Daily intake of *Lactobacillus gasseri* CP2305 ameliorates psychological premenstrual symptoms in young women: A randomized, double-blinded, placebo-controlled study

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ABSTRACT

Lactobacillus gasseri CP2305 (CP2305) ameliorates stress-induced symptoms in young adults. In this study, we evaluated the effects of CP2305 on the premenstrual symptoms of healthy young women. Fifty-six women ingested CP2305 or placebo tablets over the course of six menstrual cycles. The CP2305 group reported fewer premenstrual symptoms than the placebo group, particularly psychological symptoms, such as depressed mood and anxiety. Whereas water retention-related physical symptom scores, such as those for breast tenderness and swelling, were reduced in the placebo group, they remained unchanged in the CP2305 group. In addition, significant differences were observed in the changes from baseline levels of salivary estradiol and progesterone in the luteal phase between the two groups, resulting in sustained elevated levels of reproductive hormones in the CP2305 group. Therefore, daily intake of CP2305 tablets might improve the premenstrual psychological symptoms of healthy young women in association with changes in reproductive hormone levels.

1. Introduction

The gut–brain axis plays a crucial role in the maintenance of intestinal homeostasis and brain function through bidirectional communication. The gut microbiome modifies this communication system via immune, endocrine, and neural pathways (Muller et al., 2020; Obata & Pachnis, 2016; Rhee et al., 2009). Several lines of evidence suggest that the gut microbiome influences brain function by affecting mood, recognition, and behavior (Borre et al., 2014; Cryan & Dinan, 2012; Cryan et al., 2019). Recently, the gut microbiome has been recognized as an essential mediator in the gut–brain axis, leading to the concept of the "microbiota–gut–brain axis" (Dinan & Cryan, 2017).

Even healthy women of reproductive age may feel physiological and/or emotional discomfort in the weeks before menstruation. These symptoms generally disappear by four days after the start of the menstrual period. More than 90% of young women suffer from premenstrual symptoms to some degree with symptoms varying widely between individuals (Takeda, et al., 2006). When at least one symptom associated with economic or social dysfunction before menstruation is present for at least three consecutive menstrual cycles, it is defined as clinically significant premenstrual syndrome (PMS) (Yonkers & Simoni, 2018; Yonkers & Casper, 2019). Menstrual cycle-dependent physiological or psychological symptoms are related to periodic changes in the levels of the reproductive hormones estradiol (E2) and progesterone (P4), pituitary hormones (gonadotropins), prostaglandins, and neurotransmitters in the brain (Bäckström et al., 2011; Chan, et al., 2014; Lovick, 2013; Schmidt et al., 2017; Yonkers & Simoni, 2018). However, the causes of these symptoms are still not fully understood.

Psychological stress, including depressed mood, may affect the E2 and P4 levels through the hypothalamus–pituitary–gonadal (HPG) axis,

and consequently, influence the pathogenesis of PMS or premenstrual dysphoric disorder (Redei & Freeman, 1995; Yen et al., 2019). Gamma aminobutyric acid (GABA) neurons are distributed across many areas of the brain. This includes the hypothalamus, within which GABAergic signaling plays an important role in the neurobiological effects of stress via the control of hypothalamus–pituitary–adrenal (HPA) axis (Cullinan, et al., 2008; Marques de Souza & Franci, 2008; Sarkar et al., 2011), and the HPG axis that regulates E2 and P4 production (Watanabe et al., 2014). The cross talk between these axes may explain why stress susceptibility is sex-dependent (Melón & Maguire, 2016). Recently, growing evidence has suggested that the intestinal microbiota may also affect estrogen metabolism (Baker, et al., 2017; Chen & Madak-Erdogan, 2016), leading to subsequent downstream effects, such as cancers, metabolic syndrome, and disorders of the gut–brain axis (Baker et al., 2017).

Probiotics, defined as live microorganisms with health benefits upon ingestion, may improve a variety of physical and psychological functions, starting from the gut to the brain (Mörkl, et al., 2020), such as cognition (Rudzki et al., 2019), depression (Pinto-Sanchez et al., 2017), and sleep quality (Takada et al., 2016, 2017). In addition, a probiotic strain, *Lactobacillus rhamnosus*, alters emotional behavior through modulating the expression of GABA$_A$ and GABA$_B$ receptors in mice (Bravo et al., 2011).

Lactobacillus gasseri CP2305, originally isolated from the stool of a healthy volunteer, has exhibited stress-relieving activity (Sawada et al., 2016). Daily intake of CP2305 for four weeks ameliorated stress-associated symptoms in medical students participating in a cadaver dissection course (Sawada et al., 2017), and improved the clinical symptoms of patients with irritable bowel syndrome (Nobutani et al., 2017). Daily intake of a CP2305-containing beverage for 12 weeks reduced stress-associated mental and physical symptoms associated with the preparation for a national certification examination, attenuated stress-induced changes in salivary cortisol concentrations, and elevated the expression of stress-responsive microRNAs in the peripheral blood (Nishida et al., 2017b, 2019). These observations suggest that the administration of CP2305 may affect central nervous system functions and modulate the HPA axis, thereby making CP2305 a "psychobiotic". Based on these previous reports, and data suggesting that the gut microbiota and/or probiotics affects GABAergic signaling (Camille Melón & Maguire, 2016; Farzi et al., 2018), we hypothesized that CP2305 intake may ameliorate premenstrual symptoms, particularly psychological parameters, by modulating the HPA and HPG axes. Therefore, we designed this study to evaluate the effects of CP2305 intake on young women's premenstrual symptoms.

