A RANDOMISED CONTROL TRIAL COMPARING THE EFFECTIVENESS OF A SINGLE NUTRIENT VERSUS A BROAD SPECTRUM MICRONUTRIENT FORMULA IN THE TREATMENT OF PREMENSTRUAL SYNDROME

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Abstract

This thesis presents the Natural Treatment of Premenstrual syndrome (NTP) study, a randomised, treatment controlled trial investigating the use of a broad spectrum micronutrient formula, compared to vitamin B6, for the treatment of Premenstrual Syndrome (PMS). Seventy-eight (72 completed) participants with moderate to severe PMS were randomised to receive either a micronutrient formula containing 36 ingredients made up of vitamins, minerals, amino acids, and antioxidants or a single nutrient formula. The intervention lasted for three menstrual cycles and was followed by a naturalistic follow up three cycles post treatment. There is considerable evidence in the literature that nutrient treatments are effective for PMS, while micronutrients have demonstrated treatment efficacy in a variety of mental health disorders. Within the field of mental health, recognition of possible underlying metabolic dysfunction as a causal factor is being recognised. There are early indications that nutrient treatments, especially broad spectrum formulas, may be effective in reversing some of the damage caused by metabolic dysfunction. Therefore, the available evidence led to the hypothesis that the micronutrient formula would outperform the single nutrient treatment.

The Daily Record Severity of Problems (DRSP) was used to establish PMS as well as record change in PMS on five variables: psychological, somatic, and total symptoms, impact and worst day ratings. Linear mixed models analysis indicated both treatments produced comparable reduction in PMS symptoms with medium effect sizes (ES) across the five PMS variables (micronutrient ES=0.50-0.56; B6 ES=0.43-0.56) with no between group differences. Based on the DRSP, 72% of the micronutrient and 60% of the vitamin B6 group went into remission in PMS symptoms. A between group difference ($d=0.51$, $p<0.05$) was observed in health-related quality of life, with greater improvement in micronutrient-treated participants. Analysis of women (n=28) who met criteria for Premenstrual Dysphoric Disorder (PMDD) showed clinical (but not statistical) advantage of micronutrients on total symptoms (ES=1.67; 64% remission) over B6 (ES=0.61; 50% remission). There were no group differences in side effects, nor any serious adverse effects reported.
Both micronutrients and vitamin B6 provided similar benefit for reducing symptoms associated with PMS, with greater effect on quality of life for those consuming micronutrients as well as potential advantage of micronutrients for those with PMDD. Future research in this area should attempt to understand what changes in inflammatory markers are affected by nutrient supplementation. Replication with larger samples, specifically women with PMDD, is required.
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List of Abbreviations

ACOG: American Congress of Obstetricians and Gynaecologists
ANCOVA: Analysis of Covariance
ATP: adenosine triphosphate
BP: Bipolar Disorder
CGI: Clinical Global Impression Scale
CI: Confidence Interval
CRP: C-Reactive Protein
CNS: Central Nervous System
DRSP: Daily Record Severity of Problems
DASS: Depression, Anxiety and Stress Scale
DSM-5: Diagnostic and Statistical Manual- 5th Edition
ETC: electron transport chain
ES: Effect Size
FSH: Follicle-Stimulating Hormone
GF: germ-free
GnRH: Gonadotropin-Releasing Hormone
HPA: Hypothalamic-Pituitary-Adrenal
IL: Interleukin
IFY-γ: Interferon Gamma
ITT: Intention to Treat
LH: Luteinizing Hormone
LMM: Linear Mixed Models
MDD: Major Depressive Disorder
MBP: Modified Brinley Plots
MtDNA: mitochondrial DNA
NSSS-S: New Sexual Satisfaction Scale-Short Form
NZSEI-06: New Zealand Socio Economic Index 2006
OR: Odds Ratio
OXPHOS: oxidative phosphorylation
PMDD: Premenstrual Dysphoric Disorder
PMS: Premenstrual Syndrome
PP: Per-Protocol
PSS: Perceived Stress Scale
PSQI: Pittsburgh Sleep Quality Index
RDA: Recommended dietary allowances
RCI: Reliable Change Index
RCT: Randomised Control Trial
ROS: Reactive Oxygen Species
SES: Socio-Economic Status
SSRI: Selective Serotonin Reuptake Inhibitor
TNF-α: Tumor Necrosis Factor Alpha
WQoLQ: Women’s Quality of Life Questionnaire
Chapter 1: Premenstrual Syndrome and Treatment

This thesis presents the Natural Treatment of Premenstrual syndrome (NTP) study, a randomised, treatment controlled trial investigating the use of a broad spectrum micronutrient formula, compared to vitamin B6, for the treatment of Premenstrual Syndrome. This chapter will begin by outlining the definition, prevalence and course of the syndrome and the more symptomatic form; Premenstrual Dysphoric Disorder. I will draw links between Premenstrual Syndrome and depression and present an overview of the menstrual cycle before embarking on a lengthy discussion of the possible causes and treatment. Treatment approaches will specifically focus on pharmacological and natural-based, with a brief discussion on lifestyle which is relevant to the area of nutrition.

1.1 Definition

Premenstrual syndrome, also known as premenstrual tension, is a collection of psychological and physical symptoms that systematically occur during the luteal phase (the one to two weeks before menses), in a woman’s menstrual cycle (Hofmeister & Bodden, 2016). During their reproductive years, the majority of women will experience at least one emotional or physical complaint per cycle (Cunningham, Yonkers, O’Brien, & Erikson, 2009). Roughly, 20 to 40% of women will suffer from numerous symptoms, warranting the diagnosis of Premenstrual Syndrome (PMS) (Rapkin & Winer, 2009). However, definitions of PMS, specifically the number and type of symptoms, vary widely by organisation and by research study. The American Congress of Obstetricians and Gynaecologists (ACOG) define the disorder as present if a woman experiences; at least one physical and one affective symptom, which abate within four days of menstruation and are not present again until day 13 of the cycle, occur for two consecutive cycles of prospective recording, and cause a notable impact in work or social pursuits (American College of Obstetricians and Gynecologists, 2014).
An even more symptomatic form of PMS, known as Premenstrual Dysphoric Disorder (PMDD) is found in 1.8-5.8% of women (American Psychiatric Association, 2013). Commonly recorded symptoms of PMS and PMDD include those outlined in the Diagnostic and Statistical Manual (DSM-5); affective lability, irritability, anger, increased interpersonal conflict, depressed mood, anxiety, difficulty concentrating, fatigue, change in appetite, sleep disturbance, feelings of being out of control, less interest in normal activities as well as physical symptoms such as bloating, breast swelling, and joint pain (American Psychiatric Association, 2013). To receive a DSM-5 PMDD diagnosis, a woman must experience five or more symptoms during the luteal phase, with at least one symptom of mood alteration e.g. depressed, anxious, or irritable mood, or affective lability. Prospective daily ratings of premenstrual symptoms are to take place over two menstrual cycles to confirm the diagnosis. Much like the diagnostic criteria used by the ACOG, symptoms must be present in the week leading up to menses and occur repeatedly during this phase, resolve within a few days of menses, and cause clinically significant distress or life interference. As this cluster of symptoms must be differentiated from other psychiatric or physical disorders before a diagnosis is made, the timing of the complaints is important (Hofmeister & Bodden, 2016). While symptoms of other disorders such as anxiety or depression may worsen during the luteal phase, they can be separated from PMS due to its cyclic nature.

These monthly symptoms can present significant problems for many women. The impact is regularly seen in school and work efficiency and/or productivity, social participation, and interpersonal relationships (American Psychiatric Association, 2013). Moreover the course is persistent, lasting throughout a woman’s reproductive years and terminating only with menopause. For those affected by severe symptoms they are estimated to experience 3000 days in a symptomatic phase over their ovulating lifespan (Rapkin & Winer, 2009). A number of studies have reported on the burden of illness suffered by this patient group; most illuminating is the finding that for those with PMDD the impairment can be likened to chronic Major Depressive Disorder (Halbreich, Borenstein, Pearstein, & Kahn, 2003). Women with PMS are known to suffer from higher
work absences, larger medical expenses, and an overall lower quality of life due to health related reasons (Hylan, Sundell, & Judge, 1999).

1.2 PMS and Depression

Research suggests that there is a link between depression and reproductive lifespan milestones such as puberty, pregnancy, post-pregnancy, and pre-menopause (Harlow, Wise, Otto, Soares, & Cohen, 2003; Steiner, Dunn, & Born, 2003). Evidence indicates that even when controlling for the correlated variables of past depressive episodes and family history of depression, premenstrual depression is associated with an increased risk of developing Major Depressive Disorder (MDD) (Graze, Nee, & Endicott, 1990). Differences in mood stability (specifically irritability and depressed mood) between those with PMS and those without are not just observed during the premenstrual phase but also in phases deemed ‘asymptomatic’ (Bowen, Bowen, Baetz, Wagner, & Pierson, 2011).

Payne et al. (2007) found premenstrual mood symptoms were reported in 67.7% of women with a diagnosis of MDD or bipolar disorder (BP), while only 33.7% without a psychiatric diagnosis noticed regular mood changes during their menstrual cycle. In those with a diagnosis of MDD, premenstrual mood changes were associated with a greater chance of having both postpartum and pre-menopausal mood symptoms with an odds ratio of 2.35 (95% Confidence Interval: 0.98 to 5.63). In those with BP, this association was not seen. The results from this study confirmed the consistent finding of association between MDD and PMS and therefore suggest a trait-like expression related to the reproductive cycle. There appears to be vulnerability, in some women, towards hormonal related changes, although the mechanism(s) of action are still under consideration (as will be discussed in section 1.4).
1.3 The menstrual cycle

On average women will experience 36 reproductive years, beginning with menarche (somewhere between ages 8 to 13 years) and ending with menopause (usually around the age of 51) (Mihm, Gangooly, & Muttukrishna, 2011). Menstrual cycles (defined in length from the first day of menses to the following beginning of menses) typically last for 28 days, but are known to range from 21 to 34 days in normally menstruating women, with longer cycle lengths occurring at the beginning and end of a woman’s reproductive years. As such, determining the exact number of days that make up each stage of the cycle is inexact. Each ovarian cycle is characterised by three phases; follicular, ovulation, and luteal, while the uterine cycle is also broken into three phases; menstruation, proliferative, and secretory. In the ovarian cycle, menstruation marks the end of the luteal phase and the start of the follicular phase. Hormonal changes control each of the phases in the ovarian and uterine cycle, which collectively are known as the ‘menstrual cycle’.

The menstrual cycle is governed by a complex set of events, whereby the aim is to either prepare the womb for pregnancy or shed the lining if implantation does not occur. Beginning with the follicular phase, the follicles, located in the ovary, mature in preparation to release an egg. The follicles are stimulated by a process starting in the hypothalamus with the secretion of gonadotropin-releasing hormone (GnRH), whereby the anterior pituitary gland is then stimulated to release the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) (Hawkins & Matzuk, 2008). FSH in turn stimulates the production of oestrogen, which then downregulates the production of FSH in the anterior pituitary, thereby ensuring only one egg will mature per cycle. The high oestrogen levels subsequently produce a surge in LH which, through a series of events, leads to ovulation.

Some women experience ovulation pain, also known as mittelschmerz, in the middle of their cycle which may also cause light bleeding. During ovulation the egg is released and flows through into the fallopian tube where it will eventually pass if not fertilized. The luteal phase then begins
with a surge in progesterone caused by changes following the expulsion of the egg from the follicle. The corpus luteum disintegrates when the egg is not fertilised and levels of oestrogen and progesterone fall, stimulating changes in the endometrium (an inner layer of the uterus) and causing its functional layer to shed during menstruation. In the transition back to the follicular phase FSH levels increase, signalling the beginning of a new cycle (Chabbert-Buffet & Bouchard, 2002).

1.4 Aetiology

The cause of premenstrual syndrome is not known, although many theories have been put forward to explain the disorder. The most common hypotheses include the role of gonadal hormones, cortisol disruptions in the hypothalamic-pituitary-adrenal (HPA) axis (with a focus on neurology, neurotransmitters, and stress), and a relatively new theory; inflammation.

1.4.1 Menstrual cycle related hormonal fluctuations. As premenstrual symptoms only affect women during their reproductive years, gonadal hormones were originally thought to play a part in the cause. The use of medication to regulate oestrogen and progesterone, namely the use of a gonadotropin-releasing hormone (GnRH) agonist can eliminate PMS (Schmidt, Nieman, Danaceau, Adams, & Rubinow, 1998). Furthermore, PMS symptoms return when women are re-administered oestrogen and progesterone (Schmidt et al., 1998). Yet despite this proposedly clear influence of gonadal hormones, there are no statistical correlations between concentrations of oestrogen and progesterone and premenstrual symptoms (Jarvis, Lynch, & Morin, 2008). Moreover, additional work has not found a difference in blood plasma levels of luteinizing or follicle-stimulating hormone, testosterone-oestradiol-binding globulin, dehydroepiandrosterone sulfate, dihydrotestosterone, testosterone-oestradiol-binding globulin, prolactin, or cortisol in women with and without PMS (Rubinow et al., 1988). This would indicate either; the problem isn’t with an imbalance per se but rather an abnormal reaction to normal levels of the hormones, or something else is at the root of the cause.
It has been suggested the different response to normal levels of gonadal hormones for those experiencing PMS compared to those without the disorder may be due to phenotypical and/or genotypic differences. Rubinow and Schmidt (2006) discussed newly found polymorphisms on the oestrogen receptor (ESR1) gene, thought to be associated with PMS and a resulting differential sensitivity to oestrogen. Similarly a polymorphism of on the serotonin receptor C(-1019) allele has also been associated with PMDD and reduced serotonin transmission (Dhingra et al., 2007). More recent research in the area has led to promising findings and the identification of a complex of genes thought to be involved in PMDD. Dubey et al. (2017) have identified a different gene expression between healthy controls and women with PMDD in the Extra Sex Combs/Enhancer of Zeste ESC/E (Z) gene complex. An over expression of more than half of this gene complex was seen in PMDD diagnosed women. Oestrogen was found to decrease the expression of some of these genes in PMDD women, while progesterone increased the expression of some in healthy controls. Findings support the idea that differences in genotypic expression mediate the behavioural response to gonadal hormones. Yet more work is needed to determine ESC/E (Z) complex’s function in the brain.

An additional theory has focused on the Central Nervous System (CNS) which both produces and is acted upon by sex steroids. In the CNS model of PMDD, it is hypothesised that PMDD affected women feel the effects of oestrogen on serotonin functions more potently than those without the disorder (Hantsoo & Epperson, 2015). Serotonergic function has systematically shown alterations in response to oestrogen, such as altered gene expression on serotonin receptor and transport genes. As oestrogen levels drop in the late luteal phase, women with PMDD/ PMS have demonstrated serotonin abnormalities; they have lowered serotonin production following acute tryptophan depletion (tryptophan is a precursor in the synthesis of serotonin), which corresponds with an increase in premenstrual symptoms (Menkes, Coates, & Fawcett, 1994). Genetic polymorphisms, such as those described above, are thought to bestow the oestrogen sensitivity.
1.4.2 Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. An additional group of theories have focused on the HPA axis, using cortisol as a biomarker of the dysfunction. The HPA axis has been shown to be hyperactive in MDD (Pariante & Lightman, 2008); however, data from PMS studies have shown conflicting results. A recent review of the research area has failed to show a consistent connection between cortisol levels and premenstrual syndrome in correlational, experimental, or pharmacological studies (Kiesner & Granger, 2016). The four main theories regarding an altered cortisol response and HPA axis have focused on:

1) The similarities between depression and PMS and, due to depression’s known association with cortisol dysregulation, a possible neurological/ neuro-endocrinological common cause of both (Bancroft, Cook, Davidson, Bennie, & Goodwin, 1991).

2) Endogenous opioid withdrawal as the cause of premenstrual symptoms, with a withdrawal also affecting the HPA axis (Facchinetti et al., 1994).

3) A dysregulated stress response, associated predominantly with a history of intense stress (in particular abuse), which acts as the causal link between both PMS and altered cortisol (Girdler et al., 2007).

4) Women with PMS are hypothesised to have an altered allopregnanolone (progesterone’s main metabolite and a neuro-steroid) response to stress which modulates GABA receptors and in turn affects the HPA axis, ultimately leading to an increased anxiety response (Freeman, Frye, Rickels, Martin, & Smith, 2002). In regards to the fourth theory, women with PMDD have not shown the normal elevation in allopregnanolone in response to stress (Girdler, Straneva, Light, Pedersen, & Morrow, 2001), which would imply they are not experiencing the subsequent increase in GABA with its sedative effects (Crowley & Girdler, 2014). Animal models demonstrate that repeated stress blunts serum levels of allopregnanolone (Serra, Sanna, Mostallino, & Biggio, 2007) and
administration of allopregnanolone restores normal HPA function. So far no research has treated PMDD affected women with allopregnanolone.

Kiesner and Granger (2016) stated that there is some support, albeit extremely mixed, from the literature that women with PMS show lower levels of cortisol and a comparatively low cortisol increase in response to stress (Girdler et al., 2007; Roca et al., 2003). Research in this area has used environmental type challenges, such as social evaluative procedures or exercise, to induce a stress response in women with and without PMS and compared HPA axis responses across the two groups at different menstrual cycle stages. Confirmatory results from these environmental studies have shown women with PMS/PMDD tend to have a blunted cortisol response to stressful situations. Yet data from pharmacological and correlational studies failed to show consistent findings, or even indicate a trend towards blunted cortisol, which questions the credibility of the environment experimental studies (Kiesner & Granger, 2016).

Overall, there was little to no evidence that women with PMDD/ PMS showed differences in average mean cortisol when compared to controls, nor was there a robust cortisol response in reaction to challenges. The review of the area to date would indicate HPA axis dysfunction is not a general feature of PMS/PMDD, and thus the disorder is not induced by a stress response (Kiesner & Granger, 2016). However, there remains a possibility that specific symptom profiles such as anxiety, irritability, pain, depression etc. may be correlated with an altered cortisol response. As an individual’s symptoms may vary over the menstrual cycle there is rational for tracking changes in cortisol levels as a way of exploring symptom specific effects (Kiesner & Granger, 2016; Kiesner & Pastore, 2010).

1.4.3 Inflammation. It was suggested as early as the 1980s that the physical changes seen in the luteal phase of the menstrual cycle could be understood as an inflammatory response (Finn, 1986). More recent years have brought a focus on the role of inflammation in depression, which subsequently expanded to additional disorders, and now inflammation as a cause of PMS is
gathering momentum. In response to infection or injury our body undergoes behavioural, endocrine, and autonomic changes triggered by immune cells (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). Pro-inflammatory cytokines (interleukin-1α, β, and 6 (IL-1α, IL-1β IL-6), and tumour necrosis factor-α (TNF-α) rise in response to the perceived infection, but when our bodies are left in a state of constant immune activation another disease state may occur (Lei et al., 2015).

Studies exploring PMS and inflammation, to date, are few but there is much to suggest that in healthy, regularly menstruating women there are systematic changes in pro-inflammatory markers. These changes occur during the luteal phase with various studies showing an increase in interleukin cytokines (Faas et al.; Wolff et al., 2000), tumour necrosis factor- alpha (TNF-α) (O’Brien et al., 2007), and C-reactive protein (CRP) (Capobianco et al., 2010; Puder et al., 2006). Inflammatory markers seem to be uniquely tied to levels of oestrogen and progesterone. These sex steroids modulate the inflammatory events inside the uterus via a complicated system of immune cells and inflammatory mediators, where the purpose is to prepare the womb for implantation (for a detailed review please refer to King and Critchley (2010)). Researchers have worked hard to define the purpose of inflammatory markers within the menstrual cycle. For example, women’s ovaries will systematically produce inflammatory cytokines, some will play key roles in producing ovulation in conjunction with the surge in luteinizing hormone (Brannstrom, Bonello, Wang, & Norman, 1995).

While this evidence suggests a non-specific inflammatory response in all menstruating women, there is a little research to suggest a difference between those suffering from PMS and healthy controls.

Bertone-Johnson et al. (2014) are among the first researchers to study differences in a number of different inflammatory markers, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, TNF-α, CRP, granulocyte macrophage colony stimulating factor and Interferon gamma (IFY–γ), between women identified with moderate to severe PMS and those without. After adjusting for multiple factors known to be associated with higher inflammatory profiles such as smoking, body mass index, and age, IL-2, IL-4, IL-10, IL-12, and IFY–γ had a positive association with total symptom severity.
Blood serum levels collected in the luteal phase showed the aforementioned inflammatory markers were higher in women at the 75th versus the 25th percentile for total symptoms score. Within each cluster of symptoms (affective or physical/behavioural) some variation manifested; while both clusters were linearly associated with IL-10, specific variations persisted in the other interleukin cytokines associated with affective or physical symptoms. The authors cautioned against looking at the association of specific inflammatory markers and PMS as inflammatory markers are known to have varied and also overlapping functions (Bertone-Johnson et al., 2014). They did, however, state that this research lends support for the idea that inflammatory markers are higher in women experiencing PMS. As women with co-morbid mood disorders had been excluded from this study, the results could not have been produced by some other factor, e.g. depression, accounting for the increased inflammatory markers.

Even in women deemed to be without PMS, fluctuations in symptom profiles over the menstrual cycle map onto changes in inflammatory markers. Behaviour, mood, somatic, and pain symptoms were seen to alter in accordance with CRP levels, regardless of the changes in circulating gonadal hormone levels (Puder et al., 2006). Findings from Gold, Wells, and Rasor (2016) confirm the presence of CRP as a marker for premenstrual related symptoms. Although it must be noted this research study only looked at a prescribed amount of symptoms that did not include all of the affect symptoms commonly associated with PMS, nor was there any indication as to what time in the menstrual cycle the CPR recording was taken. While Bertone-Johnson et al. (2014) did not find a difference between symptom severity and CRP in their study, the research area is in its infancy and there is much to do before strong conclusions can be drawn. A detailed discussion of the role of inflammation and associated factors will be discussed in Chapter 2.

1.5 Treatment

Currently, the first course of treatment for severe symptoms is pharmacological in nature. The various options and their efficacy will be discussed below. Firstly, the focus will centre on
pharmacological medications before turning briefly to lifestyle factors, such as diet and exercise, and
ending with an exploration of natural-based substances.

1.5.1 Medication. No one medication exists that can effectively treat all the symptoms of
PMS. Rather, particular symptoms are targeted with available medication, which inadvertently
ignores the multi-systemic presentation of PMS as a whole (Verma, Chellappan, & Pandey, 2014).

1.5.1.1 Anti-depressants. The ACOG recommend selective serotonin reuptake inhibitors
(SSRIs) as a first line treatment in women affected by severe premenstrual symptoms (ACOG Practice
Bulletin, 2000). SSRIs are known to produce common side effects such as fatigue, nausea,
headaches, sexual dysfunction, mild tremor, insomnia, and drowsiness (Dimmock, Wyatt, Jones, &
conducted a review of 31 randomised control trials (RCTs) assessing the effectiveness of fluoxetine,
paroxetine, sertraline, escitalopram, and citalopram versus placebo. They found that the SSRIs did
indeed significantly reduce self-rated symptoms of PMS, more so than placebo. A moderate effect
size was seen between the pooled end scores for placebo versus moderate dose treatment
(Standard mean difference (SMD) -0.65, 95% CI -0.46 to -0.84), while a small effect size was
observed in those studies reporting change scores (for moderate dose SSRIs: SMD -0.36, 95% CI -
0.20 to -0.51). The authors also reported on adverse effects for the two groups, demonstrating that
they were significantly more likely to occur in the SSRI group with an odds ratio of 2.55 (95% CI 1.84
to 3.53). The adverse effects also appeared to be dose dependent, with higher doses producing
more side effects. However, one way to reduce adverse effects somewhat is to medicate only during
the luteal phase of the menstrual cycle. The almost immediate onset of therapeutic action seen in
SSRI use for PMS is thought to occur from the drugs ability to enhance the creation of neuroactive
steroids (Griffin & Mellon, 1999). SSRIs were effective in studies that applied luteal dosing and in
those that used continual dosing (Marjoribanks et al., 2013). Some symptoms of PMS such as
physical ailments and low mood may need continual dosing to see an effect (Hantsoo & Epperson,
2015). The benefits gained from medication use must be balanced against the side effect(s) produced.

Interestingly there is indication that anti-depressants may exert their effects via reduction of cytokine production. Both new wave SSRIs and older tri-cyclics have been linked with either a reduction in cytokines known to increase inflammation and/or an increase in those that have an anti-inflammatory response, specifically T-cells that regulate the immune response (Maes et al., 1999; Xia, DePierre, & Nässberger, 1996). Results from a meta-analysis of the area found evidence of a reduction in three well known inflammatory markers of depression; interluken-6 (IL), C-reactive protein, and IL-10. The former two showed a significant reduction from baseline following anti-depressant treatment, while IL-10 did not show significant change in the three studies analysed (Hiles, Baker, de Malmanche, & Attia, 2012).

1.5.1.2 Hormonal Treatment. Older forms of Oral contraceptives (OC) have shown little treatment benefit for PMS. In a RCT of a triphasic OC, women on active treatment demonstrated an improvement in breast pain and bloating but not in mood symptoms (Graham & Sherwin, 1992), meanwhile monophasic OCs didn’t fare much better (Pearlstein & Steiner, 2012). In the late 1990s a new form of Combined Oral Contraceptive (COC) showed favourable results. The newer COC contains drospirenone, a derivative of spironolactone (aldactone), and ethinyl oestradiol. A recent review of the COC (Lopez, Kaptein, & Helmerhorst, 2012) has shown it has favourable results when used in women with PMDD and compared to placebo (Weighted Mean Difference= -7.83; 95% CI -10.91 to -4.75), but when compared to other COCs for the treatment of less severe PMS there are no differences. Side effects of drospirenone COC included nausea, breast tenderness, and bleeding between menstruation phases. However, as drospirenone is known to produce fewer side effects than its older counterpart, 19-nor testosterone, it has been approved by the US Food and Drug Administration as a treatment for PMDD when combined with oestradiol (Pearlstein & Steiner, 2012).
Medications that suppress ovulation completely, namely gonadotropin-releasing hormone (GnRH) agonists, have been used in cases where premenstrual symptoms are extreme (ACOG Practice Bulletin, 2000). GnRH agonists interrupt the process of sex hormone production, essentially causing anovulation. They have proven their ability to treat both physical and emotional symptoms of PMS over and above that of placebo (Brown, Ling, Andersen, Farmer, & Arheart, 1994). Yet, their effectiveness in treating the symptom of depression is questionable, with previous studies showing no improvements in women with premenstrual depression (Brown et al., 1994). Due to serious side effects including a reduction in mineral bone density, induction of postmenopausal symptoms and additional symptoms such as headaches, lowered mood, and muscle tenderness, GnRH agonists are used sparingly (Schmidt et al., 1998). In circumstances where they are trialled as a treatment, the duration will only last between six to nine months before oestrogen and progesterone are added back medically, in an effort to avoid losses in bone mass. GnRH agonists have not been approved as a treatment for PMS by the FDA (Lynch & Morin, 2008).

1.5.1.3 Anxiolytic Medication. Anti-anxiety medication is also at times utilized as a treatment for PMS. Alprazolam, a triazolobenzodiazepine, has shown significant results when compared to placebo in a number of RCTs. Luteal phase dosing was shown to reduce total PMS scores by 50% in 37% of women in Freeman, Rickels, Sondheimer, and Polansky (1995) trial. Meanwhile, Berger and Presser (1994) suggested alprazolam may work for a certain subgroup of women with PMS, specifically only those who experience anxiety during the late luteal phase. However, alprazolam and other anxiolytic medications are usually not recommended due to their potential for addiction, tolerance, and side effects (ACOG Practice Bulletin, 2000). Buspirone, another anxiolytic drug has been studied in a small number of trials and there is potential for its use in PMS; with it performing better than placebo (Rickels, Freeman, & Sondheimer, 1989). Given the lack of risk for addiction or tolerance, this anxiolytic may prove more promising than others. Moreover, there is evidence to suggest it performs comparably well to fluoxetine, with treatment effects observable after one month of intervention (Nazari, Yari, Jariani, Marzban, & Birgandy, 2013).
1.5.2 Lifestyle. For women who are not suffering from severe symptoms, simple changes made to their lifestyle, such as their diet, are thought to alleviate problems. Dietary changes have thus far focused on increasing the intake of foods that are high in nutrients needed for the synthesis of serotonin, reducing foods high in refined sugar and salt, and reducing the overall intake of methylxanthines such as coffee and chocolate (Verma et al., 2014). However, the research evaluating dietary modification is non-existent, and suggestions have been based mainly on speculation. Since women with PMS were noted to consume more dairy, refined sugar, and foods high in sodium (Abraham, 1983) advice has been given to eliminate these foods. Likewise, caffeine intake and symptom severity are thought to be correlated (Rossignol, 1985), although more recent research has disputed this conclusion (Purdue-Smithe, Manson, Hankinson, & Bertone-Johnson, 2016). The low-fat, high fibre diet is recommended in an attempt to control oestrogen levels (Dog, 2001). Future research using an experimental approach is needed to confirm dietary modification as a viable treatment approach.

There is also evidence to suggest aerobic exercise can reduce some premenstrual symptoms (Aganoff & Boyle, 1994). In a study assessing differences between regular exercisers (defined as five hours or more of aerobic exercise a week) and non-exercisers, women in the former group showed less negative affect, impaired concentration, pain, and behaviour change than non-exercisers (Aganoff & Boyle, 1994). The increase of endorphins resulting from exercise is thought to both improve mood and fatigue symptoms. In a review of the available randomised control trials assessing exercise and PMS, Daley (2009) found positive, although modest, results from the four studies identified. While sample sizes were small and the methodology deemed poor, the emerging trend was; moderate exercise, either aerobic or anaerobic, three times a week could exert a significant effect on PMS symptomatology. Results did vary, however, with respect to the extent of symptoms positively affected by exercise. It is possible that a change in lifestyle factors may not directly impact on the PMS symptom but rather the beneficial effects may come from improved overall health.
For more on relaxation, massage, reflexology, chiropractic and biofeedback treatment please see the review by Stevinson and Ernst (2001).

1.5.3 Nutrients and diet. Before I turn to the research on the use of nutrient treatments, I will first outline what vitamins and minerals are. As we must obtain most of these substances from our diet, a discussion of the Western diet, and its shortcomings, follows. Specific attention is given to what is known about dietary changes in reproductive women, before also looking at mineral levels across the menstrual cycle.

Humans must obtain most vitamins from our diet as our bodies do not endogenously synthesize them, the exception being vitamin D, B3, and some vitamin K (Kennedy, 2016). These organic compounds are essential for our bodies to function, with 13 vitamins in total being required from food or endogenous sources. Vitamins are grouped based on solubility (fat or water soluble) and connected roles within the body. The four fat soluble vitamins are A, D, E and K (K1 is phytomenadione, K2 is menaquinones) while the nine water soluble vitamins are made up of vitamin C, B1, B2, B3, B5, B6, B7, B9, and B12. Ultimately plants provide us with vitamins but we may consume these plants in indirect ways, namely from other food sources which have originally eaten the plants. Moreover, secondary consumption is at times necessary for absorption as the vitamin may need to undergo some transformation that we humans cannot perform endogenously (Kennedy, 2016). Humans also require adequate levels of minerals to absorb vitamins. We consume minerals through plant derived products as well, which take the minerals from the soil, and these consist of metals and inorganic compounds (Gupta & Gupta, 2014). They are classified as one of three; major, secondary, or micro/trace minerals. The classification refers to their level of requirement in the human body, although all are essential for life (for a detailed mineral list please refer to Popper (2014)). Collectively, vitamins and minerals are known as ‘micronutrients’. They are essential to life due to the diverse function they carry out in almost every biological, chemical, and physiological system. They do so in conjunction with enzymes, which are molecules (usually
proteins) that speed up chemical actions. Enzymes, in general, require cofactors to help them in biochemical reactions—vitamins and minerals are either these cofactors or part of a cofactor (Popper, 2014). Minerals also contribute to the structure of enzymes or may play a part in activating them and other proteins.

