0–6 w	− 0.1 ± 0.2	0.0 ± 0.3
			Week 12	7.4 ± 0.3	7.3 ± 0.3
			⊿ 0–12 w	− 0.0 ± 0.2	0.0 ± 0.3
Albumin	g/dl	3.8-5.3	Baseline	4.7 ± 0.3	4.7 ± 0.3
			Week 6	4.7 ± 0.2	4.6 ± 0.3 *
			⊿ 0–6 w	− 0.1 ± 0.2	− 0.1 ± 0.2
			Week 12	4.7 ± 0.3 †	4.6 ± 0.3 †
			⊿ 0–12 w	− 0.1 ± 0.2	− 0.1 ± 0.2
AST (GOT)	U/L	8-38	Baseline	22.1 ± 6.1	22.0 ± 5.6
			Week 6	24.5 ± 5.9 *	23.1 ± 6.0
			⊿ 0–6 w	2.4 ± 4.3	1.0 ± 3.5
			Week 12	22.8 ± 5.4	23.1 ± 6.9
			⊿ 0–12 w	0.7 ± 5.2	1.0 ± 4.3
ALT (GPT)	U/L	4-43	Baseline	24.8 ± 14.3	23.0 ± 13.4
			Week 6	27.6 ± 11.3 †	22.6 ± 11.9
			⊿ 0–6 w	2.8 ± 7.6	− 0.4 ± 5.2
			Week 12	25.5 ± 9.1	24.5 ± 14.9
			⊿ 0–12 w	0.7 ± 9.3	1.5 ± 7.9
ALP	U/L	110-354	Baseline	196.2 ± 31.3	239.9 ± 46.9
			Week 6	189.0 ± 25.8	226.6 ± 43.1**
			⊿ 0–6 w	− 7.2 ± 21.6	− 13.3 ± 20.0
			Week 12	197.1 ± 30.2	243.6 ± 56.6
			⊿ 0–12 w	0.9 ± 25.4	3.7 ± 39.4
LD (LDH)	U/L	121-245	Baseline	180.7 ± 29.9	175.2 ± 27.9
			Week 6	192.8 ± 25.1 *	181.8 ± 27.4
			⊿ 0–6 w	12.1 ± 23.5	6.5 ± 18.9
			Week 12	178.4 ± 27.3	176.5 ± 26.8
			⊿ 0–12 w	− 2.3 ± 21.9	1.3 ± 17.1
γ -GT (γ GTP)	U/L	86 and under	Baseline	48.8 ± 49.0	31.1 ± 17.4
			Week 6	49.0 ± 49.2	29.8 ± 13.9
			⊿ 0–6 w	0.1 ± 10.9	− 1.3 ± 7.2
			Week 12	47.0 ± 40.9	30.7 ± 14.8
			⊿ 0–12 w	− 1.8 ± 21.2	− 0.4 ± 12.4
CK (CPK)	U/L	38-196	Baseline	144.6 ± 100.0	133.7 ± 62.7
			Week 6	129.1 ± 70.3	140.9 ± 74.2
			⊿ 0–6 w	− 15.5 ± 49.5	7.2 ± 40.7
			Week 12	134.4 ± 80.6	139.5 ± 72.3
			⊿ 0–12 w	− 10.3 ± 49.1	5.8 ± 40.0
Total Cholesterol	mg/dL	130-219	Baseline	207.0 ± 31.1	200.8 ± 27.9
			Week 6	204.6 ± 41.7	199.5 ± 34.0
			⊿ 0–6 w	− 2.4 ± 32.4	− 1.3 ± 15.8
			Week 12	201.0 ± 37.8	200.9 ± 28.3
			⊿ 0–12 w	− 6.0 ± 34.7	0.0 ± 18.1

Values are expressed as the mean ± SD.

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

Table 6　Changes in biochemical blood test (2)

