JOURNAL OF OLEO SCIENCE

J. Oleo Sci., Vol. **54**, No. 4, 217-224 (2005)

JOS

Efficacy of γ-linolenic Acid for Treatment of Premenstrual Syndrome, as Assessed by a Prospective Daily Rating System

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Edited by T. Itoh, Kitasato Univ., and accepted December 6, 2004 (received for review November 22, 2004)

Abstract: Evening primrose oil, which includes GLA, is one of the most popular of many treatments available for PMS. It has been reported that diagnosis of PMS on the basis of prospective daily rating is essential. However, few studies on alleviation by GLA of the symptoms of PMS have been performed using prospective daily rating. We examined the alleviating effect of GLA on symptoms of PMS diagnosed by prospective daily rating. Twenty-eight women diagnosed with PMS consumed vegetable oil containing about 180 mg/day of GLA for three luteal phases in a randomized, double-blind, parallel protocol, and were examined for duration and severity of PMS symptoms. Levels of DGLA in plasma phospholipid were significantly lower in women with PMS than in those without it (DGLA, $P < 0.01$) before treatment regardless of phase in menstrual cycle. After GLA administration, the levels of GLA and DGLA in plasma phospholipid in the GLA group were significantly higher than those both in the placebo group and before treatment. Improvement of the duration and severity of PMS symptoms as a whole, as well as that of irritability, was significantly more pronounced in the GLA than in the placebo group. These findings indicate that GLA can be effective for treating the symptoms of PMS, and that GLA and DGLA in plasma phospholipid may play a role in the onset of PMS.

Key words: γ-linolenic acid, premenstrual syndrome, prospective daily rating, plasma phospholipid, fatty acid

1 Introduction

Premenstrual syndrome (PMS) refers to a group of symptoms that appear periodically during the luteal phase of the menstrual cycle, and is common in adult

Abbreviations: PMS: premenstrual syndrome, GLA: γ-linolenic acid, DGLA: dihomo-γ-linolenic acid, PGE: prostaglandin E.

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women before menopause, reportedly affecting around 30-40% of women in the United States (1). Dalton reported that besides causing physical discomfort, PMS also has economic implications (2). No consensus yet exists concerning the etiology of this syndrome, although many hypotheses concerning it have been advanced (1). Many different treatments for PMS have correspondingly been suggested, including nutritional supplements such as vitamins (3), minerals (4) and GLA (5, 6), and medicines such as selective serotonin-reuptake inhibitors (SSRIs) (7), diuretics (8) and gonadotropin-releasing hormone (GnRH) agonists (9). Evening primrose oil (EPO), which contains GLA, is one of the most popular of many such treatments. GLA and DGLA are precursors of prostaglandin E1 (PGE1). The occurrence of psychological symptoms in PMS has been reported to result from imbalance between PGE1 and dopamine levels in brain (10). However, opinions have varied concerning the alleviating effects of GLA on the symptoms of PMS (11). The two studies by Collins *et al*. (12) and Khoo *et al*. (13) have been the best-controlled (11), and revealed no alleviation of the symptoms of PMS. It is essential to establish the diagnosis of PMS based on prospective daily records maintained by patients themselves (1, 14), since the accuracy of diagnosis on the basis of retrospective questionnaires may be limited by poor recall (15). Only the clinical study by Collins *et al*. (12) was conducted using a prospective daily rating system. Therefore, in the present studies we examined 44 healthy adult women for PMS by prospective daily rating and determined levels of fatty acids in plasma phospholipid (Study I), and investigated the alleviating effects of GLA administration in those subjects among the 44 tested in Study I who were diagnosed with PMS unclear (Study II).