Current Western diets characterised by a high consumption of dairy, eggs, processed and red meat, and refined sugars and grains, is linked with a decrease in the consumption of essential vitamins and minerals (Kennedy, 2016). Conversely, pre-agricultural diets were mainly made up of plant-based products including vegetables, nuts, and fruits with fish and meat also contributing but in lesser quantities. Epidemiological studies have consistently linked the Western diet, with its high intake of saturated fats and refined carbohydrates, to an increase in heart problems, obesity and dementia (Alwan, 2011).

Moreover, links are being made between the Western diet and mental health conditions (Lai et al., 2014). Indeed there is recent evidence illuminating the beneficial treatment effects of a Mediterranean diet on the symptoms of depression (Jacka et al., 2017). The Mediterranean diet consists of whole grains, vegetables, legumes, fruit, dairy, nuts, fish, lean red meats, chicken, eggs, and olive oil; foods that are high in vitamin and mineral content. Data from the SMILES trial (Jacka et al., 2017) indicated that mood changes were not attributable to changes in physical activity, smoking behaviour, self-efficacy or body mass index, but rather dietary changes.

Yet, a caveat must be placed on dietary sources of vitamins and minerals; research into the nutrient quality of food over the last 50 years or so has indicated a steady decline in nutrients available in a typical serving (Mayer, 1997). Despite disagreements over the concerns and causes of nutrient depletion, evidence from Marles (2017) review conceded that in crops grown for higher yields there is comparatively lower mineral concentrations than their old crop predecessors. This means, in many cases, we must eat more of the food to get the same nutritional qualities. Evidence from epidemiological studies has highlighted the prevalence of micronutrient deficiencies en masse,
1.5.3.1 Dietary patterns across the menstrual cycle. Research has indicated that all regularly menstruating women showed a change in dietary patterns based on their menstrual cycle phase. A general increase in energy intake has been observed as women move into the luteal phase, an increase seen through a larger intake of fat corresponding to 9.2g/day extra (Johnson, Corrigan, Lemmon, Bergeron, & Crusco, 1994). This increase was not attributed to increased energy expenditure and thus seemed related to changes in sex steroids. Tarasuk and Beaton (1991) found the same increase in fat consumption in the premenstrual phase and concluded either a behavioural and/or physiological force was driving the dietary change. It has been documented that the basal metabolic rate (BMR), the rate at which the body expends energy while resting, increases during the luteal phase in normally menstruating women (Solomon, Kurzer, & Calloway, 1982; Webb, 1986), with progesterone identified as the cause behind the increased energy expenditure (Webb, 1986). Further research would suggest there is a difference in food consumption between normally menstruating and PMDD affected women. Prospective food desirability ratings and a controlled laboratory food consumption experiment has shown, while food consumption increased across both groups in the luteal phase, women with PMDD consumed significantly more calories (Reed, Levin, & Evans, 2008). Moreover the desire for foods containing high fat content and the actual consumption of this food was significantly greater in those with PMDD in the luteal phase of their cycle. Much of the previous work in this area has asked women to recall their food intake, which may have hidden true group differences. However this problem was avoided in Reed et al. (2008) prospective rating based study.

1.5.3.2 Fluctuation of blood mineral levels across the menstrual cycle. It has been demonstrated that healthy women show fluctuations in blood concentrations of metallic ions including magnesium, zinc, selenium and manganese over the menstrual cycle, with zinc and
manganese lowest during the luteal phase (Das & Chowdhury, 1997). Additional research has shown high serum calcium levels in healthy women during the follicular phase and low magnesium levels in the follicular phase (Dullo & Vedi, 2008). Chuong and Dawson (1994) looked specifically at zinc and copper blood serum concentrations during the menstrual cycle in women diagnosed with PMS compared to healthy controls. They found that zinc levels were significantly lower in the PMS affected women in the luteal phase compared to their follicular phase, but this did not equate to a between groups difference. However a statistically significant between groups difference was seen in luteal phase copper levels. Moreover, research has suggested magnesium levels are low in PMS affected women, regardless of their menstrual cycle phase (Rosenstein, Elin, Hosseini, Grover, & Rubinow, 1994). To date, researchers have chosen to look at a small number of minerals in each study, which does not give a comprehensive summary of the menstrual cycle related changes. Additional research is needed to characterise possible differences between women with PMS and healthy controls and then outline the aetiology behind the possible differences in light of recent findings. If consistent mineral level fluctuations are observed, more support for the use of mineral based treatments would be gained.

1.5.4 Single nutrient and herbal supplementation for the treatment of PMS.

The following section will outline single, and in some cases tandem, vitamins, minerals, fatty acids, and plant extracts that have been studied specifically for the treatment of PMS. The results presented below are a comprehensive collection of studies retrieved from peer reviewed papers. Many of these studies have been small scale, and there is a surprising dearth of effect size measures, or equivalent, that would allow us with some certainty to quantify the impact of the treatment.

**Calcium.** Initial support in favour of the mineral came from a randomised crossover trial where participants prospectively rated their symptoms (Thys-Jacobs et al., 1989). Women were assigned to three months of placebo followed by three months of 1,000mg of calcium carbonate/day, or the reverse. Symptoms underwent a statistically significant reduction during the
luteal and menstrual phases when on treatment (p < 0.05), with specific symptoms (water retention, pain and negative affect) showing the greatest decline. However, 45% of the participants had dropped out of the trial before the midpoint and a third of those remaining had less than satisfactory treatment compliance. The principal investigator returned to the mineral a decade later, this time employing a dose of 1,200 mg of calcium carbonate/day (Thys-Jacobs, Starkey, Bernstein, & Tian, 1998). After three menstrual cycles of treatment, participants treated with calcium carbonate showed a 48% reduction on total PMS symptoms from baseline compared to a 30% reduction seen in the placebo group. Both studies have been deemed to be of good quality on the Dallhousie Assessment Instrument for Critical Appraisal of Randomized Controlled Trials of Natural Products, an instrument designed to help in the growing area of alternative treatment research (Jurgens & Whelan, 2009; Jurgens, Whelan, MacDonald, & Lord, 2009). More recent research has looked at the combined efficacy of calcium and vitamin D compared to a drospirenone COC or placebo (Shehata, 2016). Treatment was provided in 21 day cycles over three menstrual cycles, with the dose of calcium and vitamin D equating to 44mg/day and 400 IU/day respectively, a notably low dose of each. Participants were deemed to ‘improve’ (show daily rating scores on total symptoms of less than 50 in five days pre-menses, as measured by the Daily Record Severity of Problems questionnaire) most in the COC group. But the proportion of PMS reduced women was only significantly higher in the subgroup of women who had moderate PMS to begin with. Thus rates of improvement were found to be similar across the two treatment arms of the trial when participants presented with mild to moderate baseline scores. The combination of calcium and manganese has also been explored in a small scale, diet based study with non-PMS affected women (Penland & Johnson, 1993). Results suggested a high calcium diet was associated with an overall improved menstrual cycle. Specifically, women on low calcium diets reported significantly more mood, concentration and behavioural difficulties across all three stages of the menstrual cycle compared to their high calcium diet counter parts. An interaction effect was observed between a high calcium and
low manganese diet whereby increased symptoms were observed. However, changes on the PMS diary measure did not confirm these observations (Penland & Johnson, 1993).

Vitamin E. Two placebo controlled studies, both with the same primary author, have examined the use of vitamin E in PMS with favourable results. In their initial study, three different doses of vitamin E (α-tocopherol), 150IU, 300IU, and 600IU, were compared over two months of treatment (London, Sundaram, Murphy, & Goldstein, 1983). PMS symptom reduction was seen across all doses of vitamin E, with each demonstrating a statistically greater effect than placebo and 300IU/day proving most effective. In the second study women were treated with 400 IU/day or placebo for three menstrual cycles (London, Murphy, Kitlowski, & Reynolds, 1987). When compared to participants on placebo, women taking vitamin E reported less physical and emotional symptoms; however, the group differences were not statistical significant, which may be accounted for by the small sample size of 41 (out of 46) completed participants.

Vitamin D. A review of the area has demonstrated a link between low levels of 25-hydroxyvitmain D serum levels and mood disorders, including premenstrual syndrome (Murphy & Wagner, 2008). Findings from the Nurses’ Health Study II indicate that vitamin D intake is related to likelihood of developing PMS (Bertone-Johnson et al., 2005). After controlling for confounding factors, women consuming the highest amount of vitamin D, an average of 706 IU/day, had a relative risk of 0.59 of developing PMS compared to those with the lowest intake, consuming an average 112 IU/day. Higher calcium intake was also found to be inversely related to PMS risk, which was not wholly surprising given that adequate levels of vitamin D are needed to absorb calcium.

Zinc. To date, only one RCT has examined the essential trace mineral zinc. Support for this study originally came from data showing a difference in levels of zinc between those affected by PMS and those without the disorder (Chuong & Dawson, 1994). Zinc levels in control subjects have not been found to alter significantly across the menstrual cycle; however, in the luteal phase PMS affected women were found to have lower blood serum levels of zinc. Copper was also noted as
higher in women with PMS, which may have affected zinc concentrations as both minerals compete for the same binding sites. Data from the Nurses’ Health Study II indicated that higher dietary zinc levels were associated with less risk of developing PMS; an average intake of 25mg/day showed an inverse association with PMS diagnosis (Chocano-Bedoya et al., 2013). Copper levels in relation to zinc levels were also examined and shown to be important with less PMS risk among women with similar copper and zinc ratios.

Three cycles of luteal phase dosing with 50mg of elemental zinc a day produced a significant difference between the placebo and treatment group in Siahbazi, Behboudi-Gandevani, Moghaddam-Banaem, and Montazeri (2017) study. Fourteen PMS specific symptoms were recorded along with a quality of life measure. All PMS symptoms decreased significantly in the zinc group and the participants defined as having moderate to severe PMS also demonstrated a significant decrease in symptoms. Moreover, quality of life ratings showed a positive increase in the treatment group, this was however only significant in the third month of treatment. Zinc is widely involved in numerous bodily processes, many of which take place in the brain. Thus, due to its importance, including both the making and modulation of melatonin and the neurotransmitter GABA (Hosie, Dunne, Harvey, & Smart, 2003), it is hypothesized its depletion can affect neuropsychological functioning (Siahbazi et al., 2017).

**Magnesium.** A daily dose of 200mg of magnesium taken for two menstrual cycles has been shown to alleviate symptoms related to fluid retention only in a randomised, placebo controlled, cross-over trial run by Walker et al. (1998). A daily record of symptoms measured six categories; anxiety, craving, depression, hydration, other, and total overall symptoms in the 38 participants. By the second month of treatment a difference was only seen in the category 'hydration', namely swelling, breast tenderness, bloating, and weight gain, while the other categories showed no significant reduction. A further study has looked at magnesium (Mg) plus vitamin B6 supplementation in the treatment of mild PMS (2000). In this RCT, a crossover design was employed
in which participants received each of the four available treatments for one menstrual cycle; (1) 200mg Mg, (2) 50mg vitamin B, (3) 200mg Mg + 50mg vitamin B and (4) placebo. Daily record sheets again recorded the intensity of symptoms under the six categories listed above. The only treatment to show a significant result was Mg combined with vitamin B6. Symptoms falling under the category of anxiety, specifically; mood swings, irritability, nervous tension, and anxiety showed a decrease with only one month of supplementation. It is possible some results were not fully recognised given the short duration of each treatment phase, especially if magnesium levels were extremely low in the beginning or if the nutrient was poorly absorbed. Blood magnesium measurements are shown to be consistently lower in women with PMS regardless of which phase of the menstrual cycle they are measured at (Rosenstein et al., 1994).

**Chasteberry (Vitex Agnus Castus).** Chasteberry, the fruit of the chaste tree has been in medical use for centuries, most notably for gynaecological conditions. Beneficial effects from chasteberry are thought to stem from its effect on various hormones, namely prolactin and progesterone (Roemheld-Hamm, 2005). Numerous RCTs have evaluated the effectiveness of the fruit, leading the German Commission E to approve chasteberry as a treatment for PMS (Blumenthal, 2000). Benefits to both physical and emotional symptoms such as breast tenderness, pain, water retention, headaches, food cravings, mood, irritability, and anger have been observed in good quality RCTs (Jurgens & Whelan, 2009). The dosage and form of the extract can vary widely, although usually 20 to 40 mg/day appears to be effective in a fruit extract, while fluid extract and a tincture has also been used (Roemheld-Hamm, 2005).

**Ginkgo (Ginkgo Biloba).** Ginkgo, an indigenous Chinese plant used in traditional medicine, has had some success in treating PMS. One placebo controlled RCT evaluated the effectiveness of 40mg of ginkgo biloba leaf extract taken daily from day 16 of the menstrual cycle until day 5 of the following cycle for two consecutive menstrual cycles (Ozgoli, Selselei, Mohaj, & Majd, 2009). Women in the treatment group showed a reduction in psychological and physical symptoms of PMS. Baseline
overall symptom severity was 34.8% in the treatment group, reducing to 11.1% following intervention. In another RCT run by Tamborini and Taurrelle (1993), a standardised extract of ginkgo biloba leaves, named EGb761, was used to treat women with PMS. In comparison to placebo, significant reductions were seen in the symptoms of irritability, aggression, breast tenderness, and fluid retention after two cycles of treatment. In both the aforementioned studies, side effects were minimal, with 85.7% and 86% of participants, respectively, judging the tolerability of the treatment favourably.

*St John’s Wort (Hypericum Perforatum).* Few studies have assessed the use of St John’s Wort (SJW) in PMS alleviation, despite the herb’s use as a treatment for depression. 600mg of SJW extract given to participants for two menstrual cycles has shown a tendency to improve anxiety related premenstrual symptoms, yet a statistically significant result was not obtained over and above that of placebo (Hicks, Walker, Gallagher, Middleton, & Wright, 2005). In another study 900mg/day of SJW taken for two menstrual cycles demonstrated a significantly greater effect in alleviating physical and behavioural symptoms than placebo in 36 women (Canning et al., 2010). However, no significant group differences were found for either mood or pain related symptoms.

*Evening Primrose Oil.* A review of seven placebo-controlled trials of evening primrose oil (EPO) found overall little effect on premenstrual symptoms (Budein, Li Wan Po, & BDornan, 1996). However, it was noted some of the studies used a small number of participants, and thus moderate effects may have been obscured. A more recent review by Bayles and Usatine (2009), concluded EPO is likely ineffective for premenstrual syndrome, but due to methodological flaws more studies were needed to clarify this. While EPO’s use is not recommended during pregnancy, the only reported side effects when taken otherwise are headaches and gastrointestinal disturbances, indicating it is a relatively safe alternative therapy (Bayles & Usatine, 2009). Both omega-3 and omega-6 fatty acids are found in evening primrose oil, with the former to be discussed below.
1.5.5 Omega-3 fatty acids. In accordance with many of the interventions described above, omega-3 has received little attention as a treatment. The body requires a balance of essential fatty acids, yet the Western diet is found to be high in omega-6 and low in omega-3 consumption. This becomes a problem when high omega-6 levels lead to a production of type-2 prostaglandins which are pro-inflammatory. Omega-3 is known to influence the production of anti-inflammatory prostaglandins, which may be how it exerts its beneficial effects (Horrobin, 1983). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most well-known omega-3 fatty acids reported to have a crucial role in brain functioning. While the body can produce EPA and DHA it is an inefficient process, meaning that in reality we get most of our fatty acids from food sources. Fish is a good source of EPA and DHA, while flax oil contains high levels of α-linolenic acid (ALA), the overarching fatty acid in the omega-3 chain, both sources have been studied in women with PMS.

Much of the research with omega-3 has thus far focused on the physical health of patients, but a growing body of RCT research is reporting favourable results for the likes of depression, schizophrenia, bipolar disorder, attention deficit hyperactivity disorder, obsessive compulsive disorder, and borderline personality disorder (Peet & Stokes, 2005). Preliminary evidence would also suggest there is a treatment benefit in those with PMS; when compared to controls at day 45 and again at day 90, participants in a study by Sohrabi, Kashanian, Ghafoori, and Malakouti (2013) demonstrated lower psychiatric and physical symptom scores along with shorter symptom duration. Those in the treatment group received a 2g/day omega-3 supplement containing 12% DHA and 18% EPA in each of the two capsules, taken for the first 30 days. Over the following two months participants only took the supplement for eight days prior to menstruation along with two days during (10 days total). This intermittent dosing schedule has also been found to be effective in Wakeman (2013) and Sampalis et al. (2003) study, discussed below.

In another study looking only at the premenstrual symptom of mastalgia (breast tenderness), women were assigned to one of three conditions; 30g flaxseed consumed in wheat
bread, omega three pearls (the capsule contained 180mg EPA and 120mg DHA/day), or a control condition of plain wheat bread (Vaziri et al., 2014). Symptom intensity improved in both of the treatment conditions, with mixed findings for symptom duration. A treatment effect was observable more quickly in those women treated with ground flaxseed. The authors concluded this may be due to the interaction of the two elements found in flaxseed, namely omega-3 fatty acids and phytoestrogens.

Additional research suggests the source of the omega-3 fatty acid may be important. Sampalis et al. (2003) assigned participants to either a krill oil or fish oil formula. Both groups took two capsules consecutively for 30 days before taking them for ten day intervals in the following two months of treatment. While women in both treatment groups noticed statically significant improvement in physical symptoms such as swelling, weight gain and stomach pain, the emotional symptoms of PMS improved only in those taking the krill oil. Furthermore, women taking the krill oil formula used significantly fewer analgesics during the premenstrual phase. The use of krill oil as the source of omega-3 is worthy of note as it is thought DHA and EPA in the form of phospholipids, as opposed to triglycerides, are more readily absorbed by the human body (Wakeman, 2013). There a few known side effects of fish oil formulas, all of which are benign; nausea, reflux of the fish taste, and stool looseness (Peet & Stokes, 2005).

1.5.6 Soy flavonoids. Isoflavones are plant derived compounds, found predominantly in legumes, soy beans in particular. They are classed as phytooestrogens which denotes both the oestrogen-agonist and oestrogen-antagonist effect on the body (Lampe, 2003). A large body of small scale studies have focused on the use of soy isoflavones for reducing menopausal symptoms. Evidence from meta-analyses have provided promising, albeit somewhat mixed results, for phytooestrogen derived products in menopausal symptom alleviation (Roberts & Lethaby, 2014; Taku, Melby, Kronenberg, Kurzer, & Messina, 2012). Unlike menopause, the use of isoflavones in treating PMS symptoms has received little attention. Yet results from two studies assessing global
PMS symptoms have indicated this treatment may be effective. Physical symptoms of PMS have been reduced with a dose of 40mg/day of soybean isoflavones (Ishiwata, Uesugi, Uehara, Melby, & Watanabe, 2004). This effect on physical symptoms was significant when compared to placebo. Similarly, results from Bryant et al. (2005) suggested a slightly higher dose, 68mg/day, was associated with specific physical symptom reduction (headaches, cramps, breast tenderness and swelling). However, overall the placebo and active treatment group were not significantly different on total symptom and physical symptom end scores. The small sample size of 23 potentially obscured some of the results. Menopausal treatment studies have indicated that treatment length needs to be longer with soy isoflavones. Women needed 48 weeks of treatment to reach effects of 80% of the products maximum, while only 12 was required for estradiol (Li et al., 2016). There is the potential that employing longer treatment lengths will result in statistically significant findings in women with PMS, although as of yet only the physical symptoms of the disorder seem to be positively affected.

1.5.7 The research on vitamin B6. The effectiveness of vitamin B6 in treating symptoms of PMS has been established in a small number of RCTs. As little as 50mg per day was found to be effective in one study which employed a crossover design comparing vitamin B6 to placebo for moderate to severe PMS (Doll, Brown, Thurston, & Vessey, 1989). Of the total 32 women who completed the entire seven months of the study, significant symptom reduction was seen in irritability, fatigue, and depression. A higher dose of 150mg/day taken for two months, was found to decreased the specific symptoms of dizziness, vomiting, performance issues, and decreased social involvement, when compared to placebo (Kendall & Schnurr, 1987). However, this study also found many other symptoms of PMS were not decreased following active treatment. Nevertheless, additional research has indeed shown that vitamin B6 has an extensive effect on a variety of PMS ailments. For example, when comparing vitamin B6 to an extract from the Vitex Agnus Castus tree, Lauritzen, Reuter, Repges, Böhler, and Schmidt (1997) found 100mg/day of B6 produced an overall PMS score reduction from 11.9 to 5.1 (a 48% reduction). The measure used to record these changes
was a questionnaire examining 36 areas known to be affected during the PMS phase of the menstrual cycle. Furthermore, 21.3% of the 59 women taking vitamin treatment were rated as completely symptom free at the end of treatment. Moreover, B6 was effective when applied in an intermittent dosing pattern, namely for days 16 through to 35 of the menstrual cycle, for three menstrual cycles.

Much of the research that has thus far been conducted on vitamin B6 has employed flawed methodology or produced ambiguous results. Hence, some of the results must be interpreted with caution (withstanding the above detailed studies which were judged to be of average or good quality using the Dallhousie Assessment Instrument for Critical Appraisal of Randomized Controlled Trials of Natural Products (Jurgens & Whelan, 2009)). Out of 25 published trials on the efficacy of vitamin B6 as a treatment for PMS, 9 studies were included in the meta-analysis run by Wyatt, Dimmock, Jones, and Shaughn O’Brien (1999). Each study was graded by two quality assessments, the Jadad scale and another that the authors devised (for specifics please refer to the article). Of the nine studies, none met the quality standards for both of the assessments, leading the authors to conclude that there is need of a well-designed placebo controlled RCT to establish vitamin B6’s efficacy. This aside, the overall odds ratio in favour of vitamin B6, relative to placebo, was 1.57 (95% confidence interval 1.40 to 1.77). This increased to 2.32 (95% CI=1.95 to 2.54) after the removal of one trial with significant heterogeneity. As a secondary measure, the efficacy of vitamin B6 in specifically treating depressive symptoms of PMS was looked at in four trials, producing an odds ratio of 1.69 (95% CI=1.39 to 2.06). Vitamin B6 has previously been linked with a reduction in PMS related depression (Bendich, 2000), although the odds ratio of 2.32 for overall PMS symptoms would indicate the vitamin’s effect is not only apparent on the symptom of depression. Despite the lack of trials with a good quality rating used in this meta-analysis, the odds ratios do suggest that vitamin B6 is substantially more effective than placebo.
The results of the meta-analysis also suggest vitamin B6 is effective on more than simply depressive symptoms. Other studies have indeed confirmed that in comparison to placebo, vitamin B6 produces significant effects on both somatic and psychological symptoms of PMS. Kashanian, Mazinani, and Jalalmanesh (2007) assigned their participants to either placebo or 80mg/day of vitamin B6 for two menstrual cycles. Both groups showed a significant reduction in psychiatric symptoms after treatment, with the reduction being significantly greater in the vitamin B6 group than placebo. Somatic symptoms again showed a significant reduction in both groups, yet it was not greater in the vitamin B6 group. Overall PMS severity showed similar reductions in both the treatment and placebo group, but it was shown to be significantly greater in the treatment group (total PMS change score: Vitamin B6= -1.80 ± 2.36 P<0.05, placebo= -0.93 ± 2.33 P<0.05).

Wyatt et al. (1999) were additionally interested in the possibility of a dose response. Across the nine studies included in their meta-analysis doses ranged from 50mg to 600 mg. Despite the large range, no dose response emerged. However, side effects did increase with dose. Peripheral neuropathy, a tingling sensation in the limbs, is a known side effect of vitamin B6 consumption, and was noted at greater frequency in the study employing a treatment dose of 600mg/day. M. A. Cohen and Bendich (1986) concluded from their review of the safety data that doses of less than 500mg/day for up to two years were safe. Larger doses for less time, for instance 1000mg/day, were associated with adverse risks, notably peripheral neuropathy. Yet a smaller dose can also induce the same, albeit reversible, effects with as little as 200mg/day (Malmgren, Collins, & Nilsson, 1987). Given the lack of dose response and associated side effects from as dose as small as 200mg/day, there remains little reason to administer vitamin B6 at doses above 100mg/day (Wyatt et al., 1999).

Vitamin B6 remains the most widely studied vitamin for PMS. Results are generally favourable and side effects minimal to non-existent when taken at doses under 200mg/day.

1.5.8 Multi-nutrient supplementation. The ‘one disease, one treatment’ model fails to take into account the complexities of human nutrient requirements (Mertz, 1994). Hence, due to
our need for a broad selection of nutrients to ensure adequate biochemical functioning, it makes
more sense to study nutrients in combination. The following section outlines two multi-nutrient
formulas that have been trialled as PMS treatments before detailing the work around a
micronutrient supplement studied extensively in mental health more broadly.

During the 1980s a number of positive trials demonstrated the benefits of a multi-nutrient
product, Optivite, for the treatment of PMS (Chakmakjian, Higgins, & Abraham, 1985; London,
Optivite is high in magnesium and vitamin B6 and contains other essential micronutrients (for the
full list please see www.optimox.com). It is to be taken at a dose of 6 to 12 tablets per day. At the
smallest dose of 6 tablets, Chakmakjian et al. (1985) saw a significant improvement in the treat-
ment group compared to placebo in their RCT. Not all symptoms were improved at this dose, but those
that were included nervous tension, mood swings, irritability, anxiety, headaches, cravings,
increased appetite, fatigue, dizziness, heart palpitations, indicating a mix of physical and affective
symptoms could be treated with six tablets a day. London et al. (1991) observed a significant
reduction in all PMS symptoms when Optivite was administered at 12 tablets a day. In their study,
the above-mentioned symptoms were improved as well as additional symptoms of depression,
tearfulness, confusion, insomnia, forgetfulness, sensations of weight gain, swelling, bloating, and
breast tenderness. Due to the high doses of vitamin B6 and magnesium, 250mg/day and 300mg/day
at the halved dose of six tablets/day, there is conflict around the mechanism of action. Doses as high
as those observed in Optivite are stand-alone treatments, and indeed have been detailed in the
single nutrient section above. Therefore it is possible the two nutrients worked in conjunction or
even alone to produce a result, regardless of the accompanying micronutrients.

Most recently, Wakeman (2013) has begun investigating a combination treatment made up
of omega-3 fatty acids, minerals, and vitamins. Thirty-six (29 completed) women took part in the
pilot study where they consumed two capsules a day, with each capsule containing krill oil (350mg),
soy isoflavones (50mg), pyridoxine (2mg), riboflavin (1.6mg), thiamine (1.4mg), and rosemary extract (50mg). Global PMS ratings showed a significant group average reduction of 44% from baseline to end of treatment. All 29 participants showed improvement, with overall symptom reduction ranging from 10-80%. Individual symptom analysis, using data from participants who reported severe symptoms of anxiety, insomnia, headache, forgetfulness, fatigue, craving, breast tenderness and/or skin outbreaks, demonstrated strong reduction at 50% or more. Therefore it appeared the most troublesome symptoms underwent the strongest reduction. This initial open label data has provided support for further investigation into this formula, with future studies planned.

1.5.9 Research on EMPOWERPLUS. The most studied broad spectrum micronutrient formula for mental health concerns is called EMPOWERplus (EMP+) which consist of 36 ingredients, containing a variety of vitamins, minerals, amino acids, and antioxidants (although it is sold variously as EMPOWERplus Advanced, Daily Essential Nutrients and Q96). While no study has thus far investigated the use of EMP+ in PMS/PMDD, a number of studies have looked at symptoms associated with PMS.

Open label treatment in participants diagnosed with bipolar disorder (BP), over varying lengths of time, has produced a general pattern of improvement (Gately & Kaplan, 2009). Of the 15 participants previously on medication in Popper (2001) study, 11 maintained a stable prognosis while taking only the micronutrient formula. The same pattern of stability was seen in Simmons (2003) work, with all 13 previously on medication stable whilst on EMP+. In two, case controlled, within subject, cross over studies, compelling evidence was found for the treatment of mood and anger pathology. The children, whose symptoms were under investigation, both had a significant reduction in their temper outbursts and mood lability while on EMP+, which returned with each of the two subsequent ‘off’ phases (Kaplan, Crawford, Gardner, & Farrelly, 2002). Further research conducted with children and adolescents diagnosed with a variety of different disorders such as BP, Oppositional Defiant Disorder, Attention Deficit Hyperactivity Disorder (ADHD), Generalised Anxiety
Disorder and anger related symptoms of rage and mood lability, have demonstrated improvement when taking EMP+ (Kaplan, Fisher, Crawford, Field, & Kolb, 2004). Three different measures, the Child Behaviour Check List, the Young Mania Scale, and the Youth Outcome Questionnaire, recorded large reductions in negative behaviours (Cohen’s $d$ effect size < 0.8). These changes were evident not only in overt interpersonal behaviour such as aggression, but also in internalized symptoms such as irritability, low mood, and anxiety.

Micronutrient’s effect on symptoms of anxiety and low mood has been examined in a small number of studies. Following a 7.1 earthquake, participants were randomised to one of three treatment arms; a low dose of a formula equivalent to EMP+, at four capsules/day or a high dose at eight capsules/day, or a B vitamin complex, for four weeks (Rucklidge et al., 2012). Compared to baseline, all groups had improved significantly on the measures of anxiety, depression, stress, and post-traumatic stress disorder. Additionally, those taking the eight capsules/day were more likely to rate themselves as globally improved, and indeed had greater improvement than the other groups on mood, energy and anxiety measures. A group of ADHD diagnosed participants who underwent the same natural disaster were found to be significantly less stressed two weeks post-earthquake than their counterparts not receiving the formula ($d= 0.69$) (Rucklidge, Johnstone, Harrison, & Boggis, 2011). Post flood research has also highlighted the efficacy of EMP+ in treating stress and anxiety responses, even over and above that of a single nutrient treatment (Kaplan, Rucklidge, Romijn, & Dolph, 2015).

Environmentally induced stress appears to respond well to the micronutrient intervention, possibly suggesting increases in stress resilience (Rucklidge, Johnstone, et al., 2011). Work from Lothian, Blampied, and Rucklidge (2016) has also shown that general stress improves in response to EMP+ treatment also. Their work was primarily interested in insomnia, which improved significantly following eight weeks of treatment ($d= 3.45$). Secondary analyses indicated the change was also
evident in the measure of stress, anxiety and depression, with large effect sizes of $d= 2.53$, 1.36, and 1.33 respectively.

Direct investigation of both the safety and tolerability of EMP+ has been undertaken in light of the growing interest in the formula. Data from six studies comprising a total of 144 children and adults demonstrated no blood toxicity associated with the formula. Data on adverse events from 157 participants demonstrated transitory headaches and nausea were the only statistically significant adverse effects associated with the formula (Simpson et al., 2011). Tolerability of EMP+ was further enforced in a naturalistic study comparing it to pharmacological treatment. Results showed significantly fewer participants with autism reported adverse events when on the micronutrient formula compared to their medically treated counterparts (Mehl-Madrona, Leung, Kennedy, Paul, & Kaplan, 2010).

It is worthy of note, that EMP+ has not been the only micronutrient formula intensively studied for mental health treatment. A number of formulas have also shown promise in the treatment of mood, anxiety, and behavioural difficulties. For a detailed review, please refer to Rucklidge and Kaplan (2013).

The studies detailed above indicate EMP+ has a possible role to play in the PMS equivalent symptoms of affective lability, irritability, depressed mood, anxiety, energy, and sleep disturbance. While no research exists for EMP+ on the physical ailments of PMS, there is reason to suspect with its broad range of vitamins and minerals and the evidence reviewed in the single nutrient section, that these too should also be positively affected. Moreover, this formula has proven safety and tolerability, and does not contain high doses of any one nutrient that could be taken as a treatment in its own accord. Therefore, we will be able to assess the efficacy of a broad spectrum micronutrient without the confounding factors of high single nutrients or other active components such as omega 3 or isoflavones, which have been present in previous studies.
1.6 Summary of Chapter 1

Premenstrual syndrome compromises a range of physical and emotional symptoms that occur in the luteal phase proceeding menstruation. Research has failed to show a difference in hormones levels between women who experience the disorder and those who don’t, but new evidence suggests gene variations may affect how individuals respond to normal levels of gonadal hormones. Meanwhile, the HPA axis has not shown systematic dysregulation or altered cortisol response in women diagnosed with PMS compared to their healthy counterparts. A relatively new theory suggests that chronic inflammation may play a part in PMS/PMDD aetiology, with preliminary reach showing an abnormal elevation of pro-inflammatory markers.

