Clinical Nutrition ESPEN xxx (2018) e1–e9

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Randomized Controlled Trial

A lecithin phosphatidylserine and phosphatidic acid complex (PAS) reduces symptoms of the premenstrual syndrome (PMS): Results of a randomized, placebo-controlled, double-blind clinical trial

Katja Schmidt [a], Nicole Weber [a], Meir Steiner [b, c], Nadin Meyer [a], Anne Dubberke [a], David Rutenberg [d], Juliane Hellhammer [a, *]

[a] Contract Research Institute daacro, Trier, Germany
[b] Department of Psychiatry & Behavioural Neurosciences, McMaster University, Hamilton, Canada
[c] Department of Obstetrics & Gynecology, McMaster University, Hamilton, Canada
[d] Lipogen Ltd., Haifa, Israel

ARTICLE INFO

Article history:
Received 14 July 2017
Accepted 22 January 2018

Keywords:
Cortisol
Daily record of severity of problems
Phosphatidic acid
Phosphatidylserine
Premenstrual syndrome

SUMMARY

Background & aims: Many women experience emotional and physical symptoms around the time of ovulation and more so before menstruation interfering with their daily normal life also known as premenstrual syndrome (PMS). Recent observational data suggest that supplementation with Lipogen's phosphatidylserine (PS) and phosphatidic acid (PA) complex (PAS) alleviates these PMS symptoms. The aim of this study was to confirm these observations on the effects of PAS on PMS symptom severity within a controlled clinical trial setting.

Methods: Forty women aged 18–45 years with a diagnosis of PMS were assigned to either take PAS (containing 400 mg PS & 400 mg PA per day) or a matching placebo. The study comprised 5 on-site visits including 1 baseline menstrual cycle followed by 3 treatment cycles. Treatment intake was controlled for by using an electronic device, the Medication Event Monitoring System (MEMS®). Primary outcome of the study was the PMS symptoms severity as assessed by using the Daily Record of Severity of Problems (DRSP). Further, SIPS questionnaire (a German version of the Premenstrual Symptoms Screening Tool (PSST)), salivary hormone levels (cortisol awakening response (CAR) and evening cortisol levels) as well as serum levels (cortisol, estradiol, progesterone and corticosteroid binding globulin (CBG)) were assessed.

Results: PMS symptoms as assessed by the DRSP *Total score* showed a significantly better improvement ($p = 0.001$) over a 3 cycles PAS intake as compared to placebo. In addition, PAS treated women reported a greater improvement in physical ($p = 0.002$) and depressive symptoms ($p = 0.068$). They also reported a lower reduction of productivity ($p = 0.052$) and a stronger decrease in interference with relationships with others ($p = 0.099$) compared to the placebo group. No other DRSP scale or item showed significant results. Likewise, the reduction in the number of subjects fulfilling PMS or premenstrual dysphoric disorder (PMDD) criteria as classified by the SIPS did not differ between the PAS and the placebo group. For the biomarkers, the salivary cortisol percentage increase of the CAR was significantly less pronounced in the follicular phase of cycle 4 than in the follicular phase of cycle 1 for subjects taking PAS when compared to subjects taking placebo ($p = 0.018$). Furthermore, the change of serum cortisol levels between visit 1 and visit 5 differed significantly between groups ($p = 0.043$). While serum cortisol levels of PAS treated females slightly decreased between visit 1 and visit 5, cortisol levels of females treated with placebo increased. For all other biomarkers, no treatment effects were observed over the 4 cycles study period.

Abbreviations: AE, adverse event; AUC_g, area under the curve (ground); AUC_i, area under the curve (increase); BMI, body mass index; CAR, cortisol awakening response; CBG, corticosteroid binding globulin; CI, confidence interval; CLIA, chemiluminescence immunoassay; CRO, clinical research organization; DRSP, Daily Record of Severity of Problems; DSM, Diagnostic and Statistical Manual of Mental Disorders; HR, heart rate; ITT, intention to treat; MEMS, Medication Event Monitoring System; Mini-DIPS, Diagnostisches Kurz-Interview bei psychischen Stoerungen; PA, phosphatidic acid; PAS, phosphatidylserine/phosphatidic acid complex; PMDD, premenstrual dysphoric disorder; PMS, premenstrual syndrome; PP, per-protocol; PS, phosphatidylserine; PSST, Premenstrual Symptoms Screening Tool; SIPS, Screening-Instrument fuer Praemenstruelle Symptome; TSH, thyroid stimulating hormone; V, visit; WOM, word of mouth.

* Corresponding author. Contract Research Institute daacro, Science Park, Max-Planck-Strasse 22, 54295 Trier, Germany. Fax: +49 (0)651 9120 564.
E-mail address: hellhammer@daacro.de (J. Hellhammer).

https://doi.org/10.1016/j.clnesp.2018.01.067

Overall, this study confirms that a daily intake of PAS, containing 400 mg PS and 400 mg PA, can be considered as safe.

Conclusions: Results substantiate the efficacy of PAS in reducing symptoms of PMS. In view of the recent inclusion of severe PMS symptoms (PMDD) in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), the positive results of this clinical study merits consideration of developing the PAS complex as a botanical drug for treatment of PMDD.

Clinical trial registration: The study is registered at *Deutsches Register Klinischer Studien* with the registration number DRKS00009005.

1. Introduction

PMS is characterized by a cluster of somatic and psychological symptoms of varying severity. These symptoms occur only during the luteal phase of the menstrual cycle and resolve during the first days of menses [12].

The estimated prevalence of PMS varies. A systematic review reports a pooled PMS prevalence of 47.8%. This indicates that about half of women within their reproductive age experience PMS symptoms. This typically can include feeling tired, irritability, mood changes, bloating, skin irritations and breast tenderness. In addition, an increasing trend in the prevalence of PMS during 1996–2001 has been observed [8].

The etiology of premenstrual disorders is multifactorial. Precise causes and the influence of heritability are still unknown and not sufficiently explored yet [10]. The DSM-5 [2] states that the onset of PMDD, a more severe form of PMS with a prevalence of 3–8% [16,37], can occur at any point after menarche; symptoms cease after menopause but can be triggered by cyclical hormone replacement. Stress is also a factor in PMS and PMDD along with a history of interpersonal trauma and seasonal changes. Premenstrual symptoms can be improved by pharmacological interventions (e.g. oral contraceptives, antidepressants) or by nutrition.

An association between nutritional factors and PMS has been observed repeatedly (Cheng et al., 2013 [40]; Bertone-Johnson et al., 2014 [39]; Kia et al., 2015 [43]; Chocano-Bedoya et al., 2013 [41]; Gorczyca et al., 2016 [42]) and consequently the search for a nutritional supplement that is effective in alleviating PMS symptoms is of great interest. To many, this seems to be a more attractive alternative when compared to intake of pharmacological and psychiatric drugs.