2. Materials and methods

2.1. Study design

The clinical trial was approved by the Institutional Review Board of Tokushima University Hospital and was conducted according to the ethical standards established in the 1964 Declaration of Helsinki. The study was designed as a double-blind, placebo-controlled, parallel-group clinical trial, and ran from July 2017 to May 2018 (Table 1). Written informed consent was obtained from all participants. This study was registered with the University Hospital Medical Information Network (UMIN) Clinical Trials Registry as "Research on the effect of a lactic acid bacterium preparation for health conditions of females" (UMIN000027302).

The sample size calculation was performed with G*Power 3.1 using the F-test (analysis of variance (ANOVA): Repeated measures, within-between interaction). Assuming an a priori effect size of 0.175, estimated from our previous study (partial eta-squared: 0.31–0.88), an α error probability of 0.05, and a power (1 − β error probability) of 0.80, we calculated a required total sample size of 50. Taking into consideration the long duration of the intervention (approximately six months of

Table 1
Experimental schedule.

	Time (menstrual cycles) and month (2017–2018)							
	Pre-2	Pre-1	1	2	3	4	5	6
Measurements	July–Aug.		Aug.–Oct.					Feb–Apr.
Tablet intake			←――――――――――――――――――――→					
Questionnaire	●	●	●	●	●	●	●	●
Weekly diary	←―――――――――――――――――――――――――――→							
Saliva sampling		●						●

Note: Subjects started taking tablets (placebo or CP2305) just after Pre-1 menstrual cycle. Saliva was collected at pre- and post-intervention periods (details are shown in Fig. S1).

continual, daily ingestion), we estimated a dropout rate of approximately 25%. After taking into account the 25% dropout rate, we increased the required sample size to 67 subjects in this study.

The participant inclusion criteria were as follows: (i) females aged 20–35 years, (ii) provided signed informed consent and agreed to participate in this study. The exclusion criteria were as follows: (i) currently on medication, (ii) menstrual cycle is generally of atypical length (shorter than 25 days or longer than 38 days), (iii) diagnosed with a mental disorder or scored above 60 on the self-rating depression scale (SDS) (Zung, 1965). The enrolled subjects were randomly allocated to either the CP2305 or placebo group with a stratified randomization for age. The participants were instructed to ingest two tablets (placebo or CP2305) once daily over the course of six menstrual cycles (average duration of intervention, 183 days). To assess compliance, the participants self-recorded their tablet intake. During the trial, the participants were asked not to consume fermented milks, foods containing live lactic acid bacteria, or other probiotic or prebiotic products.

2.2. Preparation of tablets containing CP2305

The CP2305-containing and placebo tablets were prepared using the same procedures and formula, except for the presence or absence of heat-inactivated, washed, and dried CP2305 (1×10^{10} bacterial cells per 2 tablets). The active tablet was composed of maltose, dextrin, starch, heat-inactivated lactic acid bacteria powder, and vegetable oil. The placebo tablet was similarly composed except that the lactic acid bacteria powder was replaced with dextrin. The formula was allergen-free. These products were prepared exclusively for this study and were provided by Asahi Calpis Wellness Co., Ltd. (Tokyo, Japan).

2.3. Questionnaires to assess mental and physical states

The physical and mental health of the participants was evaluated before administration (at the time of screening) using the following questionnaires. The Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983) consists of two parts (20 items in each) to assess the general tendency to be anxious as a personality trait (STAI trait) and the degree of anxiety at a particular moment as a situation-dependent state (STAI state). The 28-item General Health Questionnaire (GHQ-28) (Goldberg & Hillier, 1979) consists of 28 items regarding emotional distress to detect those at risk of developing psychiatric disorders. The SDS (Fukuda & Kobayashi, 1983; Zung, 1965) consists of 20 items that are short self-rating scales to assess the psychological and somatic symptoms of depression. The Chalder Fatigue Scale (CFS) (Chalder et al., 1993) consists of 14 items to measure the severity of tiredness. The Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) is a self-report questionnaire that assesses sleep quality over a 1-month time interval. Before and after the start of taking the tablets, premenstrual symptoms for every menstrual cycle were evaluated using

the premenstrual tension syndrome (PMTS)-visual analog scale (VAS) questionnaire (Steiner et al., 2011). The PMTS-VAS format mirrors the symptoms of the PMTS-OR (revised version of PMTS-O), which has been implemented in many PMS clinical studies (Steiner et al., 2011). The PMTS-VAS was shown to be sensitive to variation in the severity of premenstrual symptoms among the study participants (Steiner et al., 2011). Moreover, considering that the VAS style questionnaire is generally considered more sensitive to small changes compared to simple descriptive ordinal scales in which symptoms are rated, we chose the PMTS-VAS questionnaire for this study. An original questionnaire was also used to assess detailed luteal phase symptoms using a 100-mm VAS, ranging from 0 mm to 100 mm (see each item in Fig. 3, Table S3 or S5). The participants reported their symptoms by marking the VAS every menstrual cycle during the experimental period. Subjects were also requested to keep weekly diaries during the study to keep track of whether the tablets were taken as well as to report any physical symptoms or significant life events.

