

Improvement in Symptoms and Estrogen Metabolism in Women with Premenstrual Syndrome (PMS) Using a Newly Formulated Medical Food

Research Report Number 110 – May 10, 2001

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ABSTRACT

Premenstrual syndrome (PMS) is a common condition, afflicting up to 40% of menstruating women. PMS is represented by physical symptoms, such as pain and water retention, as well as disturbances of affect, cognition and performance that occur in the luteal phase of the menstrual cycle. No standard protocols exist to clinically manage PMS symptoms. Deficiencies in nutrients have been associated with PMS and nutritional support has been shown to benefit some PMS patients; however, the mechanisms of action of these interventions are not known. Alteration in steroid hormones, in particular estrogen and progesterone, has also been shown with PMS, but the full nature of the relationship between these hormones and PMS symptomatology is unknown. We propose that a key feature of PMS is a relative imbalance in the estrogen to progesterone activity, which can occur by increased levels of estrogen and/or changes in estrogen metabolism or excretion, resulting in a relative estrogen dominance. We have formulated a medical food to nutritionally support hormone balance by promoting estrogen metabolism and increasing its excretion. We have conducted a preliminary study to assess the effects of this medical food over 2 complete menstrual cycles. Using 2 well-validated PMS symptoms-specific questionnaires, we report that nutritional support with this medical food results in significant improvement in PMS symptoms, such as pain, water retention, and affect during both premenstrual and menstrual times ($p < 0.001$). Furthermore, quality-of-life data and steroid hormone metabolism markers, such as total estrogen excretion, serum progesterone and testosterone also showed significant improvements over the course of the intervention. These observations suggest that the medical food nutritionally supports metabolic changes in hormone metabolism, which are associated with an improvement in PMS symptomatology.

INTRODUCTION

Premenstrual syndrome (PMS) refers to a group of symptoms commonly experienced as disturbances of affect, cognition or performance, or somatic discomforts that occur in the second half or luteal phase of the menstrual cycle.¹ It is estimated to afflict up to 40% of women of reproductive age, with severe effects that can compromise ability to perform daily tasks in 5% to 10% of women.² Therapies for PMS are varied and no standard approach exists. Diet has been implicated in the onset and/or magnitude of PMS symptoms, and deficiencies in several nutrients have been associated with various PMS symptoms;³⁻⁶ however, research in this area has met with uneven success, and to date the underlying mechanism(s) of these nutritional interventions has been poorly understood.⁵⁻⁹

PMS is associated with menstrual cycles, and with low circulating progesterone in some studies. Moreover, excess estrogen levels, particularly during the early luteal phase, are observed in many women with PMS, suggesting endocrine metabolism plays a role in its etiology and/or symptoms.^{10,11} Surprisingly, double-blind, placebo-controlled studies of progesterone repletion have been equivocal.¹² Furthermore, case reports suggest that supplementation with estrogen, and not progesterone, is associated with improved symptoms in some, but not all women.¹³ Therefore, the exact nature of the hormonal imbalance remains elusive, but it is generally thought that an imbalance in the activity of estrogen to progesterone, in which the activity of estrogen is dominant, is common in women with PMS.¹⁰

Several factors affect the amount of active steroid hormone in the body, one of which is the level of sex

hormone binding globulin (SHBG) that is present. SHBG is the protein that transports certain steroids, in particular estrogen, in circulation: When bound to SHBG, estrogen is not biologically active. Vegetarian diets and some phytochemicals, such as isoflavones, have been shown to increase the level of SHBG.¹⁴⁻¹⁶ For example, a recent clinical study has shown that a low-fat, vegetarian diet is associated with an increase in SHBG and a decrease in symptom duration and intensity in women with PMS.¹⁷

A primary pathway for estrogen metabolism is transformation via phase I detoxification to form the catechol estrogens, which can then be further detoxified by either methylation or conjugation with glutathione. The methylated estrogen metabolites are less active estrogens and are cytostatic and antiangiogenic.^{18,19} The methyl donor in this case is S-adenosylmethionine (SAM). The production of SAM is associated with serum homocysteine (HCys) levels; that is, the higher the level of serum HCys, the lower the level of endogenous SAM available for support of methylation reactions.^{20,21} The production of SAM is nutritionally regulated and requires vitamins B12, B6, and folate, among other nutrients.^{22,23}

Estrogen metabolites are eventually excreted in the bile or urine. High-fiber, low-fat diets have been associated with lower levels of circulating estrogen in premenopausal women, as well as with a decreased risk of breast cancer.²⁴⁻²⁷ The mechanism(s) for these relationships are not known, but may involve a decrease in colonic beta-glucuronidase activity, which would decrease the amount of estrogen that is released for recirculation by enterohepatic recycling; and/or a decrease in transit time, which would increase general excretion of steroid metabolites.²⁸⁻³⁰ Interestingly, certain types of fibers have been shown to preferentially bind steroids, in particular estrogen, suggesting that some fibers might preferentially decrease estrogen due to an increased absorptive capacity.³¹ Studies investigating the chemical nature of these fibers have shown that the component called lignin is responsible for the specificity of estrogen binding. Lignin is found at high levels in wheat and flax fibers.^{32,33}

We propose that a key feature of PMS is a relative imbalance in estrogen to progesterone activity. This

imbalance can occur with increased levels of estrogen and/or changes in estrogen metabolism or excretion. The resulting relative estrogen dominance could account for some or all of the symptomatology of PMS. Furthermore, we propose that targeted nutrient support for estrogen transport, metabolism and excretion can attenuate PMS symptoms. We have formulated a medical food using phytochemicals that have been shown to nutritionally support estrogen metabolism. We have included specific fibers, like lignin, and other phytonutrients that have been shown to increase excretion of estrogen and its metabolites as well. We have performed a preliminary clinical trial to evaluate the effects of this nutritional support approach on PMS symptoms.