PAs are the acid forms of phosphatidates, which are a part of common phospholipids. PA has different roles in the cell: It is a precursor for other lipids such as PS or phosphatidylcholine via the conversion of PA to diacylglycerol [3]. Moreover, PA influences membrane curvature [22,25] and acts as a signaling lipid [11,34]. In combination with PS, PA has shown to lower cortisol levels and enhance wellbeing under acute social stress [17]. PS, a phospholipid component is found in mammalian cell membranes. Previous studies indicated that acute and long-term administration of PS dampens cortisol responses to acute exercise and mental stress [17,26,36]. In addition, PS has shown to improve memory, learning, mood and stress management [4,17–20,38]. Further, the intake of PS has been associated with an improvement of psychiatric disorders, such as bipolar and major depressive disorders (reviewed in Refs. [1,20]) and with the prevention of inflammatory neurodegenerative events [29].

Between December 2011 and March 2012, Lipogen Ltd. performed a word of mouth (WOM) marketing campaign in the United States of America (WOM company BzzAgent, USA). 23 out of 220 women (10.45%), age <40, who consumed the product for 2 months, reported improvement in PMS. The following study was performed to confirm this effect in a single center, double-blind, placebo-controlled, randomized clinical trial.

2. Materials and methods

2.1. Study participants

Eligible participants were women aged 18–45 years with a PMS diagnosed by a gynecologist. Participants were required to have regular menstrual cycles with constant cycle duration (25–35 days) and easy access to computer and internet at home.

Participants were not eligible if any of the following exclusion criteria applied: Known allergies to ingredients of the test substance; any underlying psychiatric disorder (e.g. major depressive disorder) as assessed with the Mini-DIPS (Diagnostisches Kurz-Interview bei psychischen Stoerungen; [24]); any current/acute illness; any disease other than minor medical conditions (e.g. seasonal allergies); current intake of any drugs besides thyroid medication (TSH (thyroid stimulating hormone) values in the normal range according to lab results within the past 12 months) and blood pressure medication (stable for 6 months); intake of nutritional supplements or homeopathic remedies within the 2 weeks prior to the first visit; strict diet or excessive sport activities; smoking more than 5 cigarettes per week; working night shifts; pregnant or lactating; planning to get pregnant during the next 12 months; employee of the Sponsor or CRO (clinical research organization); investigator doubts truthfulness of self-reported health information; women otherwise apparently unsuited (lack of cognitive or verbal skills); or currently participating in another clinical study.

The study was performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonization/Good Clinical Practice (2008). The protocol received approval from the Ethics Commission of the State Chamber of Medicine in Rhineland-Palatinate (Deutschhausplatz 3, 55116 Mainz, Germany). Written informed consent was obtained from each participant before study enrollment.

2.2. Study design

This prospective, randomized, double-blind, placebo-controlled, single center study with 2 treatment arms (PAS or placebo) was conducted in Trier, Germany between July 2015 and July 2016. Each woman completed detailed assessments over a period of 4 menstrual cycles. The objective was to evaluate the effects of 3 cycles intake of PAS on PMS following a first baseline observational cycle.

The study included 5 visits. Forty eligible women were randomly assigned to either the PAS or placebo group. Eight

Please cite this article in press as: Schmidt K, et al., A lecithin phosphatidylserine and phosphatidic acid complex (PAS) reduces symptoms of the premenstrual syndrome (PMS): Results of a randomized, placebo-controlled, double-blind clinical trial, Clinical Nutrition ESPEN (2018), https://doi.org/10.1016/j.clnesp.2018.01.067

additional women were recruited to replace drop-outs. At visit 1 (V1) blood pressure, heart rate (HR) and body mass index (BMI) was measured and a blood sample for assessment of serum progesterone, estradiol, CBG and cortisol levels was drawn between 8 am and 10 am. In addition, subjects were given Salivettes® for saliva collection with instructions to collect saliva during both the follicular and luteal phases of their cycle. Medical history was assessed and subjects were trained in handling the online version of the German DRSP. The DRSP had to be completed daily in the evening. At visit 2 (V2), saliva samples were collected and a questionnaire for baseline information on PMS, the Screening-Instrument fuer Praemenstruelle Symptome (SIPS), was assessed. Subjects received 4 capsules per day for the following menstrual cycle. Capsules were packed in a container with a MEMS® track cap (Aardex Ltd, Zug, Switzerland) to monitor container openings and protocol compliance. At visit 3 (V3) and visit 4 (V4), SIPS assessment was repeated and subjects received their study treatment dose for the next complete menstrual cycle. At V4, subjects additionally received Salivettes® for saliva collection during the follicular and luteal phases of their menstrual cycle. At the final study visit (V5), saliva samples were obtained, SIPS, blood pressure, HR and BMI were assessed and a post treatment blood sample for serum progesterone, estradiol, CBG and cortisol levels was drawn between 8 am and 10 am.

All visits took place during the follicular phase of individual's menstrual cycle. To ensure exclusion of pregnant women a pregnancy test was performed at each visit. Adverse events (AE), concomitant medication and major changes in nutrition or activity level were checked at V2, V3, V4 and V5. DRSP entry and compliance of treatment intake was checked and discussed after each menstrual cycle. A general overview of the study procedure is provided in Table 1.

2.3. Laboratory analyses

Saliva samples were collected using Salivettes®, blood samples were collected using 2.7 ml serum monovettes® (both Sarstedt, Nuembrecht, Germany). Saliva samples were stored at −20 °C, blood samples were centrifuged and stored at −20 °C until all participants completed the study. Saliva cortisol levels were analyzed at the SalivaLab Trier (daacro GmbH & Co. KG, Germany) employing a high sensitivity salivary cortisol enzyme immunoassay kit (Salimetrics, LLC, USA). The intra- and inter-assay coefficients of variation were 4.40% and 10.79%, respectively. After study termination, serum cortisol, progesterone and estradiol were analyzed by Synlab GmbH (Trier, Germany) using a chemiluminescence immunoassay (CLIA). The assay is based on the emission of light (luminescence) as the result of a chemical reaction. Serum CBG samples were analyzed by IBL (Hamburg, Germany) by employing a radioimmunoassay based on the competitive binding of radiolabeled antigen and unlabeled antigen to a high-affinity antibody.

2.4. Intervention

Subjects were randomized to 2 groups (A, B) using a 1:1 assignment with randomized blocks of varying size. The randomization sequence was generated by a statistician of daacro using the program R [31]. The assignment of groups to products (test product or placebo) was conducted by the Sponsor.

Each capsule for the PAS group consisted of 100 mg PS and lysophosphatidylserine, 100 mg PA and lysophosphatidic acid, 235 mg other phospholipids and glycerides, 5 mg silicon dioxide. Each placebo capsule contained 435 mg maize starch and 5 mg silicon dioxide. Capsules were administered 3 times daily, 1 capsule in the morning, 1 in the afternoon and 2 in the evening (4 capsules/day) over 3 consecutive menstrual cycles. PAS and placebo capsules looked, smelled and tasted identical. Capsules were produced and packed by Lipogen Ltd., Israel.

2.5. Objectives

2.5.1. Daily Record of Severity of Problems

The DRSP was developed by Endicott et al. [9] to aid diagnosing and evaluating PMDD/PMS according to DSM-IV. The questionnaire comprises 24 items assessing severity of complaints on a daily basis. Subjects rate their severity on a 6-point rating scale

Table 1
Study design.