Item	Unit	Std. Value	Time points	Values	
				GOLD (n = 22)	Placebo (n = 22)
Neutral Fat (TG)	mg/dL	30-149	Baseline	175.9 ± 141.1	201.6 ± 157.1
			Week 6	147.5 ± 74.9	190.8 ± 142.7
			⊿ 0-6 w	− 28.4 ± 107.6	− 10.8 ± 83.1
			Week 12	147.0 ± 61.2	179.4 ± 139.5
			⊿ 0-12 w	− 29.0 ± 115.8	− 22.2 ± 102.1
Sodium	mEq/L	135-150	Baseline	143.1 ± 1.9	142.7 ± 1.6
			Week 6	143.0 ± 1.7	142.6 ± 1.4
			⊿ 0-6 w	− 0.1 ± 2.1	− 0.1 ± 1.4
			Week 12	143.4 ± 1.8	142.6 ± 1.4
			⊿ 0-12 w	0.2 ± 1.8	− 0.1 ± 1.9
Chloride	mEq/L	98-110	Baseline	102.8 ± 2.1	103.0 ± 1.8
			Week 6	102.9 ± 1.9	103.4 ± 2.0
			⊿ 0-6 w	0.1 ± 2.0	0.4 ± 1.6
			Week 12	103.2 ± 2.4	102.7 ± 2.1
			⊿ 0-12 w	0.5 ± 2.0	− 0.3 ± 2.0
Potassium	mEq/L	3.5-5.3	Baseline	4.0 ± 0.2	4.1 ± 0.2
			Week 6	4.2 ± 0.2 †	4.3 ± 0.3 **
			⊿ 0-6 w	0.2 ± 0.4	0.2 ± 0.3
			Week 12	4.0 ± 0.2	4.1 ± 0.3
			⊿ 0-12 w	− 0.1 ± 0.3	0.0 ± 0.3
Calcium	mg/dL	8.4-10.2	Baseline	9.7 ± 0.3	9.7 ± 0.3
			Week 6	9.7 ± 0.3	9.6 ± 0.3
			⊿ 0-6 w	0.0 ± 0.2	0.0 ± 0.3
			Week 12	9.8 ± 0.3 †	9.8 ± 0.3 *
			⊿ 0-12 w	0.1 ± 0.2	0.2 ± 0.3
Inorganic Phosphorus	mg/dL	2.5-4.5	Baseline	3.1 ± 0.4	3.2 ± 0.6
			Week 6	3.1 ± 0.5	3.0 ± 0.5 †
			⊿ 0-6 w	− 0.1 ± 0.5	− 0.2 ± 0.5
			Week 12	3.2 ± 0.4	3.2 ± 0.6
			⊿ 0-12 w	0.1 ± 0.4	0.0 ± 0.6
Urea Nitrogen	mg/dL	8.0-22.0	Baseline	13.8 ± 2.4	14.0 ± 2.9
			Week 6	14.0 ± 3.3	14.0 ± 2.9
			⊿ 0-6 w	0.2 ± 2.2	0.0 ± 3.3
			Week 12	14.3 ± 2.5	13.5 ± 3.2
			⊿ 0-12 w	0.5 ± 2.4	− 0.5 ± 2.9
Creatinine	mg/dL	0.61-1.04	Baseline	0.82 ± 0.13	0.83 ± 0.12
			Week 6	0.80 ± 0.14	0.81 ± 0.14
			⊿ 0-6 w	− 0.01 ± 0.07	− 0.01 ± 0.06
			Week 12	0.83 ± 0.12	0.83 ± 0.15
			⊿ 0-12 w	0.01 ± 0.07	0.00 ± 0.05
Blood Sugar (Serum)	mg/dL	60-109	Baseline	68.1 ± 10.9	67.9 ± 13.4
			Week 6	69.0 ± 16.6	72.2 ± 19.1
			⊿ 0-6 w	0.9 ± 16.6	4.4 ± 15.9
			Week 12	66.8 ± 14.9	67.5 ± 17.5
			⊿ 0-12 w	− 1.3 ± 14.1	− 0.3 ± 14.2

Values are expressed as the mean ± SD.

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

Table 7 Transition of urinalysis

Item	Unit	Std. Value	Time points	Values	
				GOLD (n = 22)	Placebo (n = 22)
Specific Gravity	−	1.010-1.025	Baseline	1.020 ± 0.007	1.019 ± 0.007
			Week 6	1.018 ± 0.006	1.018 ± 0.006
			⊿ 0–6 w	− 0.003 ± 0.008	− 0.001 ± 0.008
			Week 12	1.021 ± 0.007	1.018 ± 0.007
			⊿ 0–12 w	0.000 ± 0.008	− 0.002 ± 0.007
pH	−	4.5-8.0	Baseline	6.6 ± 0.6	6.5 ± 0.7
			Week 6	6.7 ± 0.8	6.3 ± 0.6
			⊿ 0–6 w	0.1 ± 0.8	− 0.3 ± 0.7
			Week 12	6.4 ± 0.7	6.4 ± 0.8
			⊿ 0–12 w	− 0.2 ± 0.8	− 0.1 ± 0.9

Values are expressed as the mean ± SD.

physiological variation (or clinically safe).

3.6. Adverse events

Any other adverse event was depicted with the test product in the course of the reporting.

4. DISCUSSIONS

We conducted a randomized, placebo-controlled, double-blind study for examining the efficacy of a supplement (GOLD) containing arginine and citrulline on the improvement of male sexual function. 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 for sexual function. 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 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 GOLD.

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). Among the GOLD, the 12-week ingestion of GOLD resulted in the significant differences in the score of #1, #2, #3, #4, #5, #8 and #10, and for intergroup comparison, the score of #1, #2 ,#3 and #4 showed significant differences. IIEF is categorized as "intercourse satisfaction" (#1, #2, #3), "orgasmic function" (#4, #5), "sexual desire" (#6, #7), "overall satisfaction" (#8, #9) and "erectile function" (#10), and the test results indicate that almost all of the categories of IIEF showed the significant difference of improvement after the 12-week ingestion of GOLD. In addition, the comparison between the GOLD and the Placebo indicates that there were significant differences in categories of "intercourse satisfaction". Based on the findings illustrated above, it can be said that from a standpoint of IIEF score, the ingestion of GOLD 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 also available for the examination of male sexual function of healthy people [17)18)]. 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 [19)20)]; 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 and citrulline contained in GOLD produce 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 [12)21)22)]. When ingested, both arginine and citrulline produce NO during their transformation and passing through urea cycle [23)]. Therefore it is considered that the simultaneous ingestion of both ingredients activated the urea cycle and increased the production of NO. The increase of the production of NO is thought to contribute to the increase of cGMP, the dilatation of blood vessels and the promotion of smooth erection (by the blood flow into the cavernous body), and