METHODS

Subjects

The clinical trial was performed at the Functional Medicine Research Center, Gig Harbor, WA. The inclusion criteria for the study were women between 21 and 45 years of age who were exhibiting significant symptoms of PMS as assessed by scores on a PMS symptoms-specific questionnaire (Shortened Premenstrual Assessment Form, described below). Exclusion criteria for the study included: evidence of untreated endocrine, neurological, or infectious disorder; pregnancy or lactation; history of diabetes, mental illness or attempted suicide, or liver, kidney or heart disease; use of oral corticosteroids within 4 weeks prior to the screening; use of prescriptive anti-arrhythmic or other cardiac medications.

Potential subjects were administered specific questionnaires during a phone interview to determine whether they were experiencing significant PMS symptoms. Candidates meeting the qualifying criteria, and who agreed to participate in the study, signed Informed Consents.

Study Design and Medical Food Intervention

The medical food used in this study was designed for nutritional support of women with symptoms associated with their hormone cycles. The nutrient profile of this medical food is shown in Table 1. Specifically, this medical food was designed with specific rice macronutrients of low-allergy potential

to provide protein and carbohydrate;³⁴ and flax meal to provide lignin, a fiber that shows specificity for binding steroid hormones,^{31,33} and lignans, a source of phytoestrogens.³⁵

Table 1. Composition of the medical food for nutritional support of symptoms related to hormone cycles,⁴⁴ provided as nutrients delivered in two servings per day.

Macronutrients	Amount per Day
Protein	30 g
Fiber	8 g
Carbohydrates	46 g
Fat	6 g
Micronutrients	Amount per Day
Vitamin A/Mixed Carotenoids	7500 IU
Vitamin C	120 mg
Vitamin D	400 IU
Vitamin E	600 IU
Vitamin K	80 mcg
Thiamin (B1)	1.5 mg
Riboflavin (B2)	1.7 mg
Niacin (B3)	20 mg
Vitamin B6	100 mg
Vitamin B12	60 mcg
Biotin	300 mcg
Folic Acid	1 mg
Pantothenic Acid	10 mg
Phosphorus	520 mg
Iron	18 mg
Calcium	700 mg
Iodine	150 mcg
Magnesium	480 mg
Zinc	15 mg
Selenium	70 mcg
Copper	2 mg
Manganese	2 mg
Chromium	200 mcg
Molybdenum	75 mcg
Isoflavones (from kudzu)	50 mg
Choline	500 mg
Curcumin	400 mg
Trimethylglycine	400 mg
N-Acetylcysteine	200 mg

The medical food was fortified with a nutrient core that included a non-soy source of isoflavones, which modifies effects of endogenous estrogen;³⁶⁻³⁸ the phytonutrient curcumin, which has been shown to act synergistically with the isoflavone genistein;³⁹ enhanced levels of B vitamins with choline, trimethylglycine and N-acetylcysteine, which support estrogen detoxification and methylation metabolic pathways;⁴⁰⁻⁴² and magnesium, calcium, and vitamin E, which have been associated with amelioration of PMS symptoms.⁴³

The study was a boxed, 2-armed trial with stratification

based on the use of oral birth control medication. Primary endpoints were monitored at the Screening Visit, Visit 1, Visit 2, and Visit 3. At Visit 1, subjects were randomized and baseline serum and urine were obtained. All visits were planned at the time when each woman was in the luteal phase of her cycle (i.e., at 75% - 80% through the subject's usual menstrual cycle). The trial lasted for 3 complete menstrual cycles.

All subjects were randomly assigned to Group A [medical food and a capsule containing the phytonutrient indole-3-carbinol (I3C)], or Group B [medical food and a placebo capsule].⁴⁴ Both clinicians and subjects were blinded regarding the randomization. (The I3C was included in this study to determine if additional benefit could be achieved from targeted nutritional modulation of detoxification activities and is discussed in greater detail in Research Report No. 111.)⁴⁵

Clinical Assessment

Two research-validated, PMS-specific questionnaires were chosen for monitoring PMS symptoms: the Shortened Premenstrual Assessment Form (SPAF) and the Menstrual Distress Questionnaire (MDQ).⁴⁶⁻⁴⁸ The SPAF rates symptoms in the second half of a woman's menstrual cycle on a scale of 1 through 6 (1= no symptoms; 6= extreme symptoms). The MDQ uses a five-point scale (0= no symptoms; 4= severe symptoms), and rates symptoms for three different stages of the menstrual cycle: premenstrual (4 days before most recent flow); menstrual (most recent flow); and intermenstrual (remainder of cycle). The MDQ data is transformed to provide a normalized score for which a population mean of 50 and a standard deviation of 10 have been determined as reference values. Scores higher than 50 ± 10 indicate PMS symptoms are present.