Treatment interventions for this patient group have typically favoured medication, specifically anti-depressants for severe PMS. Treatment results are encouraging, but side effect profiles are high. Medication, excluding hormonal treatment, usually ignores the presentation of the disorder as a whole and instead focuses on symptoms most troubling to the individual. A range of plant extract, omega 3, vitamin, and mineral treatments have been studied to varying degrees with promising results. However, the peer reviewed research in herbal and nutrient treatment has failed to produce effect size measures, excluding a meta-analysis of vitamin B6, which makes it difficult to quantify the impact these treatments are having on PMS symptoms. Despite concerns around flawed methodology, Wyatt et al. (1999) showed an odds ratio of 2.32 (95% CI=1.95 to 2.54) in benefit of B6 (after the removal of one trial with significant heterogeneity). Furthermore, it appears to be safe when taken in doses less than 200mg/day.

The rationale behind combining all nutrients into one treatment is based on evidence that many vitamins and minerals have demonstrated efficacy in treating PMS either alone or, at most, in tandem with other nutrients, they are essential for our health, can only be obtained through food sources, and a vast number of the general population are noted to be deficient (most likely in relation to Western diets. Scarce research has trialled this approach, despite the positive results
found when doing so. As the focus in mental health shifts towards an understanding of the importance of nutrition in the aetiology and/or maintenance of disorders, so this treatment approach, in general, has grown. Initial open label trials with patients diagnosed with bipolar disorder have paved the way for research using high dose vitamin and mineral treatment. A well-studied formula, EMP+, has demonstrated efficacy in the treatment of a range of disorders with symptoms similar to those seen in PMS/PMDD, prompting the suggestion it will also be effective in this disorder. In order for us to understand the rationale behind combined nutrient treatment, we must examine the linkages between mental health and chronic inflammation and disrupted energy production. This will be the focus of the next chapter.
Chapter 2: Mechanisms of action; the rationale behind micronutrient and Vitamin B6 treatment

This chapter will focus in depth on the theory of inflammation (previously discussed in Chapter 1, section 1.4.3). Inflammation, either as a cause or product of mental disturbance, will be explored in relation to mental health, mainly to Major Depressive disorder (MDD). Next, the origin of inflammation with a focus on gut dysbiosis and bacterial translocation is explored. A second form of endogenous inflammation, reactive oxygen species, and signs of their negative effects is introduced, before an extensive consideration of mitochondrial dysfunction and further links to mental health. If inflammation and/or mitochondrial dysfunction are indeed part of the cause of many of the problems we are seeing in our communities’ mental health, then possible ways to reduce or reverse the effect will be discussed throughout the chapter, which ushers in the use of vitamins and minerals. A final section will specifically explore the known role of vitamin B6 in the body, before ending with the aims and hypotheses of the current study in light of this evidence.

2.1 Metabolic Disturbances

For a small number of people, the ability to absorb nutrients will be affected by genetic mutations, which is why they may require larger than average nutrient intakes. However, for the majority of people, metabolic functioning is affected by environmental circumstances and/or mental health functioning (Ames, Elson-Schwab, & Silver, 2002). While these people may also require increased levels of nutrients, the aetiology is different. This section will begin with a discussion of inflammation before exploring the reasons behind the problem including gut dysbiosis, oxidative stress and, a further form of the problem, mitochondrial dysfunction.

In rare cases (one in every five hundred), nutrient absorption may be hindered by inborn errors of metabolism (IEM) (Saudubray, 2009). In those with IEM, genetic mutations cause an
inability for coenzymes to bind efficiently and thus effectively reduce the levels of available coenzymes. This reduces the rate of metabolic activity and impacts on the function of cells via the subsequent build-up of neurotoxins and diminished synthesis of components needed for adequate cellular function (Saudubray, 2009). Furthermore, IEM can present with a myriad of psychological features either as the first presentation of the underlying illness or as a later feature (Walterfang, Bonnot, Mocellin, & Velakoulis, 2013). By increasing the availability of micronutrients, many of which act as coenzymes, metabolic action may be increased. Current treatments include the likes of enzyme replacement or modification of the patients’ diet (Walterfang et al., 2013). However, for the majority of people with mental health concerns, metabolic functioning is not affected by genetic mutations but rather by environment and/or the disorder pathology itself.

For many decades, people were of the opinion that disrupted neurotransmitter systems were the root cause of disrupted mood states, known as the catecholamine or biogenic amine hypothesis. Although this view is still held in some circles, the evidence would suggest that while neurotransmitters do play an important part, we must also look at metabolic processes to understand the origin of mood disruption (Kaplan, Rucklidge, Romijn, & McLeod, 2015). To date, four areas of metabolic functioning have been studied in-depth with some very compelling evidence unmasked. These four areas are inflammation, the microbiome, oxidative stress, and poor mitochondrial function. As will be discussed, these four areas are not mutually exclusive but rather disruptions in one area can likely affect others in turn.

2.1.1 Inflammation. Firstly, attention must be given to chronic inflammation and its link to mental health disturbances. Inflammation, characterized by swelling, pain, redness, heat and so on is, an essential acute immune response designed to speed the healing process and fight infection. However, in cases where the process persists and becomes chronic, the effects can lead to a new disease state (Lei et al., 2015). Numerous studies have shown a strong correlation between an active immune system, shown through inflammation, and abnormal mood states (Bertone-Johnson et al.,
2014; Dowlati et al., 2012; Pitsavos et al., 2006). As such, biomarkers of inflammation have increasingly been systematically studied in participants with depression. Of these, serum levels of C-reactive protein, homocysteine (an amino acid), and any number of cytokines (protein molecules produced by cells for intracellular communication and to either protect against or enhance inflammation) have been the main areas of focus (Dowlati et al., 2012). Both tumor necrosis factors (TNF) (a subgroup of cytokines) and interleukin-6 (IL-6) cytokines have been found in significantly higher concentrations in those diagnosed with depression (Dowlati et al., 2012). Both of these proteins are produced as the body responds to immunological danger; however, as no such danger was detected in the depressed patients in Dowlati et al. (2012) study, the high levels of cytokines were classed as abnormal. A review of the area by Maes (1999) concluded that although there are some differences amongst the research regarding which exact pro-inflammatory markers are active, there is known association with increased cytokine secretion during depression.

For some time, researchers were unsure if depression caused inflammation or if inflammation caused depression. Longitudinal data from the Geelong Osteoporosis Study demonstrated, across women of all ages, that as their concentration of C-reactive protein increased so did their risk of developing depression. The hazard ratio (HR) for MDD increased by 44% for every one standard deviation increase in C-reactive protein concentration (adjusted HR= 1.44, 95% CI 1.04–1.99, p= 0.026), an association that held even after adjusting for body weight, smoking, and the use of anti-inflammatory medication (Pasco et al., 2010). Results from the Geelong Osteoporosis Study suggest that depression can indeed occur as a side effect of increased inflammation. Using inflammation and depression as a blueprint, researchers have turned their focus to other mental health disorders such as bipolar (Cunha et al., 2008), anxiety (Pitsavos et al., 2006) and schizophrenia (Sommer, de Witte, Begemann, & Kahn, 2012), amongst others. One treatment that has emerged from this work has been to add anti-inflammatory drugs to the combined treatment of schizophrenia, demonstrating a moderate overall effect size of 0.43 on change in total symptom
severity (Sommer et al., 2012). This suggests symptoms of schizophrenia may be moderately alleviated in some patients with the additional use of anti-inflammatories.

As of yet, there has been little research into PMS and inflammation. As highlighted in section 1.4.3, inflammatory markers vary during the menstrual cycle in healthy women with normal cycles, with markers such as C-reactive protein highest during the luteal and menstruating stage (Gaskins et al., 2012). The menstrual cycle is characterised as an inflammatory event. Yet preliminary research has shown increased levels of several inflammatory markers, interleukin-2, -4, -10, and -12, as well as interferon, in women with moderate to severe PMS compared to controls during the luteal phase (Bertone-Johnson et al., 2014). While all women’s inflammatory markers may increase, there is possibly a stronger inflammatory reaction in those suffering from PMS. As Bertone-Johnson et al. (2014) only took blood samples from their two groups during the luteal phase, this theory requires more research. We can conclude, however, that of the disorders that show similar symptoms to PMS/PMDD, such as anxiety and depression, pro-inflammatory markers are a feature. If indeed inflammation is causing or, at very least, part of the response to mental health problems, the next step is to understand what causes inflammation. This question ushers in the following area to consider; gut dysbiosis and the microbiome.

2.1.2 Gut Dysbiosis and the Microbiome. Humans are made of a mere 10% of our own cells, with the remaining 90% belonging to microbial cells. Most of these cells, the majority of which are bacteria, are found in the gastrointestinal system where they carry out essential biological functions including the digestion of food, energy and nutrient extraction (such as amino acids and short chain fatty acids) and at times nutrient synthesis (Carding, Verbeke, Vipond, Corfe, & Owen, 2015). Of the vast number of micro-organisms found in the upper intestine and colon, most belong to the Firmicutes and Bacteroidetes phyla although other strains are present such as Actinobacteria, Proteobacteria, Fusobacteria, Verrucomicrobia, and Cyanobacteria (J. R. Kelly et al., 2015). Generally speaking, the relationship between the host and the microbiota is a mutually beneficial one, indeed
the micro-organisms found in the gastrointestinal tract are crucial for the bidirectional communication between the gut and brain, known as the ‘gut-brain axis’. The vagus nerve allows direct communication between the gut and the brain, with microbiota communicating via endocrine (mucosal cells), immune (immune cells), and neuronal messages (Thakur, Shakya, Husain, Emerald, & Kumar, 2014). However, “dysbiosis” refers to a microbial imbalance whereby beneficial microbes become under-represented and this loss of beneficial bacteria can cause an inflammatory response in the immune system.

The cause of dysbiosis is varied, but likely includes antibiotic medication, living in environments that do not expose our bodies to enough micro-organisms (the hygiene hypothesis), psychological stress, lack of sleep and high fat/high carbohydrate/low fibre diets (Daniel et al., 2014; Rogers et al., 2016; Thaiss et al., 2014). When there is an overgrowth of toxin producing bacteria, the body will respond to the bacteria’s production of the endotoxin lipopolysaccharide (LSP), which ultimately results in inflammation. Increased levels of immunoglobulin A or M found in the plasma are thought to be a marker of this inflammation (Maes, Kubera, Leunis, & Berk, 2012).

Dysbiosis is also thought to exert its negative effects on the immune system via bacterial translocation. Bacteria, normally contained within the intestinal wall can migrate out of the intestinal tract when the tight coupling of mucosal cells providing a barrier become loose. Immune cells that are usually separated from harmful bacteria and therefore not primed for LSP will become active upon contact with bacteria, thus initiating an inflammatory response (Maes et al., 2012). There is evidence to suggest that this chronic inflammation caused by an imbalance in gut bacteria may be at the root of irritable bowel syndrome, rheumatoid arthritis, multiple sclerosis and also mental health conditions such as MDD and anxiety disorders (Fond et al., 2015; Thakur et al., 2014).

Much of the compelling causation evidence has come from animal models whereby germ-free rodents have been exposed to toxin producing bacteria or compared to non-germ-free rodents with interesting results. Neufeld, Kang, Bienenstock, and Foster (2011) detailed the differences
between germ-free (GF) mice and specific pathogen free (SPF) mice on measures of anxiety and stress reactivity. The GF mice demonstrated significantly less anxiety-type behaviour, spending significantly longer in the open arm condition of the elevated plus maze than their SPF counterparts, suggesting gut microbiota have a role in the development of behaviour. Further research has expanded on this theory by showing GF mice who received faecal transplants from patients with MDD demonstrated an increase in depressive type behaviour compared to those mice who received a faecal transplant from a healthy control individual (Zheng et al., 2016). Additionally, MDD transplantation mice demonstrated changes in amino acid and carbohydrate metabolism, specifically increased carbohydrate pathways and disturbances to the amino acid pathways, indicating a change in their metabolism that mediated the emergence of MDD type behaviours. This finding can be used to further the claims of a bidirectional gut/brain and microbiota axis.

Therefore if inflammation begins by events triggered in the microbiome, attention should be paid to lifestyle choices that may aggravate the growth of non-beneficial bacteria/ destroy the health of beneficial bacteria. One such option is the use of probiotics, ingestible microorganisms which confer health benefits to the host, which have been studied extensively in health research with positive results (for review please see Aureli et al. (2011)). Another dietary option lies in the increased uptake of foods high in fibre and low in fat such as fruits and vegetables, which coincidentally are also rich in a variety of vitamins and minerals. While a healthy microbiome may be characterised in a number of different ways, with significant differences demonstrated between healthy individuals (Hamady & Knight, 2009), diversity brings resilience. In individuals with diseases associated with inflammation, such as obesity, their microbiome has been characterised as less diverse (Turnbaugh et al., 2009), and we know we can affect our microbiome by altering the food we eat. Research has shown within 24 hours of initiating a high fat/low fibre or low fat/high fibre diet changes were observable in the microbiome of participants (Wu et al., 2011). Yet despite these rapid changes, the effects were not enough to shift the participants’ enterotype cluster (three main enterotypes have been identified which refers to the bacteriological ecosystem found in our gut.
microbiome), even after 10 days. This seemed to suggest long term diet is important for overall cluster typing. Evidence from an elderly health study demonstrated that it took a year on a residential care diet for participant’s microbiota to reflect that of other longer term participants (Claesson et al., 2012). If diverse diets are associated with more diverse, and hence healthier, microbiota, one way of ensuring an adequate intake of vitamins and minerals may lie in the use of dietary supplements.

As of yet, there is no current research linking gut microbiome disturbance to PMS. However, a study from the mid 1990s (Minelli, et al., 1996) demonstrated that severe PMS could be effectively treated through a combination of probiotic supplement (containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) and S-adenosyl methionine (SAMe). Specifically the authors posited that the probiotics were to target gastrointestinal symptoms, e.g constipation, diarrhoea and abdominal pain, while SAMe (a type of anti-depressant, discussed in section 2.2) was to target the psychological symptoms of PMS. Compared to healthy controls, at baseline, women with PMS had significantly lower bacterial concentrations of aerobes and anaerobes, as well as high concentrations of gram-positive cocci, clostridial species, and enterobacteriaceae. Despite qualitative changes to their microbiome following treatment, these did not reach statistical significance. However the sample size was small at only 16 participants taking the combined treatment, five taking probiotics only, and nine controls. For the few participants who were followed post treatment, these qualitative effects declined after treatment termination. PMS in 70% of participants was reduced from severe to mild, with a return of symptoms severity and number once treatment finished. Despite the positive results of this study, no additional research has extended the findings further. However, they lend evidence to the idea that there is a microbiome disturbance in women classified as experiencing severe PMS.

2.1.3 Oxidative Stress. The next area to consider is oxidative stress, another possible cause of chronic inflammation. Humans are part of an ‘oxygen paradox’ in that oxygen is essential for life, but the by-products are toxic to us (Kohen & Nyska, 2002). In a normal system, reactive
oxygen species (ROS), or free radicals (pro-inflammatory molecules), are produced as a by-product of metabolism. However, there is a balance between the production of ROS and the corresponding biological response that manages this inflammation, mainly through the production of endogenous antioxidant defences, this balance is known as ‘redox potential’ (Kohen & Nyska, 2002). ROS are recognized as having the potential to damage multiple areas including cellular functions, DNA, and the structure of cells and proteins. Moreover, additional research has highlighted the role certain ROS serve in proliferating inflammatory signals through their action as messenger molecules (Hensley, Robinson, Gabbita, Salsman, & Floyd, 2000). Despite the overt problems produced by high levels of ROS, some ROS is essential for adequate function (for a review please see Seifried, Anderson, Fisher, and Milner (2007)).

In a well-functioning body, the antioxidant defence system counterbalances ROS in a number of ways such as inhibiting its formation, removing it, and binding metals needed to generate ROS (Bitanihirwe & Woo, 2011). Yet the defence system cannot always manage the production of ROS and there is a strong link between oxidative stress markers such as super-oxide dismutases (SOD), nitric oxide activity, and lipid peroxidation and mental health. Studies have examined these markers in relation to ADHD (Joseph, Zhang-James, Perl, & Faraone, 2015), schizophrenia (Reddy & Yao, 1996), and bipolar disorder (BP) (Andreazza et al., 2008). Results from these studies have suggested their participant’s antioxidant production was normal, but oxidative markers were significantly increased, indicating oxidative damage was being caused by an insufficient response to the oxidative stress.

Of studies assessing oxidative stress markers and anti-oxidant levels in women with and without PMS, findings have been mixed (Bortolasci et al., 2014; Duvan, Cumaoglu, Turhan, Karasu, & Kafali, 2011; Incebiyik et al., 2015; Kalia, Sudheendran, & Rao, 2001). Work by Duvan et al. (2011) showed a significant increase in lipid hydroperoxide during the luteal phase in women with PMS. Additionally their total antioxidant capacity was shown to decrease during this time. Yet in a recent
study results failed to show a reliable difference between Paraoxonase-1 (PON-1) levels during the follicular and luteal phase in controls versus participants with PMDD. PON-1, an enzyme used as a biomarker of oxidative-stress related diseases, has been highlighted in connection with mood disorders (Bortolasci et al., 2014). Total oxidant levels were interestingly lower in the second recorded follicular phase in women with PMDD in Bortolasci et al. (2014) study. The decrease in antioxidants, recorded in both the follicular and luteal phase (Bortolasci et al., 2014; Duvan et al., 2011) is contrary to the evidence from meta-analyses of ADHD and BP, and may suggest a slightly different profile for the onset of inflammatory reactions in women with PMS/PMDD, or a null finding. Additionally, the specific markers used to identify oxidative stress have varied between studies, which may account for the variety of findings. As such this area requires substantial additional research before conclusions are drawn. It is important to note that the brain is thought to be particularly susceptible to the effects of ROS due to its high oxygen usage (and therefore the production of more toxic by-product), less antioxidant defences, large metal content (which can accelerate or cause ROS) and concentration of polyunsaturated fatty acids (which are very susceptible to ROS) (Bitanihirwe & Woo, 2011).

It is well known that ROS are involved in carcinogenesis, mutation, aging, and inflammation which highlights their involvement (either as a cause or a contributor) in numerous diseases such as cancer, cardiovascular disorders, diabetes, and neuro-generative disorders to name but a few (Kohen & Nyska, 2002). The role of vitamin C and E, and glutathione (synthesized from amino acids) as endogenous antioxidants have been outlined in the paper by Sies and Stahl (1995). Yet, the evidence from cancer and cardiovascular studies on enhancing the natural antioxidant system with dietary supplementation has been conflicting (Seifried et al., 2007). Mice studies have produced welcome results, showing that following a period of stress, DNA and oxidative changes can be reduced with micronutrient supplementation (Hasan et al., 2013). Much more understanding of the internal antioxidant system, how it interacts with ROS, and the effects of nutrient supplementation is needed (Hensley et al., 2000). Regardless of current uncertainties, for those with inadequate
baseline nutrient consumption, they are likely to benefit from an increase in dietary antioxidant consumption (Seifried et al., 2007).

ROS production naturally increases as we age as a result of impaired mitochondrial function. During this time, our ability to absorb nutrients also diminishes due to changes in our gut villae (Popper, 2014). Mitochondria have enzymatic and non-enzymatic antioxidant defence systems which remove most ROS as they are produced, but age related mutations of mitochondrial DNA and decreased enzymatic activities lead to a decrease in energy production and increased formation of ROS (Gao, Laude, & Cai, 2008). The effect of poor mitochondrial functioning is discussed in the following section.

2.1.4 Mitochondrial Dysfunction. Mitochondria are the intracellular organelles which produce energy by generating adenosine triphosphate (ATP). They are also involved in numerous cell processes including axogenesis and apoptosis as well as redox signalling and intracellular calcium regulation (Hroudová & Fišar, 2011). The formation of most ATP occurs from products produced in the tricarboxylic acid cycle (TCA cycle), otherwise known as the Krebs cycle or citric acid cycle. Glucose, derived from the breakdown of food, is metabolized into acetyl coenzyme A (acetyl-CoA). Acetyl-CoA enters the mitochondrial matrix, the enzyme filled inner membrane of the mitochondria, where it is oxidized to CO₂ (Gao et al., 2008). In the process of oxidation, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH) accept electrons from acetyl-CoA to become fully formed. NADH and FADH later donate their electrons to the electron transport chain (ETC) where further ATP is formed from adenosine diphosphate (ADP) as part of the oxidative phosphorylation (OXPHOS) process (also known as mitochondrial respiration). There are five complexes that make up the steps of the ETC. In complex V, hydrogen ions located in the intermembrane pass back into the mitochondrial matrix which produces the energy needed to synthesize ATP from ADP (Wallace, 2005). A malfunction in the biochemical cascade detailed above can damage the ETC, and may be involved in the pathogenesis of a number of psychiatric illnesses.
For ATP (energy) production to proceed properly, the Krebs cycle and ETC genesis require proper nutrient accessibility. Without adequate nutrients, enzymatic function is compromised by the unavailability of cofactors (Kaplan, Rucklidge, Romijn, & McLeod, 2015).

Mitochondria, along with providing energy for the cell through the production of the molecule ATP, have their own genome, albeit, it is very small; accounting for only 0.3% of a total DNA in a cell. Yet the mitochondrial DNA (MtDNA) is responsible for coding a total of 13 enzymes involved in ETC. Mutations of MtDNA can therefore have an effect on ETC which decreases energy production and leads to mitochondrial disease (significantly impaired energy production). It is important to note that mutations in human nuclear DNA can also cause mitochondrial dysfunction. MtDNA is inherited solely through the maternal line, with variations in MtDNA implying a sequential accumulation of mutations (Calabrese, Scapagnini, Giuffrida Stella, Bates, & Clark, 2001). MtDNA is known to easily mutate due to a range of factors including its large volume (human cells contain hundreds to thousands of mitochondria with each one containing 2-10 copies of MtDNA), lack of protective proteins, and lack of a proficient repair system (Calabrese et al., 2001). Within one cell, many MtDNA genotypes may be present, known as ‘heteroplasmy’. Mutant and wild-type MtDNA copies are able to be passed to the daughter cell at an uneven rate leading to variations in the proportion of mutated MtDNA contained within each tissue, otherwise called ‘mosaicism’. Signs of mitochondrial pathology will only become clear once the amount of mutated MtDNA reaches a compromised level (Clay, Sillivan, & Konradi, 2011).

Mitochondrial disease can have a wide-reaching effect, given the mitochondria are contained in all our cells and all tissues are dependent on the ETC process. The discovery of mitochondrial disease came about in 1958 with a patient that had, what came to be known as, Luft’s disease. In brief, this disease is characterised by hypermetabolism and abnormalities in transpiration caused by structural changes to the mitochondria (Sjöstrand, 1999). Since the discovery of Luft’s disease, over 120 other mitochondrial disease have been identified, with mtDNA mutations affecting
the brain, endocrine system, skeletal muscle, kidney, and heart (Calabrese et al., 2001). While the presentation of mitochondrial diseases are varied, some symptoms are more common such as blindness, deafness, movement dysfunction, dementia, cardiovascular disease, muscle weakness, kidney problems, and endocrine disorders, including diabetes (Wallace, 2005). Additionally, specific syndromes such as Chronic Progressive External Ophthalmoplegia (CPEO), Leber Hereditary Optic Neuropathy (LHON), Mitochondrial myopathy, Encephalopathy, Lactic Acidosis and Stroke-like episodes (MELAS), Myoclonus Epilepsy with Ragged Red Fibres (MERRF), and Kearns Sayre Syndrome (KSS) have been identified and labelled based on strong symptom similarities. However, as Taylor and Turnbull (2005) outline, it is very difficult to diagnose MtDNA mutations as the cause of certain illnesses due to the heterogeneity of their presentation. Yet there has been much interest in trying to identify specific pathogenic mutations in relation to diabetes, Alzheimer disease and Parkinson disease, especially given the known impact of ROS in the disease aetiology. Mitochondrial genome mutations conceivably can affect any of the electron transport chain proteins. Correspondingly, this would hamper the effectiveness of OXPHOS, and those tissues most reliant on OXPHOS, such as the brain (Clay et al., 2011). Our brains require roughly 20% of the energy produced by our mitochondria yet it only accounts for ~2% of total cellular mass. Given that, mitochondrial pathology would conceivably have some of its greatest effects on the brain, researchers are beginning to propose that mitochondrial dysfunction may be responsible for many mental health issues.

Structural and functional changes have been found in the mitochondria of those diagnosed with schizophrenia and bipolar disorder including changes to size, number, and location of mitochondria, and impaired OXPHOS activity leading to decreased energy production (Clay et al., 2011). Furthermore, calcium homeostasis has been found to be impaired in both disorders, with elevated calcium levels likely to place stress on cells and, coupled with decreased ATP production, put an individual at risk of a psychotic episode when additional stress is added (for a detailed review please refer to Clay et al. (2011)). Meanwhile, polymorphisms on the 5178C and 10398A MtDNA genes have been indicated as a risk factor for bipolar disorder (odds ratio of 1.3 and 1.7 respectively).
and the authors proposed an altered calcium signalling system was at the root of the increased risk (Kato & Kato, 2000). Indeed, many mood stabilizers have an effect on calcium balance via inhibiting the intracellular calcium response or inhibiting calcium influx from mitochondria (Yamaji, Kagaya, Uchitomi, Yokata, & Yamawaki, 1996). This has lead Kato and Kato (2000) to suggest a treatment strategy aimed at inhibiting calcium influx and/or increasing the calcium uptake of mitochondria. Likewise, lithium has shown positive results in enhancing ATP production, leading to calls to investigate its clinical utility in treating impaired mitochondrial function (please refer to Gardner and Boles (2011) for a review).

A substrate of those with atypical features of major depressive disorder and/or co-morbid somatic symptoms have been identified as having mitochondrial disturbances (Gardner et al., 2003). As the link between somatic symptoms of depression and mitochondrial dysfunction is so high, Gardner and Boles (2008) have created a screening tool to aid in the diagnosis of patients who may have an underlying energy production as part of their disease pathology. The specific questions were successful in dividing depressed subjects into groups who either experienced higher or lower somatic symptoms, with the former group reliably demonstrating low muscle ATP production rates. Finding from case reports on patients with mitochondrial disease show that in most instances, participants exhibited psychiatric symptoms as much as 10 years prior to the discovery of their mitochondrial dysfunction (Fattal, Budur, Vaughan, & Franco, 2006).

Numerous mental health disorders like ADHD, autism spectrum disorder, multiple anxiety and trauma based disorders, and eating disorders, as well as fibromyalgia and migraine have been linked, in some cases, to underlying mitochondrial abnormalities (Gardner & Boles, 2011). In light of this research, it appears that there is a vulnerability to psychiatric illness in a select group of those with mitochondrial dysfunction. As of yet, no research exists on the links between PMS/PMDD and mitochondrial dysfunction. Numerous physical complaints coupled with psychiatric symptoms and an abnormal psychiatric disorder course may signal a need for further investigation; specifically
inquiry into mitochondrial functioning. The treatment of mitochondrial diseases is, however, not straight forward and is indeed still relatively early in its development. Many treatment approaches will take a symptom based approach whereby individual symptoms are managed as best as possible. Overall health is also targeted along with a focus on preventative measures such as lessening the impact from physical stressors such as dehydration or an imbalance in temperature.

Parikh et al. (2009) outlined that, despite the difficulties surrounding conducting and interpreting mitochondrial treatment studies, there is support for vitamin and cofactor supplementation. Their review of the treatment options highlighted the beneficial effects found in treating individuals with coenzyme Q10 (an endogenously synthesized coenzyme which plays a crucial role in the ETC, which is important for redox shuttling and intracellular signalling along with its antioxidant properties), riboflavin (vitamin B2, a flavoprotein precursor and a cofactor in fatty acid oxidation and the TCA cycle), L-Creatine (a source of high energy phosphate and intracellular buffer), L-Arginine (classed as a semi-essential amino acid which has a number of tasks including the removal of ammonia, nitric oxide production, creatine synthesis, and involvement in growth), L-Carnitine (a cellular compound involved in the crucial transfer of fatty acids across the mitochondrial inner membrane), and folic acid (Vitamin B9) (a cofactor in metabolic reactions). While other research has been conducted on further nutrients (B1, C, and E) and alpha-lipoic acid, there is less support for them than for the treatments listed above. It is accepted that more work, including larger studies, are needed to inform clinicians if there is merit in a ‘mitochondrial psychiatry’ approach.

2.2 The role of Vitamin B6 in the body

As this study is specifically interested in the therapeutic potential of vitamin B6, the next section is devoted to an analysis of the vitamin’s importance within number of different processes. Vitamin B6 along with other B vitamins, B12 (cobalamin) and B9 (folate), are known to be important players in methyl group donation which is responsible for the synthesis of lipids, neurotransmitters,
proteins, hormones and nucleic acid (Mitchell, Conus, & Kaput, 2014). At a cellular level, B9 and B12 play a role in what is known as the *methionine cycle* whereby homocysteine undergoes changes to become methionine and later S-adenosylmethionine (SAM, otherwise known as; S-adenosyl methionine (SAMe)) (it should also be noted that vitamin B6 acts as a cofactor in this process). SAM is involved as an important co-factor in many methyltransferases after which it becomes S-adenosylhomocysteine (SAH) and then homocysteine, thus completing a cycle of methyl group transfer. Through the trans-sulfuration pathway, homocysteine becomes cysteine, from which glutathione (an antioxidant) is formed through a number of steps (requiring vitamin B6 as a cofactor) (Kennedy, 2016). Glutathione is a very capable free radical scavenger, and thus is known to have superior power in reducing the effects of oxidative stress (refer to above section on oxidative stress for a summary).

Through the methionine cycle, levels of homocysteine are effectively reduced. Without adequate levels of B vitamins, high levels of homocysteine can lead to hyper-homocysteinemia, which is observed through abnormally high levels of homocysteine in the blood. Hyper-homocysteinemia is associated with an increased risk of cardiovascular disease, cognitive impairment (dementia), and mood problems (Bender, 1999). The ‘homocysteine hypothesis’, as it is known, first grew out of the link between increased blood levels of homocysteine and cardiovascular disease. It is theorised that homocysteine exerts its detrimental effects on the brain through increasing oxidative stress (leading to mitochondrial dysfunction), causing DNA damage, programmed cell death, and nuclear disintegration (Kennedy, 2016). Furthermore, without adequate production of SAM through the methionine cycle, neurotransmitters and DNA synthesis is compromised. Interestingly, treating people with SAMe has shown positive results in alleviating the symptoms of depression, demonstrating a moderate effect size of 0.65 over placebo. Equivalent treatment effects for SAMe versus tricylic antidepressants have been observed on the Hamilton Rating score for depression, demonstrating only a small between group effect size of 0.08 (Hardy et al., 2002).
In summary, inadequate levels of B6, B9, and B12 lead to a disruption in methyl donor activity and a corresponding increase of toxic intermediates like homocysteine. Despite folate, vitamin B6, and vitamin B12’s established role in methyl group donation and the suspected effects of high homocysteine levels, results have not consistently shown supplementation to improve variables of psychological disorders or cognitive deficits (Kennedy, 2016). Effects may be better observed in more homogenous groups, namely those who have initial markers of dysfunction such as elevated homocysteine levels (Mitchell et al., 2014). Or, as Kennedy (2016) believes the focus on vitamin B6, B9 and B12 has ignored the role of the remaining five B vitamins, despite the fact that they work in concert; all B vitamins play a role, to some extent, in the methionine cycle. It is his belief that the vitamin focus must be broadened to include the whole group of B vitamins to fully understand the numerous complex interactions and mechanisms of action.

Vitamin B6 alongside its role in the methionine cycle, is known for its part in amino acid metabolism in which it is a rate-limiting cofactor. Through the decarboxylation of amino acids, B6 dependent enzymes combine with amines to produce key neurotransmitters such as dopamine, serotonin, \( \text{y-aminobutyrate acid (GABA)} \), noradrenaline, and the hormone melatonin (Bender, 2012). Some of the aforementioned neurotransmitters are more sensitive to vitamin B6 deficiency, with GABA and serotonin synthesis being down regulated even when there is only a slight deficiency. The effects are demonstrated in a loss of neural activity inhibition as well as difficulties in sleep, behaviour, cardiovascular operation, and control of hormone excretion in the hypothalamus-pituitary adrenal axis (Kennedy, 2016).