	Days since start of the individual menstruation		
	-4, -3 (luteal phase)	5 (follicular phase)	8, 9 (follicular phase)
Cycle 1		**V1:** Blood sample • Cortisol • CBG • Estradiol • Progesterone	**Salivary cortisol:** • CAR: 0, 30, 45 and 60 min after awakening • 8 pm
Cycle 2	**Salivary cortisol:** • CAR: 0, 30, 45 and 60 min after awakening • 8 pm	**V2:** SIPS	
Cycle 3		**V3:** SIPS	
Cycle 4		**V4:** SIPS	**Salivary cortisol:** • CAR: 0, 30, 45 and 60 min after awakening • 8 pm
Cycle 5	**Salivary cortisol:** • CAR: 0, 30, 45 and 60 min after awakening • 8 pm	**V5:** SIPS Blood sample • Cortisol • CBG • Estradiol • Progesterone	

DRSP was assessed every evening during the entire study.
PAS or placebo intake took place daily starting with V2.

Please cite this article in press as: Schmidt K, et al., A lecithin phosphatidylserine and phosphatidic acid complex (PAS) reduces symptoms of the premenstrual syndrome (PMS): Results of a randomized, placebo-controlled, double-blind clinical trial, Clinical Nutrition ESPEN (2018), https://doi.org/10.1016/j.clnesp.2018.01.067

ranging from 1 (not at all) to 6 (extreme). The questionnaire has shown to be a reliable and valid measure of severity of symptoms for women reporting premenstrual symptoms (Endicott et al., 2006 [9]). Psychological symptoms like depression, anxiousness, mood swings, irritability and difficulties in concentration, but also physiological symptoms like breast tenderness, headache or weight gain are recorded. Every study participant had to fill in the symptom diary online every evening during the entire study. The study compliance was monitored daily via internet. Subjects failing to complete a DRSP entry were prompted by mail or phone to comply. Severe non-compliance (e.g. no DRSP entry on 3 consecutive days) led to study exclusion. As the DRSP was assessed online, accepting only complete entries, there were no missing items and DRSP scales were only computed for complete DRSP entries.

Primary outcome of this study was the *Total score* of the DRSP questionnaire summing up 21 items. Further explorative outcomes were the DRSP subscales *Depressive symptoms, Physical symptoms* and *Anger* as well as the items *Reduced productivity, Interference with social activities* and *Interference with relationships*. The within-cycle percentage increase in symptom severity from follicular to luteal phase served as another outcome measure. The score was calculated as the sum of the DRSP scale *Total score* of the 5 late luteal worst days (luteal score) minus the sum of the DRSP scale *Total score* of the 5 cycles best days (follicular score). This value was multiplied by 100 and divided by the luteal score to get a percentage value.

2.5.2. Biomarker

Since several biomarkers are associated with PMS, this study also evaluated hormonal endpoints like salivary and serum cortisol levels, CBG, estradiol and progesterone.

Associations between PMS and cortisol levels are reported in many studies [13,28,33]. Since the CAR and cortisol evening levels are connected to some diseases [6,14], salivary cortisol was assessed during 4 times in the morning (0, 30, 45 and 60 min after awakening) and once (8 pm) in the evening on 2 consecutive days in the follicular and the luteal phase of cycle 1 and cycle 4. The dynamic of post-awakening cortisol changes was reflected using the increase, percentage increase and area under the curve with respect to increase (AUC_i), whereas information on the total post-awakening cortisol concentration was provided using the area under the curve with respect to ground (AUC_g). AUC_i and AUC_g were calculated as described by Pruessner and colleagues [30].

In a previous study [18], we had speculated that effects of PAS on cortisol levels are mediated by CBG. Therefore, serum CBG and serum cortisol levels were assessed at baseline and after 3 cycles of treatment.

Munday et al. [27] found lower progesterone levels in women with PMS in the luteal phase compared to control subjects and higher estradiol levels in PMS patients over the last 4 days of the cycle compared to control subjects. Lombardi et al. [23] found lower progesterone levels in women with PMS in both menstrual phases. To test if PAS has an effect on these hormones, serum progesterone and estradiol levels were determined before and after treatment.

2.5.3. Screening-Instrument fuer Praemenstruelle Symptome

The SIPS is the German version of the Premenstrual Symptoms Screening Tool (PSST). The PSST was developed by Bentz et al. [5] and is a short self-assessment instrument on premenstrual symptoms with 14 items. These items retrieve information on symptoms that start prior to the period and come to an end within a few days of menstrual bleedings. Interference concerning productivity, relationships and social life activities are assessed as well. Items are answered on a 4-point rating scale ranging from “not at all” to “severe”. Depending on the perceived intensity of symptoms, either PMDD or PMS can be diagnosed. Within this study the SIPS was completed 4 times (at V2, V3, V4 and V5) to measure changes in premenstrual symptoms. A subject's treatment was regarded as successful if PMS or PMDD diagnosis criteria were no longer fulfilled at the end of the study. PMS and PMDD diagnosis as assessed by SIPS served as a further exploratory outcome.

2.5.4. Medication Event Monitoring System

PAS and placebo capsules were provided in containers locked with MEMS track caps, which record the time and date of each opening. This system was implemented to enforce and to check for subjects' compliance.

To estimate treatment compliance 3 parameters were computed: the overall, daily and intake compliance. While the overall compliance was calculated as the relative amount of MEMS® openings compared with the expected amount of MEMS® openings for a given time period, the daily compliance was calculated as the relative amount of compliant days (days with at least 2 MEMS® openings) compared with the total amount of days in the given time period. To calculate the compliance based on returned products (intake compliance), the number of consumed capsules was divided by the total amount of capsules that should have been consumed and then multiplied by 100 to obtain compliance as a percentage value.

2.6. Statistical analysis

The primary efficacy analysis was performed using the intention-to-treat (ITT) and per-protocol (PP) population. All further efficacy outcomes were analyzed using the ITT population. The ITT population was defined a priori as all enrolled subjects who completed at least V1, V2 and V3, while the PP population consisted of all enrolled subjects who satisfied the inclusion/exclusion criteria, were compliant and completed at least 80% of DRSP assessments. Subjects without protocol violations and deviations for DRSP assessments were allowed in the PP population.

The sample size was calculated based on a simulation study conducted using the program R [31]. A small effect (standardized regression coefficient $\beta = 0.08$) of the test product on the DRSP scale *Total score* (cycle-treatment-interaction) was assumed. The α-error level was specified at 0.05 and the number of replications was 1500. A multilevel (random effects) model [15], with 4 cycles (14 observed diary records each) and 2 groups, was chosen as the statistical method. Within each cycle, 2 missing diary records were assumed. Results of the simulation study showed a power of 0.84 for 36 subjects. This sample size was rounded up in order to account for exclusion of subjects from analyses due to protocol deviations resulting in a total sample size of 40 subjects.