2.4. Measurements of salivary reproductive hormones, cortisol, and CgA

Saliva was collected every three days, during the pre-intervention period (one menstrual cycle before intervention; Fig. S1) and the period between the 5th and 6th menstruation from the start of the intervention, using a saliva collection aid (Salivette®, Sarstadt, Rommelsdorf, Germany). Each collection occurred for 2 min between 4 PM and 6 PM, to avoid diurnal fluctuations. Based on subjects' menstrual records, one sample was collected within 3–6 days after menstruation (follicular phase) and another was collected 3–6 days before menstruation (luteal phase; Fig. S1). The samples were then analyzed as follows. The concentrations of salivary P4 (Salivary Progesterone Enzyme Immunoassay kit; Salimetrics, Carlsbad, CA, USA), E2 (High sensitivity Salivary 17β-Estradiol Enzyme Immunoassay kit; Salimetrics, Carlsbad, CA, USA), cortisol (Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay kit; Salimetrics, Carlsbad, CA, USA), CgA (YK070 Human CgA EIA kit; Yanaihara Institute, Shizuoka, Japan), and total protein (Protein Quantification Kit-Wide Range; Dojindo, Kumamoto, Japan) were measured according to the manufacturers' instructions. The saliva samples were stored at −80 °C until analysis.

2.5. Outcome measures

The primary outcome was a change in the scale of the participants' psychological conditions during premenstrual period, which was evaluated using the PMTS-VAS questionnaire over time in the CP2305 group compared with the placebo group. The secondary outcomes were changes in the scale of the participants' physical conditions during the premenstrual period as assessed by an original VAS of luteal phase physical symptoms. In addition, changes in salivary hormone (cortisol, E2, and P4) and salivary chromogranin A (CgA) levels as the indicator for autonomic nervous activity, before and after administration in the CP2305 group, was compared to that of the placebo group.

2.6. Statistical analysis

The analysis was performed using a per-protocol analysis. Statistical analysis was performed with JMP v.13.0 (SAS Japan, Tokyo, Japan). The data are presented as the mean ± standard error of the mean (SEM). The changes in the questionnaire scores over time and the VAS scores were averaged for each menstrual cycle; the changes were analyzed using two-way ANOVA with repeated measures. Changes in the salivary marker levels were analyzed using Student's t-test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Inclusion of subjects

Eighty-three female students were recruited at Tokushima University (Tokushima, Japan). Written informed consent was obtained from each participant. Of the 83 subjects, 8 were excluded due to medication (n = 3) and abnormal length of menstrual cycle (n = 5). The remaining 75 subjects were randomly allocated to either the CP2305 (n = 37) or placebo group (n = 38) with a stratified randomization for age (Table S1). We excluded 19 subjects who failed to continue daily tablet ingestion, complete questionnaires, or provide salivary samples, with no statistical difference in drop-out numbers between the two groups (placebo, n = 7; CP2305, n = 12; χ2 test, p-value 0.5507). The reasons provided for discontinuing the trial were: (i) difficulty in taking tablets daily (placebo, n = 3; CP2305, n = 4), (ii) difficulty keeping a weekly diary (placebo, n = 2; CP2305, n = 4) and (iii) unknown (placebo, n = 2; CP2305, n = 4). However, no adverse events were observed in any of the subjects, including those that dropped out, throughout the course of the trial. The final sample population comprised 25 subjects in the CP2305 group and 31 in the placebo group (Fig. 1).

3.2. Participant demographics

Table 2 presents the background data for the 56 subjects at the time of screening for inclusion. There were no significant differences in age, body mass index (BMI), STAI state, STAI trait, or GHQ28, SDS, CFS, and PSQI global scores between the two groups. The mean scores for all questionnaires indicated similar scores to the averages reported in our previous clinical studies, as well as those of other studies on Japanese university students (Kato-Kataoka et al., 2016a, Kato-Kataoka et al., 2016b; Nishida et al., 2017b, 2019; Takada et al., 2016). The participant cohort in this study represented the average young woman with no psychiatric disorders or physical problems being treated with medical intervention. During the intervention period, subjects did not declare any unusual physical or life events that may affect their mental and/or physical conditions, in their weekly diary. The mean compliance rates were 89.7% for the placebo group and 92.7% for the CP2305 group (no significant difference by χ2 test).

3.3. Effects of CP2305 on physical and mental conditions

Fig. 2 shows the changes over time in 12 premenstrual symptoms, listed in the PMTS-VAS questionnaire (Steiner et al., 2011). Baseline scores for both groups are provided in Table S2. The CP2305 group scored significantly lower than the placebo group for the items "depressed mood," "anxious," "decreased interest in activities," "easy fatigability," and "sleep more". These data suggest that daily intake of CP2305 improved psychological and sleep-related premenstrual symptoms. However, CP2305 intake was not associated with alteration of the scores for "physical symptoms", whereas placebo intake decreased those scores.