2 Experimental

2·1 Subjects and Protocol

In Study I, PMS was diagnosed on the basis of prospective daily rating, measurement of fatty acid levels in plasma phospholipids and the investigation of daily dietary intake in female volunteers. The characteristics of the subjects in Study I are shown in **Table 1**. Forty-four healthy adult women (between 21 and 36 years of age) were asked to measure their basal body temperature daily over three menstrual cycles to determine their individual menstrual cycles. At the same time, they were instructed to record the contents of their daily diet for one week during the first luteal phase. The Cornell Medical Index (CMI) was used for evaluation of neuropathy, but none of the women were found to be in area IV. Blood samples were collected during the second follicular phase (7 ± 2 days after the start of menstruation) and the second luteal phase (7 ± 2 days before the expected start of the next menstruation).

The clinical trial in Study II was conducted to examine alleviation by GLA of the symptoms of PMS in 28 women with PMS who had participated in Study I. This clinical trial was conducted using a randomized, double-blind, placebo-controlled parallel design. The characteristics of the subjects in Study II are also shown in **Table 1**. As in Study I, the subjects were asked to record their basal body temperature and symptoms daily throughout three menstrual cycles, and to keep a diet diary for one week during the first luteal phase.

Table 1 Characteristics of Subjects in Study I and Study II.

Parameters	n (%)	Age (y)	Cycle length (d)	Luteal phase length (d)	BMI (kg/m^2)
Study I					
No PMS	6 (13.6 %)	32.6 ± 3.8	33.1 ± 5.5	12.9 ± 1.7	20.2 ± 2.2
PMS	38 (86.4 %)	28.5 ± 3.9	30.8 ± 4.1	12.6 ± 1.8	19.9 ± 1.7
Total	44 (100 %)	29.1 ± 4.1	31.2 ± 4.4	12.7 ± 1.8	20.0 ± 1.8
Study II					
Placebo group	14	28.8 ± 3.1	30.1 ± 2.6	12.7 ± 1.1	19.8 ± 2.1
GLA group	14	30.8 ± 4.2	32.2 ± 4.6	12.9 ± 1.8	20.1 ± 1.3

1) There were no significant differences between No PMS and PMS.

2) There were no significant differences between the Placebo and GLA groups.

The subjects were allocated randomly to either of two groups. The subjects in the GLA group were instructed to take capsules of edible oil containing GLA (180 mg/day) orally during the period corresponding to their individual luteal phases (12.8 ± 2 days). In the placebo group, edible oil not containing GLA was used. Blood samples were collected in each cycle on day 7 ± 2 after the start of GLA administration. To assess alleviation by GLA of the symptoms of PMS, the differences in duration and severity of symptoms between Study I and Study II were calculated for individual subjects, and the GLA and placebo groups were compared. The studies were carried out in accordance with the Helsinki declaration of 1964 (as revised in 2000) and were approved by the Ethics Committee of Ochanomizu University prior to their start. The procedures used were explained in detail to all volunteers in advance. Signed written informed consent was obtained from all volunteers prior to their participation.

2·2 Dietary Records and Diagnosis of PMS

The prospective daily rating method was used to diagnose PMS, in which subjects were requested to maintain a daily record according to the Utah PMS Calendar (16). The severity of symptoms was scored from 0 to 3. Those symptoms that appeared periodically during the luteal period, as noted in the daily records, were considered to be symptoms of PMS. To avoid confounding with menstrual symptoms, those symptoms that appeared during the luteal phase but became more severe during the menstrual period were excluded from analysis. The subjects were asked to keep a record of all they ate every day during the period indicated above and were thoroughly instructed regarding dietary records before the start of the study. Dietary records were collected and analyzed by a dietitian on the basis of the 5th Revision of the Standard Tables of Food Composition in Japan.

2·3 Analysis of Test Oils and Plasma Phospholipids

The fatty acid composition in each oil was determined by a gas-liquid chromatography system with a capillary column. The fatty acid composition of each oil is shown in **Table 2**. Plasma lipids were extracted from 500 μg of plasma according to the method of Folch *et al*. (17). Plasma phospholipid levels were determined by TLC, and the fatty acids in plasma phospholipid were identified by capillary GLC (18).

