THE ROLE OF NEUROSTEROID FLUCTUATION IN PERIMENOPAUSAL DEPRESSION

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SUPERVISORY AND EXAMINING COMMITTEE

Bethany Jane Sander, candidate for the degree of **Master of Science in Clinical Psychology,** has presented a thesis titled, ***The role of neurosteriod fluctuation in perimenopausal depression,*** in an oral examination held on **August 24, 2023**. The following committee members have found the thesis acceptable in form and content, and that the candidate demonstrated satisfactory knowledge of the subject material.

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Abstract

There is a well-established increase in depression risk during the menopause transition – known as perimenopausal depression. Increased fluctuation of estradiol has long been thought to be an important causal factor in perimenopausal depression, and recent research suggests that increases, decreases, or absolute changes in estradiol may be associated with depressive symptoms in different subsets of perimenopausal women. However, the mechanisms by which estradiol differentially influences mood are still very unclear. Preliminary evidence suggests that estradiol may increase vulnerability to developing depressive symptoms through the effects of a neurosteroid called allopregnanolone. In the current study, the role of estradiol and allopregnanolone was examined in 26 perimenopausal participants experiencing disturbed mood. Fifteen weekly measurements of depressive symptoms as well as plasma levels of estradiol, progesterone, and allopregnanolone were collected. In 10 participants with a sufficient number of weeks of hormone data available, correlational analysis revealed that every participant displayed a significant relationship between depressive symptoms and changes in at least one neurosteroid. Although mood sensitivity to progesterone was correlated with mood sensitivity to allopregnanolone, estradiol and allopregnanolone sensitivity indicators appeared not to be related. Our results suggest that fluctuations in neurosteroids in the menopause transition play an important role in perimenopausal depressed mood. Future research is needed to clarify whether estradiol and allopregnanolone changes each play an independent role in exerting effects on perimenopausal mood or work in tandem.

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Dedication

This thesis work is dedicated to those in my life who have supported me throughout this journey. To my family, friends, and boyfriend, who have encouraged me, lent a compassionate ear, and given me balance throughout this process, thank you.

Stitched throughout this thesis is also my passion and commitment for women's health. This work is dedicated to women everywhere who continue to be misunderstood, misinformed, and ignored regarding the health of their bodies and minds. My small ambition for this thesis is that this may be one more minor step in the right direction.

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CHAPTER 1: Introduction

Previous research consistently demonstrates that women have a higher risk of experiencing depression compared to men (Kessler et al., 1994; Kessler et al., 2012; Marcus et al., 2005). This heightened incidence of female depression begins with the onset of puberty (Angold et al., 1998; Kessler et al., 1993) and diminishes in advanced age when menopause commonly occurs (Bebbington et al., 1998), suggesting that the changing dynamics of female reproductive hormones throughout the lifespan may be one important contributor to the observed sex difference in depression rates. This notion is further supported by the existence of what are commonly termed “reproductive mood disorders”, which represent mood disorders coinciding with times of fluctuating reproductive hormones estradiol (E2) and progesterone (P4). These include premenstrual dysphoric disorder (PMDD), postpartum depression (PPD), and perimenopausal depression, which coincide with hormonal changes during the menstrual cycle, after childbirth, and during the menopause transition, respectively. In the following section, I will discuss each of these mood disorders and the role of reproductive hormone change in their etiology.

1.1 Premenstrual Dysphoric Disorder (PMDD)

1.1.1 The Menstrual Cycle

At menarche, humans of the female sex begin a cycle of menstruation and ovulation, which is orchestrated by cyclical fluctuations in reproductive hormones (Reed & Carr, 2015). The menstrual cycle typically repeats every 25 to 30 days - though there is considerable individual variability (for example, see (Bull et al., 2019)) - and consists of several stages and events. The early follicular phase commences with menstruation, at which time E2 and P4 levels remain relatively low and stable (see Figure 1). In the late follicular phase, E2 levels begin to climb until reaching a peak level and rapidly declining at ovulation, which on average occurs around cycle day 17 (Bull et al., 2019), though this is typically stated to occur on cycle day 14 in the prototypical 28-day cycle. Several other hormones play a role in the follicular phase. Follicle stimulating hormone (FSH) stimulates the development of mature follicles, which contain ova, and luteinizing hormone (LH) causes the release of ova during ovulation (Reed & Carr, 2015). During the follicular phase, E2 is also secreted from the ovaries and has several reproductive functions such as the thinning of cervical mucus to facilitate the passage of sperm to the fallopian tubes and supressing further LH and FSH release after ovulation. In the luteal phase, the corpus luteum, which previously housed the ovulated ovum, releases P4, which thickens the endometrium for embryo implantation. The corpus luteum eventually deteriorates, P4 and E2 levels decline, and menstruation occurs (Reed & Carr, 2015). As a result, during the luteal phase, both E2 and P4 levels gradually rise and then fall before the cycle repeats itself, beginning again with menstruation – the shedding of the endometrium or uterus lining.

As outlined above, E2 and P4, along with other reproductive hormones such as LH and FSH, have the primary biological function of facilitating conception. However, in addition to these reproductive functions, E2 and P4 also have direct effects on the brain. For example, there is evidence that these hormones play a role in neural processes such as neuroplasticity and memory (Catenaccio et al., 2016; Galea et al., 2008; Luine, 2014) as well as emotional processes involved with stress and social and emotional processing (Catenaccio et al., 2016; Kudielka & Kirschbaum, 2005; Sander, 2020). A large body of research also suggests that significant fluctuations in mood throughout the menstrual cycle may be caused by a particularly increased sensitivity to reproductive hormone fluctuation in a minority of women, such as in PMDD.