For the DRSP outcomes multilevel models were specified, comparing symptom improvement over all menstrual cycles between groups. Multilevel models take account of the correlations within the data and adjust for non-response if the data are missing at random [7]. A 3-level hierarchical structure was assumed for the DRSP *Total score*, the DRSP subscales *Physical symptoms, Depressive symptoms* and *Anger* as well as for the DRSP items *Reduced productivity, Interference with social activities* and *Interference with relationships*: the first level was menstrual cycle ($n = 2300$), the second level was day of menstrual cycle ($n = 588$) and the third level contained individual characteristics ($n = 42$). Because the model assumptions were not fulfilled for the DRSP subscales *Depressive symptoms* and *Anger* and for the DRSP items *Reduced productivity, Interference with social activities* and *Interference with relationships* despite efforts of transformation, an average score was computed for every cycle. As for the DRSP within-cycle percentage

Please cite this article in press as: Schmidt K, et al., A lecithin phosphatidylserine and phosphatidic acid complex (PAS) reduces symptoms of the premenstrual syndrome (PMS): Results of a randomized, placebo-controlled, double-blind clinical trial, Clinical Nutrition ESPEN (2018), https://doi.org/10.1016/j.clnesp.2018.01.067

increase in symptom severity, models with a 2-level hierarchical structure (level 1: menstrual cycle (n = 144 for the DRSP within-cycle percentage increase in symptom severity and n = 165 for the remaining DRSP outcomes), level 2: individual characteristics (n = 42)) were specified for these outcomes. Since a decline of PMS symptoms could only take place if PMS symptoms were present, only the last 10 days of the luteal phase and the first 4 days of the following cycle were used for inferential statistical analyses.

A two-sample t-test was used to test normal distributed biomarkers and measures of the compliance, while the Wilcoxon Rank Sum Test was used for those outcomes not normally distributed. The salivary cortisol outcomes were tested separately for the luteal and the follicular phase of menstrual cycle. Fisher tests were used to compare the number of subjects who still fulfilled the criteria for a PMS or PMDD diagnosis on V5 to the number of subjects who no longer fulfilled the criteria for a PMS or PMDD diagnosis on V5 between groups.

Additional, outlier corrected models were estimated. Two-sided hypothesis testing ($\alpha = 0.05$) was performed. As no correction for multiple testing was made, only analysis for the primary endpoint can be interpreted as a confirmatory hypothesis test. Under the assumption that any missing data are missing completely at random, missing values were not imputed in the analyses. All calculations mentioned were performed with the statistic program R version 3.2.2 [32] and were incorporated into the finalized statistical analysis plan prior to unblinding of the data.

3. Results

In this section, data are expressed as mean values (±SD).

3.1. Study participants

A total of 99 women were screened for eligibility, of which 40 completed the study. At enrollment, 51 women who failed to satisfy all inclusion criteria or presented 1 or more exclusion criteria were excluded from the study. Eight participants dropped from the study and were replaced (Fig. 1).

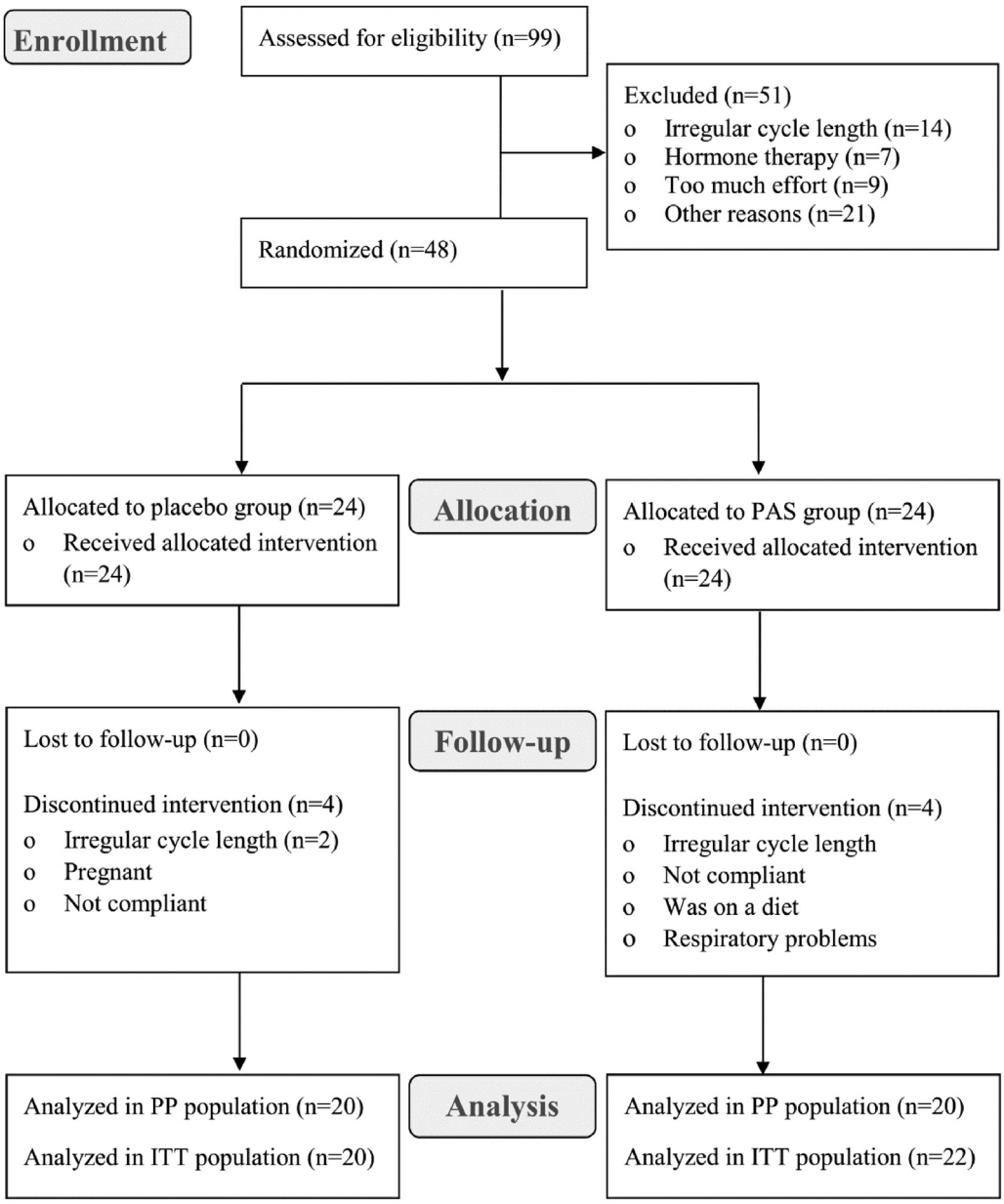

Fig. 1. Flowchart of the study participants showing the distribution of the participants from initial assessment to analysis of study data.