Subjects were also asked to fill out the Medical Outcomes Survey SF-36 questionnaire, a well-validated, quality-of-life instrument.⁴⁹ Information on symptoms and medication use, as well as assessment of compliance to the protocol, was obtained at each visit.

Laboratory Assessment

Aspartate aminotransferase, alanine aminotransferase, bilirubin, urea nitrogen, creatinine, albumin, and glucose were assessed by standard photometric methods from 10-12 hour fasting serum obtained at the Screening

Visit and Visit 3. The following were performed on 10-12 hour fasting serum from Visit 1 and Visit 3 (Laboratories Northwest, Tacoma, WA): photometric measurements of triglycerides, and total-, HDL- and LDL-cholesterol; radioimmunoassay measurements of SHBG, progesterone and testosterone; automated chemiluminescence analysis (DPC Immulite 2000) of bound estradiol; and high performance liquid chromatography quantification of homocysteine. Urinary estrogen metabolites (estradiol, estrone, and estriol) were obtained from a 24-hour urine collection at Visit 1 and Visit 3, and were quantified by gas chromatography/mass spectrophotometric methods (AAL Reference Laboratories, Santa Ana, CA). Total estrogen excretion was determined by addition of the 24-hour excretion of the 3 estrogen metabolites.

Statistical Analysis

Baseline data (the level of symptoms experienced in the preceding 2 menstrual cycles) were obtained from averaging the Screening Visit and Visit 1 values, and served as a control for non-intervention variability. Laboratory and questionnaire data were analyzed by a one-way analysis of variance (ANOVA) using JMP Statistical Package (SAS Institute, Cary, NC). Variances in laboratory analyses were determined using split sample analysis.

RESULTS

Fifty-one subjects qualified for the trial; 8 of these dropped out of the trial after the initial screening but prior to any intervention. Therefore, 43 subjects began the clinical trial; of these, 3 subjects were withdrawn from the trial during the course of the intervention (1 subject withdrew for unknown reasons, but commented on the taste of the medical food, and 2 subjects experienced adverse symptoms that included gastrointestinal pain and diarrhea). Forty subjects, between the ages of 21-45 (average 36 ± 6 years), completed the clinical trial.

Subjects showed laboratory values within the normative reference range for liver and kidney function prior to, and after the intervention with the medical food (Table 2). Alanine aminotransferase appeared to increase after intervention; however, more variability was observed in the post intervention value, and both pre- and post-intervention were well within the

reference range. Lipid panel and blood glucose assessments from 10-12 hour fasting serum were also within normative values and displayed no change following intervention.

Table 2. General laboratory markers for subjects.

	Reference Range	Mean (\pm sem)		p
		Baseline	Final	
Total cholesterol (mg/dL)*	120-200	182 (4.9)	190 (5.0)	ns
HDL (mg/dL)*	55-70	51 (2.1)	55 (2.2)	ns
LDL (mg/dL)*	80-130	111 (4.4)	115 (4.5)	ns
Triglycerides (mg/dL)*	10-175	104 (8.2)	104 (8.1)	ns
Glucose (mg/dL)†	65-120	85 (1.9)	87 (1.4)	ns
Albumin (g/dL)*	3.2-5.0	3.8 (0.04)	3.8 (0.03)	ns
Bilirubin (mg/dL)†	0.0-1.4	0.26(0.03)	0.34 (0.03)	ns
Urea nitrogen (mg/dL)†	8-24	13 (0.6)	12 (0.5)	ns
Creatinine (mg/dL)†	0.6-1.2	0.76(0.10)	0.78 (0.10)	ns
Aspartate aminotransferase (IU/L)†	10-56	22 (0.7)	24 (1.2)	0.08
Alanine aminotransferase (IU/L)†	5-60	22 (0.8)	30 (1.7)	<0.01

*N= 39; †N= 40; p= significance

The questionnaire data showed no difference between the medical food/I3C group and the medical food/placebo group (data not shown); therefore, questionnaire results for the 2 treatment groups were pooled for the purpose of analysis. Eleven patients were on oral birth control pills; however, since no differences were noted between those on birth control and those not on birth control (data not shown), these data were pooled as well.

The SPAF provides a score for total overall symptoms, as well as 3 subscores for pain, water retention, and negative affect. Subjects showed no significant change in symptoms during the 2 cycles of the baseline time course: the Screening Visit and Visit 1 average scores were 44.6 and 41.7, respectively. After beginning the intervention with the medical food, the subjects reported an average total score for symptoms of 29.3 at Visit 2, and 22.9 at Visit 3, which is a 59% reduction in symptoms with a statistical significance of $p < 0.05$. These results are graphically depicted in Figure 1. Significant decreases were consistently observed in all categories of the SPAF (Table 3), with improvements of the subscores for pain, water retention, and negative affect of 61%, 58%, and 61%, respectively, ($p < 0.05$).