1.1.2 The Role of Reproductive Hormones in PMDD

PMDD is characterized by unpleasant physical and emotional symptoms that consistently occur in the luteal phase, with the rise in levels of E2 and P4, and ameliorate with the drop in these hormones and onset of menses (American Psychiatric Association, 2013). Common symptoms of PMDD include irritability, mood lability, depressed mood, and difficulty concentrating (Hantsoo & Epperson, 2020). Prevalence of meeting criteria for PMDD, as defined in the *Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5)*, is low at about three to eight percent of women (Halbreich et al., 2003). For DSM-5 diagnosis of PMDD, an individual must fulfill at least five PMDD symptoms during the week before menses (i.e., during the late luteal phase) for the majority of cycles. Moreover, to meet criteria for this diagnosis, symptom remission must occur a few days after menses onset, once E2 and P4 have once again reached low and stable levels, and symptoms must occur with clinically significant distress or impaired functioning (American Psychiatric Association, 2013). Along with endorsing at least five PMDD symptoms, at least one mood-related symptom must be present (e.g., irritability or depressed mood) and at least one secondary symptom must be present (e.g., lethargy, appetite or sleep changes, concentration difficulties, or decreased interest in usual activities) (American Psychiatric Association, 2013). Moreover, because retrospective reports of premenstrual symptoms tend to significantly overestimate the likelihood of PMDD (Eisenlohr-Moul et al., 2017), prospective daily ratings over two consecutive

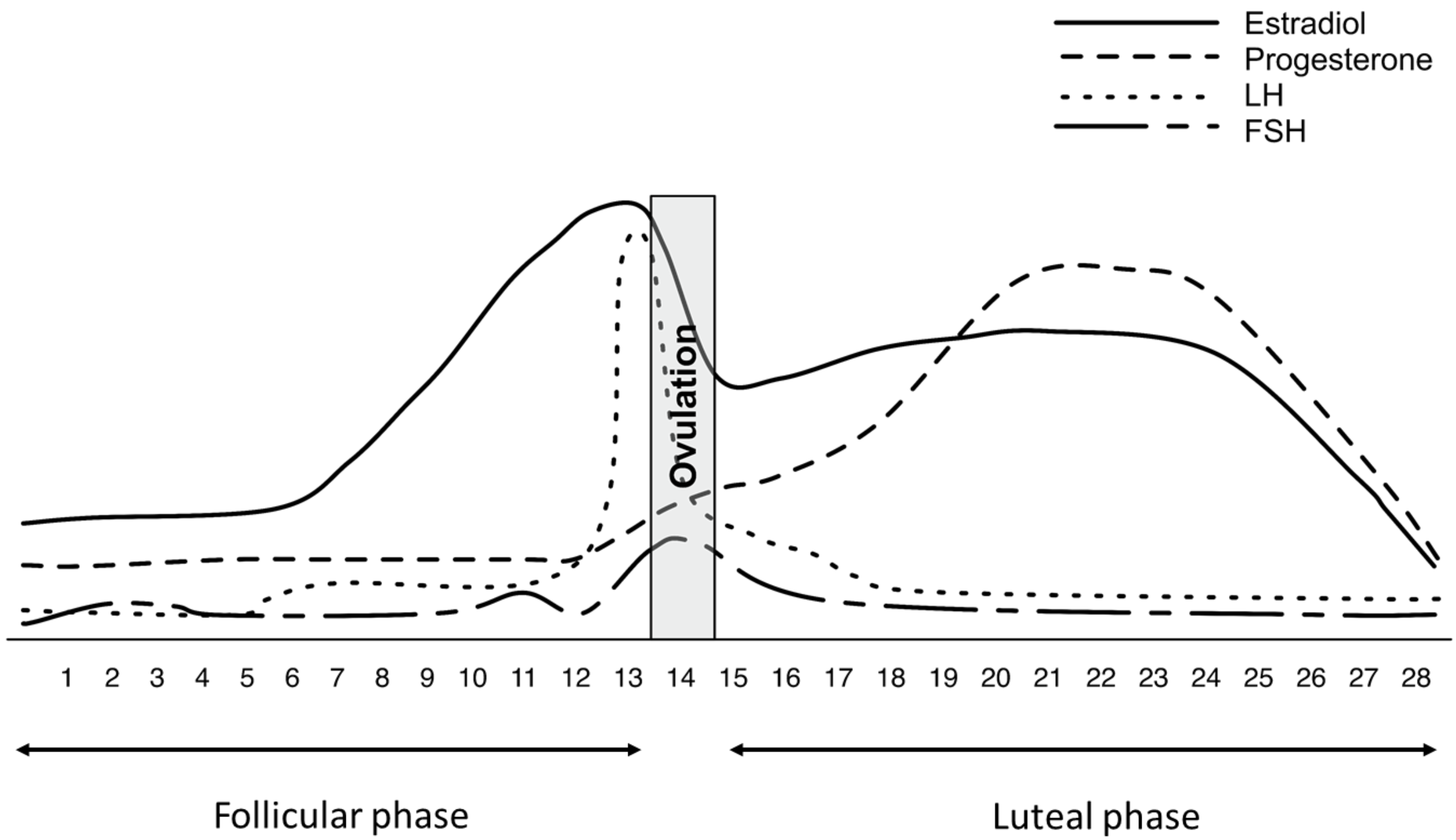


Figure 1. Estradiol and Progesterone Fluctuations during the Menstrual Cycle.

menstrual cycles are required for accurate diagnosis (American Pychiatric Association, 2013). However, the prevalence of menstrually-related mood disorders, more generally defined, may be higher. When less strict criteria are used, some research suggests a larger proportion of women experience menstrually-related mood symptoms, with one estimate finding that 13 to 18 percent of women experience premenstrual symptoms producing significant impairment and distress (Halbreich et al., 2003).

Perhaps the strongest evidence of the causal role of reproductive hormones in PMDD comes from a study by Schmidt et al (1998). In this study, the chemical suppression of endogenous sex hormones caused symptom remission in those meeting criteria for PMDD (referred to as "premenstrual syndrome" or PMS at the time), and further experimental addition of either E2 or P4 induced a return of PMS symptoms (Schmidt et al., 1998). The same hormonal manipulation did not result in mood changes in women without PMS (Schmidt et al., 1998). These findings, combined with studies confirming that E2 and P4 levels do not significantly differ in women with versus without PMDD (Thys-Jacobs et al., 2008), suggest that PMDD results from an increased mood sensitivity to normal hormonal fluctuations across the menstrual cycle. Specifically, mood symptoms in PMDD appear to occur in response to luteal *increases* in E2 and P4. However, an emerging theme in the reproductive mood disorder literature is that considering individual variability *within* these disorders is also extremely important. For instance, more recent observational research demonstrates that among individuals with PMDD, there is considerable individual variability in the timing of symptoms in relation to hormone changes (Eisenlohr-Moul et al., 2020). Specifically, in a sample of 74 participants meeting DSM-5 criteria for PMDD using prospective daily ratings across two consecutive menstrual cycles, an important degree of individual variability was seen in the timing of symptoms, with some experiencing PMDD symptoms only in the final week of the luteal phase and others showing symptoms throughout the full two weeks. As well, there was individual variability in the timing of symptom offset, with some experiencing a resolution of PMDD symptoms immediately with the onset of menstruation and others showing a delayed resolution of symptoms into the follicular phase (Eisenlohr-Moul et al., 2020). Thus, although all individuals with PMDD exhibit mood sensitivity to increases in E2 and P4, the timing of the onset and resolution in symptoms relative to hormone fluctuations may vary from person to person. This research altogether indicates that PMDD represents a group of individuals with a particular sensitivity to hormonal fluctuations, but it is still not understood what mechanisms underly this sensitivity to different hormonal triggers.