Please cite this article in press as: Schmidt K, et al., A lecithin phosphatidylserine and phosphatidic acid complex (PAS) reduces symptoms of the premenstrual syndrome (PMS): Results of a randomized, placebo-controlled, double-blind clinical trial, Clinical Nutrition ESPEN (2018), https://doi.org/10.1016/j.clnesp.2018.01.067

Table 2
Baseline demographic and clinical characteristics of the treatment groups.

	Placebo (n = 20)	PAS (n = 22)
Age [years]	32.65 (7.01)	33.64 (7.37)
Height [m]	167.80 (4.29)	167.64 (6.95)
BMI [kg/m^2]	24.64 (5.06)	24.25 (3.68)
HR [bpm]	76.75 (10.07)	72.32 (9.34)
Systolic blood pressure [mmHG]	117.60 (11.20)	115.18 (11.60)
Diastolic blood pressure [mmHG]	78.25 (11.22)	77.95 (7.52)

BMI = body mass index, bpm = beats per minute, HR = heart rate, kg = kilograms, m = meter, mmHG = millimeter of mercury, n = sample size.
Values are presented as mean (SD).

Demographic characteristics and clinical features of the trial participants at baseline were comparable between groups. Details are shown in Table 2.

3.2. Treatment adherence

During the study period, adherence was relatively high in both groups: the average daily compliance was 91.16% within the placebo group and 94.52% within the PAS group, the average overall compliance was 93.00% within the placebo group and 94.72% within the PAS group and the average intake compliance was 95.44% within the placebo group and 97.33% within the PAS group. Two participants dropped out because they were not compliant. No difference between groups in overall product intake was observed (daily compliance: p = 0.198, overall compliance: p = 0.914, intake compliance: p = 0.315).

3.3. Primary outcome

The reduction of overall symptom severity over the 4 menstrual cycles was significantly larger for subjects in the PAS group when compared to placebo (p = 0.001; Fig. 2). DRSP *Total score* decreased within the placebo group from 36.49 (±15.68) to 33.24 (±14.76) on average, while the DRSP *Total score* decreased within the PAS group from 38.24 (±14.74) to 30.82 (±10.94). This corresponds to an average symptom reduction of 8.92% within the placebo group and of 19.40% within the PAS group.

The results of the ITT population were in accordance with the results of the PP population.

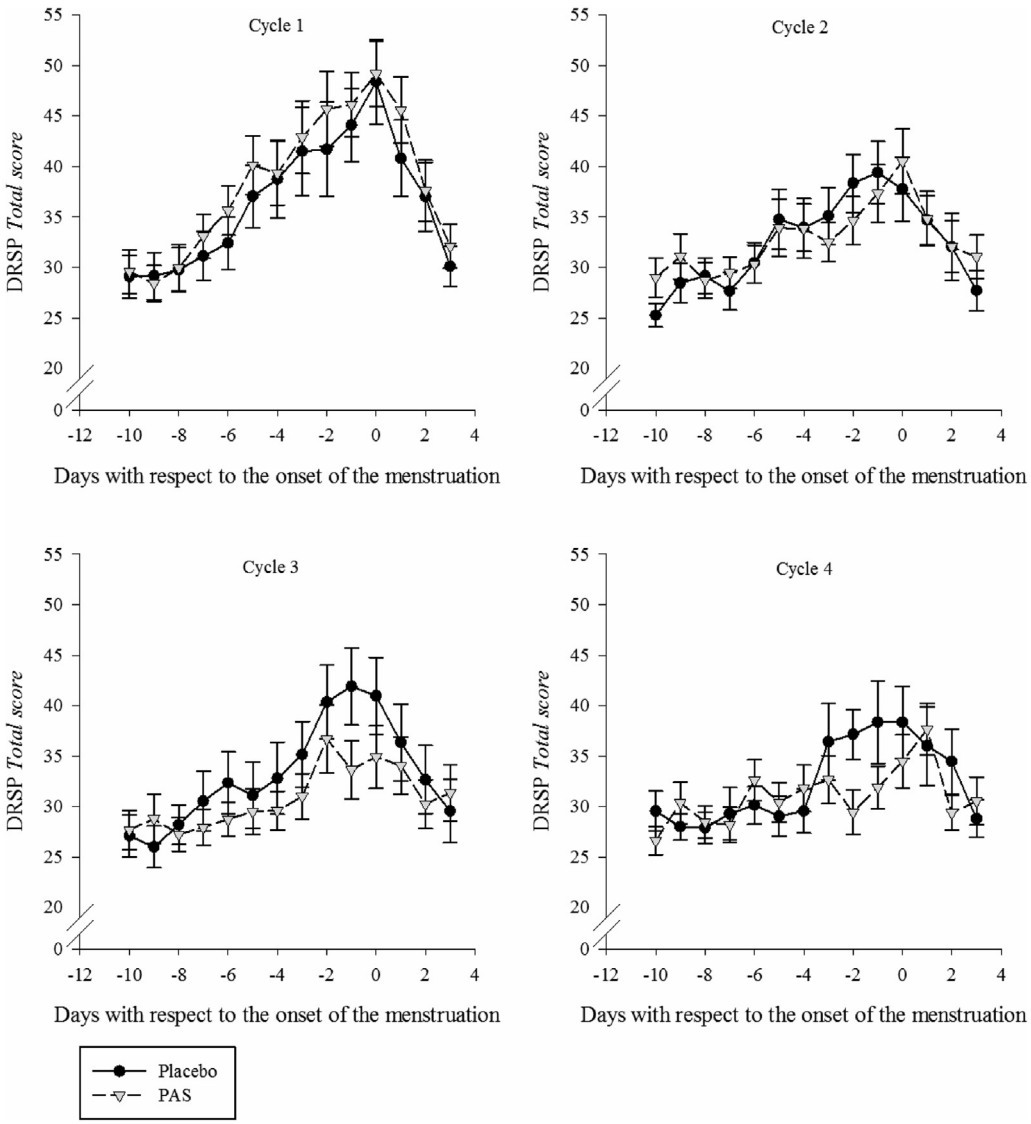

Fig. 2. PMS symptoms severity as assessed by the DRSP *Total score* during the symptomatic phase of each menstrual cycle (ITT population). There was a significant greater improvement in PMS symptoms over a 3 cycles PAS intake as compared to placebo (p = 0.001, n = 2300 observations).

Please cite this article in press as: Schmidt K, et al., A lecithin phosphatidylserine and phosphatidic acid complex (PAS) reduces symptoms of the premenstrual syndrome (PMS): Results of a randomized, placebo-controlled, double-blind clinical trial, Clinical Nutrition ESPEN (2018), https://doi.org/10.1016/j.clnesp.2018.01.067

3.4. Further analyses

Evaluation of the DRSP subscales *Physical symptoms* and *Depressive symptoms* showed a significantly greater reduction of physical symptoms ($p = 0.002$) and a marginally significant greater reduction of depressive symptoms ($p = 0.068$; Fig. 3) over the 4 menstrual cycles in the PAS group compared to the placebo group. Results indicate that PAS reduced physical symptoms by about 20.14% compared with 12.10% and depressive symptoms by about 20.18% compared with 7.11% within the placebo group. The change in the DRSP within-cycle percentage increase in symptom severity and in the DRSP subscale *Anger* over cycles did not differ between treatment groups ($p = 0.195$ and $p = 0.838$). PAS reduced the within-cycle percentage increase in symptom severity by about 36.26% and anger by about 14.13%, while there was an average reduction of 9.28% and 12.57% in the placebo group. PAS treated females reported a lower reduction in productivity and a more pronounced decrease in interference with relationships compared to the placebo group. These results reached a level of marginal significance ($p = 0.052$ and $p = 0.099$). The PAS group reported an average decrease in reduced productivity of 43.33% and an average decrease in interference with relationships of 45.09%, while the placebo group reported an average decrease of 3.36% and of 14.57%, respectively. Interference with social activities was reduced by about 42.25% in the PAS group and by about 10.71% in the placebo group. Even though there was a greater improvement in PAS treated women, this result was not significant ($p = 0.228$).