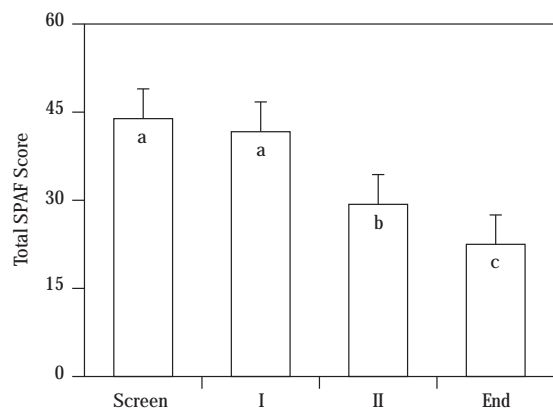


Figure 1. The total score for the Shortened Premenstrual Assessment Form (SPAF) is shown for the subjects who completed the 3-month clinical study. The Screening Visit and Visit 1, which occurred prior to intervention with the medical food, are labeled as “Screen” and “I,” respectively. Visit 2 and Visit 3, which occurred after 1-month and 2-months of intervention with the medical food, are labeled as “II” and “End,” respectively. The error bars represent a pooled evaluation of the standard deviation. The values that do not differ significantly are designated with the same letters; the values that differ significantly ($p < 0.05$) are designated by sequential letters.

Table 3. Mean changes (\pm sd) in Shortened Premenstrual Assessment Form (SPAF) scores after intervention with medical food in subjects with PMS symptoms ($n = 38$).

SPAF Category	Screening Visit	Visit 1	Visit 2	Visit 3
Affect	20.6 (2.6) ^a	18.3 (3.2) ^a	13.3 (4.9) ^b	9.6 (4.7) ^c
Pain	12.3 (3.5) ^a	11.5 (3.0) ^a	8.0 (3.2) ^b	6.6 (2.4) ^b
Water Retention	12.6 (3.6) ^a	11.8 (3.7) ^a	8.6 (3.4) ^b	6.6 (2.8) ^b
SPAF Total Score	44.6 (9.4) ^a	41.7 (8.0) ^a	29.3 (10.4) ^b	22.9 (8.3) ^c

The total SPAF score is pooled data from the 3 subsections. Incomplete questionnaires were not included in the analysis.

Entries share a superscript (a, b, or c) if they do not differ significantly ($\alpha = 0.05$) from each other. Sequential letters indicate a difference of $p < 0.05$ from the preceding value.

The MDQ provides a more detailed assessment of PMS symptoms, which are presented in 7 PMS symptom-specific subcategories (pain, water retention, autonomic reactions, negative affect, impaired concentration, behavior change, and arousal) and 1 control subcategory for 3 different times during a woman’s cycle (intermenstrual, menstrual, and premenstrual). The control category contains questions that have been reported more frequently by menopausal women but are infrequently reported by premenopausal woman and has been included in the questionnaire as

an internal control for a woman’s tendency to report symptoms that may not be related to PMS. Table 4 shows the mean (\pm sem) for the subjects’ responses to the different symptom categories of the MDQ during the intervention.

Table 4. Mean (\pm sem) of Menstrual Distress Questionnaire (MDQ) results of PMS symptoms for the 40 subjects on the medical food.

Screening Category	Visit	Visit 1	Visit 2	Visit 3	Significance (p)
Pain					
Intermenstrual	69.8 (5.2)	62.0 (3.8)	58.3 (2.8)	56.8 (3.0)	0.0753
Menstrual	73.0 (3.2) ^a	72.0 (3.4) ^a	55.4 (2.5) ^b	53.5 (2.6) ^b	< .0001
Premenstrual	81.4 (3.2) ^a	76.8 (3.5) ^a	63.1 (2.6) ^b	57.1 (2.9) ^b	< .0001
Water Retention					
Intermenstrual	69.1 (6.0)	61.1 (4.6)	55.6(3.3)	53.8 (2.7)	0.0595
Menstrual	74.6 (3.3) ^a	71.6 (3.4) ^a	56.8(2.6)	51.8 (2.3) ^b	< .0001
Premenstrual	83.4 (2.9) ^a	81.2 (3.7) ^a	64.5(3.1) ^b	58.2 (2.6) ^b	< .0001
Autonomic Reactions					
Intermenstrual	56.4 (6.9)	45.4 (3.9)	45.1(3.8)	41.4 (2.8)	0.1212
Menstrual	69.1 (4.7) ^a	64.1 (4.7) ^{ab}	53.4(3.1) ^b	50.3 (2.2) ^b	0.0014
Premenstrual	75.2 (5.2) ^a	68.7 (4.8) ^{ab}	57.5(3.5) ^{bc}	53.3 (2.5) ^c	0.0007
Negative Affect					
Intermenstrual	73.5 (5.6) ^a	64.2 (4.3) ^{ab}	54.5(2.9) ^b	56.0 (3.1) ^b	0.0045
Menstrual	78.3 (3.8) ^a	76.7 (3.6) ^a	58.4(3.1) ^b	52.8 (2.6) ^b	< .0001
Premenstrual	90.5 (2.2) ^a	84.7 (2.6) ^a	63.2(2.6) ^b	55.3(2.4) ^b	< .0001
Impaired Concentration					
Intermenstrual	68.3 (4.6) ^a	61.0 (2.8) ^{ab}	56.8(2.6) ^{ab}	54.5(2.6) ^b	0.0187
Menstrual	78.0 (5.6) ^a	79.7 (5.5) ^a	60.0(3.8) ^b	56.1(3.1) ^b	0.0002
Premenstrual	88.0 (5.5) ^a	87.5 (4.7) ^a	65.8(3.5) ^b	61.4 (3.6) ^b	< .0001
Behavior Change					
Intermenstrual	67.0 (5.4)	59.3 (3.7)	53.4(2.4)	54.4(3.0)	0.0461
Menstrual	71.4 (4.1) ^a	69.3 (4.1) ^a	53.3(2.3) ^b	48.7(2.2) ^b	< .0001
Premenstrual	86.4 (5.6) ^a	77.5 (4.4) ^a	59.5(2.9) ^b	54.5(3.2) ^b	< .0001
Arousal					
Intermenstrual	60.5 (3.1)	57.3 (2.7)	56.7(2.6)	51.4 (2.3)	0.1242
Menstrual	55.7 (2.2)	54.2 (2.3)	55.7(2.4)	49.7 (2.3)	0.2091
Premenstrual	53.9 (3.1)	56.2 (2.6)	55.4(2.4)	50.1 (2.2)	0.3519
Control					
Intermenstrual	63.6 (4.6)	58.7 (4.2)	58.4(5.5)	53.5 (3.6)	0.4723
Menstrual	62.6 (3.3)	63.7 (5.2)	53.1(3.3)	51.1 (2.2)	0.0286
Premenstrual	71.1 (4.6) ^a	70.0 (5.5) ^a	58.3(4.2) ^{ab}	53.8 (2.9) ^b	0.0111