1.1.3 The Role of Allopregnanolone (ALLO) in PMDD

Though uncertainty remains regarding the causal mechanisms underlying the relationship between hormone fluctuations and PMDD symptoms, there are several proposed mechanisms being investigated. One mechanism that is currently receiving much attention involves the P4-derived neurosteroid allopregnanolone (ALLO) (Hantsoo & Epperson, 2020). ALLO is a positive modulator of the gamma-aminobutyric acid ($GABA_A$) receptor (Morrow et al., 1987; Puia et al., 2003). In other words, ALLO works by increasing the effect of $GABA_A$ receptor activation. GABA is an inhibitory neurotransmitter (i.e., one that makes the postsynaptic neuron less likely to fire) and is often generally regarded as a neurotransmitter with a calming effect on the nervous system. In line with this, ALLO has been found to have antidepressant and anxiolytic effects in rats (Bitran et al., 1999; Shirayama et al., 2011).

However, interestingly, animal model research indicates that exposure to acute ALLO fluctuation may alter the $GABA_A$ subunit composition in such a way that ALLO's effects at the $GABA_A$ receptor are reversed; in other words, ALLO temporarily *inhibits* further GABAergic transmission and has anxiogenic rather than anxiolytic effects (Griffiths & Lovick, 2005; Lovick et al., 2005; Shen et al., 2007). It is through this mechanism that luteal increases in ALLO are believed to trigger symptoms in those with PMDD.

Thus, it is hypothesized that ALLO fluctuations, resulting from reproductive hormone fluctuations, influence mood symptoms through ALLO's downstream effects on the main inhibitory neurotransmitter receptor, the gamma-aminobutyric acid ($GABA_A$) receptor (Hantsoo & Epperson, 2020). There are several streams of research supporting this hypothesis. For instance, studies measuring saccadic eye velocity (SEV) suggest that there is dysregulated $GABA_A$ receptor activation in PMDD compared to healthy control participants. Saccades are quick eye movements initiated to focus on a visual stimulus. Benzodiazepines, which are $GABA_A$ agonists, decrease SEV (Hommer et al., 1986) and flumazenil, a $GABA_A$ antagonist, prevents benzodiazepine-induced decreases in SEV (Ball et al., 1991). Thus, SEV is an indirect measure of $GABA_A$ function. One recent study on SEV found that women exhibited altered $GABA_A$ function in response to ALLO; after ALLO administration, those with PMDD demonstrated decreased SEV in the follicular phase compared to the luteal phase – the opposite pattern that was present in the control group (Timby et al., 2016).

Moreover, research studies testing the efficacy of medications manipulating ALLO levels also suggest that ALLO plays an important role in PMDD. In one randomized, double-blind, placebo-controlled crossover study, high-dose dutasteride, a drug that inhibits the conversion of P4 to ALLO, significantly reduced premenstrual symptoms in women with prospectively-confirmed PMDD as compared to healthy controls and placebo (Martinez et al., 2016). Another medication, Sepranolone, a $GABA_A$ receptor modulating antagonist that blocks ALLO's effects at the $GABA_A$ receptor, has been tested as a treatment for PMDD. Specifically in a randomized, double-blind study, Sepranolone administered during the luteal phase effectively reduced PMDD symptoms compared to placebo in a small sample of women with prospectively-assessed PMDD (Bixo et al., 2017). A more recent randomized double-blind study with a larger sample also found that the subcutaneous injection of 10 milligrams of Sepranolone every 48 hours during the luteal phase significantly reduced symptoms, impairment, and distress compared to placebo during the nine days prior to menstruation (Bäckström et al., 2021). Thus, blocking ALLO's effects of the GABAergic system, either by preventing the production of ALLO from P4 or inhibiting ALLO's modulating effects on the $GABA_A$ receptor, seems to be an effective target for treating PMDD.

1.2 Postpartum Depression (PPD)

1.2.1 The Role of Reproductive Hormones in PPD

PPD – major depression with onset during the postpartum period after childbirth – is another disorder in which reproductive hormone fluctuations are etiologically implicated. PPD is estimated to affect approximately 14% of mothers when defined as depression with onset within three months postpartum (Shorey et al., 2018). In the DSM-

5, this disorder is diagnosed as major depressive disorder with peripartum onset – that is, a major depressive episode occurring either during pregnancy or during the first four weeks postpartum (American Psychiatric Association, 2013). However, research on PPD often extends the postpartum period up to a year after delivery (Meltzer-Brody & Kanes, 2020). The current section will primarily focus on depression in the postpartum period rather than during pregnancy, as much of the research on female reproductive mood disorders has exclusively focused on depression during the postpartum period.

Major hormonal changes occur during pregnancy and postpartum. During pregnancy, E2 and P4 levels gradually rise, reaching peak levels in the final weeks of pregnancy, before delivery (Hendrick et al., 1998). After delivery, these hormone levels rapidly decline with the removal of the hormonal contributions of the placenta (Hendrick et al., 1998). This significant hormonal withdrawal is widely believed to play a causal role in PPD. As with PMDD research, there is experimental evidence for the involvement of reproductive hormones in the pathophysiology of PPD. Specifically, in a small research sample comparing women with a history of postpartum depression to women without a history of postpartum depression, experimentally-induced withdrawal from E2 and P4 were found to trigger depressive symptoms in women with previous postpartum depression but not in those without such a history (Bloch et al., 2000). This study provides strong evidence that, as with PMDD, PPD results from an increased psychological sensitivity to reproductive hormone changes. However, while PMDD results from an increased sensitivity to increases in reproductive hormones, PPD is triggered by reproductive hormone withdrawal.