3.4. Effects of CP2305 on fluid retention-related symptoms

As shown in Fig. 2L, CP2305 intake appeared to worsen physical symptoms. In the PMTS-VAS questionnaire, participants reported a complaint if they experienced at least one of the following physical symptoms: painful or tender breasts, swelling of the abdomen, abdominal pain, headache, joint pain, water retention, or gain of body weight. To investigate in detail which physical symptoms were affected by CP2305 intake, we generated an original questionnaire to assess general physical symptoms (Fig. 3 and Table S3). Among the items probed in the questionnaire, five physical symptoms were significantly affected by the intervention (Fig. 3A-E). The scores for "breast tenderness" and "swelling," which are related to fluid retention, did not differ from those
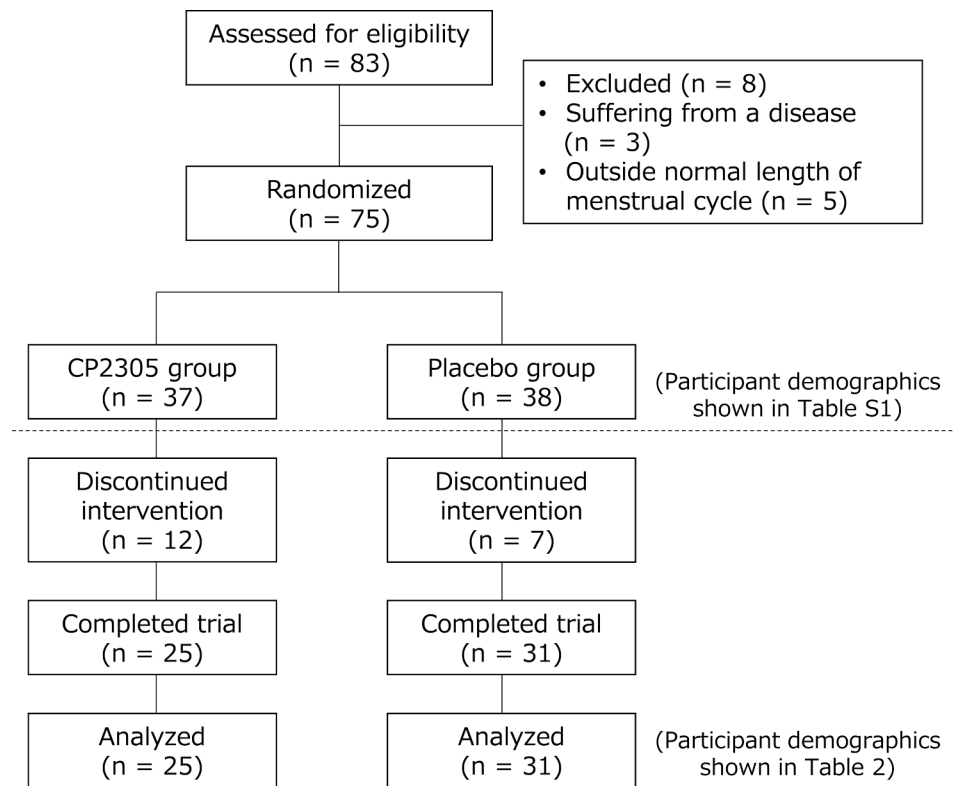
Fig. 1. Flow chart of the study design.

Table 2
Participant demographics after the completed trial.

Parameter	Placebo (n = 31)	CP2305 (n = 25)	p-value
Age (years)	21.5 ± 0.3	21.2 ± 0.3	0.459
BMI (kg/m^2)	20.3 ± 0.4	20.1 ± 0.4	0.624
Menarche (year)	12.1 ± 0.2	12.7 ± 0.2	0.067
Menstrual cycle (day)	31.1 ± 0.8	31.3 ± 0.8	0.846
Menstrual period (day)	5.9 ± 0.2	5.8 ± 0.2	0.830
Daily alcohol consumption	0/31	0/25	
Smoking	0/31	1/25	0.446
GHQ28 general	5.6 ± 0.7	6.9 ± 1.1	0.297
STAI state	43.0 ± 2.2	41.8 ± 2.2	0.685
STAI trait	45.2 ± 1.7	47.8 ± 2.0	0.321
SDS general	41.0 ± 0.9	42.0 ± 1.2	0.514
CFS general	16.7 ± 1.2	16.9 ± 1.6	0.930
PSQI global	4.0 ± 0.5	4.9 ± 0.7	0.264

The data are presented as mean ± SEM and they were analyzed with Student's t-tests. The χ2 test was used for analysis of daily alcohol consumption and smoking.
Abbreviations: BMI, body mass index; GHQ-28, 28-item General Health Questionnaire; STAI, State-Trait Anxiety Inventory; SDS, Self-rating Depression Scale; CFS, Chalder Fatigue Scale; PSQI, Pittsburgh Sleep Quality Index.

obtained at the baseline of the study in the CP2305 group, while they appeared to decrease in the placebo group during the experimental period (Fig. 3A and B). However, the scores for "constipation", "having an acne," and "vaginal discharge" were significantly lower in the CP2305 group compared with the placebo group (Fig. 3C–E). Other physiological symptoms—including headache, pain in the lower back, and stomachache—were not affected by CP2305 intake (Fig. 3F-L and Table S5). These data suggest that daily intake of CP2305 may increase fluid retention during the luteal phase.