Furthermore, B6 is known to play a direct role in inflammation as it is necessary for the production of cytokines that work to manage inflammation. Additionally B6 is linked with the proliferation of lymphocytes (white blood cells) (Morris, Sakakeeny, Jacques, Picciano, & Selhub, 2010). Low plasma concentrations of the biologically active form of vitamin B6 (pyridoxal 5'-phosphate (PLP)) have been implicated in a number of inflammatory diseases (P. J. Kelly et al., 2004).
Morris et al. (2010), in a large population based study, assessed the relationship between vitamin B6 status and inflammation. Causal inference was not permitted based on the design of the study; however, research suggested that inflammation increased the need for higher vitamin B6 intake. Sakakeeny et al. (2012) built on the finding by Morris et al. (2010), showing that inflammation had an inverse association with levels of PLP in plasma. In other words, as inflammation increases so does vitamin B-6 deficiency. There is work to be done on determining what the association is between plasma PLP levels and signs of inflammation. However, initial hypotheses have looked towards vitamin B6’s role in tryptophan metabolism. Specifically, vitamin B6 reliant enzymes are involved in the degradation of tryptophan in the kynurenine pathway. There is also reason to suspect an impact of PLP in 1-carbon metabolism, namely B6’s role as a cofactor of serine hydroxymethyltransferase (Sakakeeny et al., 2012).

Vitamin B6 has a number of highly prescribed roles in the body. It is a cofactor, along with vitamin B12 and B9 in the methionine cycle which controls levels of homocysteine, an amino acid which has been associated with an increase in physical and mental health problems. The methionine cycle is also responsible for the production of endogenous antioxidants that control the effects of ROS on the body. Furthermore during the course of this cycle SAM is produced, which has consequences for additional brain processes. Meanwhile, B6 works to fight inflammation through the production anti-inflammatory cytokines. While this vitamin does not produce its effects in isolation, it appears to be an important factor in a number of processes. Given vitamin B6’s varied and extensive role in the body, it is not surprising that low levels are linked with both negative psychological and physical health effects. Therefore B6 supplementation may reduce the symptoms of PMS through many different mechanisms, including the reduction of a possible inflammatory response.
2.3 Metabolic Pathway Conclusion

The idea that a whole system, such as the microbiome, might be pathogenic is a novel hypothesis in the mental health field, which has traditionally isolated specific brain regions or disrupted neurotransmitters as the cause of disturbance. Our microbiome is integral to our physical and mental health, with inflammation thought to originate as our body respond to an overgrowth of bad bacteria, known as gut dysbiosis, or from movement of bacteria out of the intestinal tract, known as bacterial translocation. The gut has a direct link to the brain via the vagus nerve, therefore the two can exert a bi-directional influence on one another, providing a possible link between mental health disturbances and gut dysbiosis. Research from germ free mice has indicated that not only do mice transplanted with faeces from an individual suffering from depression go on to display this disorder in their behaviour, but also their metabolisms undergo a change, even preceding the emergence of the behaviour. Therefore, strong evidence demonstrates changes to our microbiota have a subsequent effect on behaviour. Dysbiosis is hypothesised to occur in response to a number of different factors, one of which is a diet characterised as high in fat and carbohydrates and low in fibre. Diet and nutrient intake provide a, somewhat, readily available change we can make to improve not only overall health but specifically our microbiota and henceforward possibly our inflammation profile.

Oxidative stress, the name given to the process whereby ROS is created as a by-product of our metabolism, is a further form of inflammation with known health related side effects. Numerous physical health disorders such as cancer and cardiovascular disease have been linked to an increase in ROS and an inability for our endogenous antioxidant system to mitigate the negative effects. Subsequently, the focus has turned to mental health disorders and associated markers of ROS in autism spectrum, bipolar, schizophrenia, depression, and PMS. Treatment data from physical health studies has shown that dietary antioxidant supplementation requires much more research before being used as a mainstream treatment. Yet in those with lowered micronutrient intake, supplementation is warranted.
Mitochondria, the producer of energy for our cells (otherwise known as the ‘power houses’ of our cells) require an adequate amount of available nutrients for energy production to take place. Energy production may be further compromised due to mutations either in human DNA or mitochondrial DNA (MtDNA), with the latter particularly prone to mutations. MtDNA mutations may lead to specific disease states, with over 120 disorders attributed to mitochondrial dysfunction.

More recently there has been a focus on mitochondrial mutations in relation to neurodegenerative diseases and mental health, especially as the brain is reliant on 20% of the energy produced by our mitochondria. Compelling evidence from participants with schizophrenia and bipolar disorder has shown structural and functional changes in their mitochondria, resulting in a decreased energy production. Additionally, work from Gardner et al. (2003) has systematically shown a connection between depression and high somatic symptoms as indicative of an underlying energy production deficiency. This vulnerability to psychiatric illness in people with mitochondrial dysfunction does not necessarily imply that everyone with a psychiatric illness also has mitochondrial dysfunction, however, it is another potential causal link in an underlying metabolic syndrome possibly responsible for the expression of mental health disorders, including PMS. The use of ‘mitochondrial psychiatry’, which is the treatment of an underlying mitochondrial disease state, is still in its infancy. Yet current practices are focusing on general health and vitamin and cofactor supplementation.

If all the variables discussed above do indeed contribute to poor mental health, then one potential remedy lies in lifestyle changes, especially in the way of nutrition. Recommended dietary allowances (RDA) are usually government produced figures which state the dietary requirements of essential nutrients. These figures are based on population statistics, indicating they are at best a rough estimate of levels needed to keep one healthy. Despite new knowledge showing a wealth of individual differences affecting absorption and excretion of nutrients, RDA values have undergone little change for the past 40 years (Kennedy, 2016). As Ames (2004) and others have identified, while the obvious presentations of nutrient deficiency such as scurvy and rickets are abnormal in developed countries, less obvious forms of metabolic damage may be occurring within the
recommended guidelines. Moreover, the dietary guidelines do not taken into account known factors associated with need of increased nutrient intake such as antibiotic use, pregnancy, aging and the like (Popper, 2014). We may be unaware of the damage poor nutrition is having on us until our cells have undergone a long period of stress. Regardless, RDA’s do not provide guidelines on how much we should be consuming for optimal health, which, surely, should be recognised.

As discussed in section 1.5.3, vitamins and minerals are essential to our health and can only be obtained from food sources as our bodies do not endogenously synthesize them (Kennedy, 2016). The benefit of micronutrient supplementation is the ability to supply our body with a range of vitamins, minerals, and, in some instances, amino acids, in doses that are above the RDA. This approach does not suggest we forgo healthy diets in favour of micronutrients alone. There is growing interest in ‘phytonutrients’, chemicals found in plant based foods, which likely have a beneficial effect on absorption and function. This compels us to focus on nutrition in its entirety, but we must also recognise the need for supplementation. In cases where there is an underlying mitochondrial dysfunction, dietary intake of nutrients alone may not be enough to correct the energy deficiency. Moreover, as our knowledge increases so does our realisation that there is much to learn; single nutrient supplementation for the treatment of mitochondrial dysfunction with the likes of ascorbic acid or vitamin B2 may not take into account the complexities of nutrient function. While we understand, for example, that adequate levels of vitamin C are needed to properly absorb iron, there are a myriad of other functions that we are unaware of. In the emerging world of mitochondrial treatment, initial research is showing a beneficial effect of using a number of coenzymes, vitamins, and amino acids in concert (Gardner & Boles, 2005). Likewise, evidence from the field of mental health is clear that there is an effect to be gained by treating participants with micronutrient formulas (Rucklidge & Kaplan, 2013). This area may at present be small but it is robust. It provides us with part of a multifactorial approach to mental health treatment.
2.5 Overall Summary

In Chapter 1 the aetiology of PMS was discussed, including the link between inflammatory markers and the menstrual cycle. Subsequent medical treatment options have ignored the syndrome as a whole and instead focused on individual symptoms, with antidepressants the first line defence against severe PMS. A multitude of vitamins, minerals and herbs have also been explored with varying degrees of efficacy and, hence, feature sparingly as available treatment options. The little research that has combined the use of these compounds has found promising results (Chakmakjian et al., 1985; London et al., 1991; Wakeman, 2013). Currently, we are unsure if the combined treatments fare better than single nutrient options and, for unknown reasons, this treatment avenue has largely fallen out of practice. The research on broad spectrum micronutrient treatments for disorders with similar features to PMS provides the impetus to explore a new avenue of research as it relates to PMS. As described in Chapter 2, the field of mental health is making strong and widespread connections with underlying metabolic processes as potential causes or contributors to disorders. While the treatment options are still in their infancy, there appears to be a role for nutrition in modifying the microbiome and with that, subsequent inflammation. Meanwhile, nutrient supplementation has shown promising results in the treatment of underlying mitochondrial dysfunction.

Premenstrual complaints have received little attention as a disorder of metabolic processing, although there may be reason to suspect it is affected as blood mineral concentrations fluctuate over the menstrual cycle, dietary patterns have been shown to be different in those affected with PMS, and preliminary work indicates that inflammatory markers are more pronounced in women with PMS in the luteal phase. Evidence, albeit scarce, has already linked modest PMS improvement to nutrient supplementation. If research can show broad spectrum micronutrient formulas decrease premenstrual symptoms over and above the effect of single nutrient supplements, this may widen the research field into metabolic processes and PMS aetiology.
2.6 The Current Study

The following chapters present the procedure and outcomes of a randomised control trial assessing the efficacy of a broad spectrum micronutrient compared to a single nutrient for the treatment of PMS.

2.6.1 Aims and hypotheses. This trial aimed to strengthen the field of research on nutrient interventions by comparing a well-known single vitamin treatment to an experimental micronutrient treatment. We wanted to see if a high dose, broad spectrum formula could address the symptoms of PMS as a whole.

Based on the existing literature demonstrating the widespread effect of micronutrient formulas on a host of symptoms similar to those seen in PMS, it was hypothesised that:

1. Participants with moderate to severe PMS who were administered the micronutrient formula would demonstrate a greater reduction in premenstrual symptoms compared to participants taking vitamin B6;
2. This would correspond to a greater number of treatment ‘responders’ in the micronutrient treated group, as assessed by the percentage of participants no longer meeting study criteria for PMS and DSM-5 criteria for PMDD (American Psychiatric Association, 2013);
3. Group differences would also emerge on secondary variables measuring associated problems of stress, anxiety, depression, sexual functioning, sleep quality, and health related quality of life;
4. No differences would emerge on the tolerability and side effects of the two treatment formulas.
Chapter 3: Method

3.1 Study Design

The study followed women for a total of eight menstrual cycles; two cycles of pre-treatment, three cycles of active treatment, and a three-cycle natural follow up. It was a randomized, double blind, treatment-controlled trial of nutritional supplement interventions for moderate to severe PMS. Seventy-eight women were enrolled from predominantly the greater Canterbury region (New Zealand). Recruitment and intervention occurred over a two year time frame. Participants were randomized 1:1 to receive either a micronutrient formula, EMPowerplus Advanced (EMP+), or an active comparator treatment, vitamin B6.

3.2 Participant Eligibility

All participants had to be over 18 years of age, experience regular menstrual cycles (lasting between 21–35 days), not be pregnant or breast feeding or attempting to become pregnant and could not be taking other medications for the treatment of PMS. Participants using sex hormones other than the contraceptive pill were also excluded (these included gonadotropin-releasing hormone agonists, and other exogenous hormones specifically targeting PMS symptoms); however, if their contraceptive pill dosage stayed the same throughout the study they were eligible to participate. Final exclusion criteria included the presence of a current mood disorder (other than PMDD), a neurological disorder involving brain or other central nervous system function (e.g., epilepsy), evidence of untreated or unstable thyroid disease, any known abnormality of mineral metabolism (e.g., Wilson’s disease, hemochromatosis), at serious risk of suicide or violence (clinically judged), or currently taking medication with primary central nervous system influence.

1 The majority of the information included in this Methods section has been published in a protocol paper; Retallick-Brown, Rucklidge, and Blampied (2016)
3.3 Sample Recruitment

Participants were recruited via community-based strategies. Methods of recruitment included posters around Christchurch, at public health centers, and at the University of Canterbury, as well as advertising on social media outlets such as Facebook.

Prospective participants completed an initial screening questionnaire via the study website (http://bit.ly/nutritionandPMS). This questionnaire contained information on the study, as well as questions assessing inclusion/exclusion criteria, current and history of mental illness, PMS history, demographic information (available in Appendix 1). If the prospective participant met the preliminary inclusion criteria they were invited to attend further face-to-face screening at the university-based laboratory, or if this was not possible a phone interview was conducted.

3.4 Interventions

Participants were randomized to receive either vitamin B6 or EMP+, the full list of ingredients and doses for both formulas can be viewed in Table 1. Numerous studies have used EMP+ for the treatment of mental health concerns, with no occurrence of significant adverse outcome (Simpson et al., 2011). Vitamin B6, at very high doses, 2000 mg/day has been shown to cause peripheral neuropathy (Joint Food Safety and Standards Group, 1997). However, the same, but reversible effects have been noted in participants taking 200 mg/day (Malmgren et al., 1987). Therefore, the current study used 80 mg/day, a dose that has proven effective on both physical and emotional symptoms of PMS without co-occurring side effects (Kashanian et al., 2007).
Table 1. Nutrient information for the broad-spectrum micronutrient formula and vitamin B6 capsules.

<table>
<thead>
<tr>
<th>EMPowerPlus Advanced ingredients</th>
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<tbody>
<tr>
<td>Amount per 8 capsules</td>
</tr>
<tr>
<td>Vitamin A (as retinyl palmitate)</td>
</tr>
<tr>
<td>Vitamin C (as ascorbic acid)</td>
</tr>
<tr>
<td>Vitamin D (as cholecalciferol)</td>
</tr>
<tr>
<td>Vitamin E (as d-alpha tocopheryl succinate)</td>
</tr>
<tr>
<td>Thiamin (as thiamin mononitrate)</td>
</tr>
<tr>
<td>Riboflavin</td>
</tr>
<tr>
<td>Niacin (as niacinamide)</td>
</tr>
<tr>
<td>Vitamin B6 (as pyridoxine hydrochloride)</td>
</tr>
<tr>
<td>Folic acid</td>
</tr>
<tr>
<td>Vitamin B12 (as methylcobalamin)</td>
</tr>
<tr>
<td>Biotin</td>
</tr>
<tr>
<td>Pantothenic acid (as calcium pantothenate)</td>
</tr>
</tbody>
</table>

Propriety blend: Choline bitartrate, DL-phenylalanine, citrus Bioflavonoids, Inositol, L-Glutamine, L-Methionine, Grape seed extract, Gingko biloba leaf extract, germanium sesquioxide, Boron (as chelate), Vanadium (as chelate), Nickel (as chelate).

Other ingredients: capsule shell (gelatin, titanium dioxide) microcrystalline cellulose, glycine, citric acid, magnesium stearate, silicon dioxide, mineral wax

**Vitamin B6 Supplement Facts**

<table>
<thead>
<tr>
<th>Amount per 8 capsules</th>
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</thead>
<tbody>
<tr>
<td>Vitamin B6 (as Pyridoxicne HCl)</td>
</tr>
<tr>
<td>Acacia Gum</td>
</tr>
</tbody>
</table>

Other ingredients: maltodextrin, and steric acid

3.5 Study Procedure

3.5.1 Screening Assessment. Prospective participants completed a modified version of the Daily Record of Severity of Problems (DRSP) questionnaire online to confirm the presence of premenstrual symptoms in the luteal phase of their menstrual cycle. They had to meet the following to be included: (1) during the mid-follicular phase (day 6–10 after the onset of menses), their symptoms had to be no more than “mild” (unless reasonably explained by another disorder e.g. chronic fatigue syndrome; however that symptom would have to show a worsening during the PMS phase to count as a symptom); (2) in the six days prior to menses, there had to be an increase in at least one emotional symptom i.e., depression, anxiety, affective liability, or anger had to be rated “moderate” or higher; (3) during this phase they had to show a worsening in at least three out of the
eleven symptoms covered in the DRSP questionnaire; (4) and finally these symptoms had to have a “moderate” impact on their quality of life, as indicated by the participant’s endorsement of one of three questions in the DRSP. These initial screening criteria were again to be confirmed during the double cycle baseline period. Judgments of “mild”, “moderate” and so on were based on the numerical value the participant ascribed to her symptom, where; 1 = not at all, 2 = minimal, 3 = mild, 4 = moderate, 5 = severe, 6 = extreme.

Women who meet the above criteria were invited to attend a meeting where current and past mood disorders were assessed using a psychiatric diagnostic interview (Structured Clinical Interview for DSM-5, Research Version) (First, Williams, Karg, & Spitzer, 2015). Any participants who were deemed to have a current mood disorder, other than Premenstrual Dysphoric Disorder (PMDD), were excluded at this point. In instances where mood disorders were detected the clinical psychologist overseeing the study was notified and referrals were made to appropriate services, as deemed necessary by the psychologist. Likewise, any concerns arising during the baseline or treatment phase were managed in the same manner; however, if a participant developed a mood disorder during the treatment phase they were not excluded from the study at that point. In one instance a participant became clinically depressed during the treatment stage, as she chose to take psychoactive medication she was withdrawn from the study. For an overview of the study process, please refer to Figure 1.
3.5.2 Randomization and Allocation. If an individual met inclusion criteria, consented to participate in the study, and completed the two cycles of baseline, they were then allocated the next available randomized participant number from a previously generated list. Participants were randomized in a 1:1 ratio to the micronutrients or vitamin B6 by a research assistant using block randomization in blocks of four (Dallal, 2013). The pharmacist was in possession of this randomization list which they used to package all capsules (micronutrient and vitamin B6) in plain
white containers, labelled with the participant number and daily dose requirements. A sealed envelope containing the treatment allocation for each participant was kept in a secure location, only to be opened in the case of an emergency (e.g., a serious deterioration in the participant’s health), meaning the blind would have been broken for that participant only. A separate randomization sheet containing the labels A and B for the two groups was kept by the research assistant and given to the primary researcher for the analysis of the primary and secondary outcome at the end of data collection.

3.5.3 Blinding. Both participants and researchers were blind to the treatment condition of everyone. Randomization was conducted by a research assistant who was not involved in any other aspect of the study. There was no difference between the appearance of the two supplements, and both contained riboflavin to ensure the change in urine colour this vitamin causes was universal across the conditions. A dose of 80mgs of vitamin B6 was distributed across 8 pills such that the group randomized to vitamin B6 consumed the same number of capsules as those in the micronutrient group. To fill the capsules, other inert ingredients were included alongside vitamin B6 including; acacia gum, cocoa powder, maltodextrin, and steric acid, none of which were derived from wheat, so the formula was safe for coeliacs to consume. Once the analysis of the primary outcome measure was complete the blind was broken, and participants were informed of their treatment allocation.

3.5.4 Intervention phase. Participants received their first cycle supply of nutrients near the completion of the baseline phase. They were instructed to begin capsule consumption on the first day of menstruation which is considered the first day of a new cycle. Capsule dose was titrated up over six days to the full dose of eight capsules/day (two doses of four capsules taken with water and food). Participants consumed the capsules for three menstrual cycles, with the amount of time a participant was on active treatment varying as a function of their menstrual cycle length. Based on previous clinical trials, participants usually described a gradual effect of micronutrient supplements
that reached full effect within four weeks, therefore the three-cycle treatment phase should, theoretically, have afford enough time for the treatments to reach their full effect.

Nearing the end of each treatment cycle participants came to the University to complete questionnaires, receive new capsules and return old capsules and their Daily Record Severity of Problems diary. When this was not possible, participants completed the questionnaires online and new capsules were posted to them. For each on site visit participants received a NZD $10 voucher to cover travel-related costs. Treatment compliance was measured via counting and recording capsule consumption, whereby less than 80% consumption of the assigned dose was considered non-compliance. In some cases, compliance could not be determined via pill counts and instead self-report of missed doses was used.

3.5.5 Natural Follow Up. At the end of the intervention phase, participants were able to decide on their own course of treatment; continuing with one of the treatments used in the current study, using another active treatment of their choosing, or discontinuing treatment altogether. Information on how to obtain vitamin B6 or EMP+ was provided to participants following completion of the intervention. Three cycles later, participants were contacted via email to complete a modified version of the DRSP online, as well as answer questions about their current PMS management (including treatment use over the preceding three cycles). The modified version of the DRSP asked participants to rate their symptoms as usual on the 6-point scale; however, instead of completing the diary for each day of their PMS phase, they instead gave an overall rating for each symptom at the end.

3.6 Data Collection and Outcome Measures

Table 3 displays the outcome measures and time points at which they were collected. Premenstrual symptoms were recorded daily via a validated diary measure. Self-report questionnaires for secondary outcomes were obtained from each participant at baseline, and during each cycle of treatment to assess depressive, anxious, and stress symptoms, sleep quality, sexual
satisfaction, and quality of life as well as general information on food, drug and alcohol intake. Third party observers answered their questionnaires pre and post intervention, while participants rated their impressions of improvement at the end of the treatment phase. Questionnaires (excluding the daily diary during baseline and treatment), for all involved, were collected online through the web-based survey tool Qualtrics (www.canterbury.qualtrics.com).

3.6.1 Primary Outcome Measure. Over the course of the study daily data on premenstrual symptoms was recorded via the Daily Record of Severity of Problems (DRSP) questionnaire (Endicott, Nee, & Harrison, 2006). The DRSP is a commonly used screening tool for PMDD (Lustyk & Gerrish, 2010) and it maps directly onto the DSM-5 diagnosis of PMDD. If a participant met criteria for a diagnosis of PMDD during the Structured Clinical Interview, this was later confirmed using prospectively collected diary data from the two cycles of baseline. The measure provided a severity rating from 1 (not at all) to 6 (extreme) for 11 questions assessing premenstrual symptoms and asked a further three that looked at the impact the symptoms had in terms of productivity, participation in normal activities, and relationship harmony (Endicott et al., 2006) (for the daily version used in this study please refer to Appendix 2a).

Participants completed the diary each day for five cycles, two during baseline and three during treatment. The six days preceding menstruation were recorded as the PMS stage uniformly. Missing data from any of the six days would result in non-inclusion of that cycle. At natural follow up, women completed a modified version of the DRSP that they completed only once. In the modified version they were asked to rate the utmost severity of each symptom in the preceding six days, using the same one to six scale (available in Appendix 2b). The diary produced five primary outcome measures assessing Psychological Symptoms (4 questions), Somatic Symptoms (7 questions), Total Symptoms (the combination of psychological and somatic, totalling 11 questions), Impact Ratings (3 questions) and Worst Day Ratings (the day with the highest combined symptom
and impact scores, all 14 question, from the six day period). The possible range for each dependent variable is presented in Table 2.

Table 2. Range of possible scores for each dependent variable in the primary outcome measure.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Period of measurement</th>
<th>Possible Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychological symptoms</td>
<td>6 days</td>
<td>24-144</td>
</tr>
<tr>
<td>Somatic symptoms</td>
<td>6 days</td>
<td>42-252</td>
</tr>
<tr>
<td>Total symptoms</td>
<td>6 days</td>
<td>66-396</td>
</tr>
<tr>
<td>Impact symptoms</td>
<td>6 days</td>
<td>18-108</td>
</tr>
<tr>
<td>Worst day ratings</td>
<td>1 day</td>
<td>14-84</td>
</tr>
</tbody>
</table>

Worst Day Ratings were used to gain insight into continued severity of PMS, which, due to the six days of measurement, the other outcomes may have missed had a participant’s symptoms lasted for a short duration. The remaining four outcome measures indicated duration as well as severity (to a lesser extent).

3.6.2 Secondary Outcome Measures

Table 3. Outline of questionnaire schedule including type of measurement and time point at which it was to be completed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Instrument</th>
<th>Time Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-report</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenstrual symptoms</td>
<td>DRSP</td>
<td>Two cycle baseline, each treatment cycle, a modified version at natural follow up</td>
</tr>
<tr>
<td>Psychiatric status (mood disorders only)</td>
<td>Structured Clinical Interview (for DSM-5)-Research Version (SCID-1/RV) *</td>
<td>Screening</td>
</tr>
<tr>
<td>Depression, anxiety, stress</td>
<td>Depression and Anxiety Stress Scales-42 (DASS-42)</td>
<td>Baseline, each treatment cycle</td>
</tr>
<tr>
<td>Quality of life, wellbeing</td>
<td>Women’s Quality of Life Questionnaire (WQoLQ)</td>
<td>Baseline, each treatment cycle</td>
</tr>
<tr>
<td>Sleep quality</td>
<td>Pittsburgh Sleep Quality Index (PSQI)</td>
<td>Baseline, each treatment cycle</td>
</tr>
<tr>
<td>Contextual stress</td>
<td>The Perceived Stress Scale (PSS)</td>
<td>Baseline, each treatment cycle</td>
</tr>
<tr>
<td>Sexual satisfaction</td>
<td>The New Sexual Satisfaction Scale-Short From (NSSS-S)</td>
<td>Baseline, each treatment cycle</td>
</tr>
<tr>
<td>Diet quality</td>
<td>Food Questionnaire</td>
<td>Baseline, each treatment cycle</td>
</tr>
<tr>
<td>Treatment side effects</td>
<td>Side Effects Questionnaire</td>
<td>Baseline, each treatment cycle</td>
</tr>
<tr>
<td>Treatment efficacy</td>
<td>Clinical Global Impression scale (CGI scale, Improvement scale)</td>
<td>End of treatment</td>
</tr>
<tr>
<td>Observer report</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in premenstrual symptoms</td>
<td>Modified DRSP</td>
<td>Baseline, end of treatment</td>
</tr>
</tbody>
</table>

* Clinician administered.
At baseline and the following three treatment cycles, participants completed the following questionnaires:

- **Depression and Anxiety Stress Scales-42 (DASS-42).** The DASS-42 is a 42 item self-report instrument designed to measure three related negative emotional states; depression, anxiety, and tension/stress (P. F. Lovibond & S. H. Lovibond, 1995). It is used across a variety of clinical studies and as women with PMS/PMDD commonly experience symptoms measured by the DASS-42 it provides an additional useful tool for tracking symptom change.

- **The Perceived Stress Scale (PSS).** Common contextual every day stress was measured by the PSS 10-question version (S. Cohen, Kamarck, & Mermelstein, 1983). Participants were asked to rate the stressfulness of several events from the past week of their life in a modified version of the PSS. While there are three versions of the PSS, this study used the 10-question version which has been well validated (Fliege et al., 2005). Questions were rated on a 0–4 point scale, focusing on relatively general stressors that pertained to every day events as opposed to large-scale incidents such as death or environmental disaster (S. Cohen et al., 1983). The PSS has been found to be a good predictor of both health and health-related outcomes, and while it is correlated with depressive symptomatology it is still known to measure an independent construct. There are two factors which make up the questionnaire: perceived helplessness and perceived self-efficacy (Roberti, Harrington, & Storch, 2006). In a sample of college students, the PSS was found to have good test-retest reliability (r = 0.85), an important factor for this study.

- **Women’s Quality of Life Questionnaire (WQoLQ).** The WQoLQ was specifically designed to assess a health related woman’s quality of life in four domains: physical, psychological, social, and spiritual wellbeing (Gehlert, Chang, Bock, & Hartlage, 2006). Forty questions have three possible answers; Yes, No, or Not Applicable, with higher overall scores indicating a better quality of life. Quality of Life scales are frequently used in chronic illness and disease studies as a measure of disease impact and treatment response (Rapkin & Winer, 2009).
• *The New Sexual Satisfaction Scale-Short form (NSSS-S).* The NSSS-S is comprised of 12 items which are measured on a 5-point scale (1= not at all satisfied – 5= extremely satisfied), with higher scores indicating greater sexual satisfaction (Stulhofer, Busko, & Brouillard, 2011). Two factors are covered in this questionnaire; personal experiences and sensations, and partner’s general behaviours and sexual activity (Stulhofer et al., 2011). The form was modified slightly in time; instead of asking about sexual satisfaction over the last six months, participants assessed their satisfaction over the last week. Only those women who were currently in a sexually active relationship were asked to fill out the questionnaire.

• *Pittsburgh Sleep Quality Index (PSQI)*. Pittsburgh Sleep Quality Index (PSQI) measures sleep quality and disturbances over a one month period (Buysse, Reynolds, Monk, Berman, & Kupfer, 1988). There are 19 questions which yield seven different measures; subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. These scores then sum together to give an overall sleep quality total, with scores >5 established to indicate poor sleep quality (possible component score range: 0-21). Due to the difficulties involved in modifying this questionnaire to refer only to the last week, the questionnaire was left to focus on sleep over the one-month period. The PSQI has been found to have sound reliability and validity (Buysse et al., 1988).

• *Food Questionnaire.* Questions regarding dietary patterns over the previous week were assessed using a questionnaire modified from Baker, Little, and Brownell (2003). In this study, a healthy eater was defined as someone who ate in a balanced way, ate breakfast, didn’t eat too much junk food, ate moderate amounts, and stopped eating when full. Participants were asked to indicate from 1 (<once a week) to 5 (daily) how often over the previous week they ate breakfast, ate a balanced meal, ate even when full, ate lots of fruits and vegetables, and ate fast foods or snack foods such as potato chips or candy bars. They were specifically asked about average daily servings of fruit and vegetables (from 1 (<one serving) to 5 (4 or more servings)), and to indicate from 1 (not very healthy) to 7 (very healthy) how healthy they thought their diet was. Total
scores had a possible range from 8 to 41, with a higher score indicative of a healthier diet. The specific questions used in the food questionnaire were recently adapted and validated by Kuijer and Boyce (Kuijer & Boyce, 2014). In the validation study, Kuijer and Boyce showed that the questions were correlated with a two-week diary report of those eating behaviours. Moreover, the retrospective recall was found to be a fairly accurate estimate of the eating behaviours as reported during the diary period. The summing of the items has been used successfully in other studies, such that a higher score on the summed scale indicates healthier eating behaviours (Cronbach’s alpha 0.67) (Kuijer & Boyce, 2014).

- **Side effect questionnaire.** A scale assessing common side effects was administered throughout the study, including baseline. Participants were asked to indicate whether any symptoms commonly identified as side effects were experienced during treatment and then asked what actions they took to remedy them (a copy of the side effect questionnaire can be found in appendix 3). If one of the symptoms on the scale was indicated as present during baseline and remained for the duration of the study, it was not considered a side effect. However, if a symptom was endorsed at baseline, was absent, and then reoccurred it was considered a side effect. A number of the symptoms such as headache, weight gain, change in appetite, abdominal pain, lethargy, agitation, and anxiety were known premenstrual symptoms, therefore we expected many ‘side effects’ to be endorsed.

  *Clinical Global Impression scale (CGI scale)* (Guy, 1976). The CGI scale is used in numerous clinical studies to evaluate the global efficacy and safety of a medication (Lauritzen et al., 1997). At the end of the intervention phase participants judged their improvement in PMS symptoms, sleep, anxiety/stress and overall improvement on the Clinical Global Impression-Improvement scale (CGI-I). Answers could range from one (very much improved) to seven (very much worse).

  *Third party symptom ratings:* Participants, who agreed, were asked to provide details of a third-party observer who was able and willing to comment on the participant’s PMS symptoms. Observers completed a modified version of the DRSP questionnaire online twice in the luteal phase; once at
baseline and again at the end of the treatment. Only participants who completed the treatment intervention were eligible for third party ratings. The DRSP was modified to only include those questions which would be observable to an outside observer. Subsequently, questions that were deemed subjective such as pain, tiredness, reduced productivity/efficiency and less participation were removed to leave a remainder of 10 diary questions (please refer to appendix 2c). The observer was asked to rate the severity of these 10 questions based on the last week (the premenstrual phase). They needed access to email and an internet capable device to complete the questionnaire online.