it can be said that this mechanism supports the test results of this study. 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]. In addition, since amino acids such as arginine or citrulline are principles of protein, they can contribute to the improvement of muscle strength and fatigue recovery, and can also affect the function of unstriated muscle when an erection occurs [24]. These functions are different from those of curative medicine for ED which has functions of inhibiting PDE5 that biodegrades cGMP and maintaining an erection. Therefore, GOLD 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 the medicine.

Secondary Findings

In this study we observed a significant difference of the blood flow velocity in the GOLD compared to the Placebo, after 6-week and 12-week ingestion. On the other hand, the score of the male hormone testosterone did not show a significant change. These results illustrate that while arginine and citrulline contained in GOLD contributed to the improvement of the blood flow velocity, these two ingredients, which function as the substances for secreting testosterone [25], were not sufficient enough to stimulate its secretion. The variation of the blood velocity 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. The results showed that in the GOLD 9 items (of the questionnaire) showed the significant differences, and the comparison between the GOLD and the Placebo indicated the significant differences on 3 items. Among the category of POMS, we mainly found the significant difference in "vigor-related" categories such as "vigor lively" and "active", and these results may indicate that the improvement of sexual function brought by the ingestion of GOLD also improved their level of QOL.

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 GOLD. In the blood test, although significant difference was observed in the changes of AST and LD (LDH), the difference was a minor one and the investigator judged it as the range of physiological variation. During the test period five subjects discontinued the test. Four of them stopped the test because of their personal reason such as impossibility of continuing the test due to their business, and the remaining one stopped due to his illness (catching a cold). Therefore, their discontinuance has nothing to do with the ingestion of GOLD and we could rule out any harmful influence against biochemical and/or physiological matters of the subjects. These results indicated the safety of the ingestion of GOLD for the 12-week test period.

General Information

There is an opinion that the loss of confidence among men 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 [26]. For men, the matter of the male sexual function (i.e. an erectile function) not only involves countermeasures to the falling birth rate in which the improvement of the male sexual function is considered as a key factor, but also relates to "three major desires (food appetite, sleep drive and sexual appetite)". The sexual appetite can be regarded as one of the important factors for pursuing QOL, since it significantly affects their own confidence and motivation, and their partnership as couples. For men, visiting a doctor for the prescription of the curative medicine for ED may involve a mental distress, since they have to confess an actual condition of their sexual function to the doctor (a third party). If they are able to build confidence toward their sexual function by simple and convenient methods without any side effects, such as obtaining supplements, it should become highly possible to facilitate the partnership in their daily sex life.

Limitations

In order to evaluate the 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 citrulline, there is also a report that points out arginine is not functional for the mixed-type impotence [27], 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, citrulline and the other ingredients contained in GOLD, and this point should be further scrutinized.

5. CONCLUSION

We found out that the ingestion of the supplement GOLD containing arginine and citrulline 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

All parts of this study were funded by INSPIRE CO. LTD. Ryo Kohiyama is the principal. All authors state that the study was conducted in the absence of any other relationships that could be interpreted as a conflict of interest.

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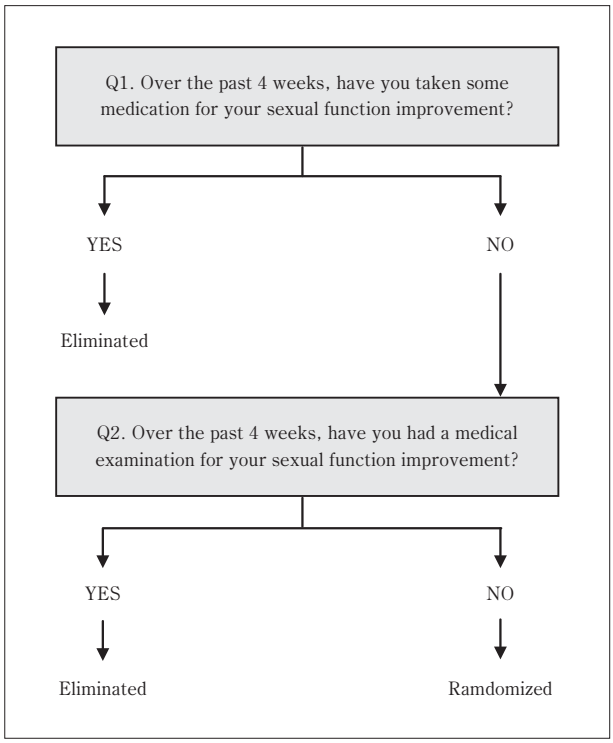
***Appendix 1*. Questionnaire for screening**



***Appendix 2*. 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 3*. 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