1.2.3 The Role of ALLO in PPD

As in PMDD, mounting research suggests that altered responses to fluctuations in ALLO may underlie the negative mood effects of reproductive hormone changes in PPD. However, in contrast to PMDD, it is withdrawal from ALLO following childbirth is thought to trigger PPD symptoms (Meltzer-Brody & Kanes, 2020). The most compelling evidence supporting ALLO's role in PPD comes from clinical research on the efficacy of a compound called Brexanolone, a synthetic form of ALLO. Three double-blind, randomised, placebo-controlled trials have found that a 60-hour continuously administered intravenous dose of Brexanolone in mothers with PPD within six months postpartum effectively reduced depressive symptoms in the short term (i.e., at 60 hours) and at longer-term follow up (i.e., at 30 days) (Kanes et al., 2017; Meltzer-Brody et al., 2018). Thus, reversing the ALLO withdrawal that is experienced in the postpartum period appears to resolve PPD symptoms. As a result of the above research, Brexanolone was FDA approved for the treatment of PPD in March of 2019 (Scott, 2019).

1.3 Perimenopausal Depression

1.3.1 The Hormonal Environment of the Menopause Transition

The menopause transition – or perimenopause – is another time during which reproductive hormone fluctuations have been observed to influence mood. Perimenopause represents the time of increasing menstrual cycle irregularity and hormonal changes prior to the complete cessation of ovulatory menstrual cycles. Perimenopause typically lasts five to six years (Avis & McKinlay, 1995; Oldenhave et al., 1993; Treloar, 1981) and is commonly classified using the Stages of Reproductive Aging Workshop (STRAW + 10) criteria (Harlow et al., 2012). In this system, early perimenopause is defined by the appearance of consecutive menstrual cycles that are seven days shorter or longer than usual while late perimenopause is defined by the appearance of 60 days of amenorrhea. Twelve months of amenorrhea (i.e., absence of menstruation) marks the onset of the postmenopausal period (Harlow et al., 2012).

Profound hormonal changes accompany this transition, particularly changes in levels of E2. Human females are born with a limited number of E2-releasing ovarian follicles, which begin to rapidly decline in midlife (Richardson et al., 1987). Once a critically low number of follicles is reached, a cascade of hormonal changes is triggered, including erratic fluctuations in FSH. These fluctuations in FSH can trigger the exaggerated growth of ovarian follicles in the luteal phase, as well as an increased incidence of polyvovulation (the release of several ova at a time), resulting in E2 peaks that can reach 2-3 times higher levels than those seen in the premenopausal phase (Baerwald et al., 2018; Brink et al., 2013; Hale et al., 2009; Hale et al., 2007; O'Connor et al., 2009). Additionally, there is an increasing frequency of anovulatory cycles, which are characterized by extended periods of very low E2 levels, in the late menopause transition (Burger et al., 2005; Santoro et al., 2017). Thus, the menopause transition is a time that is marked by both abnormally high and low levels of E2. Anovulatory cycles are also characterized by low levels of P4 as the luteal increase in P4 typically seen across the menstrual cycle does not occur (Lee et al., 1988; Longcope et al., 1986; Miro et al., 2004; Santoro et al., 2004; Sherman et al., 1976). However, greater attention has been paid to E2 fluctuation in research on perimenopausal depression (Gordon & Sander, 2021).

1.3.2 Depressive Mood in the Menopause Transition

The hormonal changes of perimenopause have been long accepted to be responsible for many physical symptoms that often occur during perimenopause, such as vasomotor symptoms (i.e., night sweats and hot flashes), headaches, sleep disturbances, and sexual dysfunction (Monteleone et al., 2018). However, the notion that this changing hormonal environment may influence psychological wellbeing as well is somewhat more recent.

As outlined in a recent review, the heightened risk of depressive symptoms during perimenopause is well-established, with longitudinal studies finding an increase in the occurrence of clinically significant depressive symptoms by a factor of 1.3-1.6 in the early transition and 1.7-2.9 in the late transition (Maki et al., 2019). Overall, approximately 45 to 68% of women are estimated to suffer from elevated depressive symptoms during the menopause transition according to cross-sectional studies (Maki et al., 2019). However, it should be noted that an increased risk of major depressive disorder, as defined by the DSM-5, may only be elevated among those with a premenopausal history of major depressive disorder (Bromberger et al., 2015).

Previously, researchers have posited that the physical symptoms of perimenopause are responsible for the increased depression risk during perimenopause. The “domino theory” held that physical discomfort caused by vasomotor symptoms contributed to sleep disturbances, which in turn caused depressed mood (Bachmann, 1999; Campbell & Whitehead, 1977). Although depressive symptoms are indeed increased in perimenopausal women with physical complaints such as vasomotor symptoms (i.e., hot flashes and night sweats) (Bosworth et al., 2001; Joffe et al., 2002), research has not consistently supported the domino theory. For instance, estrogen therapy has been demonstrated to effectively treat perimenopausal depression independently of vasomotor symptoms (Schmidt et al., 2000), and depressed mood in the menopause transition often occurs prior to onset of physical symptoms (Freeman et al., 2009). Therefore, while it is acknowledged that poor sleep resulting from night sweats may contribute to negative mood in some individuals, many researchers currently believe that perimenopausal hormonal changes, especially in E2, may also directly contribute to perimenopausal depressive symptoms at the level of the brain.