3.5. Effects of CP2305 intake on salivary hormone levels

Several previous studies suggest that abnormal secretion of

reproductive hormones is associated with premenstrual symptoms. CP2305 intake reduces salivary cortisol and/or CgA levels under various stressful conditions (Nishida et al., 2017b, 2019; Sawada et al., 2019). We measured the concentrations of E2, P4, cortisol, and CgA by ELISA in salivary samples collected during the luteal phase (3 to 6 days prior to menstruation) and the follicular phase (3 to 6 days after menstruation). As shown in Table 3, neither salivary E2, nor P4 post-menstruation levels measured during the follicular phase were affected by CP2305 administration. Luteal phase P4 levels were higher and E2 levels were maintained in the CP2305 group compared with the baseline levels. Salivary cortisol and CgA levels were not affected by CP2305 administration. These data suggest that daily intake of CP2305 may affect E2 and P4 secretion during the luteal phase, thereby ameliorating premenstrual symptoms.

4. Discussion

This study aimed to assess the effects of CP2305 on premenstrual symptoms, which are common among women. Interestingly, we found that daily administration of CP2305 significantly improved psychological premenstrual symptoms and increased luteal phase levels of reproductive hormones. These findings provide new insight into CP2305 function in alleviating premenstrual symptoms.

L. gasseri CP2305 is a unique strain that was isolated from the stool sample of a healthy volunteer in 1994 (Sugawara et al., 2016). CP2305 exhibits pleiotropic functions in human clinical studies: stress relief (Nishida et al., 2017a, 2017b, 2019; Nobutani et al., 2017; Sawada et al., 2017), improvement of sleep quality (Nishida et al., 2017a, 2017b, 2019; Sawada et al., 2017), and enhancement of recovery from fatigue (Sawada et al., 2019). These studies revealed three important features of CP2305. First, CP2305 appears to affect the central nervous system, resulting in attenuation of the stress-induced HPA response and improvement of sleep quality. Second, CP2305 may alter gut microbiota composition, resulting in increased short-chain fatty acid (SCFA) production. Third, viable and killed CP2305 exert similar effects, as do

Table 3

Concentration of reproductive hormones, cortisol and CgA in the saliva.

Phase	Parameters	Treatment	Baseline (Pre)	Post	Changes	p-value#
follicular	E2	Placebo	0.98 ± 0.15	1.14 ± 0.21	0.16 ± 0.19	0.386
	(pg/ml)	CP2305	0.81 ± 0.11	0.77 ± 0.12	−0.04 ± 0.12	
	P4	Placebo	83.65 ± 19.67	89.91 ± 16.95	6.25 ± 16.87	0.529
	(pg/ml)	CP2305	57.34 ± 11.23	50.36 ± 13.25	−6.98 ± 12.61	
luteal	E2	Placebo	1.57 ± 0.14	1.16 ± 0.12	−0.41 ± 0.11	0.010*
	(pg/ml)	CP2305	1.21 ± 0.13	1.26 ± 0.11	0.05 ± 0.12	
	P4	Placebo	202.80 ± 35.09	124.38 ± 27.57	−78.42 ± 50.27	0.045*
	(pg/ml)	CP2305	148.71 ± 26.85	188.19 ± 28.79	39.48 ± 29.34	
	Cortisol	Placebo	0.21 ± 0.04	0.14 ± 0.03	−0.07 ± 0.05	0.161
	(µg/dl)	CP2305	0.11 ± 0.01	0.12 ± 0.02	0.01 ± 0.03	
	CgA/TP	Placebo	4.17 ± 1.30	3.27 ± 0.61	−0.91 ± 1.34	0.408
	(pmol/mg)	CP2305	5.09 ± 1.77	6.17 ± 3.32	1.12 ± 1.97	

Data are presented as the mean ± SEM.

Abbreviations: E2, estradiol; P4, progesterone; CgA, chromogranin A; TP, total proteins.

Changes between the placebo and CP2305 groups analyzed using Student's *t*-test.