3.6.3 Demographic and Premenstrual Syndrome Information. In the screening questionnaire, participants provided their date of birth, ethnicity, occupation, household income, age of first menses and time taken from first menses to PMS onset. The New Zealand Socio Economic Index 2006 (NZSEI-06) was used to determine a participants socio-economic status (SES) based on their occupation or, when unavailable, on their highest level of education (Milne, Byun, & Lee, 2013). The occupation ‘student’ and those under 21 years of age are not included in the index as it is thought their jobs will not reflect their education and/or skill level. For the 22 participants who were either under 21 or a student at the time of the screening questionnaire, their SES was an estimate based on their highest level of education. This method of estimation is thought to provide a fairly accurate representation of current status and was chosen instead of relying on family or partner income which is not considered as accurate (Milne et al., 2013). The NZSEI-06 scores could be divided into six socioeconomic groups which closely relate to the Elley and Irving scale that was historically used in this country (Elley & Irving, 1972). While the cut points were chosen arbitrarily, the distribution of scores over the six groups resembles a bell curve; roughly 10%, 15 %, 21 %, 29%, 12%, and 11% of the population fall into the six groups. Therefore, the middle socioeconomic groups contain most of the population, as can be seen in the table presented below.
Table 4. Socioeconomic status (SES) group divisions based on the six-group scale, demonstrating the percentage of the population who fall into each group.

<table>
<thead>
<tr>
<th>SES Group</th>
<th>NZSEI-06 range</th>
<th>Percent of Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>71-90</td>
<td>10.8</td>
</tr>
<tr>
<td>2</td>
<td>62-70</td>
<td>15.2</td>
</tr>
<tr>
<td>3</td>
<td>45-61</td>
<td>21.5</td>
</tr>
<tr>
<td>4</td>
<td>34-44</td>
<td>29.4</td>
</tr>
<tr>
<td>5</td>
<td>25-33</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>10-24</td>
<td>11.1</td>
</tr>
</tbody>
</table>

3.6.4 Mental health and medication(s). Potential participants indicated whether they had suffered from a mental illness (e.g. depression, drug and alcohol dependence) in the past or currently during the screening questionnaire. They also commented on the use of any psychoactive medications, as well as specific medications for premenstrual symptoms, and contraceptive use.

3.7 Data Management and Study Integrity

Study data was kept in locked storage systems; either a password protected computer system at the University of Canterbury or on a web-based data collection system (www.canterbury.qualtrics.com) for electronic documents. Hard copies were kept in secure filing cabinets at the university.

The trial was prospectively registered under the Australian and New Zealand Clinical Trials Registry (ANZCTR). Trial Identification = ACTRN12615000131550.

Universal Trial Number (UTN) = U1111-1164-2407.

Ethics approval was granted through the University of Canterbury Human Ethics Committee: HEC 2014/129, on 13 November 2014. Approval was updated on 4 February 2015 following trial amendments. Written informed consent was granted by all participants before entry into this study.
3.8 Sample Size

Determining the sample size was challenging based on the limited studies that had thus far been conducted. The aim was to recruit 40 participants per group, making a total of 80 participants. Final sample size calculations used an effect size (ES) estimate of 0.36 as vitamin B6’s effect on the psychological symptoms of PMS (Kashanian et al., 2007). A conservative ES estimate of EMP+’s effect on mood symptoms (in a sample not recruited for PMS treatment) in a blinded trial comparing EMP to placebo was 0.64 (Rucklidge, Frampton, Gorman, & Boggis, 2014). Yet in an open label trial, where participants again were not recruited for PMS treatment, the reported ES pre to post treatment was 1.96 (Rucklidge, Taylor, & Whitehead, 2011). To detect a medium between group ES it was determined that a total of 72 participants was needed. This number was increased to 80, 40 per-group, to allow for attrition. A medium effect was chosen as it was believed this would be clinically relevant for those women choosing to use nutritional supplements for PMS treatment. A small group difference would have less clinical relevance. Based on the lack of nutrient comparison studies for PMS, it was difficult to determine if this number of participants would allow for a significant between groups effect to be seen. However, current estimates for mood in blinded, placebo-controlled, micronutrient trials appeared to be medium to large (Rucklidge, Frampton, et al., 2014) whereas they have been estimated to be small for B6.

3.9 Statistical Analysis

Statistical analyses were run using SPSS IBM® Statistics 22. Significance tests, excluding the ANCOVA, were two-tailed and a p-value of less than 0.05 was used to define a statistically significant result.

3.9.1 Intention to treat analyses. The data were initially analysed according to Intention To Treat (ITT) principals, whereby all participants, regardless of whether they supplied all data or dropped out, were included. If a participant was missing data for the final month of treatment, then their last available data point was used i.e. for participants with only baseline data
this became their treatment cycle three data as well. The full ITT sample included 78 participants for the secondary analyses and 76 for the primary diary analysis (two participants never completed any diary data). Further ITT analyses were conducted with the 28 participants who met criteria for PMDD.

3.9.2 Per-protocol analyses. Per protocol analyses were conducted with the 56 participants meeting the per-protocol criteria. The criteria excluded participants who dropped out (n= 6), with less than 80% pill adherence (n= 10), who were found to have been on psychoactive medication (n= 1) and those whose PMS symptoms failed to meet initial severity as based on their prospective baseline diaries (n= 5).

3.9.3 Demographic and PMS information. Baseline characteristics (mean, standard deviation, range, and percentage) for both treatment groups were established to compare the two randomised groups. Chi-square tests of association ascertained whether any group differences were statistically relevant based on frequency of observations. The effect size of the association between group and the variable in question was determined via the Cramner’s V statistic, with Cohen (1988) establishing 0.1 as a small effect, 0.3 a moderate effect, and 0.5 a large effect.

3.9.4 Primary Measure Analyses. The primary outcome measure, established a priori, was the DRSP questionnaire which measured five areas; Psychological Symptoms, Somatic Symptoms, Total Symptoms, Impact Ratings, and Worst Day Ratings. The changes from baseline to end of intervention were analysed using a Linear Mixed Models approach, accounting for variation in initial severity and differences between speeds of change through the addition of random factors. ANCOVA analyses, using baseline as the covariate, provided supplementary assurance of the outcomes produced in the mixed models approach.

Additional procedures determined the percentage of each treatment group who no longer: a) met criteria for PMDD, and b) met entry criteria used in this study for a PMS diagnosis. From this
the Number Needed to Treat (NNT) was established. Specifically, the number of participants who
would need to take the micronutrient formula to get a positive result (no longer meeting criteria for
a PMS diagnosis) over and above that of the vitamin B6 group was calculated.

Modified Brinley Plots provided a visual analysis of individual pre to post treatment effect
over the five PMS variables. Three sets of plots are presented; one displaying change in participants
with data from baseline plus at least one treatment cycle, the second set displaying change in
women without a PMDD diagnosis and with full diary data, and the final set displaying outcomes for
participants diagnosed with PMDD who had also provided complete diary data. Scores for each
individual were plotted on the X-axis (time one) and Y-axis (time two) with deviation from the 45
degree line of no change signalling a treatment effect (Blampied, 2017). The Reliable Change Index
(RCI), where possible, was calculated for each measure. If a deviation from the 45-degree line was
greater than the computed RCI then we could be more satisfied it indicated a true treatment change
as opposed to possible measurement error. Cohen’s $d$ effect sizes were calculated to determine the
standard mean difference between pre and post treatment, with the aid of ESCII Software
developed by Cumming (2013). Specifically the ES used was the within subject version of $d$, also
known as $d_{av}$ (Lakens, 2013). The ES and corresponding 95% confidence interval (CI), in which we
could be 95% sure the true ES lies, is displayed on the first set of Modified Brinley Plots.

3.9.5 Secondary Measures Analyses. ITT and per-protocol analyses were run with the
secondary questionnaire measures, including third party ratings and follow up data. ANCOVA
analyses assessed mean change score differences between the groups while controlling for baseline
values (a change score was established for each participant by subtracting the final treatment score
from their baseline value). Post hoc tests, with Bonferroni adjustments, were computed if the
ANCOVA revealed significant between groups differences. Cohen’s $d$ effect sizes were computed to
understand the magnitude of the between groups difference, whereby .2 was a small effect, .5 a
medium effect, and .8 a large effect (J. Cohen, 1988). Chi-square tests for independence were used
to analyse third party ratings, CGI-I and side effect data, while follow up data was analysed using t-tests. All expected cell counts for chi-square analyses were greater than 5 unless otherwise stated.

On occasion, in the ANCOVA analysis, outliers were found in the standardised residual of the change score (±3 standard deviations). The ANCOVA was run with and then without the outlier, but as this made no change to the statistical significance the original model has been reported. Additionally, Shapiro-Wilk tests at times indicated non-normality of the within group residuals. As the one-way ANCOVA is known to be robust to deviations of normality and the Type 1 error rate is usually not significantly affected, the decision was made to continue despite slight deviations. Furthermore square-root transformations over-corrected the residual skew, meaning the data was unable to undergo transformation.

A questionnaire error meant a small number (n=4) of baseline reports were invalid for the sleep quality questionnaire. In the cases where this occurred, data from the first cycle of treatment was taken as the baseline value. This had the potential to somewhat dilute the results, as there may have been treatment effects by the end of the first cycle. Never-the-less this conservative approach was undertaken to include as much data as possible in the analysis.
Chapter 4: Results

4.1 Study sample

Between March 2015 and August 2016 194 potential participants completed screening questionnaires. Of these, 106 women (age ≥18 years) with moderate to severe premenstrual symptoms completed informed consent and began the baseline phase. Twenty-eight women did not complete baseline, leaving a total of 78 women who were randomized to treatment following the two-cycle baseline phase. Of these 78, six women withdrew from the study, corresponding to a dropout rate of 8%, while the per-protocol analyses included 56 women deemed to have met overall study requirements (see Figure 2).

4.1.2 Demographic information. Demographic information for the 78 participants who were allocated to either vitamin B6 or a micronutrient formula is presented below (see Table 5). The two groups were found to be comparable on both demographic and clinical variables, indicating no systematic differences between the two groups. The one exception was rates of past bipolar disorder, as measured by the structured clinical interview (First et al., 2015). There was an association between the micronutrient intervention and baseline levels of bipolar disorder, \( \chi^2 = 4.672, p = .031 \), with more participants being identified as having bipolar disorder in the B6 group as compared with the micronutrient group.
Figure 2. CONSORT flow diagram tracking participants through the phases of the study for the two treatment groups and indicating reasons for inclusion and exclusion from analyses.
Table 5. Baseline demographic and clinical characteristics of both treatment groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Micronutrients (n= 41)</th>
<th>Vitamin B6 (n= 37)</th>
<th>Between groups p-value</th>
<th>Total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years: mean (SD)</td>
<td>36.16 (7.92)</td>
<td>34.32 (10.17)</td>
<td>.71</td>
<td>35.16 (9.07)</td>
</tr>
<tr>
<td></td>
<td>Range: 19.43-48.68</td>
<td>Range: 18.78-54.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household income, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low $0-40,000</td>
<td>11 (26.8)</td>
<td>10 (27.0)</td>
<td>21 (26.9)</td>
<td></td>
</tr>
<tr>
<td>Middle $40,000-80,000</td>
<td>15 (36.6)</td>
<td>9 (24.3)</td>
<td>24 (32.1)</td>
<td></td>
</tr>
<tr>
<td>Upper +$80,000</td>
<td>15 (36.6)</td>
<td>18 (48.6)</td>
<td>33 (41.0)</td>
<td></td>
</tr>
<tr>
<td>Socioeconomic status, mean (SD)</td>
<td>53.1 (14.7)</td>
<td>54.4 (14.5)</td>
<td>.71</td>
<td>53.7 (15.57)</td>
</tr>
<tr>
<td></td>
<td>Range: 28-74</td>
<td>Range: 31-74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnic origin, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>.41</td>
</tr>
<tr>
<td>New Zealanders of European descent</td>
<td>35 (85.4)</td>
<td>28 (75.7)</td>
<td>63 (80.8)</td>
<td></td>
</tr>
<tr>
<td>Maori (indigenous people of New Zealand)</td>
<td>1 (2.4)</td>
<td>3 (8.1)</td>
<td>4 (5.1)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>5 (12.2)</td>
<td>6 (16.2)</td>
<td>11 (14.1)</td>
<td></td>
</tr>
<tr>
<td>Previous psychiatric disorder, self-reported, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety disorder</td>
<td>8 (19.5)</td>
<td>10 (27.0)</td>
<td>.43</td>
<td>18 (23.1)</td>
</tr>
<tr>
<td>Major depressive disorder</td>
<td>6 (14.6)</td>
<td>6 (16.2)</td>
<td>.84</td>
<td>12 (15.4)</td>
</tr>
<tr>
<td>Persistent depressive disorder</td>
<td>2 (4.9)</td>
<td>1 (2.7)</td>
<td>.62</td>
<td>3 (3.8)</td>
</tr>
<tr>
<td>Bipolar disorder</td>
<td>0</td>
<td>1 (2.7)</td>
<td>.30</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Current psychiatric disorder, self-reported, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety disorder</td>
<td>2 (4.9)</td>
<td>3 (8.1)</td>
<td>.56</td>
<td>5 (6.4)</td>
</tr>
<tr>
<td>Structured Clinical Interview for mood disorders, past disorder diagnosis, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major depressive disorder</td>
<td>16 (39.0)</td>
<td>16 (43.2)</td>
<td>.71</td>
<td>32 (41.0)</td>
</tr>
<tr>
<td>Bipolar I disorderab</td>
<td>0</td>
<td>4 (10.8)</td>
<td>.03*</td>
<td>4 (5.1)</td>
</tr>
<tr>
<td>Persistent depressive disorder</td>
<td>6 (14.6)</td>
<td>5 (13.5)</td>
<td>.89</td>
<td>11 (14.1)</td>
</tr>
<tr>
<td>Current PMDD diagnosisc</td>
<td>13(31.7)</td>
<td>15 (40.5)</td>
<td>.41</td>
<td>28 (36.8)</td>
</tr>
<tr>
<td>Age of menstruation, n (%)</td>
<td></td>
<td></td>
<td>.37</td>
<td></td>
</tr>
<tr>
<td>Age 9</td>
<td>-</td>
<td>1 (2.7)</td>
<td>1 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Age 10</td>
<td>1 (2.4)</td>
<td>2 (5.4)</td>
<td>3 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Age 11</td>
<td>14 (34.1)</td>
<td>7 (18.9)</td>
<td>21 (36.9)</td>
<td></td>
</tr>
<tr>
<td>Age 12</td>
<td>10 (24.4)</td>
<td>12 (32.4)</td>
<td>22 (28.2)</td>
<td></td>
</tr>
<tr>
<td>Age 13</td>
<td>8 (19.5)</td>
<td>7 (18.9)</td>
<td>15 (19.2)</td>
<td></td>
</tr>
<tr>
<td>Age 14</td>
<td>5 (12.2)</td>
<td>4 (10.8)</td>
<td>9 (11.5)</td>
<td></td>
</tr>
<tr>
<td>Age 15</td>
<td>1 (2.4)</td>
<td>4 (10.8)</td>
<td>5 (6.4)</td>
<td></td>
</tr>
<tr>
<td>Over age 15</td>
<td>2 (4.9)</td>
<td>-</td>
<td>2 (2.6)</td>
<td></td>
</tr>
<tr>
<td>PMS onset, n (%)</td>
<td></td>
<td></td>
<td>.53</td>
<td></td>
</tr>
<tr>
<td>With menstruation onset</td>
<td>9 (22.0)</td>
<td>4 (10.8)</td>
<td>13 (16.7)</td>
<td></td>
</tr>
</tbody>
</table>
Most of the sample began menstruating at 11 (26.9%), 12 (28.2%), or 13 (19.2%) years of age. Premenstrual symptoms most frequently presented 1-5 years after menstruation onset (35.9%), although there was a large variation in this range; from as much as 16 or more years to immediate onset. Similar numbers per group met criteria for a past diagnosis of major depressive disorder and/or persistent depressive disorder as measured by the Structured Clinical Interview.

Likewise, the frequency of participants per group currently suffering from Premenstrual Dysphoric Disorder (PMDD), as determined via the interview and prospective diary data collection, were nearly equivalent. Participants in both treatments reported comparable use of analgesics to alleviate symptoms of premenstrual syndrome.

<table>
<thead>
<tr>
<th>Time Period After Menstruation Onset</th>
<th>Endometriosis, n (%)</th>
<th>On Contraception, n (%)</th>
<th>Type of Contraception, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Contraceptive pill</td>
</tr>
<tr>
<td>1-5 years after menstruation onset</td>
<td>16 (39.0)</td>
<td>8 (19.5)</td>
<td>5 (12.2)</td>
</tr>
<tr>
<td>5-10 years after menstruation onset</td>
<td>12 (32.4)</td>
<td>9 (24.3)</td>
<td>4 (10.8)</td>
</tr>
<tr>
<td>10-15 years after menstruation onset</td>
<td>28 (35.9)</td>
<td>30 (1.3)</td>
<td>0 (2.7)</td>
</tr>
<tr>
<td>15+ years after menstruation onset</td>
<td>17 (21.8)</td>
<td>17 (1.3)</td>
<td>1 (1.3)</td>
</tr>
</tbody>
</table>

Type of contraception, n (%):

- **Contraceptive pill**: 5 (12.2), 9 (19.5), 9 (11.5)
- **IUD**: 3 (7.3), 3 (8.1), 6 (7.7)
- **Rod i.e jadelle**: 0, 3 (8.1), 3 (3.9)
- **Taking health supplements at time of screening questionnaire, n (%)**: 17 (41.5), 10 (27.0), 27 (34.6)
- **Taking medication for PMS/PMDD at time of screening questionnaire, n (%)**: 13 (31.7), 12 (32.4), 25 (32.5)
- **Consuming alcohol in the week preceding the baseline questionnaire, n (%)**: 22 (53.7), 22 (59.5), 44 (56.4)
- **Consuming cigarettes in the week preceding the baseline questionnaire, n (%)**: 5 (12.2), 4 (10.8), 9 (11.5)

Abbreviations: PMDD= Premenstrual Dysphoric Disorder, PMS= Premenstrual Syndrome

- a. Based on the New Zealand Socio-Economic Index 2006 (NZSEI-06) (Milne et al., 2013)
- b. Specifically, in remission, and hence eligible to participate
- c. Including two months of perspective screening using the daily diary measure
- d. Medication refers to analgesic medication e.g panadol, ibuprofen
4.2 Results: Primary outcome measures

4.2.1 Treatment response. Diary data from the second and third cycle of treatment was analysed to see a) if participants who met study entry criteria for PMS were still experiencing the syndrome and b) whether those diagnosed with PMDD were still meeting criteria.

All participants, excluding five who handed in their baseline dairies post randomisation and two who did not provide dairy data, met the diagnosis of PMS used in this study. Specifically, during the premenstrual phase participants experienced a worsening in a least one mood symptom, had a deterioration in three symptoms overall, and rated the impact in at least one area as moderate or higher. Fifty-nine had diary data available from treatment cycle two or three from which their PMS diagnosis could be assessed (micronutrient, n= 29, vitamin B6 n= 30). Data presented in Table 6 show similar numbers of participants per group had switched from a PMS diagnosis to non-PMS status. Chi-square analyses showed no group differences in the frequency with which participants responded to treatment, $\chi^2 (1) = 1.014, p = .314$.

From the available data, including those with an initial PMDD diagnosis, 72% of participants randomized to micronutrients and 60% randomized to vitamin B6 were PMS free by cycle three of treatment. This corresponded to an increased success of 12.4% for taking micronutrients, and a number needed to treat of eight. Specially, one in every eight meeting a diagnosis of PMS and taking micronutrients will benefit over and above the improvement expected of the vitamin B6 group. However, of the 71 participants meeting PMS criteria at baseline, 12 were not included in the above calculations; five were drop outs while the remaining seven did not provide sufficient diary data to estimate PMS status. If a conservative estimate of PMS improvement per group was to be made, in which the 12 were assumed to still meet criteria for PMS, then 50% of the vitamin B6 and 60% of the micronutrient treated participants would be PMS diagnosis free.
At baseline, 28 women, out of 76 who provided diary data, in addition to being included in the PMS diagnosed group, were identified as having PMDD; 15 in the vitamin B6 group, 13 in the micronutrient group. Final diary data was available for 12 and 11 participants respectively. Chi-square analysis again showed no difference in remission in PMDD diagnosis between treatment groups, $\chi^2 (1) = .434, p = .510$, with both treatments appearing to perform equally well. However, due to the small sample size some expected cell counts were fewer than five. Of the 23 with final diary data, 64% of the micronutrient sample and 50% of the vitamin B6 sample no longer met a diagnosis of PMDD. The NNT was similar to the previous group, with one in seven women with PMDD likely to respond to micronutrients relative to those receiving vitamin B6.

Table 6. The relationship between treatment group and premenstrual syndrome and/or premenstrual dysphoric diagnosis following treatment.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Micronutrient</th>
<th>Vitamin B6</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMS Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (%)</td>
<td>8 (27.6)</td>
<td>12 (40.0)</td>
</tr>
<tr>
<td>No (%)</td>
<td>21 (72.4)</td>
<td>18 (60.0)</td>
</tr>
<tr>
<td>PMDD Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (%)</td>
<td>4 (36.4)</td>
<td>6 (50.0)</td>
</tr>
<tr>
<td>No (%)</td>
<td>7 (63.6)</td>
<td>6 (50.0)</td>
</tr>
</tbody>
</table>

4.2.2 PMS diary results: Linear Mixed Model analysis. To investigate the outcomes measured by the primary diary data, a Linear Mixed Model (LMM) analysis was employed using SPSS IBM® Statistics 22. This analysis was chosen because of the high frequency of missing diary data. Furthermore, the LMM approach had the advantage of greater model flexibility in which population (fixed) and participant (random) effects could be computed for greater accuracy. All 76 participants (40 in the micronutrient group, 36 in the vitamin B6 group) with baseline diary data were included in the analysis despite varying frequencies of missing diary entries across the three treatment cycles. Analysis of the pattern and characteristics of those with missing data indicated that data were missing at random and therefore a LMM analysis was appropriate. Of the 76 participants, 40 responded at each treatment wave, while 36 had diary data missing for at least one
treatment cycle. Chi-square analyses indicated there were no statistically significant differences between the participants who completed their diaries versus those who supplied incomplete diaries, in terms of; income ($\chi^2(2) = .388, p = .824$), treatment type ($\chi^2(1) = .803, p = .370$), overall treatment compliance ($\chi^2(1) = 2.459, p = .117$), PMDD diagnosis ($\chi^2(1) = .016, p = .900$), and ethnicity ($\chi^2(2) = 1.294, p = .524$) (however, some expected cell counts were under five due to the small number of those whose ethnicity was not New Zealand European).

For each of the five outcome (dependent) variables, namely; Psychological Symptoms, Somatic Symptoms, and Total Symptoms, Impact Ratings and Worst Day Ratings, possible models were fitted within a Maximum Likelihood (ML) estimation. The ML estimation was used so that whilst the LMM was being built through the addition of random factors, the different models could be compared by the log-likelihood change value. A further reason for using ML was the suggestion by Twisk (2006) that ML provides a superior analysis for those models where the main outcome interest is in the fixed regression values, as ML is believed to be best at estimating these in comparison to the Restricted Maximum Likelihood (REML) method.

The final model contained time as a covariate, the random effect of participant and of time and participant by time interaction, the fixed effects of group, time, and group by time interaction. The model had both a random intercept and a random slope, which took into account baseline variability among participants and individual differences in response to the treatment on the variable in question. An unstructured covariance structure was used to model the random intercept and slope. Initially, the model included only fixed effects, which assumed there was no individual-specific effect of time (i.e., the slope), or of baseline starting point (i.e., the intercept). By adding both a random slope and intercept to the model, the model fit for each of the five dependent variables was improved, as measured by the log likelihood change ($p < 0.05$). Akaike’s Information Criterion (AIC) was selected by likelihood ratio comparisons to provide the best model fit (demonstrated by the smallest log likelihood value out of the four adjusted versions of the log
likelihood) for each of the dependent variables. The AIC compares competing models using a parsimony criterion whereby the number of parameters estimated and the corresponding likelihood of the model gives an indication of the goodness of fit (with smaller values indicating a more parsimonious model) (Field, 2014).

The relationship between time and each PMS variable was assessed using growth models, which assessed linear, quadratic and cubic trends, so as to explore the effect of time. As a second order polynomial best described the change over time in all of the outcome measures, growth curves were fitted allowing for both random intercepts and slopes; however, the chi-square change value did not indicate that this led to a better fit so the final model contained random factors for the linear trend only. Raw scores for each dependent variable over the four time points (baseline, then treatment cycles 1, 2, and 3) are displayed in Figure 3. Participants’ ratings over the six day premenstrual phase were totalled, added together, and then averaged by the number of responders to give a six day group average which was then displayed per group at each time point. The only exception to this was in the worst day ratings, whereby ratings came from one day of the premenstrual phase as opposed to six. Due to missing data from the treatment cycles, there are varying numbers of responders at each time point.
Psychological Symptoms over 6 days

Somatic Symptoms over 6 days

Impact Ratings over 6 days
The mixed model analysis supported the trend for improvement over time seen in Figure 1 in all five of the primary outcome measures for both groups. The variable of time significantly predicted change in psychological symptoms \( F(1, 66.253) = 20.803, p < .001 \), somatic symptoms \( F(1, \ 65.790) = 20.877, p < .001 \), total PMS symptoms \( F(1, 66.452) = 22.803, p < .001 \), impact ratings \( F(1, 
66.622) = 18.245, p < .001 \), and worst day ratings \( F(1, 65.323) = 29.249, p < .001 \). Information presented in Table 1 shows that with each unit increase in time (i.e., stepping from baseline through each treatment cycle) average raw scores underwent a similar reduction in both treatment groups; total scores underwent a reduction of roughly 11 points, psychological symptoms were reduced by 4 points, somatic scores by 7 points, impact ratings decreased by 3 points and the worst day rating decreased by 3 points for every step in time. The possible score range over the six days for each PMS variable is as follows: 24-144 for Psychological Symptoms, 42-252 for Somatic Symptoms, 66-396 for Total Symptoms, 18-108 for Impact Ratings, and 14-84 for Worst Day Ratings. Table 1 details the exact score reductions per unit increase in time for each group. Additionally, the initial starting point, or baseline group average, is represented by the intercept, with those in vitamin B6 group having a slightly higher (i.e., more severe) average starting point in the full sample data.
The variables in the model were then standardized to represent the reduction per unit increase in time as an r effect size. Table 8 documents the consequent effect size measure with the corresponding 95% confidence interval, along with Cohen’s $d_{\text{within}}$ effect size (ES) estimates provided through Lenhard and Lenhard (2016). According to J. Cohen (1988), a $d$ effect of .2 is considered small, .5 medium, and >.8 is large. The symptom reduction across both groups and all variables was consistently expressed as a moderate ES.
Table 7. Fixed effects table showing the intercept (baseline value) and decrease in raw scores per unit increase in time for the micronutrient and vitamin B6 treatment groups. Data are presented separately for the full sample and for the subgroup of participants meeting criteria for PMDD.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Treatment</th>
<th>Fixed Effect</th>
<th>$b$</th>
<th>SE*</th>
<th>P value</th>
<th>95% Confidence Interval**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
</tr>
<tr>
<td>Total symptoms</td>
<td>Micronutrient</td>
<td>Intercept</td>
<td>167.10</td>
<td>10.93</td>
<td>&lt;0.001</td>
<td>145.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-12.29</td>
<td>3.32</td>
<td>&lt;0.001</td>
<td>-18.92</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Intercept</td>
<td>180.16</td>
<td>11.60</td>
<td>&lt;0.001</td>
<td>138.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-11.00</td>
<td>3.57</td>
<td>&lt;0.001</td>
<td>-18.13</td>
</tr>
<tr>
<td>Psychological</td>
<td>Micronutrient</td>
<td>Intercept</td>
<td>60.15</td>
<td>4.27</td>
<td>&lt;0.001</td>
<td>51.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-4.73</td>
<td>1.33</td>
<td>&lt;0.001</td>
<td>-7.39</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Intercept</td>
<td>66.94</td>
<td>4.53</td>
<td>&lt;0.001</td>
<td>57.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-4.19</td>
<td>1.43</td>
<td>&lt;0.001</td>
<td>-7.05</td>
</tr>
<tr>
<td>Somatic symptoms</td>
<td>Micronutrient</td>
<td>Intercept</td>
<td>106.92</td>
<td>7.15</td>
<td>&lt;0.001</td>
<td>92.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-7.55</td>
<td>2.14</td>
<td>&lt;0.001</td>
<td>-11.83</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Intercept</td>
<td>113.20</td>
<td>7.59</td>
<td>&lt;0.001</td>
<td>98.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-6.82</td>
<td>2.30</td>
<td>&lt;0.001</td>
<td>-11.42</td>
</tr>
<tr>
<td>Impact ratings</td>
<td>Micronutrient</td>
<td>Intercept</td>
<td>40.36</td>
<td>3.28</td>
<td>&lt;0.001</td>
<td>33.82</td>
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<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-3.19</td>
<td>1.05</td>
<td>&lt;0.001</td>
<td>-5.29</td>
</tr>
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<td></td>
<td>Vitamin B6</td>
<td>Intercept</td>
<td>42.77</td>
<td>3.48</td>
<td>&lt;0.001</td>
<td>35.83</td>
</tr>
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<td></td>
<td></td>
<td>Time</td>
<td>-3.42</td>
<td>1.13</td>
<td>&lt;0.001</td>
<td>-5.67</td>
</tr>
<tr>
<td>Worst day ratings</td>
<td>Micronutrient</td>
<td>Intercept</td>
<td>45.30</td>
<td>2.67</td>
<td>&lt;0.001</td>
<td>39.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-3.49</td>
<td>.88</td>
<td>&lt;0.001</td>
<td>-5.24</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Intercept</td>
<td>48.47</td>
<td>2.84</td>
<td>&lt;0.001</td>
<td>42.81</td>
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<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-3.49</td>
<td>.94</td>
<td>&lt;0.001</td>
<td>-5.37</td>
</tr>
<tr>
<td>PMDD subgroup</td>
<td>Micronutrient</td>
<td>Intercept</td>
<td>257.53</td>
<td>17.59</td>
<td>&lt;0.001</td>
<td>222.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-32.165</td>
<td>6.29</td>
<td>&lt;0.001</td>
<td>-44.91</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Intercept</td>
<td>218.01</td>
<td>16.99</td>
<td>&lt;0.001</td>
<td>183.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-14.536</td>
<td>6.289</td>
<td>.026</td>
<td>-27.26</td>
</tr>
<tr>
<td>Somatic symptoms</td>
<td>Micronutrient</td>
<td>Intercept</td>
<td>157.76</td>
<td>11.85</td>
<td>&lt;0.001</td>
<td>133.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-18.74</td>
<td>3.78</td>
<td>&lt;0.001</td>
<td>-26.59</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Intercept</td>
<td>135.47</td>
<td>11.75</td>
<td>&lt;0.001</td>
<td>111.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-8.43</td>
<td>3.91</td>
<td>&lt;0.001</td>
<td>-16.49</td>
</tr>
<tr>
<td>Impact ratings</td>
<td>Micronutrient</td>
<td>Intercept</td>
<td>62.20</td>
<td>4.92</td>
<td>&lt;0.001</td>
<td>51.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-8.34</td>
<td>1.56</td>
<td>&lt;0.001</td>
<td>-11.60</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Intercept</td>
<td>54.69</td>
<td>4.88</td>
<td>&lt;0.001</td>
<td>44.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>-5.22</td>
<td>1.62</td>
<td>.004</td>
<td>-8.58</td>
</tr>
</tbody>
</table>

* SE is the standard error associated with the beta (b) value
**95% CI is associated with the fixed effect
Abbreviations: PMDD= Premenstrual Dysphoric Disorder
The LMM analyses also confirmed what is evident in Figure 3, namely, that there were no statistically significant group by time interactions (psychological symptoms \( F(1, 66.253) = .077, p = .782 \), somatic symptoms \( F(1, 65.790) = .054, p = .816 \), total symptoms \( F(1, 66.452) = .070, p = .793 \), impact ratings \( F(1, 66.622) = .021, p = .816 \), worst day ratings \( F(1, 65.323) = .000, p = .997 \)), nor did one treatment statistically significantly differ from the other on any of the dependent measures (psychological symptoms \( F(1, 75.131) = 1.192, p = .278 \), somatic symptoms \( F(1, 74.762) = .363, p = .548 \), total symptoms \( F(1, 75.154) = .672, p = .415 \), impact ratings \( F(1, 73.172) = .253, p = .617 \), worst day ratings \( F(1, 75.097) = .658, p = .420 \)).