2·4 Statistical Analysis

Results are presented as mean value ± SD. The significance of differences between the two groups was evaluated by the Mann-Whitney U test or Wilcoxon rank-sum test. Statistical analysis was performed with SPSS for Windows (version 10.0j; SPSS Japan Inc., Tokyo, Japan). *P* values less than 0.05 were considered to indicate statistical significance.

3 Results

3·1 Study I

Thirty-eight of the 44 healthy adult women volunteers were diagnosed with PMS. As shown in **Table 3**, the levels of stearic acid, oleic acid and DGLA in phospholipid extracted plasma during the follicular and luteal phases were significantly lower in women with PMS than in those without it. The levels of palmitic acid and GLA in plasma phospholipid during the follicular phase were significantly lower in women with PMS than in those without it, and lower than during the luteal phase. The phospholipid levels in plasma were

Table 2 Fatty Acid Compositions of Test Oils.

Fatty acid (%)	Placebo	GLA
8：0	0.8	8.8
10：0	0.6	3.6
12：0	0.3	0.4
14：0	0.7	0.2
16：0	19.3	8.6
16：1	0.6	0.1
18：0	2.7	3.2
18：1	23.6	14.6
18：2	49.9	33.2
18：3 (n-6)	0.0	19.0
18：3 (n-3)	0.7	0.2
20：0	0.4	0.2
20：1	0.2	3.8
20：2	0.0	0.2
22：0	0.2	0.1
22：1	0.0	2.3
24：0	0.0	0.0
24：1	0.0	1.5
Total (%)	100.0	100.0

Table 3 Levels of Fatty Acids in Plasma Phospholipid during the Follicular and Luteal Phases (Study I).

Fatty acids (μg/g)	Follicular phase		Luteal phase	
	No PMS (n = 6)	PMS (n = 38)	No PMS (n = 6)	PMS (n = 38)
16：0	259.8 ± 16.7	224.6 ± 35.3 †*	245.7 ± 50.2	210.3 ± 38.3
18：0	145.7 ± 12.3	119.5 ± 20.1 ††**	135.2 ± 24.1	110.2 ± 21.8*
18：1	121.7 ± 12.0 †	95.2 ± 19.4 ††**	105.0 ± 18.8	87.8 ± 17.1*
18：2 (n-6)	217.9 ± 34.6 †	186.9 ± 32.8 ††	201.6 ± 39.7	172.3 ± 31.0
18：3 (n-6)	0.9 ± 0.7	0.3 ± 0.7 †*	0.3 ± 0.4	0.1 ± 0.4
20：3 (n-6)	23.6 ± 3.6	13.7 ± 3.7**	22.1 ± 5.2	13.2 ± 4.0**
20：4 (n-6)	86.7 ± 23.3	69.0 ± 12.9	80.6 ± 16.6	70.7 ± 14.5
18：3 (n-3)	2.9 ± 1.3	2.1 ± 0.8	2.2 ± 0.7	1.8 ± 0.5
20：5 (n-3)	16.1 ± 6.9	17.6 ± 10.5	20.4 ± 10.8	18.5 ± 9.7
22：5 (n-3)	9.9 ± 2.2	8.5 ± 2.6	8.6 ± 1.6	8.1 ± 1.7
22：6 (n-3)	59.7 ± 11.8	51.8 ± 14.8	58.5 ± 18.5	51.2 ± 13.0

1) Vales are presented as the mean ± SD and mean contents per plasma during the second cycle in Study I.
2) † $P < 0.05$, significantly different from the luteal phase by Wilcoxon rank-sum test.
3) †† $P < 0.01$, significantly different from the luteal phase by Wilcoxon rank-sum test.
4) * $P < 0.05$, significantly different from No PMS by Mann-Whitney U-test.
5) ** $P < 0.01$, significantly different from No PMS by Mann-Whitney U-test.

significantly also lower in women with PMS than in those without it, regardless of phase in menstrual cycle. The lower level of total cholesterol in women with PMS was significantly lower than in those without it (data not shown). The dietary survey revealed no significant difference between women with and without PMS in energy, fat, protein, carbohydrate, vitamins or mineral consumption in Study I (**Table 4**). The subjects with PMS reported a variety of symptoms, 38 in all. Irritability was the most common symptom, followed in order by swelling of the breasts, drowsiness, increased appetite and facial eruption (**Table 5**). Symptomatic severity was highest for increased appetite, followed in order by drowsiness, edema, swelling of the breasts and constipation (**Table 5**).