The data are presented for the 7 categories of PMS symptoms and the control category, which rates symptoms not generally associated with PMS as an internal control for intermenstrual, menstrual, and premenstrual times during each cycle. The scores are presented as T-scores, which for the population have a mean of 50 and a standard deviation of 10. The significance (p) was obtained from ANOVA analysis. Entries within a symptom class that share a superscript do not differ significantly from each other at ($\alpha = 0.05$, as determined by using the Tukey-Kramer honestly significant difference (HSD) analysis.

As assessed by the MDQ, subjects reported significant improvement ($p < 0.0002$) in pain, water retention, negative affect, impaired concentration, and behavior change during the menstrual and premenstrual times

after intervention with the medical food. Subjects reported significant improvement in negative affect and behavior change ($p < 0.005$ and $p < 0.05$, respectively) during the intermenstrual time as well. Improvement was also noted in autonomic reactions. The control symptoms showed some improvement, but not nearing the level of significance of those of the other categories (Table 4; Fig. 2), whereas little change was reported for the arousal symptoms category.

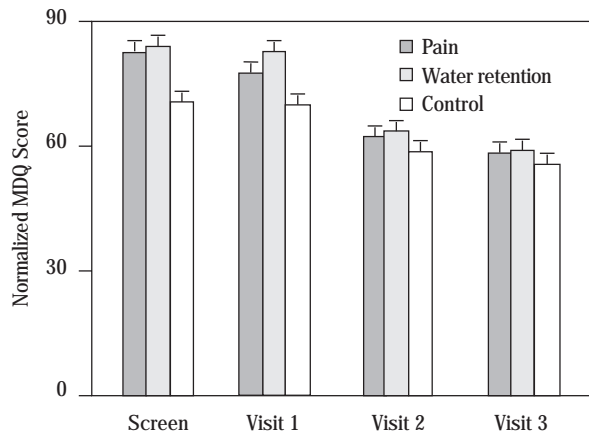


Figure 2. Scores from representative categories of the MDQ are shown for all subjects who completed the clinical study. The data represents scores for the premenstrual time of the subjects' cycle for the categories of pain (dark grey bars), water retention (light gray), and control (open bars). The normative score for all categories is 50 ± 10 , in which a higher score represents an increase in symptoms. Highly significant decreases in symptoms were observed from the Screening Visit to Visit 3 for the pain and water retention symptoms categories, $p < 0.0001$, whereas the change for the control category was more modest ($p = 0.0111$). The full data set is presented in Table 4.

The SF-36 quality-of-life assessment reports general health and well-being as two scores: the Physical Component Score (PCS), an indication of physical pain and ability to function; and the Mental Component Score (MCS), an indication of mood and affect. The PCS and MCS are normalized to 50, which is the average score observed in a healthy population. At initiation of the trial, the subjects rated $51.2 (\pm 1.2)$ on the PCS, which remained constant throughout the trial ($p = 0.9773$). The initial MCS scores were $38.8 (\pm 1.6)$ and $38.9 (\pm 1.6)$ for the Screening Visit and Visit 1, respectively, well below the mean, suggesting compromised mental well-being at initiation of the

trial; the MCS scores were significantly increased by the end of the trial to $47.0 (\pm 1.5)$ and $48.5 (\pm 1.4)$; $p < 0.0001$ for Visit 2 and Visit 3, respectively. These results are graphically depicted in Figure 3.