* Significant difference (p < 0.05).
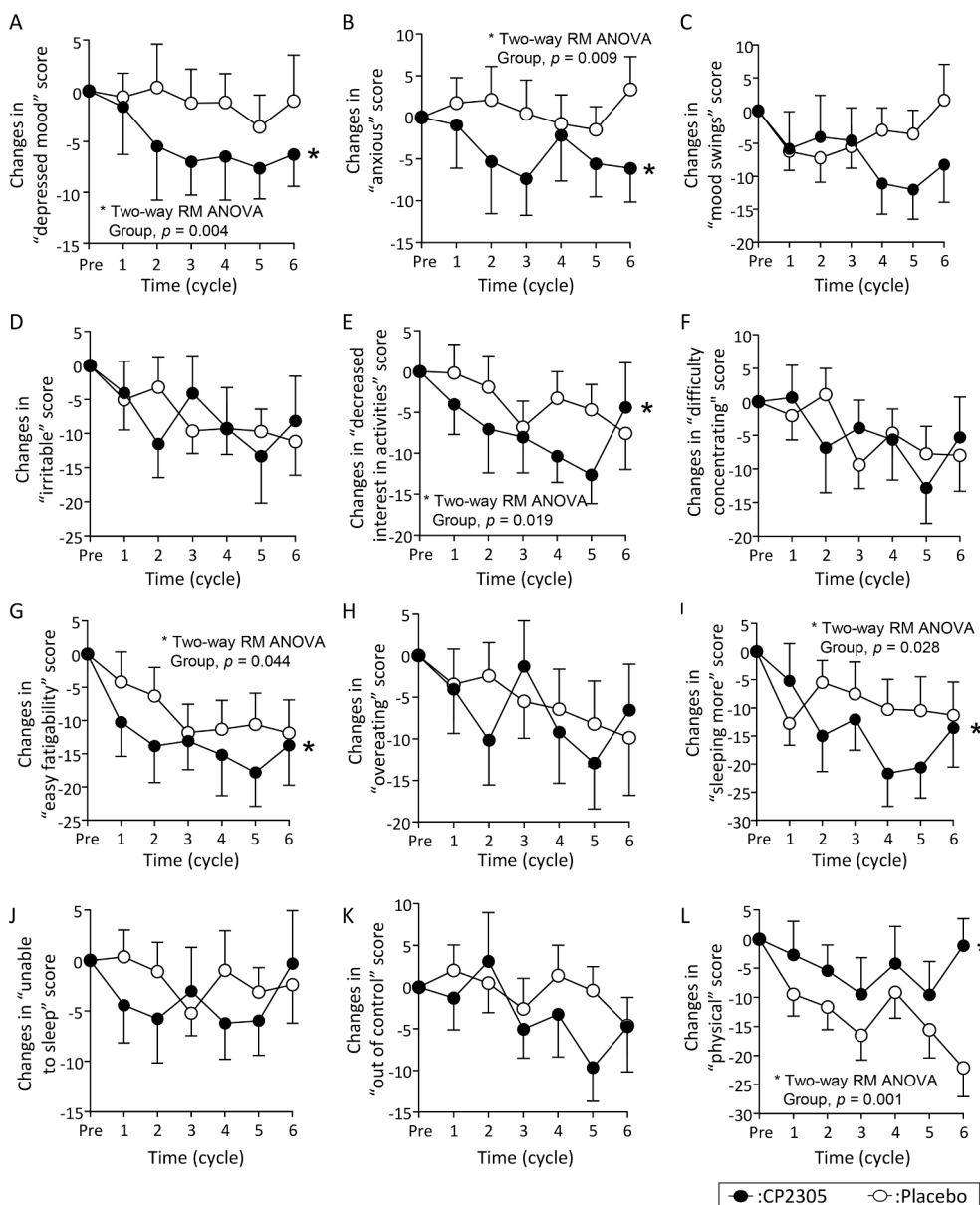
Fig. 2. Changes in premenstrual symptoms as reported in the PMTS-VAS questionnaires. (A–L) Time-dependent changes in the premenstrual symptom scores are shown. The data are expressed as the mean ± SEM. The data were analyzed with two-way repeated measures (RM) ANOVA and the *p*-value is shown in each panel. *represents statistical difference between groups (detailed statistical results in Table S4). Open circles (○) represent the placebo group, and closed circles (●) represent the CP2305 group.
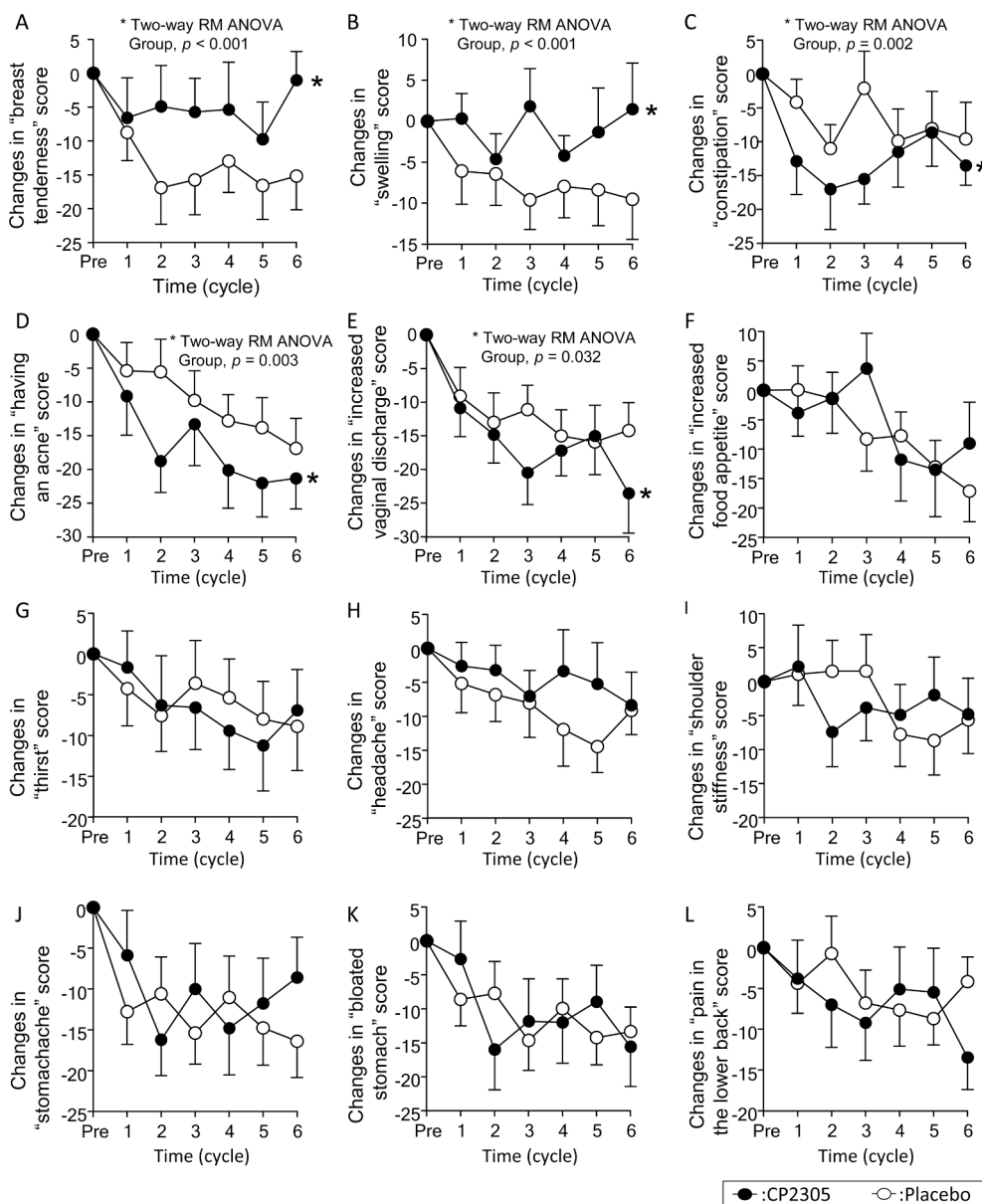
Fig. 3. Changes in physical symptoms during the luteal phase. (A–L) Changes in the physical symptom scores over time are indicated. The data are expressed as the mean ± SEM. The data were analyzed by two-way repeated measures (RM) ANOVA between the groups and the p-value is shown in each panel. *represents statistical difference between groups (detailed statistical results in Table S5). Open circles (○) represent the placebo group, and closed circles (●) represent the CP2305 group.