3·2 Study II

Study II revealed progressive increases in the levels of GLA and DGLA in plasma phospholipid in the GLA group. Significant differences from the placebo group in these levels were confirmed during the third cycle after the start of GLA administration (**Table 6**). Improvement of the duration and severity of PMS symptoms overall, (physical, mental, and social), as well as that of irritability, became greater in the GLA group than in the placebo group during the third cycle after the start of GLA administration ($P < 0.05$, **Table 7**).

4 Discussion

The objective of this study was to investigate the alleviation by GLA of symptoms of PMS diagnosed on the basis of prospective daily rating. Improvement of the duration and severity of PMS symptoms as a whole (physical, mental, and social), as well as that of irritability, became greater in the GLA group than in the placebo group during the third cycle after the start of GLA administration ($P < 0.05$, **Table 7**). Additionally, the levels of stearic acid, oleic acid and DGLA in plasma phospholipid were significantly lower in the women with PMS than in those without it, regardless of phase in the menstrual cycle. Administration of GLA yielded progressive increases in the levels of GLA and DGLA in plasma phospholipid compared with before treatment. Significant differences from the placebo group in these levels were confirmed during the third cycle after the start of GLA administration (**Table 6**).

Dietary supplements that have been evaluated in women with PMS include vitamins (3), calcium, mag-

Table 4 Nutrient Intake Calculated Based on Dietary Records.

Nutrients	Before treatment		During treatment	
	Placebo group	GLA group	Placebo group	GLA group
Energy	1893 ± 322	1809 ± 282	1827 ± 283	1745 ± 341
Protein	69.0 ± 11.4	69.3 ± 12.5	67.0 ± 10.5	65.0 ± 12.1
Fat	63.2 ± 14.9	59.8 ± 12.0	63.1 ± 15.0	58.3 ± 13.4
Carbohydrate	246.3 ± 50.2	233.4 ± 44.9	237.5 ± 43.4	224.9 ± 47.2
Ash	17.0 ± 3.3	16.3 ± 3.4	17.2 ± 3.1	15.9 ± 3.1
Total fatty acids	50.7 ± 11.8	48.4 ± 9.7	51.3 ± 12.4	47.4 ± 10.5
SFA	16.9 ± 6.3	17.3 ± 4.4	18.0 ± 5.9	16.3 ± 4.5
MFA	19.7 ± 4.1	18.5 ± 3.6	19.7 ± 4.8	18.8 ± 4.6
PUFA	14.1 ± 2.9	12.6 ± 2.5	13.6 ± 2.8	12.3 ± 2.7
UFA	33.8 ± 6.5	31.1 ± 5.7	33.3 ± 7.0	31.1 ± 6.8
n-6 Fatty acids	11.4 ± 2.5	10.3 ± 2.2	10.8 ± 2.5	9.9 ± 2.3
n-3 Fatty acids	2.7 ± 0.6	2.3 ± 0.5	2.8 ± 0.4	2.4 ± 0.7

1) There were no significant differences among groups.

2) Before treatment: mean intake for one week during the first luteal phase in Study I,
During treatment: mean intake for one week during the first luteal phase in Study II.

3) Unit of energy: kcal/day, other components: g/day.

Table 5 Incidence and Severity of PMS Symptoms (Study I).