Table 8. Effect size estimates (\( r \)) and their 95% confidence intervals for the fixed effect of time, using data from both the full sample and the PMDD subgroup.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Treatment</th>
<th>Fixed Effect</th>
<th>( r ) Lower bound</th>
<th>Upper bound</th>
<th>( \text{Cohen's } d ) equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total symptoms</td>
<td>Micronutrient</td>
<td>Time</td>
<td>-.24</td>
<td>-.38</td>
<td>-.20</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Time</td>
<td>-.23</td>
<td>-.38</td>
<td>-.08</td>
</tr>
<tr>
<td>Psychological symptoms</td>
<td>Micronutrient</td>
<td>Time</td>
<td>-.24</td>
<td>-.37</td>
<td>-.10</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Time</td>
<td>-.21</td>
<td>-.35</td>
<td>-.06</td>
</tr>
<tr>
<td>Somatic symptoms</td>
<td>Micronutrient</td>
<td>Time</td>
<td>-.24</td>
<td>-.37</td>
<td>-.10</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Time</td>
<td>-.21</td>
<td>-.36</td>
<td>-.07</td>
</tr>
<tr>
<td>Impact ratings</td>
<td>Micronutrient</td>
<td>Time</td>
<td>-.24</td>
<td>-.39</td>
<td>-.08</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Time</td>
<td>-.25</td>
<td>-.42</td>
<td>-.08</td>
</tr>
<tr>
<td>Worst day ratings</td>
<td>Micronutrient</td>
<td>Time</td>
<td>-.27</td>
<td>-.41</td>
<td>-.13</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Time</td>
<td>-.27</td>
<td>-.42</td>
<td>-.12</td>
</tr>
<tr>
<td><strong>PMDD subgroup</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total symptoms</td>
<td>Micronutrient</td>
<td>Time</td>
<td>-.64</td>
<td>-.88</td>
<td>-.40</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Time</td>
<td>-.29</td>
<td>-.53</td>
<td>-.05</td>
</tr>
<tr>
<td>Somatic symptoms</td>
<td>Micronutrient</td>
<td>Time</td>
<td>-.54</td>
<td>-.76</td>
<td>-.31</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Time</td>
<td>-.24</td>
<td>-.47</td>
<td>-.01</td>
</tr>
<tr>
<td>Impact ratings</td>
<td>Micronutrient</td>
<td>Time</td>
<td>-.56</td>
<td>-.78</td>
<td>-.34</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Time</td>
<td>-.35</td>
<td>-.57</td>
<td>-.13</td>
</tr>
</tbody>
</table>

Abbreviations: PMDD= Premenstrual Dysphoric Disorder

There was a large component of individual variance in the random time by subject interaction (see Table 9). Most of the total variance in the random factors was explained by this interaction, highlighting its importance within the model and indicating there was large between-participants variability in baseline ratings and subsequent treatment effects. Further exploration of
the rate of change in the PMS variables over time using a growth model demonstrated there was both a linear (psychological symptoms $F(1, 66.366) = 21.252, p < .001$, somatic symptoms $F(1, 65.734) = 21.202, p < .001$, total symptoms $F(1, 66.455) = 23.216, p < .001$, impact ratings $F(1, 66.450) = 18.304, p < .001$, worst day ratings $F(1, 65.125) = 29.448, p < .001$) and quadratic trend in change over time (psychological symptoms $F(1, 121.590) = 14.043, p < .001$, somatic symptoms $F(1, 119.079) = 19.851, p < .001$, total symptoms $F(1, 120.164) = 19.258, p < .001$, impact ratings $F(1, 119.574) = 18.579, p < .001$, worst day ratings $F(1, 121.598) = 14.914, p < .001$). This reflected what is evident in Figure 1 through a) a decline in total symptoms over time, and b) a tendency for the decline to be most rapid in the first month of treatment. Based on the chi-square log likelihood change value ($p < .01$) the quadratic trend best described the data (see Table 10). Specifically, symptoms underwent their largest decline in the first cycle of treatment. At treatment cycle two and three this effect began to even out, with scores increasing slightly on average in specific dependent variables and treatment groups. This trend is, however, an approximation, and therefore may not entirely reflect the symptom change uniformly. Consistency of change over time at the individual level is further analysed using modified Brinley Plots (see section 4.3.4).

Results from the LMM analyses were confirmed with intention to treat ANCOVA analyses comparing pre-treatment to total change scores (the change score was calculated as the baseline value minus the final treatment cycle score). All five primary outcomes (psychological symptoms $F(1, 74) = .448, p = .505$, partial $\eta^2 = .006$, somatic symptoms $F(1, 74) = 0.53, p = .818$, partial $\eta^2 = .001$, total symptoms $F(1, 74) = .005, p = .944$, partial $\eta^2 = .000$, impact ratings $F(1, 74) = .044, p = .834$, partial $\eta^2 = .001$, and worst day ratings $F(1, 74) = .077, p = .934$, partial $\eta^2 = .000$) demonstrated no statistically significant between treatment group differences.
Table 9. Intraclass Correlation Coefficient’s (ICC) for random effects in the full data model.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Total variance estimate</th>
<th>Parameter variance</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total symptoms</td>
<td>4263.76</td>
<td>Variance of intercepts</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Covariance of slopes and intercepts</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance of slopes</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Residual</td>
<td>33%</td>
</tr>
<tr>
<td>Psychological symptoms</td>
<td>618.04</td>
<td>Variance of intercepts</td>
<td>54%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Covariance of slopes and intercepts</td>
<td>6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance of slopes</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Residual</td>
<td>39%</td>
</tr>
<tr>
<td>Somatic symptoms</td>
<td>1878.50</td>
<td>Variance of intercepts</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Covariance of slopes and intercepts</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance of slopes</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Residual</td>
<td>30%</td>
</tr>
<tr>
<td>Impact scores</td>
<td>425.96</td>
<td>Variance of intercepts</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Covariance of slopes and intercepts</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance of slopes</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Residual</td>
<td>26%</td>
</tr>
<tr>
<td>Worst day scores</td>
<td>232.66</td>
<td>Variance of intercepts</td>
<td>48%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Covariance of slopes and intercepts</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance of slopes</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Residual</td>
<td>45%</td>
</tr>
</tbody>
</table>

4.2.3 Premenstrual dysphoric Disorder subgroup analysis. A further LMM analysis was run with data from the subgroup of women who met criteria at baseline for a diagnosis of PMDD (15 in the vitamin B6 group, 13 in the micronutrient group). The model, including fixed and random effects and the covariance structure, was kept the same as that for the whole data set; however, due to the small sample size, the Restricted Maximum Likelihood (REML) estimation was used to produce a more conservative estimate and thus reduce bias (Field, 2014). Somatic Symptoms, Total Symptoms, and Impact Ratings were analysed in the LMM, but the Psychological Symptoms and Worst Day Ratings could not be computed as the model was unable to converge for these data.
Figure 4. Average raw score ratings of premenstrual symptoms from baseline to three cycles of treatment, shown separately for the micronutrient and vitamin B6 groups. Error bars denote the standard error for all values.

Data from the full sample showed baseline average symptom severity was higher across all PMS variables in the vitamin B6 treated group; however, this was reversed in the PMDD subgroup. The overall pattern of results in the PMDD subgroup remained largely unchanged from the full
sample, in that time was still statistically significantly associated with symptom improvement in both treatment groups (somatic symptoms $F(1, 22.498) = 24.977, p < .001$, total symptoms $F(1, 38.276) = 27.544, p < .001$), impact ratings $F(1, 21.448) = 36.258, p < .001$). There were no statistically significant treatment group differences (somatic symptoms $F(1, 24.889) = 1.785, p = .194$, total symptoms $F(1, 53.093) = 2.610, p = .112$), impact ratings $F(1, 22.965) = 1.172, p = .290$) or group by time interactions, (somatic symptoms $F(1, 22.498) = 3.597, p = .071$, total symptoms $F(1, 38.276) = 3.925, p = .055$), impact ratings $F(1, 21.448) = 1.915, p = .181$, this was despite a near significant result on the total symptoms by time measure where the micronutrient treated group appeared to have a larger symptom decrease over time (please refer to Figure 4).

Despite no statistically significant effects, there was a difference in the rate of average symptom decrease. With each unit increase in time, participants taking the micronutrient formula had a larger decrease in their symptom ratings (see Table 7). For Somatic Symptoms, Total Symptoms, and Impact Ratings the ES was large in the micronutrient group and moderate in the vitamin B6 group (see Table 8). This between-group difference was further investigated in the Modified Brinley Plots (see below).

Growth curve modelling demonstrated that a quadratic trend provided the best fit for the subgroup data (somatic symptoms $F(1, 41.964) = 9.686, p = .003$, total symptoms $F(1, 56.818) = 7.159, p = .010$), impact ratings $F(1, 41.714) = 7.851, p = .008$). The plots in Figure 4 show a general pattern of large symptom decrease until cycle two, followed by a tapering off, and in some cases a small increase, in symptoms by cycle three; a pattern that was consistent with the underlying quadratic tend.

Finally, ANCOVA analyses provided confirmation of the LMM results for the PMDD subgroup in that there were no statistically significant differences between treatment groups when baseline scores were compared to total score change (somatic symptoms $F(1, 25) = 1.791, p = .193$, partial
\eta^2 = .067, \text{ total symptoms } F(1, 25) = .903, p = .351, \text{ partial } \eta^2 = .035, \text{ impact ratings } F(1, 25) = 1.827, p = .189, \text{ partial } \eta^2 = .068).

Table 10. Growth curve modelling demonstrating the -2 log likelihood change between the linear and quadratic trends for dependent variables in the full sample and PMDD sample data.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Growth trend</th>
<th>-2 Log Likelihood</th>
<th>\chi^2 Change</th>
<th>Change p-value (1 df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total symptoms</td>
<td>Linear</td>
<td>2594.344</td>
<td>17.296</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Quadratic</td>
<td>2577.048</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychological symptoms</td>
<td>Linear</td>
<td>2152.945</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quadratic</td>
<td>2139.865</td>
<td>13.08</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Somatic symptoms</td>
<td>Linear</td>
<td>2374.490</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quadratic</td>
<td>2356.836</td>
<td>17.654</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Impact scores</td>
<td>Linear</td>
<td>1972.763</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quadratic</td>
<td>1955.881</td>
<td>16.882</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Worst day scores</td>
<td>Linear</td>
<td>1936.878</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quadratic</td>
<td>1923.126</td>
<td>13.752</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Total symptoms</td>
<td>Linear</td>
<td>2551.166</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quadratic</td>
<td>2532.314</td>
<td>18.853</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>PMDD subgroup</td>
<td>Total symptoms</td>
<td>Linear</td>
<td>957.763</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quadratic</td>
<td>946.023</td>
<td>11.74</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Somatic symptoms</td>
<td>Linear</td>
<td>907.567</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quadratic</td>
<td>895.162</td>
<td>12.405</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Impact ratings</td>
<td>Linear</td>
<td>749.707</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quadratic</td>
<td>740.362</td>
<td>17.654</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Abbreviations: PMDD= Premenstrual Dysphoric Disorder

### 4.2.4 PMS diary results: Modified Brinley Plot Analyses.

Further analysis of the primary outcome measure was undertaken at an individual level through examining participant’s symptom ratings at each study point. Modified Brinley Plots (MBP) (Blampied, 2017) permit changes reported in the diaries to be tracked across cycles for each individual participant who had provided a diary record at baseline and for at least one cycle (n= 65). A subset of analyses was also performed for those women who had completed diaries at baseline and for all three treatment cycles (n=40) as this provided information about consistency of treatment effects over time. Note that the MBPs could not include data from those women who only completed the diary at baseline.

As described in section 3.9.4, for each PMS variable an individual’s data from treatment cycle 1, 2, or 3 is plotted on the Y-axis against baseline plotted on the X-axis, as a coordinate point.
Data points that lie off the diagonal line of no effect indicate change from baseline to that time point. The Reliable Change Index (RCI) was computed, where appropriate psychometric data was available, and provides a guide by which to judge the extent of change; scores outside of the RCI boundaries indicate a change larger than that expected due to measurement error alone. For the dairy outcome measures, scores that were below the line of no change indicated improvement (see Figure 5 for the interpretation of these graphs). In the figures that follow, the vitamin B6 group is denoted by black circles, while the micronutrient group are open circles with a black outline.

![Modified Brinley Plot](image)

**Figure 5.** The figure illustrates how modified Brinley plots should be interpreted when the clinical direction of change in scores is downwards, as indicated by the arrow. The solid diagonal line from the origin is the line of no change; any data points on or about that line have shown no change from time 1 to time 2. The dashed lines parallel to the line of no change show the upper and lower boundaries of the Reliable Change Index respectively. The vertical and horizontal lines show the clinical cut offs for the measure (if available). The zones on the graph formed by the cut off lines and the diagonal line have clinical meaning, as indicated.

The modified Brinley plots are in three sets. The first set presents all cases where a participant supplied a diary for baseline plus at least one treatment cycle ($n=65$). Thus the number of participants represented at each time point differs slightly from cycle to cycle. For each symptom cluster (Psychological Symptoms, Total Symptoms, etc) the two treatment groups are displayed side by side for each treatment cycle, with cycles one to three displayed down the page. Effect sizes (Cohen’s $d_{av}$; (Lakens, 2013)) for each plot are displayed in the top left hand corner along with the 95% confidence interval of $d$ (calculated using software provided by Cumming (2013)).
Figure 6. Modified Brinley plots showing total symptom change from baseline to treatment cycle three in both treatment groups.
Figure 7. Modified Brinley plots showing psychological symptom change from baseline to treatment cycle three in both treatment groups.
Figure 8. Modified Brinley plots showing somatic symptom change from baseline to treatment cycle three in both treatment groups.
Figure 9. Modified Brinley plots showing impact rating change from baseline to treatment cycle three in both treatment groups.
Figure 10. Modified Brinley plots showing worst day rating change from baseline to treatment cycle three in both treatment groups.
Overall, the patterns of change in response to treatment and over time were similar across variables and treatment groups. Figure 6, 7, 8, 9, and 10 demonstrate the change in symptoms for each individual in comparison to her own baseline score. In both treatment groups, symptoms are most reduced during the second cycle of treatment, with effect sizes consistently higher in the micronutrient treated group for cycle two (other than for Worst Day Ratings). Yet, by the final cycle of treatment effect sizes were in the moderate range for both micronutrient ($d= 0.55$ to $0.71$) and vitamin B6 ($d= 0.51$ to $0.72$) treated participants. More participants experienced reliable change (i.e., their data points lie below the lower RCI boundary) when on micronutrients. In comparison to their baseline, a small number of participants from both treatment groups have data points above the line of no change, indicating a worsening in that PMS variable relative to baseline. Of these, a very small number show reliable deterioration, with points sitting above the upper RCI boundary. There appears to be a differential treatment effect based on symptom cluster; psychological symptoms show more positive reliable change than somatic symptoms in both treatment groups.

Further investigation of individual change was completed with the 40 women who provided full diary data. This group was divided into those who met criteria for PMDD and those who did not. Circles with a + in the middle denote participants who, while they completed their diary, were not fully compliant with the treatment protocol, i.e., their symptoms did not meet the diagnosis of PMS used in this study, or they did not consume at least 80% of capsules supplied. In this set of figures each treatment group is displayed in a separate graph, with the inclusion of follow up data on the variable of worst day. Because the numbers are small, Cohen’s $d_{av}$ has not been calculated for this data set.
Figure 11. Modified Brinley plots showing change across all symptom clusters from baseline to treatment cycle three in the non-PMDD Vitamin B6 group. Note that in order to keep the scale legible, Total Symptom scores were divided by 3 before plotting.
Figure 12. Modified Brinley plots showing change across all symptom clusters from baseline to treatment cycle three in the non-PMDD micronutrient group. Note that in order to keep the scale legible, Total Symptom scores were divided by 3 before plotting.

Figure 11 and 12 display outcome data for the participants who did not meet PMDD criteria. This includes 11 participants in Figure 11 taking vitamin B6 and 14 in Figure 12 taking micronutrients.

As can be seen from Figures 11 and 12, both groups have lower baseline symptomatology, indicated
by the cluster of points towards the bottom left corner. There is little to no reliable change in the vitamin B6 group on the variables of Psychological, Somatic, and Total symptoms. There is slightly more frequent reliable change in the micronutrient group, with Psychological and Total symptoms showing the most improvement. Follow up worst day ratings are varied which may relate to the difference between those who continued to take treatment and those who did not (this will be examined in more detail in later analyses).
Figure 13. Modified Brinley plots showing change across all symptom clusters from baseline to treatment cycle three in the PMDD Vitamin B6 group. Note that in order to keep the scale legible, Total Symptom scores were divided by 3 before plotting.
Figure 14. Modified Brinley plots showing change across all symptom clusters from baseline to treatment cycle three in the PMDD micronutrient group. Note that in order to keep the scale legible, Total Symptom scores were divided by 3 before plotting.

Figures 13 and 14 show data from those with a PMDD diagnosis, six receiving vitamin B6 and nine micronutrients. These figures show that women with PMDD showed the most reliable change in Psychological, Somatic and Total symptom clusters in the micronutrient group. Overall,
there was a clear trend of improvement in both treatments, with plots more consistently falling below the 45-degree line of no change than in comparison to the full sample data. By treatment cycle three all participants rated their symptoms as the same or better than baseline. The only exception to this was on Psychological Symptoms where one participant from each treatment had slightly worse symptoms. Compliance rates were good across both groups, with only one participant from the micronutrient treated group noted to have less than 80% capsule compliance (she did none-the-less see an improvement). Follow-up worst day ratings, collected three cycles post final treatment cycle, showed mixed results. Some participants’ symptoms remained improved while others showed a worsening (this will be further explored in the follow up section presented under the secondary analyses).

4.3 Results: Secondary outcome measures

4.3.1 DASS-42, PSS-10, WQoLQ, PSQI, NSSS-S, and Food Questionnaire outcomes. Multiple ANCOVA’s were conducted using ITT data to determine the effect of micronutrients and vitamin B6 on final treatment cycle outcomes. Baseline values were used as the covariate.

After adjustment for baseline ratings, there were no statistically significant differences between the mean change scores of the two treatments on any DASS-42 variable (anxiety: \( F(1,75)=2.539, p= .115, \) partial \( \eta^2= .033 \), depression: \( F(1,75)= 1.123, p= .293, \) partial \( \eta^2= .015 \), stress: \( F(1,75)= .612, p= .473, \) partial \( \eta^2= .008 \)). An interpretation of the pre to post treatment change based on scoring guidelines (S. H. Lovibond & P. F. Lovibond, 1995) showed the average baseline depression scores (micronutrients: Mean (M)=9.59, Standard Deviation (SD)= 8.64, vitamin B6; M= 11.11, SD= 8.58) fell within the ‘mild’ range, and by end of treatment both groups had average means equating to ‘normal’ (micronutrients: M=3.56, SD= 4.17, vitamin B6; M= 5.05, SD= 5.73). Anxiety scores stayed within the normal range pre and post treatment but the reduction was still roughly half of the baseline score (micronutrients: pre-M= 6.51, post-M= 2.90, vitamin B6; pre-
M=6.27, post-M= 3.76). Stress scores were initially of moderate severity (micronutrients: M=20.24, SD= 8.91, vitamin B6; M= 19.84, SD= 9.01), yet by end of treatment both groups had a reduced average mean score that placed them within the ‘normal’ range (micronutrients: M=10.39, SD= 6.67, vitamin B6; M= 9.22, SD= 6.06).

Likewise, the PSS-10 showed no outcome differences between the two treatments, $F(1,75)= 6.04, p= 0.44$, partial $\eta^2= .008$, yet final treatment scores for the micronutrient group (M= 15.29, SD= 7.19) and vitamin B6 group (M= 16.89, SD= 5.72) were lower than that reported for a non-clinical female college student sample used in the norming data (M= 18.4, SD= 6.5) (Roberti et al., 2006). Results from the DASS-42 and PSS-10 indicated there were no significant between group differences, nevertheless average within-group scores indicated meaningful change in stress, depression, and anxiety related symptoms with both treatments.

Group differences did emerge on the measure assessing women’s health-related quality of life. After adjusting for the baseline values as a covariate, those treated with a micronutrient formula had a statistically significantly greater average change score of 10.69 points (Standard Error (SE) = .99) compared to the change of 7.56 (SE= 1.01) seen in the vitamin B6 group (higher scores indicate better quality of life). This corresponded to an average mean difference of 3.13 points (95% CI: .30-5.95), $F(1,75)= 4.861, p= .031$, partial $\eta^2= .061$, and a moderate between groups ES ($d=.51$). Information from the initial WQoLQ development studies showed the average post treatment score of 30.18 seen in the micronutrient group was comparable to the score of 30.45 recorded for women during their late luteal phase who had never experienced PMS (Gehlert et al., 2006).

After controlling for baseline values, no group differences were statistically significant on the measure of sleep quality, $F(1,75)= 2.542, p= 0.816$, partial $\eta^2= .001$. Initially, before baseline values were accounted for, vitamin B6 appeared to create a larger average decrease (M= 6.91, SD= 3.49) than the micronutrient treatment (M= 4.80, SD= 3.56). As shown by the values in Table 11, this difference was mitigated once baseline values were controlled for. Both groups had pre-treatment
scores that were in the poor sleep quality range (>5), but by the third cycle of treatment ratings on the seven components of the questionnaire (subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleeping medication and daytime dysfunction) improved. This led to a clinically important average mean decrease that placed each group within the non-dysfunctional range (micronutrient: pre M= 6.4, post M= 1.6, vitamin B6: pre M= 8.5, post= 1.6).

Table 11. Between groups ANCOVA results using change score intention to treat data from secondary measures. Results are displayed for the vitamin B6 and micronutrient treated group.

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Micronutrient (n=41)</th>
<th>Vitamin B6 (n=37)</th>
<th>Between groups p-value</th>
<th>Effect Size (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DASS-42 Depression</td>
<td>6.50(.63)</td>
<td>5.24-7.76</td>
<td>5.52(.67)</td>
<td>4.20-6.85</td>
</tr>
<tr>
<td>Anxiety</td>
<td>3.55(.42)</td>
<td>2.72-4.37</td>
<td>2.59(.44)</td>
<td>1.71-3.46</td>
</tr>
<tr>
<td>Stress</td>
<td>9.70(.96)</td>
<td>7.80-11.61</td>
<td>10.79(1.01)</td>
<td>8.79-12.79</td>
</tr>
<tr>
<td>WQoLQ</td>
<td>10.69(.99)</td>
<td>8.72-12.66</td>
<td>7.56(1.01)</td>
<td>5.55-9.58</td>
</tr>
<tr>
<td>PSS-10</td>
<td>5.32(1.01)</td>
<td>3.31-7.34</td>
<td>4.18(1.07)</td>
<td>2.06-6.30</td>
</tr>
<tr>
<td>PSQI</td>
<td>5.85(.36)</td>
<td>5.34-6.36</td>
<td>5.76(.22)</td>
<td>5.22-6.30</td>
</tr>
<tr>
<td>NSSS-S</td>
<td>3.03(1.86)</td>
<td>0.71-6.76</td>
<td>5.01(1.83)</td>
<td>1.34-8.68</td>
</tr>
<tr>
<td>Food Q</td>
<td>2.05(.59)</td>
<td>0.87-3.23</td>
<td>1.65(.62)</td>
<td>0.40-2.89</td>
</tr>
</tbody>
</table>

*Significant at the 0.05 level

Abbreviations: SE= Standard Error; 95% CI= Confidence Interval of adjusted mean; ES= Effect Size; DASS-42= Depression and Anxiety Stress Scales-42; WQoLQ= Women’s Quality of Life Questionnaire; PSS-10= The Perceived Stress Scale-10; PSQI = Pittsburgh Sleep Quality Index; NSSS-S= the New Sexual Satisfaction Scale-Short form

Overall diet quality improved somewhat on average for participants in both groups, showing no treatment difference, $F(1, 75)= .225, p=.636, partial \eta^2 = .003$. Scores on the 8-41 point scale increased by 2.05 points in the group treated with micronutrients and 1.65 points in the group receiving vitamin B6. Average group results indicate there was not a large improvement in diet quality while participants were on active treatment.
Participants who were in a sexually active relationship and comfortable completing a questionnaire measuring their own and their partner’s sexual satisfaction completed the NSSS-S (n=55). Again, neither treatment produced a statistically different change score to the other when controlling for baseline values, $F(1, 52) = .580, P = .450$, partial $\eta^2 = .011$. Pre to post scores increased roughly by three points in both treatments (micronutrient: pre M= 35.0, post M= 37.9, vitamin B6: pre M= 34.7, post= 37.8) indicating an increase in overall average sexual satisfaction. With a possible score range of 12-60, a change of three points was not notable.

### 4.3.2 Per-protocol analyses for DASS-42, PSS-10, WQoLQ, PSQI, NSSS-S, and Food Questionnaire.

Subsequent per-protocol ANCOVA’s revealed no significant mean group differences on most of the outcome measures, which was in line with findings from the prior ITT analyses (DASS-42; Stress: $F(1, 53) = .395, p = .533$, partial $\eta^2 = .007$, Depression: $F(1, 53) = 2.701, p = .106$, partial $\eta^2 = .048$, Anxiety: $F(1, 53) = .713, p = .402$, partial $\eta^2 = .013$ PSS-10: $F(1, 53) = .566, p = .455$, partial $\eta^2 = .011$, NSSS-S: $F(1, 36) = .660, P = .422$, partial $\eta^2 = .018$, Food Questionnaire: $F (1, 53) = .069, p = .794$, partial $\eta^2 = .001$, PSQI: $F(1, 53) = 1.337, p = .562$, partial $\eta^2 = .006$). However, for the quality of life measure the between group difference remained statistically significant, increasing to a 4.12 (95% CI 0.94-7.31) point difference between the two treatment’s mean change score, $F(1, 53) = 6.742, p = .012$, partial $\eta^2 = .113$. Thus, following three cycles of active treatment, there was a large between group difference ($d= .71$) in favour of the micronutrient treatment.
Table 12. Between groups ANCOVA results using change score per-protocol data from secondary measures. Results are displayed for the vitamin B6 and micronutrient treated group.

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Micronutrient Adjusted mean change (SE)</th>
<th>(n=41) 95% CI</th>
<th>Vitamin B6 Adjusted mean change (SE)</th>
<th>(n=37) 95% CI</th>
<th>Between groups p-value</th>
<th>Effect Size (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DASS Depression</td>
<td>7.78(.73)</td>
<td>6.34-9.24</td>
<td>6.05(.75)</td>
<td>4.54-7.56</td>
<td>0.11</td>
<td>.45</td>
</tr>
<tr>
<td>Anxiety</td>
<td>4.34(.42)</td>
<td>3.49-5.19</td>
<td>3.82(.44)</td>
<td>2.94-4.70</td>
<td>0.40</td>
<td>.23</td>
</tr>
<tr>
<td>Stress</td>
<td>11.31(1.06)</td>
<td>9.19-13.42</td>
<td>12.26(1.09)</td>
<td>10.07-14.45</td>
<td>0.53</td>
<td>.21</td>
</tr>
<tr>
<td>WQoLQ</td>
<td>13.04(1.10)</td>
<td>10.83-15.25</td>
<td>8.92(1.14)</td>
<td>6.63-11.21</td>
<td>0.01*</td>
<td>.71</td>
</tr>
<tr>
<td>PSS-10</td>
<td>6.62(1.21)</td>
<td>4.18-9.05</td>
<td>5.30(1.26)</td>
<td>2.78-7.82</td>
<td>0.46</td>
<td>.21</td>
</tr>
<tr>
<td>PSQI</td>
<td>6.55(.22)</td>
<td>6.11-6.99</td>
<td>6.74(.22)</td>
<td>6.28-7.20</td>
<td>0.56</td>
<td>.16</td>
</tr>
<tr>
<td>NSSS-S</td>
<td>4.36(2.29)</td>
<td>0.29-9.01</td>
<td>6.96(2.23)</td>
<td>2.43-11.49</td>
<td>0.42</td>
<td>.27</td>
</tr>
<tr>
<td>Food Q</td>
<td>2.07(.76)</td>
<td>0.56-3.61</td>
<td>1.80(.78)</td>
<td>.22-3.38</td>
<td>0.79</td>
<td>.06</td>
</tr>
</tbody>
</table>

*Significant at the 0.05 level

Abbreviations: SE= Standard Error; 95% CI= Confidence Interval of adjusted mean; ES= Effect Size; DASS-42= Depression and Anxiety Stress Scales-42; WQoLQ= Women’s Quality of Life Questionnaire; PSS-10= The Perceived Stress Scale-10; PSQI = Pittsburgh Sleep Quality Index; NSSS-S= the New Sexual Satisfaction Scale-Short form

4.3.3 Clinical Global Impression- Improvement (CGI-I) Scale. A total of 70 participants completed the CGI-I at the end of the intervention phase. Of the original 78 participants, two did not complete this measure in the final set of questionnaires while six dropped out. Participants who rated themselves as ‘very much improved’ or ‘much improved’ (either a 1 or 2 on the scale) were considered to be ‘responders’. The eight participants with no data were delegated as ‘non responders’ for the ITT analysis. The responders for each group are presented in Table 13 below.
Table 13. The number and accompanying group percentage of participants from each group who rated themselves as either much improved or very much improved on the CGI-I scale, using ITT data.

<table>
<thead>
<tr>
<th></th>
<th>Micronutrient</th>
<th>Vitamin B6</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMS Improvement (%)</td>
<td>18 (43.9)</td>
<td>23 (62.2)</td>
</tr>
<tr>
<td>Mood Improvement (%)</td>
<td>18 (43.9)</td>
<td>20 (54.1)</td>
</tr>
<tr>
<td>Anxiety/Stress Improvement (%)</td>
<td>17 (41.5)</td>
<td>12 (32.4)</td>
</tr>
<tr>
<td>Sleep Improvement (%)</td>
<td>9 (22.0)</td>
<td>9 (25.0)</td>
</tr>
<tr>
<td>Overall Improvement (%)</td>
<td>17 (41.5)</td>
<td>21 (56.8)</td>
</tr>
</tbody>
</table>

Chi-square tests of independence demonstrated that there were no significant associations between treatment group and ‘responder’ likelihood on all five variables (\(p > 0.05\)). A further analysis run with the group of participants who were identified as having premenstrual dysphoric disorder at baseline, again showed no significant association between treatment group and self-rated improvement (\(p > 0.05\)). Similarly, per protocol analyses did not reveal any significant associations.