1.3.3 The Role of Estradiol (E2) in Perimenopausal Depression

Many early observational studies failed to find a significant correlation between E2 and perimenopausal depressive symptoms, which may have been due to infrequent annual hormone sampling (Avis et al., 2001; Bromberger et al., 2011; Woods et al., 2008). As outlined above, perimenopause is a time of dynamic and complex hormonal changes; thus, infrequent sampling may not accurately capture the relationship between hormonal changes and mood. Indeed, more recent studies using more frequent assessments of E2 levels and mood have found an association between E2 variability – typically measured as the standard deviation in E2 levels measured over time – and depressive symptoms (Freeman et al., 2006; Gordon et al., 2016; Joffe et al., 2020; Willi et al., 2021). There has also been some experimental work in this area. Particularly, one influential study experimentally inducing E2 withdrawal in postmenopausal women found that withdrawal triggered significant increases in depressive symptoms in women with previously diagnosed perimenopausal depression that had been responsive to estrogen therapy but not in women without such a history (Schmidt et al., 2015). This experimental study provided strong evidence that increased mood sensitivity to E2 changes in the menopause transition is an important etiological factor in the development of perimenopausal depressive symptoms. Consistent with this, research suggests that stabilizing E2 levels using hormone therapy is effective in preventing or treating depression in the menopause transition (Cohen et al., 2003; de Novaes Soares et al., 2001; Gordon et al., 2018; Schmidt et al., 2000).

Also consistent with the above research is a recent study by our team, which measured levels of a urinary metabolite of E2, estrone glucuronide (E1G), and depressive symptoms weekly in 101 perimenopausal women, that revealed considerable individual variability in the relationship between depressive symptoms and E1G. Specifically, a subset of women exhibited depressive symptoms during times of E1G withdrawal (7%), others reported more depressive symptoms following increases in E1G (12%), and still others developed depressive mood following changes in E1G in either direction (20%) (Gordon et al., 2021). Sixty-one percent of the sample was found to not display a significant relationship between E1G and mood. The magnitude of the relationship between E1G and depressive mood in E1G-sensitive individuals also predicted the occurrence of clinically significant depressive symptoms in the following nine months (Gordon et al., 2021). Altogether this research on the menopause transition suggests that perimenopausal depression, similar to PMDD and PPD, results from an increased mood sensitivity to reproductive hormone change. But while PMDD symptoms are triggered by increases in reproductive hormones and PPD symptoms triggered by hormone withdrawal, perimenopausal depressive symptoms can be triggered by hormone changes in either direction, depending on the individual.

1.3.4 The Proposed Role of ALLO in Perimenopausal Depression

Similar to the previously described research on other female reproductive mood disorders, the neurosteroid ALLO is hypothesized to play an important role in the relationship between E2 and depressed mood in perimenopause (Gordon et al., 2015). First of all, perimenopausal fluctuation in E2 would be expected to result in parallel changes in ALLO. Animal research indicates that E2 positively modulates ALLO levels by regulating the enzymes that convert P4 to ALLO (Pluchino et al., 2009). Consistent with this, E2 therapy significantly increases ALLO levels in postmenopausal women (Bernardi et al., 2003). Furthermore, considering the confirmed role of ALLO fluctuation in the etiology of both PMDD and PPD symptoms, there is good reason to believe that ALLO fluctuation resulting from the hormonal changes of the menopause transition also plays a role in triggering perimenopausal depressive symptoms.

Also supporting the notion that the etiological mechanisms underlying PMDD and PPD are involved in perimenopausal depression is the fact that women experiencing perimenopausal depression report higher rates of premenstrual mood disturbance (Bromberger et al., 2003; Dennerstein et al., 2004; Freeman et al., 2006; Freeman et al., 2004; Morse et al., 1998; Stewart & Boydell, 1993) and histories of PPD (Freeman et al., 2006; Stewart & Boydell, 1993; Woods & Mitchell, 1996; Woods et al., 2008). These disorders may therefore represent different manifestations of the same underlying sensitivity to ALLO fluctuation. However, unlike PMDD and PPD, there has been a considerable lack of research investigating the role of ALLO in perimenopausal depression. In fact, to my knowledge, there is only one such study, which found some evidence of a relationship between low ALLO levels and depressive symptoms only in early postmenopausal women. In this study, ALLO and depressive symptoms were only measured once in 140 late perimenopausal and early postmenopausal women. Unfortunately, frequent assessments of mood and hormones were not taken, making it impossible to investigate potential individual variability in the relationship between hormones and mood, and individuals in the early menopause transition were excluded (Slopien et al., 2018). Thus, an investigation into the role of ALLO in perimenopausal depression utilizing frequent assessments and analytical methods that allow for the detection of individual variability in hormone sensitivity is very much warranted.

1.4 Rationale and Purpose of the Current Study

1.4.1 The Relationship between E2, ALLO, and Depressive Mood in Perimenopause

Though the increased lifetime rate of depression observed in women is likely due to a complex variety of social, psychological, and biological factors, the evidence summarized thus far suggests that sex hormone fluctuations may play a key role in the development of mood disorders for a subset of hormone-sensitive individuals. In the case of PMDD and PPD, there is compelling evidence that changes in ALLO mediate the effect of reproductive hormones on mood. However, ALLO's involvement in triggering perimenopausal depressive symptoms has not yet been appropriately examined.

The purpose of the current study is therefore to conduct a preliminary exploration of ALLO's potential role in triggering perimenopausal depressive symptoms using frequent assessments of hormones and depressive symptoms in an observational longitudinal design. Specifically, weekly assessments of ALLO and depressive symptoms in women reporting an increase in depressive mood since the onset of the menopause transition allows for the examination of within-person effects of ALLO on weekly mood.

Simultaneous assessments of E2 furthermore allow for the examination of the relationship between mood sensitivity to ALLO and mood sensitivity to E2, testing the hypothesis that the effect of E2 changes on mood is, at least in part, mediated by changes in ALLO. In other words, it was hypothesized that the within-person correlation between weekly E2 and depressive symptoms across the 15 weeks would be moderately related to the within-person correlation between weekly ALLO and depressive symptoms. Given that ALLO is a metabolite of P4 and P4 has been demonstrated to play a role in other female reproductive mood disorders, P4 was also measured weekly and examined in relation to weekly mood, though its effects were not the primary focus of this study.