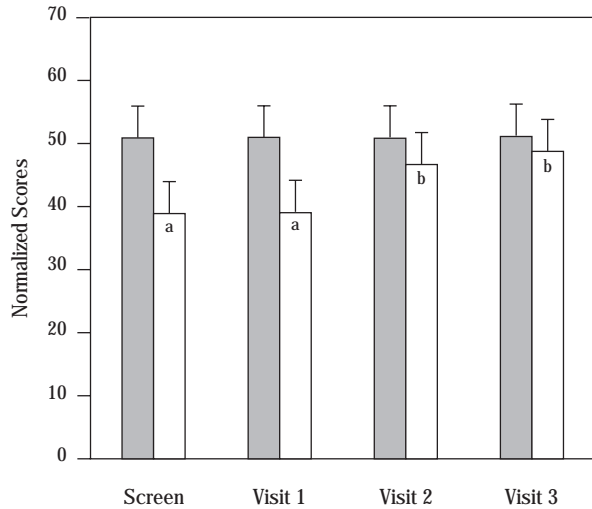


Figure 3. The scores for the quality-of-life assessment using the SF-36 questionnaire are shown for the subjects who completed the 3-month clinical study. The scores are provided as the Physical Component Score (PCS) and the Mental Component Score (MCS). The mean (\pm sd) scores for the Screening Visit and Visit 1, which occurred prior to intervention, and Visit 2 and Visit 3, which occurred after intervention for the PCS (shaded bars) are $51.2 (7.2)$, $51.6 (7.2)$, $51.7 (6.6)$, and $51.8 (5.8)$, respectively ($p = 0.9773$). The mean (\pm sd) scores for the Screening Visit, Visit 1, Visit 2, and Visit 3 for the MCS (open bars) are $38.8 (9.7)$, $38.9 (9.7)$, $47.0 (9.5)$, and $48.5 (8.5)$, respectively ($p < 0.0001$). The error bars represent a pooled evaluation of the standard deviation. The values that do not differ significantly are designated with the same letters; the values that differ significantly ($p < 0.05$) are designated by sequential letters.

The total excretion of estrogen metabolites, as assessed by a 24-hour urinary excretion of estrone, estradiol, and estriol was significantly increased after the intervention with the medical food ($p < 0.005$) when data from all subjects were analyzed (Table 5). When total estrogen excretion was analyzed using the geometric mean (90% confidence), an increase was observed from $49.3 (43.1 - 56.5) \mu\text{g}/24$ hours initially to $69.7 (59.4 - 81.7) \mu\text{g}/24$ hours after the intervention with the medical food. Some beneficial changes were noted in serum steroid hormone metabolism markers as well, such as a decrease in HCys and testosterone and an increase in progesterone, but when data from all subjects were analyzed no significant changes were observed.

Table 5. Serum and urinary markers associated with hormone transport, metabolism, and excretion for all subjects who completed the trial.

	Reference Range	Mean (\pm sem)		p
		Baseline	Final	
Homocysteine ($\mu\text{mol/L}$)*	< 9.0	7.3 (0.3)	6.6 (0.2)	0.07
Total testosterone (ng/dL)*	15-70	28.6 (2.1)	28.5 (1.9)	ns
Free testosterone (pg/mL)*	1.0-8.5	4.2 (0.4)	3.8 (0.3)	ns
Progesterone (ng/mL)*	0.2-28	8.8 (1.3)	11.4 (1.6)	ns
SHBG (nM)*	17-120	82.2 (11.0)	81.4 (10.2)	ns
Bound estradiol (pg/mL)*	60-130	58.8 (8.7)	65.3 (9.0)	ns
Excreted estrogen (μg) ^{†,§}	18-162	53.5 (4.0)	77.6 (6.6)	< 0.005

*N= 39; [†]N= 35; [§]Normative data are for estrogen excretion during the luteal phase.

Total estrogen excretion includes estrone, estradiol, and estriol excreted over 24 hours.

Although no significant changes in serum markers were noted when all data were analyzed, when the data were stratified based upon whether the subject showed initial values near the limit or outside of the normative range, significance was established, as shown in Table 6. Twenty-eight women presented with low bound estradiol, as compared to the reference range (< 60 pg/mL); a significant increase in bound estradiol to 63.7 (\pm 10.3) pg/mL was observed in these women after the intervention ($p=0.002$). The 16 women who presented with elevated unbound testosterone, defined as > 1.5% free testosterone, showed a statistically significant decrease in serum testosterone ($p<0.001$). The 26 women with low initial serum progesterone, (< 10 ng/mL), responded to the intervention with a statistically significant increase in serum progesterone to 10.2 (\pm 2.01) ng/mL ($p<0.005$; Fig. 4). Likewise, the 12 women with elevated HCys (> 8 mol/L; Fig. 5) at the start of the trial responded with a statistically significant decrease in serum HCys ($p<.001$). SHBG also showed an increase from pre- to post-intervention in the 20 individuals who had initially low values (< 5.5 nmol/L) from 39.9 (\pm 2.0) to 43.3 (\pm 2.7) nmol/L, respectively, but the increase was not statistically significant.

Table 6. Mean (\pm sem) serum hormone metabolites of subjects for whom initial laboratory values were either at the limits of, or not within reference range.

	Criterion	N	Baseline	Final	p
High free testosterone	> 1.5%	16	1.90 (0.09)	1.53 (0.04)	< 0.001
Low progesterone	< 10 ng/mL	26	4.1 (0.44)	10.2 (2.01)	< 0.005
Low SHBG	< 55 nmol/L	20	39.9 (2.0)	43.3 (2.7)	0.07
Low bound estradiol	< 60 pg/mL	28	31.3 (2.7)	63.7 (10.3)	0.002
High homocysteine	> 8 $\mu\text{mol/L}$	12	9.4 (0.4)	7.3 (0.3)	< 0.001

Data are provided for Baseline (prior to medical food intervention) and Final (after 2 months of medical food intervention) values, in addition to the criterion used to select data for each analysis.