Serum cortisol levels increased about 19.77% within the placebo group and decreased about 2.72% within the PAS group. This result reached statistical significance ($p = 0.043$; Table 3). Furthermore, the salivary cortisol percentage increase of the CAR was significantly less pronounced in the follicular phase of cycle 4 than in the follicular phase of cycle 1 for subjects taking PAS when compared to subjects taking placebo ($p = 0.018$; Table 4). No significant differences between groups were found for any of the further salivary cortisol measures (Table 4), CBG, estradiol and progesterone (Table 3) as well as for the SIPS outcomes (number of PMS diagnoses: $p = 0.387$; number of PMDD diagnoses: $p = 1.000$).

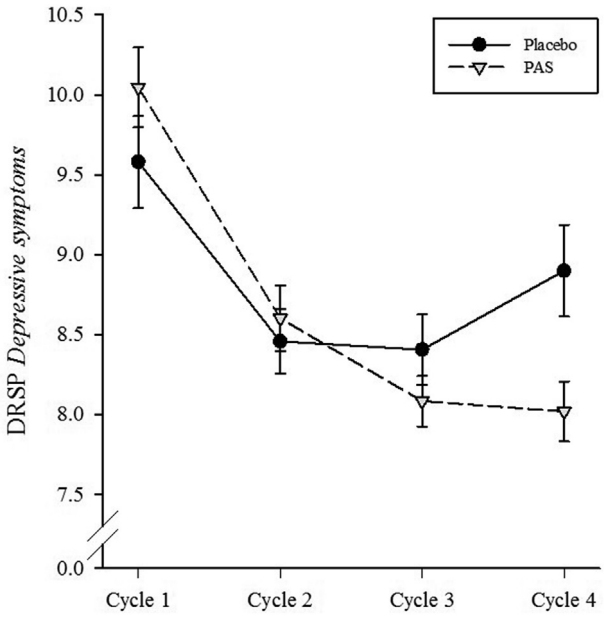

Fig. 3. Average score of the severity of depressive symptoms for each menstrual cycle. The reduction of depressive symptoms was marginally significant greater in the PAS group when compared to placebo ($p = 0.068$, $n = 165$ observations).

3.5. Safety analyses

A total of 338 AEs were reported by 22 subjects in the placebo group (136 AEs) and 22 subjects in the PAS group (202 AEs). One AE in the PAS group was classified as serious. This AE was reported due to appendicitis with a following appendectomy and was considered unrelated to PAS. Causality was evaluated as not related for 317 AEs (129 in the placebo group and 188 in the PAS group), as unlikely causal for 18 AEs (6 in the placebo group and 12 in the PAS group), as possible causal for 2 AEs in the PAS group (tender and aching breasts - worsening of PMS symptoms) and as probably causal for 1 AE in the placebo group (skin impurities in the face).

4. Discussion

This study substantiates beneficial effects of a daily intake of a phosphatidylserine & phosphatidic acid (1:1) complex (PAS) over 3 cycles on symptom levels as assessed by several well-recognized instruments for PMS evaluation. Even though results do not reach significance on all (sub-)scales, they convincingly point in the same direction. This does not apply to the measured biological parameters. Our hypothesis that the effects of PAS on cortisol levels, which were found in previous studies [18], are mediated by CBG, could not be confirmed by the current study. One reason for not seeing group differences for CBG, estradiol and progesterone could be that women suffering from PMS do not have abnormal hormone levels, but are rather over-sensitive to hormonal fluctuations as hypothesized by Soares and Zitek [35]. This needs to be further investigated. A reason for not seeing group differences for cortisol during the luteal phase of menstrual cycle could be that the analysis of salivary cortisol was confounded by sampling time. The salivary cortisol sampling during the luteal phase should have taken place on the 4th and 3rd day before the onset of the menses, but actually the measurements took place with deviations of up to 8 days from cycle day -4 and -3. Unfortunately, this was unavoidable due to very irregular cycle lengths in this population. The inaccurate timing of sampling may account for no observable differences in cortisol levels during the luteal phase. However, salivary cortisol levels of the follicular phase as well as serum cortisol levels showed significant results. While the percentage increase of the CAR remained almost stable from cycle 1 to cycle 4 in the placebo group, it decreased in women taking PAS from higher values on cycle 1 to levels comparable with the placebo group. The significant group differences may be attributed to the significant baseline

Table 3
Change in blood parameters between V1 and V5.

	V5 − V1		
	Placebo (n = 20)	PAS (n = 20)	p-value[a]
Serum cortisol [ng/ml]			
Mean (SD)	23.52 (45.49)	−3.77 (36.32)	0.043
95% CI	[3.22, 43.82]	[−20.77, 13.23]	
CBG [µg/ml]			
Mean (SD)	−5.70 (11.74)	−2.98 (6.21)	0.482
95% CI	−11.19, −0.21]	[−5.75, −0.21]	
Estradiol [pg/ml]			
Mean (SD)	−4.98 (82.93)	23.81 (77.85)	0.398
95% CI	[−43.80, 33.83]	[−10.93, 58.55]	
Progesterone [ng/ml]			
Mean (SD)	0.59 (1.83)	0.19 (0.32)	0.617
95% CI	[−0.27, 1.44]	[0.04, 0.33]	

CI = confidence interval, µg = microgram, ml = milliliter, n = sample size, ng = nanogram, pg = pictogram, V = visit.
[a] Obtained by using a two-sample t-test or alternatively a Wilcoxon Rank Sum Test.

Please cite this article in press as: Schmidt K, et al., A lecithin phosphatidylserine and phosphatidic acid complex (PAS) reduces symptoms of the premenstrual syndrome (PMS): Results of a randomized, placebo-controlled, double-blind clinical trial, Clinical Nutrition ESPEN (2018), https://doi.org/10.1016/j.clnesp.2018.01.067

Table 4
Change in measures of salivary cortisol between Cycle 1 and Cycle 4.