CP2305 administered as a beverage or tablet; and therefore, CP2305 is considered a paraprobiotic (de Almada et al., 2016). Our findings in this study provide new evidence for the unexplored ability of CP2305 to ameliorate psychological premenstrual symptoms.

Premenstrual symptoms are often observed in young women, but the causes are not fully understood. Researchers have hypothesized that fluctuations in reproductive hormones (E2 and P4) trigger the symptoms (Yen et al., 2019). CP2305 administration did not affect salivary reproductive hormone levels during the follicular phase and increased or inhibited the reduction of those hormone levels during the luteal phase, compared to the levels in response to placebo intake. From a different perspective, placebo intake seemed to reduce salivary reproductive hormone levels. This may have been caused by changes in the circumstances of the subjects. For instance, most subjects started taking CP2305 or placebo tablets between August and September (during summer vacation) and finished in February or March, during which time they may have experienced stress in the latter months of the intervention period (January to March), because they had to qualify to advance to the

next university grade. These stressful conditions may account for the changes in the levels of the reproductive hormones in the placebo group. However, salivary cortisol levels, a well-known stress biomarker, showed no significant difference between the groups (Table 3). We could not evaluate whether CP2305 intake attenuates stress-induced HPA responses during the intervention period in the current study, as previously reported.

Another possible explanation is the seasonal changes in reproductive hormone levels. Some reports have shown that E2 and P4 levels in human plasma are relatively higher in the summer and lower in the winter (Bjørnerem et al., 2006; Santi et al., 2020). Similar to reproductive hormones, seasonal variations occur in psychological symptoms, which are more common in women than men (Lyall et al., 2018). Indeed, the significant effects of time on certain psychological and physical symptoms were observed in the two-way repeated measures ANOVA (Table S4 and Table S5). Seasonal changes may partially influence the fluctuations in symptoms. Taken together, CP2305 intake may have conferred protection against stress-related or seasonal

reductions in reproductive hormone levels.

The maintenance of P4 and E2 levels may have influenced the participants' physical symptoms, as these hormones are known to affect fluid regulation (Stachenfeld, 2008). The physical symptoms related to fluid retention were higher in the CP2305 group than in the placebo group; and therefore, CP2305 intake seemed to worsen tissue swelling and tenderness. However, it appears as though the placebo group did show a reduction in these symptoms (Fig. 3). The possibility that a group bias could influence changes in the scores for the physical symptoms, related to fluid retention, cannot be excluded, because raw scores of "breast tenderness" and "swelling" at the baseline of the study tended to be (not significantly) higher in the placebo group (Table S3).