1.4.2 Secondary Aims

The current study's repeated assessment of mood and menopausal symptoms such as sleep disturbance and vasomotor symptoms (i.e., hot flashes and night sweats) also allowed for a unique opportunity to examine the within-person effects of menopausal symptoms on weekly mood. As well, I sought to examine a number of baseline characteristics in relation to depressive symptom severity, menopausal symptom severity, and mood sensitivity to ALLO and E2. These included basic demographic characteristics but also prior history of major depressive disorder, which has been identified as a risk factor for perimenopausal depression (Bromberger et al., 2015; Willi et al., 2021) and prior history of anxiety disorders in light of ALLO's known effects on anxiety (Schüle et a., 2014). Exposure to stressful life events occurring within the past 6 months was also examined as a predictor of symptoms and hormone sensitivity as past research has found life stressors to moderate the relationship between E2 changes and depressive mood (Gordon et al., 2016)

CHAPTER 2: Methods

2.1 Participants

The sample consisted of 26 perimenopausal individuals living in the cities of Regina and Saskatoon in the age range of 42 to 55 years who reported experiencing increased depressive symptoms or mood lability since entering the menopause transition. Perimenopausal status was determined based on the Stages of Reproductive Aging Workshop (STRAW + 10) criteria (Harlow et al., 2012). Specifically, participants must have reported either having at least two consecutive menstrual periods occurring one week earlier or later than typical (early perimenopausal stage) or at least 60 days but less than one year of amenorrhea (late perimenopausal stage). As I was aiming to target those with depressive mood specifically tied to the menopause transition, participants were only included if they answered yes to the question "Have you noticed an increase in depressive symptoms or mood swings since you began experiencing changes in your menstrual cycle?" Moreover, participants were only included if they endorsed clinically significant depressive symptoms on a self-report depression questionnaire, defined as obtaining at least a score of 16 on the Centre of Epidemiological Studies – Depression scale (CES-D).

Prospective participants were excluded for the following: 1) self-reported history of diagnosed bipolar disorder or a psychotic disorder, 2) current use of any medication affecting mood or reproductive hormones, 3) current participation in psychotherapy for depression, 4) diagnosis of any condition affecting ovarian hormone levels (e.g., polycystic ovary syndrome), 5) current pregnancy or nursing, and 6) reports of active suicidal thoughts within two weeks of study enrollment. Participants reporting suicidal ideation in the past two weeks in the online eligibility survey completed the Suicide

Behaviours Questionnaire-Revised (Osman et al., 2001), and those who scored above the cut-off score of seven were excluded from participating. Instead, the research supervisor (Jennifer Gordon, a registered Clinical Psychologist) scheduled a phone call to assess risk and refer to appropriate mental health services. As menopausal status was defined using menstrual bleeding patterns, participants without a uterus (i.e., due to hysterectomy) or without at least one ovary (i.e., due to bilateral oophorectomy) were also excluded.

2.2 Procedure

2.2.1 Enrollment

Prospective participants were recruited using social media advertisements that included a brief study description (Appendix A) and an invitation to contact the research team if interested. Additionally, physicians from the Women's Mid-Life Health Centre in Saskatoon provided study information (Appendix B) to their potentially eligible patients. Interested participants were assigned a participant number and sent a link to an eligibility survey (Appendix C). Eligible and interested individuals then completed a Zoom-facilitated enrollment session (Appendix D) during which the study procedures and consent form were explained and the Structured Clinical Interview for the DSM-5 – Research Version (SCID-V-RV) modules for anxiety and depression were administered to assess the presence of past and current depressive and anxiety disorders only after receiving oral informed consent (Appendix E). Participants were then emailed a survey link containing the baseline survey, which included questions on sociodemographic characteristics, medical history, experiences of stressful life events, baseline sleep quality, and vasomotor symptoms (Appendix G). Ahead of the enrollment session, participants were emailed a copy of the consent form and an enrollment package

(Appendix F) containing instructions for completing the weekly surveys as well as explaining the procedures followed for blood collections, which the research assistant also reviewed with the participant during the enrollment session. Additionally, kits containing materials necessary for participation (e.g., hot flashes tracking sheets) were delivered to the participants' homes before they begun the weekly measurement phase.

2.2.2 Weekly Measures

After completing all baseline questionnaires, participants began weekly mood and hormone measures. One evening per week for 15 weeks on a consistent weekday of their choosing, participants were sent a survey (Appendix H) assessing past-week depressive symptoms via the Centre of Epidemiological Studies Depression as well as sleep quality for the previous night. A question asking participants if and when they had a menstrual period during the last week was also included to keep track of participants' menstrual cycles and perimenopausal stage status. The day of the weekly mood measure, participants also had their blood collected (Appendix J) by a trained and certified phlebotomist to allow for the measurement of neurosteroids. Several certified phlebotomists and nurses performed blood draws after receiving training in research ethics (e.g., protecting participant confidentiality) and the specifics of the protocol guidelines. Blood collections occurred in a private location that was convenient for the participant, usually in the participant's home, and occurred at a consistent time each week. One day prior to the blood draw and mood survey, participants were sent an email reminder of their blood collection appointment for the next day. Starting at 8:00 AM on the day of the blood collection and mood survey, participants were asked to begin tracking any experiences of hot flashes or night sweats using the hot flash tracking sheet (see Appendix K) until 8:00 AM the following day, at which point participants were emailed a brief follow-up survey (see Appendix I) asking them report the total number of mild, moderate, and severe hot flashes counted in the hot flash tracking sheet. The hot flashes tracking sheet includes the following definitions of severity: mild was defined as the sensation of heat without sweating or dampness or noticing dampness after waking up in the morning, moderate as the sensation of sweating or dampness or waking up at night due to being hot, and severe as the sensation of intense heat with sweating causing disruption of current activity or requiring action (e.g., removing clothes).

For completion of the entire research study, participants received $400 in compensation, prorated as follows: $25 for the psychiatric screening and baseline questionnaires and $25 for completing each week of surveys and biosample collections. These research methods have been approved by the Saskatchewan Health Authority and the University of Regina Research Ethics Board (file number 2022-038).

2.2.3 Biosample Collection and Assay Procedure

An EDTA-treated Vacutainer tube (Beckman Dickinson and Company, Franklin Lakes, NJ) was used to collect 6 ml of blood. Samples were then transported over ice to either the Women's Mid-Life Health Centre in Saskatoon or the University of Regina's Reproductive Mental Health Research Unit in Regina. Samples were then centrifuged at 1500 rpm for 10 minutes in a refrigerated centrifuge. Plasma was extracted and stored in 0.5 ml aliquots at -80°C before eventually being transported to the University of Regina Institute of Environmental Change and Society Laboratory to be assayed.