	Cycle 4 − Cycle 1		
	Placebo (n = 20)	PAS (n = 22)	p-value[a]
Luteal phase			
AUC_i			
Mean (SD)	−130.02 (449.04)	32.12 (288.89)	0.184
95% CI	[−340.18, 80.13]	[−96.80, 161.03]	
AUC_g			
Mean (SD)	−30.98 (369.95)	−60.11 (257.13)	0.925
95% CI	[−204.12, 142.16]	[−174.85, 54.63]	
Cortisol increase [nmol/l]			
Mean (SD)	−3.22 (10.76)	0.80 (6.34)	0.160
95% CI	[−8.26, 1.81]	[−2.02, 3.63]	
Percentage cortisol increase			
Mean (SD)	−36.32 (192.56)	18.73 (139.94)	0.547
95% CI	[−126.44, 53.80]	[−43.72, 81.18]	
Evening cortisol [nmol/l]			
Mean (SD)	0.66 (3.48)	−0.47 (1.69)	0.841
95% CI	[−0.97, 2.28]	[−1.23, 0.28]	
Follicular phase			
AUC_i			
Mean (SD)	−11.08 (230.64)	−108.02 (207.83)	0.177
95% CI	[−119.43, 97.27]	[−200.76, −15.28]	
AUC_g			
Mean (SD)	−64.97 (254.76)	10.39 (482.66)	0.813
95% CI	[−184.65, 54.71]	[−204.99, 225.77]	
Cortisol increase [nmol/l]			
Mean (SD)	−0.94 (5.60)	−2.71 (5.85)	0.340
95% CI	[−3.57, 1.70]	[−5.32, −0.10]	
Percentage cortisol increase			
Mean (SD)	−13.97 (88.08)	−114.59 (214.55)	0.018
95% CI	[−55.35, 27.41]	[−210.33, −18.85]	
Evening cortisol [nmol/l]			
Mean (SD)	1.11 (2.78)	0.13 (1.98)	0.749
95% CI	[−0.19, 2.42]	[−0.75, 1.02]	

AUC_g = Area under the curve (ground), AUC_i = Area under the curve (increase), CI = confidence interval, n = sample size, nmol/l = nanomol per liter.
[a] Obtained by using a two-sample t-test or alternatively a Wilcoxon Rank Sum Test.

differences. When controlling for the baseline percentage cortisol increase of the CAR no significant group differences were seen any more. Regarding serum cortisol, there was an increase in cortisol levels in the placebo group, while cortisol levels slightly decreased in women taking PAS. The fact that there was only a change in cortisol levels of the placebo group, while cortisol levels in women taking PAS remained almost constant makes this result hard to interpret. It may indicate that this effect is not a treatment effect of PAS. To conclude, no evidence was found, that treatment with PAS changes cortisol levels of women with PMS during both phases of the menstrual cycle. Our results were in line with a systematic review of the associations between PMS/PMDD and cortisol levels performed by Kiesner and Granger [21]. They concluded that overall, there is very little evidence that women with and without PMS/PMDD demonstrate systematic and predictable mean-level differences in cortisol. The authors found this conclusion supported by the predominance of studies showing null effects, and by the lack of consistency in the direction of effects that were found.

The current study clearly substantiates the effective and safe treatment of PMS with a complex of phosphatidylserine and phosphatidic acid. The PAS complex alleviated the PMS symptoms, providing a safe alternative to standard pharmacological treatment. In view of the recent inclusion of PMDD in the DSM-5, the positive results of this clinical study merits consideration of developing the PAS complex as a botanical drug for treatment of PMDD.

Conflict of interest statement and funding sources

This study was financed by Lipogen Ltd. and performed by daacro GmbH & Co. KG, a clinical research organization. DR discloses a commercial interest. JH, KS, NW, AD, NM and MS declare that they have no competing interest.

Statement of authorship

JH and DR were responsible for the conception and design of the study. KS analyzed data and drafted the manuscript. NW and NM organized and performed the clinical trial. As head of the Saliva Lab AD contributed in writing the manuscript. MS critically revised drafts of the manuscript and helped with the interpretation of results. All authors have approved the final article.

Acknowledgements

The authors would like to thank Trier's resident gynecologists for their assistance in recruiting PMS diagnosed patients. Also, we are grateful to all the anonymous participants for their valuable contribution to this study.

References

[1] Appleton KM, Rogers PJ, Ness AR. Is there a role for n-3 long-chain polyunsaturated fatty acids in the regulation of mood and behaviour? A review of the evidence to date from epidemiological studies, clinical studies and intervention trials. Nutr Res Rev 2008;21:13–41.
[2] American Psychiatric Association. Diagnostic and statistical manual of mental disorders, (DSM-5®). American Psychiatric Pub; 2013.
[3] Athenstaedt K, Daum G. Phosphatidic acid, a key intermediate in lipid metabolism. Eur J Biochem 1999;266(1):1–16.
[4] Benton D, Donohoe RT, Sillance B, Nabb S. The influence of phosphatidylserine supplementation on mood and heart rate when faced with an acute stressor. Nutr Neurosci 2001;4(3):169–78.
[5] Bentz D, Steiner M, Meinlschmid G. SIPS – Screening-Instrument für prämenstruelle Symptome. Nervenarzt 2012;83:33–9. https://doi.org/10.1007/s00115-010-3210-6.
[6] Chida Y, Steptoe A. Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. Biol Psychol 2009;80:265–78.
[7] Clarke P, Hardy R. Methods for handling missing data. In: Pickles A, Maughan B, Wadsworth M, editors. Epidemiological methods in life course research. Oxford: Oxford University Press; 2007. p. 157–80.
[8] Direkvand-Moghadam A, Sayehmiri K, Delpisheh A, Sattar K. Epidemiology of Premenstrual Syndrome (PMS)-a systematic review and meta-analysis study. J Clin Diagn Res 2014;8(2):106–9. https://doi.org/10.7860/JCDR/2014/8024.4021.
[9] Endicott J, Nee J, Harrison W. Daily record of severity of problems (DRSP): reliability and validity. Arch Womens Ment Health 2006;9(1):41–9. https://doi.org/10.1007/s00737-005-0103-y.
[10] Epperson CN, Steiner M, Hartlage SA, Eriksson E, Schmidt PJ, Jones I, et al. Premenstrual dysphoric disorder: evidence for a new category for DSM-5. Am J Psychiatry 2012;169(5):465–75. https://doi.org/10.1176/appi.ajp.2012.11081302.
[11] Foster DA. Phosphatidic acid signaling to mTOR: signals for the survival of human cancer cells. Biochim Biophys Acta Mol Cell Biol Lipids 2009;1791(9):949–55.
[12] Freeman EW, Sondheimer SJ. Premenstrual dysphoric disorder: recognition and treatment. Prim Care Companion J Clin Psychiatry 2003;5(1):30–9. Retrieved from, http://www.ncbi.nlm.nih.gov/pubmed/15156244.
[13] Girdler SS, Straneva PA, Light KC, Pedersen CA, Morrow AL. Allopregnanolone levels and reactivity to mental stress in premenstrual dysphoric disorder. Biol Psychiatry 2001;49(9):788–97.
[14] Giubilei F, Patacchioli FR, Antonini G, Monti MS, Tisei P, Bastianello S, et al. Altered circadian cortisol secretion in Alzheimer's disease: clinical and neuroradiological aspects. J Neurosci Res 2001;66(2):262–5.
[15] Goldstein H. Multilevel statistical modelsvol. 922. John Wiley & Sons; 2011.
[16] Halbreich U, Borenstein J, Pearlstein T, Kahn LS. The prevalence, impairment, impact, and burden of premenstrual dysphoric disorder (PMS/PMDD). Psychoneuroendocrinology 2003;28:1–23.
[17] Hellhammer J, Fries E, Buss C, Engert V, Tuch A, Rutenberg D, et al. Effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on the endocrine and psychological responses to mental stress. Stress 2004;7(2):119–26. https://doi.org/10.1080/10253890410001728379.