E2 and P4 can also influence women's emotions and sleep patterns. E2 regulates various neurotransmitter systems, including serotonin and dopamine, which affect mood (Wharton et al., 2012). Reproductive hormones have been shown to modulate women's sleep patterns throughout adulthood (Gervais et al., 2017). These hormones also affected anxiety-like behavior in a rodent model (Flores et al., 2020). Therefore, changes in the premenstrual symptoms observed in this study may have been caused by changes in E2 and P4 levels.

Recently, the gut microbiota has been recognized as a principal regulator of circulating estrogen (Baker et al., 2017). Previously, we found that CP2305 intake increases the diversity of the gut microbiota, and particularly increases the representation of bacteria that produce SCFAs (Sawada et al., 2019). In this study, changes in the gut microbiome by CP2305 intake were not assessed. However, CP2305 intake ameliorated constipation and reduced the incidence of acne (Fig. 3), which are both related to the gut microbiome ecosystem (Ohkusa et al., 2019; Salem et al., 2018), suggesting that CP2305 intake may have altered the gut microbiome composition. Gut microbiota can regulate estrogens through secretion of β-glucuronidase, an enzyme that deconjugates estrogens into their active form (Baker et al., 2017). Gut metabolites produced by microbiota, such as SCFAs, may also regulate E2 and P4 synthesis by the granulosa cells (Lu et al., 2017). Therefore, alteration of the gut environment by CP2305 intake may influence E2 and P4 metabolites, resulting in fluctuation of premenstrual symptoms. In addition, gut microbiota have reportedly demonstrated seasonal variations (Davenport et al., 2014; Koliada et al., 2020). Given the seasonal effects observed for some of the symptoms in this study, it is of interest to investigate correlations between seasonal variations in gut microbiota composition and fluctuations in premenstrual symptoms. Unfortunately, this study did not include analyses of gut microbiota composition or gut metabolites. Further investigations are warranted to elucidate whether alteration of the microbiota by CP2305 is associated with changes in the levels of reproductive hormones.

Reproductive hormones may be a novel target of *Lactobacillus gasseri* CP2305 through the microbiome-gut–brain axis. CP2305 improved the gut environment (reduced constipation), leading to changes in reproductive hormone metabolism. These hormonal changes may subsequently reduce anxiety or depressed moods and improve sleep status, in addition to preventing seasonal changes in water retention-related symptoms. However, considering that the subjects in this study did not experience severe symptoms that interfered with their daily lives, we could not assess the clinical significance of changes in physical symptoms caused by CP2305. Further studies focusing on PMS would reveal the clinical significance of the effects elicited by CP2305 for premenstrual physical symptoms.

We measured salivary reproductive hormone levels (P4 and E2) in this study. To avoid confounding changes caused by menstrual cycles or circadian rhythms, the salivary samples were collected every three days between 4 PM and 6 PM. However, the dynamic and seasonal changes discussed above may have affected the results. To develop a clearer understanding of the mechanisms underlying CP2305-mediated effects on reproductive hormone levels or the HPG axis, serum levels of these hormones and gonadotropins should be evaluated in future studies.

The study has several potential limitations. First, we included only one university-based population; therefore, our results may not be generalizable to other populations with different backgrounds. Second, specialized medical practitioners, such as gynecologists, or sexual & reproductive health professionals, did not confirm the PMS diagnosis. Third, the study population comprised healthy subjects; therefore, the results may not apply to all young menstruating women, particularly those who require medical intervention for severe PMS.

5. Conclusions

CP2305 ameliorated some of the psychological premenstrual effects in young women. Clinical trials targeting the gut–brain axis using various probiotics, similar to *L. gasseri* CP2305, have reported antidepressant effects (Liu et al., 2019; Reis et al., 2018). However, to the best of our knowledge, this is the first study to evaluate the effects of probiotics or paraprobiotics on premenstrual symptoms. Further mechanistic studies will more completely reveal the impact of daily intake of paraprobiotic CP2305 on young women's mental health.

Ethics statement

The authors ensure that the current clinical trial has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The protocol was approved by the Institutional Review Boards of Tokushima University Hospital, Tokushima, Japan. This study was registered with the UMIN Clinical Trials Registry as UMIN000027302 (Title; Research on the effect of a lactic acid bacterium preparation for health conditions of females), and was conducted in compliance with the protocol. Written informed consent was obtained from all subjects prior to enrolment.

CRediT authorship contribution statement

Kensei Nishida: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing - original draft. **Daisuke Sawada:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. **Toshiyuki Yasui:** Supervision, Writing - review & editing. **Yuki Kuwano:** Conceptualization, Data curation, Investigation, Writing - review & editing. **Kazuhito Rokutan:** Conceptualization, Funding acquisition, Resources, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [D.S. is an employee of Asahi Quality & Innovations, Ltd., related to Asahi Group Holdings, Ltd. The other authors declare that the research was conducted in the absence of any financial relationships that could be construed as a potential conflict of interest.]

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104426.

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