Incidence (%)		Severity (score)	
Irritability	43.1	Increased appetite	7.3
Swelling of the breasts	29.5	Drowsiness	6.3
Drowsiness	27.3	Edema	5.6
Increased appetite	25.0	Swelling of the breasts	5.2
Facial eruption	22.7	Constipation	5.0

Scores are means for 3 cycles.

nesium (4), multivitamin/mineral supplements and EPO containing GLA (12-13). There have been surprisingly few clinical trials investigating the effect of GLA on PMS. The only two such clinical studies, by Collins *et al*. (12) and Khoo *et al*. (13), were each conducted with a well-controlled design and failed to reveal any beneficial effects of GLA. Two factors may explain the alleviating effect of GLA in the present study: diagnosis based prospective daily rating, and the proportion of subjects with dysmenorrhea. In making the diagnosis of PMS, it is important to use prospective daily records kept in diaries, since retrospective questionnaires may be limited by poor recall (14,15). The diagnosis of PMS by prospective daily rating was not used in the clinical study performed by Khoo *et al*. (13). Only in the study by Collins *et al*. (12), which failed to demonstrate the effectiveness of GLA (540 mg/day), was the diagnosis of PMS based on a prospective daily rating system. In that study, a large proportion (70%) of subjects suffered from dysmenorrhea. In contrast, only a small proportion of subjects (20%) in our study had dysmenorrhea. Although PMS and dysmenorrhea are two different entities, the severity of dysmenorrhea may influence the symptomatic severity of PMS (19). Women with dysmenorrhea may also suffer psychological and social symptoms during the perimenstrual phases. The difference in the results between our study and that of Collins et al might therefore be related to the difference between them in incidence of dysmenorrhea in study subjects. However, it did not appear that diagnosis of PMS by prospective daily rating and the lower proportion of subjects with dysmenorrhea in our study clearly enhanced the alleviating effect of GLA. It seems the accuracy of a statistical analysis has been risen more by

Table 6 Levels of Fatty Acids in Plasma Phospholipid (Study II).

Fatty acids (μg/g)	Placebo (n = 14)		GLA (n = 14)	
	Before treatment	After treatment	Before treatment	After treatment
16：0	222.1 ± 36.4	209.8 ± 36.5 †	235.3 ± 33.9	226.6 ± 36.3
18：0	116.1 ± 18.1	108.5 ± 17.9 †	113.8 ± 19.4	111.5 ± 18.9
18：1	87.8 ± 17.0	87.3 ± 20.0	92.9 ± 18.2	88.3 ± 12.4
18：2 (n-6)	160.1 ± 21.2	177.1 ± 31.4 ††	180.4 ± 33.0	178.4 ± 33.3
18：3 (n-6)	0.5 ± 0.6	0.7 ± 0.6	0.6 ± 0.5	1.4 ± 0.6 ††**
20：3 (n-6)	12.2 ± 3.1	12.3 ± 2.7	12.0 ± 3.8	15.1 ± 3.8 ††*
20：4 (n-6)	64.8 ± 14.5	65.8 ± 9.3	61.1 ± 10.0	68.0 ± 17.9
18：3 (n-3)	1.6 ± 0.4	1.8 ± 0.6	1.9 ± 0.6	1.6 ± 0.4
20：5 (n-3)	17.7 ± 8.9	15.6 ± 9.0	14.6 ± 10.8	19.1 ± 12.3
22：5 (n-3)	6.9 ± 1.1	7.1 ± 1.5	6.9 ± 1.8	7.8 ± 2.7
22：6 (n-3)	44.7 ± 9.7	46.0 ± 11.0	43.8 ± 11.9	48.6 ± 17.0

1) Values are presented as the mean ± SD and mean contents per plasma during the third luteal phase.
2) †: $P < 0.05$, significantly different from before treatment by Wilcoxon rank-sum test.
3) ††: $P < 0.01$, significantly different from before treatment by Wilcoxon rank-sum test.
4) *: $P < 0.05$, significantly different from the placebo group by Mann-Whitney U-test.
5) **: $P < 0.01$, significantly different from the placebo group by Mann-Whitney U-test.