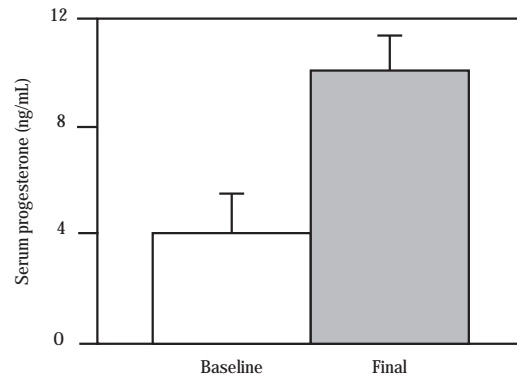


Figure 4. The means (\pm sem) are graphically shown for initial and final serum progesterone for the 26 subjects who showed initial serum progesterone values of below 10 ng/mL. In these subjects, serum progesterone significantly increased from 4.1 (0.44) to 10.2 (2.01) over the course of the 2-month intervention ($p<0.005$; pooled sem = 1.45).

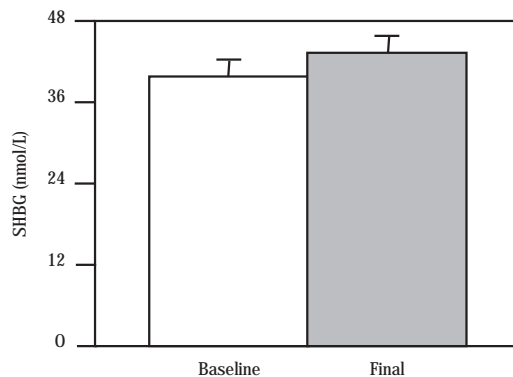


Figure 5. The means (\pm sd) are graphically shown for initial and final sex hormone-binding globulin (SHBG) for the 20 subjects who showed initial SHBG values of below 55 nmol/L. In these subjects, SHBG significantly increased from 39.9 (2.0) to 43.3 (2.7) over the course of the 2-month intervention (pooled sem = 2.38).

DISCUSSION

We have conducted a preliminary study to assess the effects of a newly formulated medical food for the nutritional support for symptoms related to hormone cycles, with or without the phytonutrient I3C, over 2 complete menstrual cycles on PMS symptomatology. The primary endpoint for this trial was subjective improvement of PMS as determined by 2 well-validated PMS symptoms-specific questionnaires: the SPAF and the MDQ. The results of the SPAF and MDQ suggest that consumption of this medical food nutritionally supported significant improvement in PMS-specific symptoms such as pain, water retention, affect and mood. Furthermore, quality-of-life data and laboratory markers, such as total estrogen excretion, serum progesterone and testosterone, also showed significant improvement over the course of the intervention. These observations suggest that the medical food nutritionally supports metabolic changes in hormone metabolism that are associated with improvement in PMS symptomatology.

Data from subjects on and not on oral contraceptives were pooled due to failure to find distinction. Data between the 2 groups in the trial, the medical food/I3C and medical food/placebo group, were also pooled since no differences in the primary end-points were noted between the 2 groups. The inability to distinguish between the 2 treatment groups argues only that I3C treatment had no additional effect on the resolution of PMS symptoms over that of the medical food alone. Data on estrogen metabolism suggests differences did occur in estrogen metabolites with the I3C and, consistent with published literature, that inclusion of I3C with the medical food may promote higher levels of the safer estrogenic metabolite, 2-hydroxyestrone (2OH-E).^{45,50} The role of the estrogenic metabolites, such as 2OH-E, in etiology or enhancement of symptoms remains unclear; however, 2OH-E is considered a safer estrogen because higher levels of 2OH-E are associated with a decrease in risk of hormone-dependent cancers, such as breast cancer.⁵¹⁻

One hypothesis for the biochemical imbalance underlying PMS symptomatology is an imbalance in the activity of estrogen to progesterone. This relative increase in estrogen activity has been termed estrogen dominance. High estrogen activity may be due to a

low level of overall excretion of the estrogen metabolites, a decrease in SHBG with high serum (free) levels of estrogen, and/or an increase in the more estrogenic metabolites over the less estrogenic metabolites. The medical food was designed, in part, to nutritionally support an increase in estrogen excretion by providing fibers that preferentially bind sex hormones, including estrogen. Fiber can also facilitate excretion of estrogen by its effect on increasing transit through the colon. Data on estrogen excretion suggests that consumption of the medical food did result in a significant increase in excretion of estrone, estrinol, and estradiol in the subjects on the trial ($p < 0.005$).

The amount of estrogen and testosterone available to cells is influenced by the amount of SHBG present in circulation. SHBG can bind free estrogen or testosterone and, while bound, these hormones are not active. About half of the circulating testosterone and approximately 80% to 90% of circulating estrogen is bound to SHBG under optimal conditions.¹⁶ SHBG is produced in the liver, and its production is regulated by steroidal and peptidic hormones, and by dietary factors.^{15,16} In particular, dietary isoflavones and lignans have been shown to significantly increase the production of SHBG.^{15,54,55} In this study, consumption of the medical food resulted in an increase in SHBG levels in those individuals who initially presented with the lowest levels of SHBG ($p = 0.07$). A moderate, but non-significant decrease in free testosterone was noted when data from all subjects were analyzed, whereas no change in serum testosterone was observed; however, a significant decrease in free testosterone was observed when the data from subjects who presented with the highest levels of free testosterone were reviewed ($p < 0.001$). A significant increase in bound estradiol was also observed in the 28 women who presented with low bound estradiol ($p = 0.002$). Taken together, these observations suggest that SHBG levels were increased as a result of the medical food intervention.