[18] Hellhammer J, Vogt D, Franz N, Freitas U, Rutenberg D. A soy-based phosphatidylserine/phosphatidic acid complex (PAS) normalizes the stress reactivity of hypothalamus-pituitary-adrenal-axis in chronically stressed male subjects: a randomized, placebo-controlled study. Lipids Health Dis 2014;13:1–11. https://doi.org/10.1186/1476-511X-13-121.

[19] Hellhammer J, Waladkhani AR, Hero T, Buss C. Effects of milk phospholipid on memory and psychological stress response. Br Food J 2010;112(10):1124–37. https://doi.org/10.1108/00070701011080258.

[20] Kidd PM. Omega-3 DHA and EPA for cognition, behavior, and mood: clinical findings and structural-functional synergies with cell membrane phospholipids. Altern Med Rev 2007;12(3):207–27. Retrieved from, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18072818.

[21] Kiesner J, Granger DA. A lack of consistent evidence for cortisol dysregulation in premenstrual syndrome/premenstrual dysphoric disorder. Psychoneuroendocrinology 2016;65:149–64.

[22] Kooijman EE, Chupin V, de Kruijff B, Burger KN. Modulation of membrane curvature by phosphatidic acid and lysophosphatidic acid. Traffic 2003;4(3):162–74.

[23] Lombardi I, Luisi S, Quirici B, Monteleone P, Bernardi F, Liut M, et al. Adrenal response to adrenocorticotropic hormone stimulation in patients with premenstrual syndrome. Gynecol Endocrinol 2004;18(2):79–87. Retrieved from, http://www.ncbi.nlm.nih.gov/pubmed/15195499.

[24] Margraf J. Mini-DIPS. Diagnostisches Kurz-Interview bei psychischen Stoerungen. Testmappe mit Handbuch und Interviewleitfaden. 1994.

[25] McMahon HT, Gallop JL. Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 2005;438(7068):590–6.

[26] Monteleone P, Beinat L, Tanzillo C, Maj M, Kemali D. Effects of phosphatidylserine on the neuroendocrine response to physical stress in humans. Neuroendocrinology 1990;52(3):243–8.

[27] Munday MR, Brush MG, Taylor RW. Correlations between progesterone, oestradiol and aldosterone levels in the premenstrual syndrome. Clin Endocrinol (Oxf) 1981;14(1):1–9. Retrieved from, http://www.ncbi.nlm.nih.gov/pubmed/7194756.

[28] Odber J, Cawood EH, Bancroft J. Salivary cortisol in women with and without perimenstrual mood changes. J Psychosom Res 1998;45(6):557–68. Retrieved from, http://www.ncbi.nlm.nih.gov/pubmed/9859857.

[29] Pandey NR, Sultan K, Twomey E, Sparks DL. Phospholipids block nuclear factor-kappa B and tau phosphorylation and inhibit amyloid-beta secretion in human neuroblastoma cells. Neuroscience 2009;164(4):1744–53.

[30] Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 2003;28(7):916–31. https://doi.org/10.1016/S0306-4530(02)00108-7.

[31] R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2015. https://www.R-project.org/.

[32] R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2016. https://www.R-project.org/.

[33] Rabin DS, Schmidt PJ, Campbell G, Gold PW, Jensvold M, Rubinow DR, et al. Hypothalamic-pituitary-adrenal function in patients with the premenstrual syndrome. J Clin Endocrinol Metabol 1990;71(5):1158–62.

[34] Sergeant S, Waite KA, Heravi J, McPhail LC. Phosphatidic acid regulates tyrosine phosphorylating activity in human neutrophils: enhancement of Fgr activity. J Biol Chem 2000;276(7):4737–46.

[35] Soares CN, Zitek B. Reproductive hormone sensitivity and risk for depression across the female life cycle: a continuum of vulnerability? J Psychiatry Neurosci: JPN 2008;33(4):331.

[36] Starks MA, Starks SL, Kingsley M, Purpura M, Jager R. The effects of phosphatidylserine on endocrine response to moderate intensity exercise. J Int Soc Sports Nutr 2008;5(11). https://doi.org/10.1186/1550-2783-5-11. 1550-2783-5-11 [pii].

[37] Sveindóttir H, Bäckström T. Prevalence of menstrual cycle symptom cyclicity and premenstrual dysphoric disorder in a random sample of women using and not using oral contraceptives. Acta Obstet Gynecol Scand 2000;79(5):405–13.

[38] Vakhapova V, Cohen T, Richter Y, Herzog Y, Korczyn AD. Phosphatidylserine containing omega-3 fatty acids may improve memory abilities in non-demented elderly with memory complaints: a double-blind placebo-controlled trial. Dement Geriatr Cogn Disord 2010;29(5):467–74. https://doi.org/10.1159/000310330. 000310330 [pii].

[39] Bertone-Johnson ER, Hankinson SE, Forger NG, Powers SI, Willett WC, Johnson SR, et al. Plasma 25-hydroxyvitamin D and risk of premenstrual syndrome in a prospective cohort study. BMC Women Health 2014;14(56).

[40] Cheng S-H, Shih C-C, Yang Y-K, Chen K-T, Chang Y-H, Yang Y-C. Factors associated with premenstrual syndrome – a survey of new female university students. Kaohsiung J Med Sci 2013;29(2):100–5.

[41] Chocano-Bedoya PO, Manson JAE, Hankinson SE, Johnson SR, Chasan-Taber L, Ronnenberg AG, et al. Intake of selected minerals and risk of premenstrual syndrome. Am J Epidemiol 2013;177(10):1118–27.

[42] Gorczyca AM, Sjaarda LA, Mitchell EM, Perkins NJ, Schliep KC, Wactawski-Wende J, et al. Changes in macronutrient, micronutrient, and food group intakes throughout the menstrual cycle in healthy, premenopausal women. Eur J Nutr 2016;55(3):1181–8.

[43] Kia AS, Amani R, Cheraghian B. The association between the risk of premenstrual syndrome and vitamin d, calcium, and magnesium status among university students: a case control study. Health Promot Perspect 2015;5(3):225–30.

Please cite this article in press as: Schmidt K, et al., A lecithin phosphatidylserine and phosphatidic acid complex (PAS) reduces symptoms of the premenstrual syndrome (PMS): Results of a randomized, placebo-controlled, double-blind clinical trial, Clinical Nutrition ESPEN (2018), https://doi.org/10.1016/j.clnesp.2018.01.067