Table 7 Efficacy of GLA for Treatment of PMS Symptoms Overall and Irritability in the Clinical Study (Study II).

	Symptoms of overall			Irritability	
	n	Δ Duration	Δ Severity	n	Δ Duration	Δ Severity
GLA	14	−1.4 ± 1.6*	−1.5 ± 1.3	6	−2.5 ± 3.9*	−3.3 ± 5.3*
Placebo	14	−0.1 ± 1.7	−0.1 ± 2.4	11	1.7 ± 2.4	2.6 ± 5.1

1) Values are presented as the mean ± SD.
2) Differences in duration and severity of symptoms between Study I and Study II were calculated for individual subjects.
3) *: $P < 0.05$, significantly different from the placebo group by Mann-Whitney U-test.

the difference of protocols.

Horrobin *et al*. and other groups suggested (20-26) that elevation of prolactin level in blood may cause PMS, since many of the features of PMS are similar to the effects of injection of prolactin. However, it subsequently became apparent that most women with PMS do not have elevated prolactin levels. It is therefore highly unlikely that elevation of prolactin levels is a general cause of PMS. Horrobin et al. also found that one of the effects of prolactin was stimulation of the formation of PGE1 by mobilizing the PGE1 precursor DGLA (27). Brush *et al*. (28) reported that women suffering from PMS exhibit partial blockade of delta-6-desaturase enzyme activity, leading to abnormalities in plasma levels of n-6 essential fatty acids. It has been hypothesized that abnormal fatty acid metabolism can result in increased sensitivity to luteal phase prolactin and other hormones. As suggested by Horrobin (6) and Horrobin and Manku (29), it seems to us that bypassing delta-6-desaturase blockade by administration of GLA should facilitate normal biosynthesis of essential fatty acids. The dietary survey in Study I revealed no significant differences between women with and without PMS, and plasma levels of the essential fatty acids such as DGLA were lower in those with PMS than without it. Significant increases in plasma levels of GLA and

DGLA were found in Study II following GLA administration. The levels of phospholipids and total cholesterol in plasma were lower in women with PMS than in those without it. These findings suggest that abnormal lipid metabolism may play a role in the onset of PMS. In the present study, irritability, one of the most typical symptoms of PMS, was particularly reduced in women given GLA. The occurrence of psychological symptoms in PMS has been reported to result from impairment of balance between PGE1 and dopamine levels in the brain (10). Thus, GLA supplementation may help to improve this balance.

All of the studies performed to date on the effect of EPO treatment on PMS suffer from shortcomings. Open studies of PMS have been particularly unreliable, since participants were subject to placebo effect. The two particularly well-controlled studies (12-13) were small, and were performed using a method of diagnosis of PMS different from ours and with groups of subjects with different incidences of dysmenorrhea. Beneficial effects of GLA on PMS can therefore not be excluded. We believe that the present findings might provide the basis for larger-scale trials, and be useful for identifying the causes of PMS and for developing effective methods for preventing and treating it.

5 Conclusion

Levels of GLA and DGLA in plasma phospholipid were significantly lower in women with PMS than in those without it (GLA, $P < 0.05$, DGLA, $P < 0.01$). Daily intake of vegetable oil containing 180 mg GLA significantly alleviated the symptoms of PMS overall, as well as irritability, and elevated levels of GLA and DGLA in plasma phospholipid. These findings indicate that GLA can be effective for treating the symptoms of PMS, and that GLA and DGLA in plasma phospholipid may play a role in onset of PMS.

Acknowledgments

The authors thank all the volunteers, and also all those who supported and collaborated with us. We especially wish to thank K Kawase for guidance regarding psychological aspects of PMS, H Takeuchi for valuable advice concerning preparation of the manuscript and Y Hirasawa for enthusiastic cooperation in the field work. This study was funded by The Nisshin OilliO Group, Ltd.

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