One pathway for metabolism of the estrogen metabolites involves methylation by the catechol-O-methyltransferase enzyme, which uses the methyl-donor SAM. The methylated estrogens show low estrogenic activity, are considered anti-estrogenic, and are rapidly excreted.¹⁹ The methylated estrogen

derivative of 2OH-E has been shown to inhibit the growth of breast cancer cells, have antiangiogenic activity, and inhibit adipocyte proliferation, suggesting it may be a protective estrogen.⁵⁶⁻⁵⁹ Thus, nutritional support for production of SAM, and therefore for methylation itself, may positively influence estrogen metabolism. Nutrients that support SAM production included in the medical food are vitamins B6, B12, and folate, as well as choline and trimethylglycine. It is unknown whether these nutrients resulted in an increase in methylation of estrogen in this trial; however, a quarter of the subjects presented with high circulating HCys levels, which is an indication of compromised methylation. The level of HCys was significantly decreased over the course of the intervention in these subjects ($p < 0.001$), suggesting that methylation was improved.

Estrogen dominance can occur when estrogen metabolism is normal and progesterone production is low. Over half of the subjects in this trial presented with low or low-normal initial serum progesterone levels, and the serum progesterone was significantly increased over the course of the intervention in these subjects ($p < 0.005$). Few data have been reported on the role of nutritional support for progesterone production, and its role in PMS symptomatology is controversial. For example, although the most popular theory of hormone involvement in PMS symptoms implicates low progesterone during some phase of the cycle, placebo-controlled trials with progesterone supplementation have not unequivocally ameliorated symptoms and, therefore, have not supported this hypothesis.^{10,13} Furthermore, increased levels of luteal phase estrogen have been associated with more intense PMS symptoms.^{11,12} Thus, it would appear that estrogen makes PMS symptoms worse.

In contrast to the observations that high levels of estrogen are associated with more intense PMS symptoms, estrogen supplementation has been shown to attenuate PMS symptoms.¹³ Therefore, the role of estrogen and progesterone in PMS symptomatology is unclear. We propose that the important factor is not just the absolute levels themselves, but the ratio of estrogen to progesterone, and possibly the nature of the estrogen metabolites within this ratio. The observed increase of progesterone in individuals who initially

displayed the lowest serum progesterone levels could have resulted in reestablishment of a more balanced, beneficial estrogen-to-progesterone ratio. Alternatively, increases in serum progesterone may have occurred from an increase in ovulatory cycles, which can also affect the ratio of estrogen to progesterone in the luteal phase of the menstrual cycle.

PMS symptoms show a strong placebo effect. The preliminary clinical trial reported in this paper did not contain a control group, and therefore, placebo effect should be considered in evaluating these data. The MDQ contains a control category that allows an estimation of placebo effect, since it reflects symptoms not generally associated with PMS that should be equally responsive to placebo as PMS-specific symptoms. We did observe some change in symptoms in the control category of the MDQ. The MDQ control category includes the symptoms of chest pains, feelings of suffocation, ringing in the ears, heart pounding, numbness and tingling, and effects on vision.⁴⁷ Although these symptoms are not generally associated with PMS, some of them are associated with early perimenopause, which has similar hormonal fluctuations as PMS. The overlap of symptoms may explain why a significant change was observed in this category for menstrual and premenstrual symptoms ($p < 0.03$). However, this change was not as highly significant as the changes in pain, water retention, affect, concentration, and behavior for menstrual and premenstrual symptoms ($p < 0.0001$). Moreover, laboratory markers show significant changes, which would be unlikely to result from a placebo effect alone. Therefore, taken together, these data are fully concordant and suggest that the medical food, via nutritional modulation of hormone metabolism, significantly reduces PMS symptoms.

ACKNOWLEDGEMENTS

We thank Tracey Irving and Dave Lindhorst for preparation of the medical food product. We also thank Julie Triggs, Jill Edwards and Nancy Chatfield for clinical support, and Mabel Lorenzi-Albe and Lisa Leupp for editorial comments and critiques.

NOTE

Financial support for this study provided by Metagenics, Inc. Dan Lukaczer, ND, is the Director

of Clinical Research; DeAnn J. Liska, PhD, is the Director of Technical Information and Research Publications; Gary Darland, PhD, is the Director of Applied Science; Barbara Schiltz, MS, CN, RN, is a Clinical Research Associate; and Matt Tripp, PhD, is the Vice President of Research and Development for the Functional Medicine Research Center, the research arm of Metagenics, Inc. Jeffrey S. Bland, PhD, is President of Metagenics, Inc. and President of the Board of Directors for the Institute for Functional Medicine, a non-profit educational organization, Gig Harbor, WA. Robert H. Lerman, MD, PhD is the Medical Director for the Functional Medicine Research Center, Metagenics, Inc. and the Director of Medical Education for the Institute for Functional Medicine.

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