Levels of E2, P4, and ALLO were quantified from plasma using liquid chromatography with tandem mass spectrometry. Neurosteroids were extracted by solid phase extraction (Waters OASIS HLB) according to manufacturer's instructions. OASIS HLB cartridges were conditioned with methanol and equilibrated with water. Plasma samples were diluted with 1% formic acid, and 1 mL of each sample was loaded onto cartridges. Subsequently, the column was washed with 5% methanol in water and the analytes were eluted with acetonitrile. The sample was dried in a concentrator and reconstituted in 50% acetonitrile with 0.1% formic acid.

For liquid chromatography, separation was conducted on a Waters I-Class UPLC with Waters Acquity UPLC BEH C-18 column (2.1 mm x 50 mm; 1.7 µm particle size). The mobile phases consisted of LC-MS grade water and acetonitrile, both supplemented with 0.1% formic acid. The gradient began at 20% acetonitrile to 75% acetonitrile in 6 minutes, moving to 95% acetonitrile for a one-minute flush then returning to 20% acetonitrile for minute of equilibration. The flow rate was kept at 0.3 mL/ minute and the column temperature was kept at 35°C for the duration of the run.

The Waters Synapt XS was used to quantify the analytes in positive electrospray ionization mode a at capillary voltage of 500 V in MSE mode. A ramp in the transfer cell from 20 to 30 V was run throughout the scan with a scan time of 0.3 s in sensitive mode. Neurosteroids were identified based on retention time and transitions based on injected reference material and/or transition data from literature.

2.3 Measures

2.3.1 Eligibility Survey

2.3.1.1 Centre of Epidemiological Studies – Depression (CES-D) Scale. The CES-D was used to measure baseline depressive symptomology and determine eligibility. The CES-D is a 20-item self-report questionnaire in which participants are asked to rate whether depressive symptoms (e.g., “I was bothered by things that usually don't bother me”) occurred within the last week rarely (0), some or a little of the time (1), occasionally or a moderate amount of time (2), or most or all of the time (3) (Radloff, 1977). Higher scores indicate more severe depressive symptomology, and several items are reverse-scored (e.g., “I felt hopeful about the future”). A score of 16 points or more is a common threshold to identify potential clinical depression (Boyd et al., 1982); a score in this range is predictive of later diagnosis of major depressive disorder (Thomas et al., 2001). Individuals were only included if they met this threshold. This commonly-used questionnaire has established validity and reliability for measuring depressive symptoms in clinical and general populations (Radloff, 1977) and in midlife women (Knight et al., 1997). Internal reliability was also good for the current sample at baseline with a coefficient alpha of .73.

2.3.1.2 Suicide Behaviours Questionnaire – Revised (SBQ-R). In the eligibility survey, in order to exclude (and direct resources to) those with moderate-to-severe suicidality, participants answering yes to experiencing suicidal ideation in the past two weeks were administered the SBQ-R, and those scoring at least the cut-off score of seven were excluded. The SBQ-R consists of four items assessing one’s thoughts and behaviours related to suicide and demonstrates adequate to moderately high internal reliability (Osman et al., 2001). Specifically, the coefficient alpha for the SBQ-R ranged from .76 to .88 depending on the population, with the lowest estimate being for an undergraduate student sample and the highest estimate being for an adolescent inpatient population. Adult psychiatric inpatient and high school populations had a coefficient alpha of .87. The SBQ-R also demonstrates good criterion validity. Using receiver operating characteristic (ROC) analysis, for non-clinical samples, a cut-off score of seven most accurately discriminated between suicidal and non-suicidal groups (compared to a cut-off score of eight for clinical samples) (Osman et al., 2001). Considering that not all participants in the proposed study have a psychiatric diagnosis, we utilized the cut-off score of seven.

2.3.2 Baseline Questionnaire

2.3.2.1 Demographics and Health History. In the baseline survey, participants were asked to report their age, relationship status, ethnicity, education level, and approximate income. Questions on reproductive health history (i.e., how many biological children one has had) and medical history were also included. Finally, to assess potential eligibility for a future follow-up study that will include experimental manipulation of E2, known contraindications to hormone therapy were assessed.

2.3.2.2 Life Experiences Survey. A modified version of the Life Experiences Survey (Sarason & Johnson, 1976; Sarason et al., 1978) was used to assess recent stressful life events (within 6 months) at baseline. The modified version only includes 28 events, which are the events identified as moderately to severely stressful based on previous research (Leserman et al., 2008; Leserman et al., 2002; Leserman et al., 1997). The events are classified into five categories: relationships; death or illness among family or close friends; work and financial issues; personal illness, accidents, injury, safety; crime and legal problems; and other life changes.

2.3.2.3 Hot Flash Related Daily Interference Scale (HFRDIS). Used to assess hot flashes severity at baseline, the HFRDIS measures the impact of hot flashes on nine specific areas of activity (e.g., work, social activities, sleep, sexuality) as well as overall quality of life (Carpenter, 2001). A total score is computed by computing the mean from the 10 items. Internal reliability estimates were exceptional, with an alpha coefficient of .96 (Carpenter, 2001). Good construct validity of the HFRDIS is evident in the measure's moderate to high convergent and known-groups validity. The HFRDIS also demonstrated sensitivity to change over time, with HFRDIS scores correlating with changes in hot flash frequency between two time points (Carpenter, 2001). Internal consistency in the current study was found to be excellent for this measure with a coefficient alpha of .90.

2.3.2.4 Pittsburgh Sleep Quality Index (PSQI). The PSQI is a 19-item measure of subjective sleep quality, sleep duration, habitual sleep, and sleep disturbances (Buysse et al., 1989). Scoring for this measure consists of summing individual items to compute seven different component scores ((1) subjective sleep quality, (2) sleep latency, (3) sleep duration, (4) sleep efficiency, (5) sleep disturbance, (6) use of sleep medication, (7) daytime dysfunction), and further summing those scores to compute a global indicator. Each component score is on a scale of zero to three, and the total indicator has a maximum score of 21. Higher scores indicate more sleep problems. This widely-used scale has good reliability, with a coefficient alpha estimate of .80 (Carpenter & Andrykowski, 1998), and good validity in measuring overall sleep quality as demonstrated by high convergent and discriminant validity (Carpenter & Andrykowski, 1998) and high sensitivity (89.6 %) and specificity (86.5%) for known-groups validity in distinguishing between good and poor sleepers (Buysse et al., 1989). Internal consistency in the current study was found to be acceptable with a coefficient alpha of .63.