4.3.4 Third Party Ratings. Demographics of the participants who had a third-party observer rate their symptoms pre and post intervention were compared to those who either declined to participate or whose third party did not respond (please see Table 14). Chi-square tests for independence showed there to be a significant association between overall compliance and group (\(\chi^2 (1) = 4.206, p = .04\)) and likelihood of suffering from current anxiety and group (\(\chi^2 (1) = 4.860, p = .027\)), but no other associations were significant (\(p < 0.05\)). Both of these associations were small, with a Cramer’s V of .242 and .260 respectively. Analysis of the expected versus observed frequencies indicated overall compliance was higher in participants without a third-party rating, while baseline anxiety levels were greater than expected in those with observer ratings.
Table 14. Demographic and clinical characteristics of participants with third party responses compared to those without.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Third party (n=25)</th>
<th>No third party (n=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years: mean (SD)</td>
<td>33.1 (8.9)</td>
<td>36.6 (9.2)</td>
</tr>
<tr>
<td>On micronutrient intervention, n (%)</td>
<td>11 (44)</td>
<td>27 (57.4)</td>
</tr>
<tr>
<td>On Vitamin B6 intervention, n (%)</td>
<td>14 (56)</td>
<td>20 (42.6)</td>
</tr>
<tr>
<td>Socioeconomic status^a^, mean (SD)</td>
<td>55.8 (14.3)</td>
<td>53.2 (14)</td>
</tr>
<tr>
<td>Ethnic origin, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealanders of European descent</td>
<td>21 (84)</td>
<td>37 (78.7)</td>
</tr>
<tr>
<td>New Zealand Maori</td>
<td>1 (4)</td>
<td>3 (6.4)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (12)</td>
<td>7 (14.9)</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met criteria for PMDD^b^, n (%)</td>
<td>9 (36)</td>
<td>18 (38.3)</td>
</tr>
<tr>
<td>Past diagnosis of any mood disorder^c^, n (%)</td>
<td>13 (52)</td>
<td>19 (40.4)</td>
</tr>
<tr>
<td>Anxiety disorder^d^, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>4 (16)</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>Past</td>
<td>6 (24)</td>
<td>8 (17)</td>
</tr>
<tr>
<td>Overall intervention compliance, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compliant</td>
<td>16 (64)</td>
<td>40 (85.1)</td>
</tr>
<tr>
<td>Non-compliant</td>
<td>9 (36)</td>
<td>7 (14.9)</td>
</tr>
</tbody>
</table>

Abbreviations: PMDD= Premenstrual dysphoric disorder

- a. Based on the New Zealand Socio-Economic Index 2006 (NZSEI-06) (Milne et al., 2013)
- b. Met DSM-5 criteria for a premenstrual dysphoric disorder at baseline (American Psychiatric Association, 2013)
- c. Confirmed using the Structured Clinical Interview for mood disorders (First et al., 2015)
- d. Established from self-report at screening

Paired t-tests for the 25 participants with complete pre and post-test observer ratings were compared on the variables of psychological symptoms, somatic symptoms, total scores, relationship impact, and overall rating (in a modified version of the DRSP, description available in section 2.9.4). The mean decrease from pre to post ratings along with the 95% confidence interval and corresponding p-value and effect size (ES) (Cohen’s d) are presented in Table 15.
Table 15. Decrease in symptoms from baseline to end of treatment rated by outside observers on measures of psychological, somatic and total symptoms and relationship impact and overall rating.

<table>
<thead>
<tr>
<th>Symptom (Category)</th>
<th>Micronutrient</th>
<th>Vitamin B6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Psychological</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean change (SD)</td>
<td>4 (5.4)</td>
<td>2.1 (5.5)</td>
</tr>
<tr>
<td>95% CI</td>
<td>.4 to 7.6</td>
<td>-1.1 to 5.2</td>
</tr>
<tr>
<td>p-value</td>
<td>.033*</td>
<td>.179</td>
</tr>
<tr>
<td>E.S</td>
<td>.74</td>
<td>.38</td>
</tr>
<tr>
<td><strong>Somatic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean change (SD)</td>
<td>2.6 (6.2)</td>
<td>1.4 (6.2)</td>
</tr>
<tr>
<td>95% CI</td>
<td>-.16 to 6.8</td>
<td>-.22 to 4.9</td>
</tr>
<tr>
<td>p-value</td>
<td>.191</td>
<td>.429</td>
</tr>
<tr>
<td>E.S</td>
<td>.42</td>
<td>.23</td>
</tr>
<tr>
<td><strong>Total Scores</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean change (SD)</td>
<td>6.6 (10.8)</td>
<td>3.4 (11)</td>
</tr>
<tr>
<td>95% CI</td>
<td>-.6 to 13.9</td>
<td>-.29 to 9.7</td>
</tr>
<tr>
<td>p-value</td>
<td>.068</td>
<td>.266</td>
</tr>
<tr>
<td>E.S</td>
<td>.61</td>
<td>.31</td>
</tr>
<tr>
<td><strong>Relationship Impact</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean change (SD)</td>
<td>.73 (1.8)</td>
<td>.86 (1.6)</td>
</tr>
<tr>
<td>95% CI</td>
<td>-.51 to 2</td>
<td>-.04 to 1.8</td>
</tr>
<tr>
<td>p-value</td>
<td>.221</td>
<td>.061</td>
</tr>
<tr>
<td>E.S</td>
<td>.41</td>
<td>.54</td>
</tr>
<tr>
<td><strong>Overall rating</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean change (SD)</td>
<td>14 (23)</td>
<td>7.7 (22.9)</td>
</tr>
<tr>
<td>95% CI</td>
<td>-1.5 to 29.5</td>
<td>-5.5 to 21</td>
</tr>
<tr>
<td>p-value</td>
<td>.072</td>
<td>.230</td>
</tr>
<tr>
<td>E.S</td>
<td>.61</td>
<td>.34</td>
</tr>
</tbody>
</table>

*Significant at the 0.05 level

While the averaged results show a decrease in symptom ratings made by third party observers, none of the measures showed reliable change from pre to post as the confidence intervals contained zero. The exception to this was the psychological symptom change in the micronutrient group which underwent a reduction of 4 points ($p = .033$) corresponding to a moderate effect size ($d = .74$). ANCOVA analysis did not reveal a statistically significant group difference on the psychological symptoms change score after controlling for baseline values, $F(1, 22) = .951$, $p = .340$, partial $\eta^2 = .041$. 

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4.4 Results: Natural follow up

Of the 72 participants who completed the intervention phase, only 48 (67%) completed the three cycle follow up questionnaire. Possible differences between those who responded at follow up and those who did not were investigated using chi-square tests of independence. These demonstrated that treatment group had no impact on response rates, $\chi^2 (1) = .738, p = .390$, but overall treatment compliance did, $\chi^2 (1) = 8.815, p = .003$. Analysis of the expected versus observed frequencies, indicated there was less overall compliance in the group lost to follow up, corresponding to a small association, Cramer’s $V = .341$. Additionally, more people were compliant than expected in the followed up group. Collectively this indicated overall study compliance was, unsurprisingly, statistically lower in those not supplying follow-up data.

The three cycle follow up employed a modified version of the DRSP. Again participants were asked to rate their symptoms on the one to six scale; however, they rated the symptom overall based on its most severe point in the last six days of premenstrual complaints. This corresponded most closely with the measure of ‘worst day’ from the DRSP baseline data.

Only seven of the participants contacted at three cycles post intervention had continued to take some form of treatment, other than analgesics, for premenstrual syndrome. Two had moved to psychoactive medication, citalopram and zopiclone (and vitamin B6), while the other five were taking nutrient supplements (EMP+, B vitamin complex, and/or vitamin B6). At the end of the intervention, many women, in personal communication to the researcher, had expressed a desire to discontinue treatment and see if their symptoms reverted. As follow up ratings were consistent with baseline scores, it would appear the average participant did experience symptom reversion, see Figure 15. For those participants who had chosen to take some form of treatment in the natural follow up period their symptoms, as a whole, decreased further. From the final treatment cycle to follow up, the five women taking nutrient treatment(s) had a non-significant average mean decrease of 8.2 points (95% CI -3.19 to 19.59, $t(4) = 1.998, p = .116$). Baseline scores to follow up therefore
naturally showed a larger average mean decrease of 24.3 points (95% CI 6.32 to 42.28), which was significant ($t(4) = 3.752, p = .02$).

![Figure 15. Worst day ratings from baseline, the final treatment cycle, and three cycle natural follow up for the 48 responders. Results are split by treatment group and those five who continued to take a treatment during the natural follow up phase. Participants in the Micronutrient and Vitamin B6 group discontinued treatment in the natural follow up stage.](image)

For the remaining 41 participants who discontinued all treatment, the paired t-test analyses showed a different pattern of results. Between baseline and follow up, those allocated to micronutrients during the intervention had a non-significant increase of 3.9 points (95% CI, -11.98 to 44.08, $t(18) = -1.033, p = .315$), while those allocated to B6 saw a non-significant decrease of 1.3 points (95% CI, -5.20 to 7.81, $t(23) = .416, p = .681$). Results from final treatment cycle to follow up (using ITT data for the final treatment cycle data) showed both groups had reverted nearer to baseline. At three cycles post intervention, scores had increased by 8.8 points (95% CI, .32 to 17.3) in those who had been treated with micronutrients and this increase was significant ($t(18) = 2.180, p = .043$). There was also a similar increase of 9.8 points (95% CI, 2.90 to 16.67) observed in those participants who had been taking the vitamin B6 formula, and this increase was also significant ($t(23) = 2.180, p = .007$). Furthermore, when the analysis was run with participants who had followed protocol (and provided diary data for the final treatment cycle), PMS ratings rose by 12.8 points.
(95% CI 4.42 to 21.18) in the vitamin B6 group at follow up ($t(14) = 3.277$, $p = .006$). Likewise, there was a further small increase in the micronutrient treated group, to 9.2 points (95% CI -0.86 to 19.24), which, while significant, was not reliable due to the confidence interval ($t(15) = 1.947$, $p = .07$).

### 4.5 Adverse outcomes

Side effect analyses were performed using data from 76 participants as two participants provided no data past baseline. As suspected, due to the similarities between some common side effects and premenstrual syndrome, many symptoms were endorsed. Yet, as the symptoms were present at baseline and remained for the duration of the study, we could conclude they were not induced by treatment. Chi-square analyses indicated there was no association between treatment type and specific side effects. Despite the larger endorsement of ‘tingling in limbs’ experienced by people taking vitamin B6, the association did not reach significance, $\chi^2(1) = 3.007$, $p = .083$.

The common side effects accounted for and their frequency in each group are presented in Table 16 along with the results of the significance test for association. All adverse events that did occur were classed as ‘mild’. Specifically, the magnitude of the effect did not require the blind to be broken, hospitalisation, or any other serious action to be taken. The majority of adverse events required no intervention while the actions undertaken to remedy those that did ranged from the use of analgesics to varying the dose and timing of the nutrient intake.
Table 16. Recorded adverse events for study participants based on treatment group.

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Micronutrient (n = 40)</th>
<th>Vitamin B6 (n = 36)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tingling in limbs (%)</td>
<td>4 (10)</td>
<td>9 (25)</td>
<td>0.08</td>
</tr>
<tr>
<td>Constipation (%)</td>
<td>10 (25)</td>
<td>10 (27.8)</td>
<td>0.78</td>
</tr>
<tr>
<td>Sedation/lethargy (%)</td>
<td>11 (27.5)</td>
<td>10 (27.8)</td>
<td>0.98</td>
</tr>
<tr>
<td>Sleep disturbance (%)</td>
<td>8 (20)</td>
<td>10 (27.8)</td>
<td>0.43</td>
</tr>
<tr>
<td>Nightmares (%)</td>
<td>13 (32.5)</td>
<td>9 (25)</td>
<td>0.47</td>
</tr>
<tr>
<td>Change in appetite (%)</td>
<td>7 (17.5)</td>
<td>12 (33.3)</td>
<td>0.11</td>
</tr>
<tr>
<td>Skin rash (%)</td>
<td>4 (10)</td>
<td>4 (11.1)</td>
<td>0.88</td>
</tr>
<tr>
<td>Weight gain (%)</td>
<td>12 (30)</td>
<td>10 (27.8)</td>
<td>0.83</td>
</tr>
<tr>
<td>Headache (%)</td>
<td>8 (20)</td>
<td>12 (33.3)</td>
<td>0.19</td>
</tr>
<tr>
<td>Nausea (%)</td>
<td>11 (27.5)</td>
<td>10 (27.8)</td>
<td>0.98</td>
</tr>
<tr>
<td>Gastrointestinal disturbance/diarrhoea (%)</td>
<td>9 (22.5)</td>
<td>14 (38.9)</td>
<td>0.12</td>
</tr>
<tr>
<td>Abdominal pain (%)</td>
<td>11 (27.5)</td>
<td>13 (36.1)</td>
<td>0.42</td>
</tr>
<tr>
<td>Loss of libido (%)</td>
<td>10 (25)</td>
<td>6 (16.7)</td>
<td>0.37</td>
</tr>
<tr>
<td>Dry mouth (%)</td>
<td>10 (25)</td>
<td>7 (19.4)</td>
<td>0.56</td>
</tr>
<tr>
<td>Urinary retention (%)</td>
<td>4 (10)</td>
<td>6 (16.7)</td>
<td>0.39</td>
</tr>
<tr>
<td>Blurred vision (%)</td>
<td>6 (15)</td>
<td>8 (22.2)</td>
<td>0.42</td>
</tr>
<tr>
<td>Agitation (%)</td>
<td>9 (22.5)</td>
<td>8 (22.2)</td>
<td>0.98</td>
</tr>
<tr>
<td>Anxiety (%)</td>
<td>9 (22.5)</td>
<td>11 (30.6)</td>
<td>0.43</td>
</tr>
<tr>
<td>Other (%)</td>
<td>5 (12.5)</td>
<td>9 (25)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

4.6 Summary of results

The Natural Treatment of Premenstrual syndrome study (NTP) recruited 78 participants who were randomised to receive either a broad-spectrum micronutrient formula or a single nutrient comparator treatment for three menstrual cycles. Of the 72 participants who completed the treatment phase, 48 of them responded at three cycles post-intervention for the natural follow up phase.

For the intervention phase, no group differences were found on the primary PMS diary outcome measure. Nor were there differences in the number of participants per-group who no longer meet criteria for PMS and/or PMDD. Both treatments produced a moderate effect on PMS Psychological, Somatic and Total Symptoms as well as Impact and Worst Day Ratings. The majority of the micronutrient (72%) and vitamin B6 (60%) group were classed as PMS free by the end of treatment cycle two and three.
For participants diagnosed with PMDD, the micronutrient formula produced larger ESs on the diary data outcomes, yet this did not reach statistical significance as a between group difference. MBP analyses further confirmed this finding with PMDD participants in the micronutrient group more likely to show reliable change in their symptoms. The ESs for the 65 participants with sufficient dairy data for these analyses, indicated results were similar for both interventions.

For most of the secondary measures no group differences emerged after controlling for baseline values as a covariate. The exception to this was on the quality of life measure in the intention-to-treat and per-protocol analyses. In both instances, the participants in the micronutrient group were found to have a significantly greater average mean difference score when compared with the vitamin B6 group. These findings showed the beneficial treatment effect on health-related quality of life was significantly greater in women treated with micronutrients. Within group analyses showed women in both treatments saw statistically and, where questionnaires made it possible to estimate, clinically significant change from baseline on measures of stress, anxiety, depression, quality of life, sleep quality and sexual functioning.

Treatment compliance and adherence to the study protocol were good. No adverse events were statistically found to occur more often in either group, although 25% of the vitamin B6 sample experienced a tingling sensation in the limb(s), a common side effect associated with that treatment. The dropout rate during the treatment phase was 8%, with equal numbers of drop outs per treatment group.

A low rate of response to a request to supply follow-up data at three cycles post intervention meant only 67% of the eligible sample responded at follow up. Of the 48 who responded, seven had taken some form of PMS medication over the preceding cycles and the five who were taking a nutritional treatment, on average, saw further improvement in their PMS severity. The remaining 41 participants who stopped the treatment saw a return to baseline levels of severity.
Chapter 5: Discussion

This thesis reports a randomised, controlled, treatment trial to test whether a broad-spectrum micronutrient formula can outperform a single nutrient formula for moderate to severe PMS. This chapter will begin by restating the study aims and hypotheses before exploring the results based on the stated hypothesis. Next the strengths and limitations of the study will be assessed before ending with the implications of this research and possible future directions for investigation.

The current literature suggests a variety of mental health complaints are associated with inflammation caused via imbalances in our microbiome or through oxidative stress, which is further associated with mitochondrial dysfunction. The range of disorders thought to be affected by these underlying metabolic pathways and the links with inflammation and oxidative stress makers in PMS/PMDD in particular, lead to the proposal made by this study, namely that PMS/PMDD could be successfully treated with micronutrients.

5.1 Thesis Aims and Hypotheses

The current study sought to increase the field of knowledge about nutritional treatments for moderate to severe premenstrual syndrome, specifically focused on a comparison of single nutrient versus multi-nutrient treatment. The primary aim was to assess the impact of a relatively experimental treatment, a broad-spectrum micronutrient formula, to an already established single nutrient treatment on symptoms seen in the premenstrual phase. The study further aimed to establish the effectiveness of the two treatments via the number of treatment responders. Finally investigations also focused on the wider effect of the treatment on secondary symptoms known to be affected by PMS.

Based on existing literature outlining the efficacy of single nutrient treatments, the similarities between symptoms of PMS and other disorders effectively treated with micronutrient
formulas, the growing knowledge of the impact of the inflammation and its associated effects on the body, and the role of diet on the microbiome, the following hypotheses were proposed:

5. Participants with moderate to severe PMS who were administered the micronutrient formula would demonstrate a greater reduction in premenstrual symptoms compared to participants taking vitamin B6;

6. There would be a greater number of treatment ‘responders’ in the micronutrient treated group, as assessed by the percentage of participants no longer meeting study criteria for PMS and DSM-5 criteria for PMDD (American Psychiatric Association, 2013);

7. Group differences would also emerge on secondary variables measuring associated problems of stress, anxiety, depression, sexual functioning, sleep quality, and health related quality of life;

8. No differences would emerge on the tolerability of the two treatment formulas.

5.2 Summary of findings

The current sample reflected a group of women, predominantly from the greater Christchurch (New Zealand) region, experiencing moderate to severe premenstrual symptoms. Seventy-eight (72 completed) participants were randomly allocated to a micronutrient formula (EmPowerPlus advanced (EMP+)) or vitamin B6. In the vitamin B6 group, four participants reported having had a past episode of bipolar disorder vs none for EMP+; otherwise the two groups were comparable on both baseline demographic and clinical variables.

The primary PMS symptom analyses were conducted using a Linear Mixed models (LMM) and modified Brinley Plot (MBP) approach. Using Intent-to-treat (ITT) data for the LMM, all 76 participants (40 in the micronutrient group, 36 in the vitamin B6 group) with baseline diary data were included in the analysis. Forty completed the diary at each treatment wave (completers), while 36 had data missing for at least one treatment cycle (incomplete). Chi-square analyses indicated
there were no statistically significant differences between the completer and incomplete participants, fulfilling the assumption of data ‘missing at random’ (indicating that this did not impact on the analysis). The MBP analyses included 65 participants who had provided a diary record at baseline and for at least one treatment cycle. A Reliable Change Index (RCI) was computed for these analyses which provided a guide by which to judge the extent of change; scores outside of the RCI boundaries indicated a change larger than that expected due to measurement error alone. Further MBP were run with the 40 completers, separated into two groups; those with PMDD and those without. These further analyses did not provide effect size (ES) measures due to the small sample sizes per group.

5.2.1 Hypothesis 1 and 2: Micronutrient superiority in the treatment of premenstrual symptoms, corresponding to a greater number of treatment ‘responders’. The findings for Hypothesis 1 were generally consistent that no treatment was superior to the other. However, the MBP analyses did demonstrate some additional findings. The first hypothesis was not supported by the LMM analyses. LMM analyses indicated both treatments produced comparable reduction in PMS symptoms with medium ESs across the five PMS variables (micronutrient ES=0.50-0.56; vitamin B6 ES=0.43-0.56) and no between group differences (p> 0.05).

In agreement with the above finding, MBP analyses confirmed that by treatment cycle three, both groups were improving and showing changes consistent with medium ESs (micronutrient ES=0.55-0.71; vitamin B6 ES=0.51-0.72). ES were almost consistently higher during the second cycle of treatment, with medium to large effects observed in both groups (micronutrient ES=0.77-0.87; vitamin B6 ES=0.51-0.92). This was not surprising however, as LMM growth curve modelling had already demonstrated there was a quadratic trend in the data i.e., a non-linear, U-shaped, pattern of symptom reduction, corresponding with greatest symptom reduction in the second cycle, provided the best fit for the data. The reason for the increased ES and corresponding larger decrease in PMS
symptoms during treatment cycle two is unclear. It may be that both treatments lose some of their efficacy by cycle three, or it may be due to the impact of missing data for treatment cycle two, whereby those with higher scores (less of a treatment effect) did not provide data for cycle two. Of the 65 participants included in the MPB ES calculations, not all provided complete diary data, meaning the individuals in each plot change from treatment cycle to treatment cycle (full data were provided by 40/65 participants). Given that the published literature show that if people stay on micronutrients their symptom improvement is maintained or further improved (Rucklidge, Blampied, Gorman, Gordon, & Sole, 2014; Rucklidge, Taylor, et al., 2011), the drop in effect from cycle two to three is more likely due to the effect of the missing data.

The finding that was idiosyncratic to the MPB analyses and provided support for Hypothesis 1 was centred on the RCI. MBPs showed a larger number of participants in the micronutrient group had reliable reduction in Psychological, Somatic and Total Symptom PMS variables. Specifically, this indicated the magnitude of the micronutrient participant’s treatment response was larger than that expected from possible measurement error alone. Therefore, based on this result, we can be more confident EMP+ produces genuine reduction in premenstrual symptoms.

Additionally, the MBP analyses suggested a differential treatment effect based on symptom clustering. That is the psychological symptoms of PMS/PMDD (depression, anxiety, irritability/anger, and affective lability) are more greatly improved when compared with physical symptoms. In both treatments, the RCI demonstrated psychological symptoms were more reliably reduced compared to somatic symptoms. This did not equate with larger ES estimates for Psychological Symptoms relative to Somatic Symptoms in either the MBP or LMM analyses. There is some research to suggest vitamin B6 is more effective on psychological symptoms than somatic symptoms when compared to placebo (Kashanian et al., 2007). Vitamin B6 is known to have particular efficacy in treating depressive premenstrual symptoms, with an Odds Ratio (OR) in favour of the vitamin, when compared to placebo, of 1.69 (95% Confidence Interval: 1.39 to 2.06) (Wyatt et al., 1999). However, the overall
OR from Wyatt et al. (1999) meta-analysis indicated Vitamin B6 reduces more than just depressive symptoms. Much of the research with EMP+ has focused specifically on mental health, and thus on psychological symptoms resembling the psychological symptoms of PMS. However, micronutrients are an effective insomnia treatment (Lothian et al., 2016). The unique RCI finding from the MBP analyses highlights the nuances that are to be found when tracking change at the individual level as opposed to relying on group averages, although more work is needed to confirm if it is a consistent result.

A small, but clinically relevant, treatment difference did emerge in the group of participants diagnosed with PMDD (n=28). LMM analyses looking at the dependent variables of Somatic and Total Symptoms, and Impact Ratings showed women with PMDD, treated with micronutrients, consistently showed a larger average symptom reduction. While this did not equate to a statistically significant group difference, effect size measures were large (ES=1.28-1.67) for the micronutrient treated group and moderate (ES=0.50-0.75) for the vitamin B6 group on all three dependent variables.

Overall the data from the LMM and MBP analyses suggest both treatments are effective in reducing the symptoms of PMS, with neither treatment demonstrating superior ability overall. The RCI in the MBP analyses suggest that participants taking the micronutrient formula are more likely to experience genuine change in Psychological, Somatic and Total Symptoms. Both treatments, according to the RCI, also produce more genuine change on the psychological symptoms of PMS over and above that of somatic symptoms. Support for Hypothesis 1 comes from the subgroup of participant’s diagnosed with PMDD. LMM analyses suggested there was a stronger reduction in symptoms in the micronutrient group, equating to large ES.

Hypothesis 2 was not supported by the data. More of the participants randomized to micronutrients (n=21, 72%) had become PMS free by cycle two or three versus those randomized to vitamin B6 (n=18, 60%); however, this difference was not statistically significant, $X^2 (1) = 0.375, p =$
0.540. Even if a conservative PMS diagnosis estimate was produced, including the missing 12 participants as ‘PMS positive’, 60% of the micronutrient treated participants and 50% of the vitamin B6 participants would be in PMS remission (also equating to no statistical difference). A similar pattern emerged with the 28 women diagnosed with PMDD. Data was available for 23 participants and indicated 64% ($n=7$) of the micronutrient sample and 50% ($n=6$) of the vitamin B6 sample no longer met a diagnosis of PMDD by cycle two or three. Using the PMS sample’s data, the number needed to treat (NNT) was eight, indicating that for every eight women with PMS randomly allocated to treatment by either micronutrients or vitamin B6, one more receiving micronutrients benefitted relative to the vitamin B6 group. When a treatment is compared to a placebo it naturally shows a smaller NNT, for example SSRIs demonstrate a NNT of three (Freeman et al., 1995). The larger NNT in this study is a result of using a treatment-controlled design.

As this study was the first of its kind to compare a micronutrient formula to a single nutrient for the treatment of PMS, there were no prior data to suggest one treatment would fare better than the other. However, the prediction that there would be a beneficial effect of micronutrient treatment was based on the research on micronutrients, specifically the formula used in this study, showing wide ranging effects on a variety of mental health disorders (Rucklidge & Kaplan, 2013). In fact, given the extent of symptoms observed in PMS and the similarities between these symptoms and those that had successfully been treated with broad-spectrum nutritional interventions, the prediction was made that micronutrients would perform better. Yet this expectation was not borne out in the available data. Rather, it appears vitamin B6 and EMP+ are equally efficacious in treating PMS, with a clinically relevant difference observed only in the subgroup of women with PMDD (where micronutrients proved more effective).

It is possible that both treatments exerted their effects through a reduction in pro-inflammatory molecules. Rising interest into metabolic dysfunction has highlighted the link between numerous mental health concerns including depression (Maes, 1999), anxiety (Pitsavos et al., 2006),
bipolar disorder (Andreaazza et al., 2008), schizophrenia (Reddy & Yao, 1996) ADHD (Joseph et al., 2015) and inflammation. Further studies have explored the connection between signs of mitochondrial dysfunction and the emergence of both physical and mental health disorders (Calabrese et al., 2001; Gardner & Boles, 2011). Vitamin B6’s importance is recognised in the methionine cycle through which homocysteine is reduced and glutathione (an antioxidant) is formed (Kennedy, 2016). High levels of homocysteine are known to induce oxidative damage and mitochondrial dysfunction through its detrimental effects on the brain. The vitamin also plays another key role in metabolic functioning through the production of cytokines that work to manage inflammation (Morris et al., 2010).

The menstrual cycle has been classed as an inflammatory event, and there is now reason to suspect that a difference exists between women with PMS in their levels of pro-inflammatory molecules compared to healthy controls (Bertone-Johnson et al., 2014). It is possible a greater inflammatory response may be evident in women with PMDD, although research has yet to confirm this speculation. If an inflammatory response is indeed causally implicated then micronutrient treatment may show more promising results over vitamin B6 due to an ability to supply the body with a range of vitamins and minerals, used in endogenous defence systems (Hasan et al., 2013; Parikh et al., 2009). Additionally, in cases where inflammation is caused by an imbalance in the host’s microbiome as opposed to mitochondrial energy depletion or reactive oxygen species related inflammation, micronutrients may prove more effective than vitamin B6 alone in treating the underlying imbalance. Dietary changes have been shown to effect the gut microbiome in as little as 24 hours (Wu et al., 2011). Therefore, nutrient supplementation may work through the same pathways, although research needs to quantify the specific effects supplement interventions have on the microbiome.

5.2.2 Hypothesis 3: Secondary variables. Overall, the data did not generally support the third hypothesis although there was one exception. No group differences emerged on any of the
secondary variables, excluding one; the women’s quality of life measure. Both ITT and per-protocol analyses revealed significant between groups differences on health based ratings of life quality. The average mean difference for each analysis equated to a moderate between groups effect size ($d= .51$ and $d= .71$, respectively) in favour of the micronutrient treated participants. Moreover, post-treatment average mean scores indicated participants in the micronutrient group were experiencing comparable quality of life to women who had never experienced PMS complaints (Gehlert et al., 2006). Health related quality of life is known to be significantly affected in women with PMS. They are particularly burdened in the areas of efficiency, productivity, social participation and interpersonal relationships (American Psychiatric Association, 2013). This is correlated with higher work absences and larger medical expenses (Hylan et al., 1999). Given the above, it is noteworthy that micronutrients have the ability to improve quality of life over and above that of vitamin B6. The questionnaire used to measure health related quality of life (Gehlert et al., 2006) assessed four domains of functioning; physical, psychological, social, and spiritual (this focused on overall emotional wellness rather than spirituality per se). This broad focus on health-related functioning allowed inferences to be made regarding a participant’s overall wellbeing. Previous micronutrient studies have indicated the treatment has broad effects, with improvements seen not only in the primary symptoms but also secondary areas (Rucklidge & Kaplan, 2013). As such, if the treatment is positively influencing a number of areas it is likely this will be reflected in quality of life ratings. It is important to note that the vitamin B6 treated participants also had significantly higher ratings post treatment, which fits with the finding of an overall benefit of this treatment too.

No group difference was reported on measures of depression, stress, and anxiety, sleep quality, perceived stress, and sexual functioning. Average mean change scores indicated both groups were improving on these measures, with some noteworthy improvements; on components of the Depression Anxiety Stress Scale-42 (DASS-42) and Pittsburgh Sleep Quality Inventory (PSQI) both group averages moved from the clinical range to non-clinical from pre to post treatment. Findings from the anxiety, stress and depression scales of the DASS-42 added to the growing body of
literature showing a clinically significant impact of micronutrients on these symptoms (Kaplan et al., 2002; Lothian et al., 2016; Rucklidge, Johnstone, et al., 2011). While B-vitamin complexes have demonstrated efficacy with symptoms of anxiety, stress and depression (Rucklidge et al., 2012), there is a lack of research into vitamin B6 alone as a treatment. However, based on B6’s efficacy with depressive symptoms of PMS (Wyatt et al., 1999), it is not surprising that positive outcomes were observed on the DASS-42. These findings demonstrate the interventions are having a quantifiable impact on affective symptoms.

Prior research has indicated broad-spectrum micronutrient formulas have a positive impact on insomnia (Lothian et al., 2016), and there is some evidence and rationale behind the use of vitamin B6 in sleep given its connections to serotonin synthesis and melatonin secretion (Peuhkuri, Sihvola, & Korpela, 2012). The findings from this study confirm there is a clinically significant impact of nutrient treatments on sleep quality. Additionally, despite the lack of clinical cut offs for the perceived stress and sexual functioning questionnaires, pre to post scores indicated there was a notable improvement in both measures for both groups.

There was no statistically significant difference between the groups on post treatment food quality as measured by the food questionnaire adapted from Baker et al. (2003). Moreover, the average mean change in the ITT data, after controlling for baseline starting values was roughly 2 points for each treatment which, on the scale of 8-41, was a negligible change. Therefore, we can be certain that any change attributable to the nutrients was not confounded by a group wide change in overall food intake quality. This allows us, with some credibility, to conclude that both treatments are effective on symptoms of stress, anxiety, depression, sexual functioning, sleep and quality of life (with better results on this measure following micronutrient treatment) and that this is not better explained by a change in diet quality during the trial.

5.2.3 Hypothesis 4: Side effects. The final hypothesis was supported by the findings of this study; no one treatment group had statistically higher occurrences of specific side effects than
the other. This finding was despite the fact that 25% of the vitamin B6 group experienced a tingling sensation in their limb(s). This neared a significant difference to the 10% of tingling recorded in the micronutrient participants \((p = 0.08)\). Previous research would suggest peripheral neuropathy is experienced in vitamin B6 doses as low as 200mg/day, but the effect is reversible (Malmgren et al., 1987). None of the participants who experienced these sensations chose to modify the study protocol in any way, indicating the sensations were not severe enough to warrant action. The low dropout rate (8%) and good pill compliance (86%) (number not pill adherent= 10), suggests these treatments were generally acceptable.

Many of the symptoms recorded as possible side effects were also symptoms regularly seen in the premenstrual phase, which made it difficult to decipher what was a true side effect. Nevertheless, it was determined apriori to not to record a symptom as a side effect unless it was either a) the first occurrence of the symptom post baseline, or b) a return of a symptom that had been absent for at least one cycle. Of the recorded side effects, none required serious intervention further than increasing water intake, decreasing nutrient dose, or changing the timing of ingestion of the capsules. Moreover, no one withdrew from the study due to adverse side effects. The findings from this study add to the current literature showing the safety of micronutrient formulas, specifically EMP+ (Simpson et al., 2011), and supports the use of 80mg/day of vitamin B6 as a safe and effective dose.

### 5.3 Research Strengths

This study employed a rigorous design to ensure the validity of its findings. It was treatment controlled, randomised, and double blinded using appropriate methods. Furthermore both the study protocol and trial registration were completed prior to data analysis, which is a traceable way to hold the study accountable to its original aims, hypotheses, and methods. The nature of a treatment-control study is also worthy of mention; as well as giving us the ability to offer all participants a potential remedy, we held the micronutrient treatment up to a higher standard than
that of a placebo controlled trial. The, hypothesised, superior treatment (micronutrients) had to out-perform a recognised treatment. This study design was chosen so we could ensure a) a more comprehensive formula was indeed superior to a current single nutrient option, b) either validate or invalidate vitamin B6 as a treatment for PMS, c) in general, provide more information on the ability of nutritional treatments to effectively treat PMS, and d) offer all participants a potential treatment.

In regards to the final point listed above, a large benefit of the current study was that participants were offered a treatment and a large majority improved. The number of responders, those deemed to no longer meet study criteria for PMS were 60% or above per group (based on participants with available data from treatment cycle two or three). Specifically 72% of the micronutrient group and 60% of the vitamin B6 group saw a clinically relevant reduction in their PMS symptoms. Moreover, the use of two questionnaires with clinical cut offs, the DASS-42 and the PSQI, allowed us to trace the clinical impact on specific symptoms. Depression, stress, and sleep quality reduced to the non-clinical range following both nutrient treatments.

Part of the study design included a two cycle baseline phase, whereby potential participants completed a daily dairy recording their PMS symptoms. This, along with the Structured Clinical Interview for DSM-5 (SCID), enabled us to confirm the presence of PMDD. This allowed for certainty when making a diagnosis of PMDD, as opposed to basing the diagnosis on retrospective ratings which have shown inconsistency (Endicott & Halbreich, 1982). There was also added security that the study was indeed treating PMS/PMDD and not the symptoms of another mood disorder, as the SCID was used to exclude participants if they were experiencing a current major mood disorder. Thus, this allowed the nutrients’ effect on PMS to be measured more conclusively.

The broad inclusion criteria, allowing women on oral contraceptives and other forms of contraception, as well as those with co-morbid mental health concerns (other than an active mood disorder) to participate is an additional strength of this study. With a more varied sample, the generalisability of the findings is increased. It is also unlikely that oral contraceptives significantly
affected treatment results as participants still met entry criteria for PMS, indicating the contraception was not affecting, or at least not fully treating, their PMS symptoms. Previous research with vitamin B6 has shown no difference in treatment outcomes between women taking oral contraceptives and those who were not (Doll et al., 1989; Williams, Harris, & Dean, 1985).

Further, our inclusion criteria allowed for participants with active mental health disorders, other than a mood disorder, and those with a history of mood disorders to participate. There is reason to suspect women with PMS are more likely to experience mood instability regardless of their menstrual cycle stage (Bowen et al., 2011). Premenstrual mood fluctuations are particularly common in women with major depressive disorder and bipolar disorder (Payne et al., 2007). Therefore, had we excluded participants based on non-active disorder diagnoses we may have precluded many women from participating. Additionally we would have failed to provide treatment for a group that, potentially, needs it the most.

5.4 Research Limitations

A large limitation of the present study was the amount of missing data on the primary outcome measure. Only 40 participants had complete diary data for each cycle of the study, with some participants only supplying baseline data. Despite the ability of the LMM analyses to compute scores for missing values, statistical analyses are most accurate when data are complete. The missing data may have affected the ability to fully observe the nuances of the treatment effects. This is especially true of the individualised Brinley Plot analyses which tracked individual’s responses to treatment over the course of time. Moreover, we were unable to assess final PMS/PMDD diagnosis in all of the 71 participants diagnosed at baseline. This directly impacted on our ability to calculate response rates and the subsequent number needed to treat analysis. It appears likely that participants found the diary measure burdensome as adherence rates for the secondary questionnaires, which were completed on a monthly basis, were better.
The dropout rate for the active treatment phase was small, equating to 8% of total participants. However, 33% were lost to follow up three cycles post intervention. This limits the ability to generalise these results as bias may be a factor. It is possible participants who were much better or who were taking standardised treatments such as antidepressants did not respond to follow-up requests for data. The reverse is also possible. Hence our lack of knowledge as to why people did not complete the follow up questionnaires presents as a limitation. Additionally, in an attempt to reduce the data reporting burden, we employed a modified version of the diary which assessed severity of impact and symptoms but not each dependent variable used in the main analyses. Therefore we could compare participant’s severity (worst day ratings) across baseline, end of treatment, and follow up but we could not assess how specific symptom clusters had changed.

While the positive of providing all study participants a potential treatment is acknowledged in Research Strengths, there is also a possible downside. That is that expectations of benefit may be elevated, corresponding with inflated treatment results. Open label (OL) trials consistently show higher ESs than Randomised Controlled Trials (RCT) for the same treatment. For example, recent research using a broad-spectrum micronutrient treatment in children diagnosed with ADHD has shown a significant ES decrease from OL to RCT, possibly explained by participants’ awareness they had a 50% chance of receiving a placebo pill (Gordon, Rucklidge, Blampied, & Johnstone, 2015; Rucklidge, Eggleston, Johnstone, Darling, & Frampton, 2017). In the current study, participants knew they would be randomised to either an experimental micronutrient treatment or an established single nutrient treatment. While there is much evidence to suggest vitamin B6 outperforms placebo for PMS (Wyatt et al., 1999), therefore ameliorating the possibility of a pure placebo effect, we cannot rule out a treatment expectation effect as a by-product of the study design. There is likely to have been a placebo effect operating within both treatments, which, in the absence of an inert placebo condition, cannot be quantified.
Participant/researcher contact is often mentioned as a study limitation due to the therapeutic effect participation in a trial and regular contact with a researcher can engender. In an attempt to limit contact, participants were only seen a total of four to five times (depending if they came in for the end of treatment meeting or opted to do this via email/phone) during the course of the active five months. Meetings, other than pre-baseline, were kept short (generally less than 10 minutes as participants often chose to complete online questionnaires at home). The follow up phase was completed entirely online, reducing not only the researcher contact but also the impact on the participants’ time. It is, however, possible an element of therapeutic contact influenced results.

The vitamin B6 formula contained additional ingredients such as acacia gum, riboflavin, cocoa powder, maltodextrin, and steric acid (please refer to Table 1 for a description of the quantity). Some of these ingredients have themselves been associated with improvement in PMS symptoms (Chocano-Bedoya et al., 2011, Houghton et al., 2017). However, the doses used in this study had not previously been associated with therapeutic potential and therefore it is unlikely they affected the results obtained with the vitamin B6 formula.

There were no previous multi-nutrient versus single nutrient comparator studies to use as a resource when conducting the sample size calculations for this study. It is possible that the sample size was too small to detect a significant between groups difference. This is particularly relevant in the group of women diagnosed with PMDD. Although the p-value approached significance, for the reduction in total symptoms, it did not reach it. However, given that a small group difference for those with PMS carries little clinical significance, conducting a much larger study in order to detect what appears to be a small group difference may not be a worthwhile investment of scarce research funds. A focused study on PMDD would perhaps be a more strategic focus of future research.

Finally, as we did not assess pro-inflammatory and oxidative stress biomarkers we were unable to make inferences about the interventions’ possible mechanisms of actions. As discussed in
Chapter 2, premenstrual symptoms have been linked to increased pro-inflammatory biomarkers. Preliminary evidence has shown a link between interferon gamma, a number of interleukin cytokines and signs of oxidative stress (Bertone-Johnson et al., 2014; Duvan et al., 2011). To the best of our knowledge, we are unaware of any previous studies that have assessed biomarkers pre and post treatment for PMS/PMDD.

5.5 General Implications

The current study has a number of implications for further practice and research, with the latter to be discussed in the following section. Firstly, this research contributes to the field of broad spectrum micronutrient research by showing it is a safe, viable treatment option for PMS, with greater clinical utility in those diagnosed with PMDD. This idea is consistent with the knowledge we have of vitamins and minerals working in concert with one another. Namely, we require sufficient levels of certain nutrients to enable absorption of others, and they often join as cofactors in enzymatic reactions (Popper, 2014). Moreover, as we cannot endogenously synthesise most of these essential nutrients, they must be sourced from our diet.

Secondly, the findings reiterate that vitamin B6, at a dose of 80mg/day, is a safe and effective treatment for PMS. Despite previous concerns over flawed methodology impacting earlier claims (Wyatt et al., 1999), this research has shown clinically relevant change, and moderate effect sizes from pre to post intervention with vitamin B6.

While this study was specifically interested in the comparison of a multi-nutrient versus single nutrient treatment for PMS, our findings lend some support to the idea that there is an underlying metabolic dysfunction in PMS. As micronutrients and vitamin B6 are able to remedy symptoms of PMS, possibly increased inflammation and/or mitochondrial dysfunction are the cause of the disorder. This hypothesis requires considerable investigation. The present research has
provided the ground work for future studies to investigate the therapeutic mode of action of nutrients in reducing the symptoms associated with PMS/PMDD.

An additional discussion must be had around the financial differences between micronutrient treatment and standard medical care. To date, two case studies have examined the monetary value to be gained from using broad spectrum micronutrient formulas as opposed to in/out-patient mental health care. The price comparison between unsuccessful medical care of a mentally unwell child, which included inpatient hospitalisation, and that of successful EMP+ treatment is staggering. Rodway et al. (2012) found the cost of micronutrient treatment was less than 1% of the cost of mental healthcare. Furthermore, in this case, the child did not benefit from the six months of inpatient care he received, yet he showed a remarkable turnaround once he had transitioned fully into micronutrient treatment. The second case involving an adult female, documented substantial unsuccessful government-funded care and the widespread change in her symptoms following micronutrient treatment (Kaplan, Isaranuwatchai, & Hoch, 2017). Again the cost benefit was staggeringly large in favour of micronutrients.

If we assume that the medical costs of treating women with PMS are substantial relative to the cost of nutritional treatment such as EMP+ (or vitamin B6), the same economic arguments in favour of the nutritional treatments apply. Studies reporting on the cost associated with PMS/PMDD in European and Latin American settings, have indeed demonstrated there is a large cost associated with medical treatment (Lowin et al., 2009; Schiola, Lowin, Lindemann, Patel, & Endicott, 2011). This cost has been shown to increase as the severity of the disorder increases. Using predefined severity categories Lowin et al. (2009) showed across nine OECD countries the average annual cost associated with the highest symptom ratings was, in Swedish Krona, 48,201kr (95% CI 26,103, 75,635). This equates to roughly $7,730 per year in New Zealand currency. As of yet, the New Zealand government does not fund broad spectrum micronutrient formulas, meaning if someone were to choose this less traditional course of treatment they would be self-funded. Findings from
this study, and the case studies listed above, suggest nutrients may prove a more cost-effective treatment compared to medical care.

In New Zealand, as the number of people in need of mental health intervention grows, we are observing increasingly long waiting lists for government funded aid and choosing to (having the ability to) use private rather than public services is associated with considerable cost. Furthermore, there are known side effects of pharmacological treatment for PMS which, sometimes, outweigh the benefits (Marjoribanks et al., 2013). Thus, the ability to provide participants with relief from their symptoms with nutrient formulas, as the results from this study demonstrate, surely suggests this approach as a cost effective, safe treatment option.

5.6 Directions for Future Research

Worldwide, as our rates of mental health disorders rise and our ability to effectively treat them falters (Whitaker, 2005), we need to search for alternative options. Vitamin B6 has already proven effective in a number of trials, and despite warnings about the quality of these studies, enough evidence is presented to suggest it as a viable treatment (Wyatt et al., 1999). The findings from this study would again suggest it is effective and at a dose of only 80mg/day. In their systematic review, Wyatt et al. (1999) concluded there was no apparent dose effect; specifically, higher doses did not ensure better treatment outcomes. However, due to the authors’ reservations about the qualities of the studies reviewed, future research could compare treatment effects between different levels of vitamin B6 (within a safe range). Moreover, 80mg/day was found to be effective on the psychological symptoms of PMS over and above that of placebo in Kashanian et al. (2007) study, but it was not more effective than placebo on physical symptoms. Therefore a well-designed study to re-test dose response is warranted.

Luteal phase dosing with anti-depressants has proven as effective as continual dosing for PMS/PMDD (Marjoribanks et al., 2013). Usually this process is standardised as treatment from,
roughly, day 14 of the menstrual cycle to day 2 of the following cycle (Freeman, Rickels, Sondheimer, Polansky, & Xiao, 2004). Similar results have been shown when using nutrients/ herbal extracts in an luteal dosing pattern to treat PMS, including vitamin B6, vitex agnus castus (Lauritzen et al., 1997) and zinc (Siahbazi et al., 2017). Furthermore, intermittent dosing (specifically, continuous dosing for one to two months followed by luteal phase dosing) has shown promising results in PMS treatment. Much of the research with omega 3 treatment has followed this approach (Sampalis et al., 2003; Sohrabi et al., 2013), while antidepressants are also known to be effective when applied in an intermittent fashion (Marjoribanks et al., 2013).

Luteal phase or intermittent dosing is an additional treatment option for both broad spectrum micronutrient formulas and vitamin B6. While compliance for the present study was good at 86%, it is acknowledged that asking women to consume eight capsules a day is more burdensome than most medications. Therefore, if one of the dosing options proves effective, it may address not only non-compliance but also nutrient cost. Dosing patterns are plentiful in PMS research. Therefore, there is much scope to replicate and modify doses based on previous research. One such available option may lie in an individually dictated response-to-treatment strategy. Anti-depressant research has shown that if luteal phase dosing does not prove effective by the first cycle of treatment then the dose may be increased for more effectiveness in following luteal phases (Freeman et al., 2004). This individually tailored method has already been trialled with micronutrient research. Anecdotal reports from the Mental Health and Nutrition Research Group at the University of Canterbury have indicated increasing nutrient dose at times of high stress has helped to manage participant’s escalating symptoms. This approach complements findings from an RCT assessing micronutrient dose response following a natural disaster. The greatest improvement in stress, mood, and energy was seen in participants consuming a larger dose of nutrients (Rucklidge et al., 2012). Therefore, there is reason to suggest increasing the dose in response to a stressful event or increasing symptoms, is beneficial.
It would be beneficial to understand what moderates treatment response. Personal characteristic such as high trait anxiety and neuroticism may decrease an individual’s treatment response. Likewise cultural factors such as socialization, personal learning, coping experiences and so on may also determine who is most likely to see a benefit from nutrient treatment. If an individual is likely to respond poorly to nutrient treatment based on the above factors, alternate treatment options such as the combination of talking therapy, must be considered.

No between groups treatment differences emerged on the primary diary data, despite a trend towards a statistically significant group difference in the subgroup of women diagnosed with PMDD. It is likely this trend would have proved statistically significant had the study had sufficient power. This study was based on the notion that multi-nutrient formulas are more effective than single nutrient and indeed there is evidence to suggest they are. Specifically in relation to symptoms observed in PMS, research has shown affective symptoms i.e., mood, stress and anxiety are significantly improved by a B-vitamin complex or a broad spectrum micronutrient formula when compared to vitamin D supplementation alone (Kaplan, Rucklidge, Romijn, & Dolph, 2015). Therefore it is recommended that in future research larger samples are employed in order to address the power limitation that may have impaired the ability to detect a group difference between vitamin B6 and micronutrients in those women who met criteria for PMDD.

The limitations of the current study could be remedied in future research; inflammatory markers in the follicular and luteal phase should be recorded pre and post treatment, sample sizes should aim for a larger percentage of women diagnosed with PMDD, and compliance on daily diary measures should be addressed. It is possible the use of electronic apps for monitoring might improve participant’s compliance in providing necessary data on symptoms during the PMS phase.

It is also worth considering if the interventions themselves could be enhanced, such as supplementing micronutrients with omega-3 and/or a probiotic formula. Preliminary evidence would suggest omega-3 has a beneficial effect on physical and psychological symptoms of PMS (Sohrabi et
While a growing body of RCT research suggests omega-3 has a role in the treatment of mental health disorders (Peet & Stokes, 2005). Improving our mental health may not be the scope of one nutrient based treatment but rather a collection to ensure we are adequately supplying the body with all the essential elements. As we rely on our gut microbiota to absorb and synthesise nutrients (Carding et al., 2015), we might enhance the effectiveness of supplements by further treating gut dysbiosis. The underlying imbalance might best be treated simultaneously through the use of micronutrients and either probiotics or dietary modifications. However, the use of probiotics is not straight forward (Sun & Chang, 2014), hence extensive research into this area, and potentially personalised bacterial strains, would need to be employed before combining supplements. As our knowledge of metabolic dysfunction increases, so does our ability to remedy underlying disease states.

5.7 Conclusion

The current study was the first to compare a broad spectrum micronutrient formula to a single nutrient for the treatment of premenstrual syndrome. The findings show that micronutrients and vitamin B6 are effective treatments for the relief of PMS as well as secondary areas associated with the disorder. Despite indications the micronutrient formula was performing better than vitamin B6, these findings were not statistically significant on the majority of measures. Quality of life ratings improved across both groups pre and post intervention, with significantly greater improvement in the micronutrient group. In the small subgroup of women diagnosed with PMDD, the micronutrient formula produced larger effect sizes corresponding with better clinical utility over and above that of the vitamin B6. It is not unreasonable to expect that if this sample had been larger, statistical significance would have been achieved. Regardless of the absence of a between group effect, excluding findings from the quality of life measure, this research does support the use of both vitamin B6 and micronutrients in the treatment of premenstrual syndrome.
In summary, vitamin B6 and a broad-spectrum micronutrient formula are near equally efficacious in the treatment of PMS. For women with PMDD, they are likely to benefit more from micronutrient treatment.
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depression and bipolar disorder. *Journal of Affective Disorders, 99*(1–3), 221-229. doi: [http://dx.doi.org/10.1016/j.jad.2006.08.013](http://dx.doi.org/10.1016/j.jad.2006.08.013)


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Appendices

1. Screening Questionnaire

2. a) Daily Record Severity of Problem questionnaire
   b) Modified Daily Record Severity of Problem questionnaire
   c) Third Party symptom ratings on a modified Daily Record Severity of Problem questionnaire

3. Side effect questionnaire
Appendix 1. Screening Questionnaire

The following questions are being asked in order to assess your eligibility and to gain some background information on the types of people who will take part in the study. This research is aimed at exploring the effect of a micronutrient supplement on premenstrual symptoms. We want to know if this micronutrient formula exerts a similar effect on symptoms as an already proven vitamin treatment (vitamin B6).

You are now invited to answer a series of questions. The questions will take approximately 20-30 minutes to complete. The information gained from this survey will be kept confidential.

Your specific contact details are being requested in order for us to contact you if you are eligible, they will not be used for any other purposes.

I understand that the information that I provide will be used for the purposes of assessing my eligibility to take part in this study and that all personal information gained about me will be kept strictly confidential. I agree to answer the following questions about myself, and my premenstrual symptoms.

Disagree
Agree

What is your gender?

Male
Female

Are you aged 18 years or older?

Yes
No

This study will last 5 months as it will include a two month baseline period, and three months of medication.
Are you willing to participate in the study for this amount of time?

Yes
No

Please complete the following contact details so that we can contact you about the study.

First Name
Surname

Address

Home phone

Cell phone

E-mail address

What is your date of birth (dd/mm/yyyy)

Does your usual menstrual cycle last between 21-35 days? (i.e length is calculated from the first day of your period to the first day of your next period. Somewhere between 21-35 days is considered a 'normal' menstrual length).

☐ Yes

☐ No

Are you pregnant or breastfeeding?

☐ Yes

☐ No

Are you attempting/planning to become pregnant in the next 6months?

☐ Yes

☐ No

Are you on contraception?

☐ Yes

☐ No

Do you suffer from endometriosis?

☐ Yes

☐ No

Are you currently taking any psychiatric medication, including anti-depressants, anti-anxiety drugs and so on.

☐ Yes

☐ No

If ‘Yes’, what medication are you taking?

Do you currently suffer from a neurological disorder? (e.g., epilepsy, MS, narcolepsy)
Do you have any known abnormality of mineral metabolism? (e.g. Wilson's disease, haemochromatosis?).

Please indicate if you have experienced in the past or are currently suffering from any of the following:

<table>
<thead>
<tr>
<th>Disorder</th>
<th>You used to experience</th>
<th>You are currently suffering</th>
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<tbody>
<tr>
<td>Anxiety disorder</td>
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<tr>
<td>Major Depressive disorder</td>
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<td>Dysthymia</td>
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<tr>
<td>Bipolar disorder (mania depression)</td>
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<td>Psychotic disorder (schizophrenia)</td>
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<tr>
<td>Behavioural problems (e.g., ADHD)</td>
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<tr>
<td>Problems with drugs and/or alcohol</td>
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</table>

Are you currently taking medication for Premenstrual syndrome (PMS)? (this includes pain killers)

If 'Yes', what medication are you taking?

Are you currently taking any herbal or nutritional supplement (e.g vitamins, omega 3s, melatonin, St John's Wort)?
No

If 'Yes'; Please list which nutritional supplements you are taking, and how long you have been taking them.

Click to write Choice 1

Click to write Choice 2

Click to write Choice 3

Click to write Choice 4

Click to write Choice 5

Do you have thyroid problems?

Yes

No

Please rate the following questions based on how they are for you in the WEEK BEFORE your menstrual period begins

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all</th>
<th>Minimal</th>
<th>Mild</th>
<th>Moderate</th>
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<tbody>
<tr>
<td>Felt depressed, sad, &quot;down&quot;, or felt hopeless; or felt worthless or guilty</td>
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<td>Felt anxious, tense, &quot;keyed up&quot;, or &quot;on edge&quot;</td>
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<td>Had mood swings (i.e. suddenly feeling sad or tearful), or was sensitive to rejection, or feelings were easily hurt.</td>
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<td>Felt angry, or irritable</td>
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<td>Had less interest in usual activities (work, university, friends, hobbies)</td>
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<td>Had difficulty concentrating</td>
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<tr>
<th>Question</th>
<th>Not at all</th>
<th>Minimal</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
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<td>Felt lethargic, tired, or fatigued; or had lack of energy</td>
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<tr>
<td>Had increased appetite or overate; or had craving for specific foods</td>
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<td>Slept more, took naps, found it hard to get up when intended; or had trouble getting to sleep or staying asleep</td>
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<td>Felt overwhelmed or unable to cope; or felt out of control</td>
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<td>Had breast tenderness, breast swelling, bloated sensation, weight gain, headache, joint or muscle pain, or other physical symptoms</td>
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<tr>
<td>At work, university, home, or in daily routine, at least one of the problems noted above caused reduction of productivity or efficiency</td>
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<td>At least one of the problems noted above caused avoidance of or less participation in hobbies or social activities</td>
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<td>At least one of the problems above interfered with my relationship with others</td>
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Please rate the following questions based on how they are for you in the WEEK AFTER your menstrual period ends

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all</th>
<th>Minimal</th>
<th>Mild</th>
<th>Moderate</th>
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<tr>
<td>Felt depressed, sad, &quot;down&quot;, or felt hopeless; or felt worthless or guilty</td>
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<td>Symptom</td>
<td>Not at all</td>
<td>Minimal</td>
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<tr>
<td>Felt anxious, tense, &quot;keyed up&quot;, or &quot;on edge&quot;</td>
<td>Not at all</td>
<td>Minimal</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
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<tr>
<td>Had mood swings (i.e. suddenly feeling sad or tearful), or was sensitive to rejection, or feelings were easily hurt.</td>
<td>Not at all</td>
<td>Minimal</td>
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<td>Moderate</td>
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<td>Felt angry, or irritable</td>
<td>Not at all</td>
<td>Minimal</td>
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<tr>
<td>Had less interest in usual activities (work, university, friends, hobbies)</td>
<td>Not at all</td>
<td>Minimal</td>
<td>Mild</td>
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<td>Severe</td>
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<tr>
<td>Had difficulty concentrating</td>
<td>Not at all</td>
<td>Minimal</td>
<td>Mild</td>
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<td>Felt lethargic, tired, or fatigued; or had lack of energy</td>
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<td>Felt overwhelmed or unable to cope; or felt out of control</td>
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<td>Mild</td>
<td>Moderate</td>
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At least one of the problems above interfered with my relationship with others

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<tr>
<th>Not at all</th>
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Where did you hear about this study? (Tick all that apply)

- [ ] Facebook
- [ ] Website
- [ ] Friend
- [ ] Media
- [ ] Poster
- [ ] Other (please specify)

It is important for us to gather the following data in order to see if this sample of women is representative of the general New Zealand female population, please answer each question to the best of your abilities.

Please indicate which of the following ethnic groups you belong to (you may select more than one).

- [ ] NZ European/Pakeha
- [ ] NZ Maori
- [ ] Samoan
- [ ] Tongan
- [ ] Niuean
- [ ] Chinese
- **Indian**
- **Other**

What is your occupation?

Please indicate which of the following best describes your total household income before tax (include income from all sources):

- □ less than $20,000
- □ from $20,000 to $40,000
- □ from $40,000 to $60,000
- □ from $60,000 to $80,000
- □ more than $80,000

How old were you (roughly) when you began menstruating?

- □ Under 8 years of age
- □ 9
- □ 10
- □ 11
- □ 12
- □ 13
- □ 14
- □ 15
- □ Over 15 years of age
- □ Don’t know

How many years had you been menstruating when you felt that your PMS was bad?

- □ As soon as I began menstruating
- □ 1 to 5 years after the onset of my period
- □ 5 to 10 years after the onset of my period
- □ 10 to 15 years after the onset of my period
- □ More than 15 years after the onset of my period
If you are eligible to take part in this study, we will be inviting you to meet us at the university at some point in the next few weeks, to answer some questions and begin your participation in the study. Please indicate which times you would be available to come to this initial appointment. You can select more than one time option (the following question will ask you to indicate preferred days).

- ☐ Mornings 9-12 noon
- ☐ Afternoons 12-3pm
- ☐ Evenings 3-6 pm

Please indicate your preferred days to come in to meet with us.

- ☐ Monday
- ☐ Tuesday
- ☐ Wednesday
- ☐ Thursday
- ☐ Friday

- Thank you very much for taking the time to complete this study - your participation is very important to us.
- We will contact you if you are eligible for this study.

- Please, visit our website http://bit.ly/UCnutritionresearch for information on counselling services in the Christchurch area.

Appendix 2. Daily Record Severity of Problems questionnaires

a) Daily Record Severity of Problems questionnaire

Name...........................................................................
Date of first entry..............................................

Please fill in this questionnaire at the end of every day.

Put a number in the box which corresponds to the severity: 1- not at all, 2-minimal, 3-mild, 4-moderate, 5-severe, 6-extreme.

<table>
<thead>
<tr>
<th>Enter day (Monday= “M”)</th>
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<tr>
<td>Note spotting by entering “S”</td>
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<td>Notes menses by entering “M”</td>
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<tr>
<td>Enter calendar date (e.g 1st, 5th)</td>
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</table>

Felt depressed, sad, “down”, or “blue” or felt hopeless; or felt worthless or guilty

Felt anxious, tense, “keyed up” or “on edge”

Had mood swings (i.e. suddenly feeling sad or tearful) or was sensitive to rejection or feelings were easily hurt

Felt angry, or irritable

Had less interest in usual activities (work, university, friends, hobbies)

Had difficulty concentrating

Felt lethargic, tired, or fatigued; or had lack of energy

Had increased appetite or overate; or had cravings for specific foods

Slept more, took naps, found it hard to get up when intended; or had trouble getting to sleep or staying asleep

Felt overwhelmed or unable to cope; or felt out of control

Had breast tenderness, breast swelling, bloated sensation, weight gain, headache, joint or muscle pain, or other physical symptoms
At work, university, home, or in daily routine, at least one of the problems noted above caused reduction of productivity or inefficiency
At least one of the problems noted above caused avoidance of or less participation in hobbies or social activities
At least one of the problems noted above interfered with relationships with others

b) Modified Daily Record Severity of Problem questionnaire

We want to see how your symptoms have been over the last six days (over your last PMS phase). Rate the questions on the 6-point scale below for how they compare to how you
**usually are** e.g. if you usually feel tired but this last week has been worse than normal for you, you would rate it at a 4-6, even if it was only one day you felt very tired for.

<table>
<thead>
<tr>
<th>Feeling</th>
<th>1 Not at all</th>
<th>2 Minimal</th>
<th>3 Mild</th>
<th>4 Moderate</th>
<th>5 Severe</th>
<th>6 Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Felt depressed, sad, “down”, or “blue” or felt hopeless; or felt worthless or guilty</td>
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<tr>
<td>Felt anxious, tense, “keyed up” or “on edge”</td>
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<tr>
<td>Had mood swings (i.e. suddenly feeling sad or tearful) or was sensitive to rejection or feelings were easily hurt</td>
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<tr>
<td>Felt angry, or irritable</td>
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<tr>
<td>Had less interest in usual activities (work, university, friends, hobbies)</td>
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<tr>
<td>Had difficulty concentrating</td>
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<tr>
<td>Felt lethargic, tired, or fatigued; or had lack of energy</td>
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<tr>
<td>Had increased appetite or overate; or had cravings for specific foods</td>
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<tr>
<td>Slept more, took naps, found it hard to get up when intended; or had trouble getting to sleep or staying asleep</td>
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<tr>
<td>Felt overwhelmed or unable to cope; or felt out of control</td>
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<tr>
<td>Had breast tenderness, breast swelling, bloated sensation, weight gain, headache, joint or muscle pain, or other physical symptoms</td>
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<tr>
<td>At work, university, home, or in daily routine, at least one of the problems noted above caused reduction of productivity or inefficiency</td>
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</tbody>
</table>
At least one of the problems noted above caused avoidance of or less participation in hobbies or social activities

At least one of the problems noted above interfered with relationships with others

c) Third Party symptom ratings on a modified Daily Record Severity of Problem questionnaire

The following questions ask you to rate how your partner's/ friend's/ relative's premenstrual symptoms have been in the previous week.

The symptoms do not need to be present every day but they should be noticeable to you or they should be a change from normal for you to rate them highly.

Name of your friend /partner/ relative in the study?

Please indicate if any of the following symptoms have been present in the last week, and note how severe they have been on a 6 point scale from 1 (not at all) to 6 (extreme).

<table>
<thead>
<tr>
<th>Symptom Description</th>
<th>1- not at all</th>
<th>2- minimal</th>
<th>3- mild</th>
<th>4- moderate</th>
<th>5-severe</th>
<th>6- extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noticed they felt depressed, sad, “down”, or “blue” or felt hopeless; or felt worthless or guilty</td>
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<td></td>
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<td></td>
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<tr>
<td>Noticed they felt anxious, tense, “keyed up” or “on edge”</td>
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<tr>
<td>Noticed they had mood swings (i.e. suddenly feeling sad or tearful) or were sensitive to rejection or feelings were easily hurt</td>
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<tr>
<td>Noticed they felt angry, or irritable</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Noticed they had less interest in usual activities (work, university, friends, hobbies)</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Noticed they had difficulty concentrating</td>
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<tr>
<td>Issue</td>
<td>Boxes</td>
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<td>-----------------------------------------------------------------------</td>
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<tr>
<td>Noticed they felt lethargic, tired, or fatigued; or had lack of energy</td>
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<tr>
<td>Noticed they had increased appetite or overate; or had cravings for specific foods</td>
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<tr>
<td>Noticed they felt overwhelmed or unable to cope; or felt out of control</td>
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<tr>
<td>Noticed at least one of the problems noted above interfered with their relationships with others</td>
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</tbody>
</table>

**Appendix 3. Side effect questionnaire**

Please indicate if you have felt any of the following symptoms in the past four weeks.
<table>
<thead>
<tr>
<th>Symptom</th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tingling in your limb(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td></td>
<td></td>
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<tr>
<td>sedation/lethargy</td>
<td></td>
<td></td>
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<tr>
<td>Sleep disturbance</td>
<td></td>
<td></td>
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<tr>
<td>Nightmares</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in appetite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin rash</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Weight gain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal disturbance/diarrhoea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
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<td></td>
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<tr>
<td>Loss of libido</td>
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<td></td>
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<tr>
<td>Dry mouth</td>
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<td></td>
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<tr>
<td>Urinary retention</td>
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<tr>
<td>Blurred vision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight gain</td>
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<td></td>
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<tr>
<td>Agitation</td>
<td></td>
<td></td>
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<tr>
<td>Anxiety</td>
<td></td>
<td></td>
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<tr>
<td>Other Symptoms</td>
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</tr>
</tbody>
</table>

What action(s) did you take to alleviate your symptoms?