2.3.2.5 Structured Clinical Interview for the DSM-5 – Research Version (SCID-V-RV). The SCID-V-RV is a guide for a semi-structured interview to assess criteria for disorders present in the *Diagnostic and Statistical Manual of Mental Disorders – 5th Edition (DSM-5* (First et al., 2015)*)*. The following disorders were assessed for current and previous symptom criteria: major depressive disorder, persistent depressive disorder, panic disorder, agoraphobia, social anxiety disorder, specific phobia disorder, generalized anxiety disorder, and obsessive-compulsive disorder. The SCID-5-RV was administered via Zoom by a masters-level clinical psychology graduate student or a bachelors-level research assistant who received training and supervision from a doctoral-level registered clinical psychologist (Dr. Gordon).

2.3.3 Weekly Questionnaire

2.3.3.1 Centre of Epidemiological Studies – Depression scale (CES-D). The CES-D, described above, was also repeated during each weekly survey. Internal consistency for the weekly measure in the sample was high, with a coefficient alpha of .90. Scores of 16 or above were classified as clinically significant depressive symptoms.

2.3.3.2 Vasomotor Symptoms and Sleep Disturbance. The vasomotor symptoms diary assessed the frequency of mild, moderate, and severe hot flashes and night sweats as based on methods commonly used in research on perimenopausal hot flashes (Sloan et al., 2001). The weekly sleep questionnaire consisted of questions retrieved from the PSQI that assessed sleep duration and sleep quality adapted to assess sleep over the previous night instead of the previous month. However, for the current investigation, responses to a single item asking about subjective sleep quality were used. This question was phrased as follows: “How would you rate the quality of your sleep?” Response items included “Very good” (scored a 1), “Fairly good” (scored a 2), “Fairly bad” (scored a 3), and “Very bad” (scored a 4).

2.4 Statistical Analyses

A variety of descriptive statistics were performed on variables measured at baseline to characterize the sample. Descriptive statistics were also conducted for weekly symptoms and hormone levels.

Multilevel models were then conducted using PROC MIXED in SAS 9.4 examining participant characteristics assessed at baseline such as stressful life events and mental health history in predicting weekly sleep quality, vasomotor symptoms, and depressive symptoms, each as univariate models. All significant predictors were then entered simultaneously into a multivariate model to identify the strongest predictor(s) of each dependent variable of interest. In the case of depressive symptoms, this multivariate model included both weekly sleep quality and moderate-severe hot flashes as covariates. In the case of sleep, only number of moderate-severe hot flashes was included as a covariate while in the case of vasomotor symptoms, sleep quality was the only covariate included. In all cases, models were fitted using a Restricted Maximum Likelihood (REML) estimation method, and a first-order autoregressive covariance structure for within person error was applied. Degrees of freedom was calculated using the Kenward-Rogers correction.

The primary analyses examined the extent to which increased mood sensitivity to E2 mirrors sensitivity to ALLO. As a first step, using statistical methods used previously (Gordon et al., 2021), Pearson correlations between person-centred levels of E2 and depressive symptoms (i.e., CES-D scores) were calculated for each individual participant. Correlations between the absolute value of person-centred E2 and mood measures were also calculated. The correlation coefficient of greatest magnitude (correlation between E2 and CES-D versus |E2| and CES-D) determined that participant's overall E2 sensitivity strength. Appendix L provides a clear definition of these various sensitivity indicators. The same correlational analyses were then repeated with ALLO and P4. Finally, the correlation between each E2, P4, and ALLO sensitivity indicator was calculated, as well as the correlation between mean levels of E2, P4, and ALLO and their sensitivity indicators.

General linear models (PROC GLM in SAS 9.4) were then conducted examining participant characteristics in predicting E2, P4, and ALLO sensitivity strength. Finally, secondary analyses explored whether baseline stressful life events or depression and anxiety disorders were correlated with mood sensitivity to E2 and ALLO.

2.5 Power Calculations

Using G*Power (Faul et al., 2009), it was determined that 29 participants would allow for the detection of a moderate correlation (r = 0.5) between mood sensitivity to E2 and mood sensitivity to ALLO, setting alpha at 0.05 and power at 80%.

CHAPTER 3: Results

3.1 Study Compliance

A total of 31 participants attended an enrollment session and provided informed consent to participate. However, two participants dropped out after the enrollment session, prior to beginning weekly measures, and a third withdrew after completing one week of measures. An additional two participants dropped out of the study after weeks three and five due to difficulties or discomfort during blood collections. Thus, the final sample included 26 participants. Of the completed sample, participants completed an average of 14.5 weeks of blood, 14.5 weeks of CES-D and sleep data, and 14.4 weeks of vasomotor symptom diaries. During the weekly measures phase, study pauses occurred due to phlebotomist and participant availability. The average participant completed the weekly measures phase in 16.8 weeks (SD = 2.7) and the entire study in 21.0 weeks (SD = 4.5).

Throughout the weekly measures phase, participants were permitted to reschedule the occasional blood draw according to the following guidelines: (1) the blood draw was not rescheduled for more than two days earlier or later than the original appointment and (2) the blood draw was not rescheduled for more than 2 hours earlier or later in the day than the original appointment time. Despite our best efforts, eight appointments (2% of all blood draws) had to be rescheduled beyond these time guidelines to ensure participant completion of the study. Participants were instructed to complete the mood survey the same evening of the blood draw and the vasomotor symptom survey the next morning (after the 24-hour tracking period for vasomotor symptoms was complete). If the blood draw day was changed, survey completion day was changed accordingly as well. Surveys were considered late if they were not completed on the appropriate day. Surveys were checked frequently and participants were sent additional reminders if they did not complete their surveys on time. Participants on average completed 3.9 mood and sleep surveys (*SD* = 3.1) and 2.4 vasomotor symptom surveys (*SD* = 2.3) late. Most surveys that were completed late were completed on the next day (58% of late mood and sleep surveys and 53% of late vasomotor symptoms surveys). An additional 16% of late mood and sleep surveys and 26% of late vasomotor symptoms surveys were completed 2 days late, and an additional 7% and 10% 3 days late. This leaves 19% of late mood and sleep surveys and 11% of late vasomotor symptom surveys more than 3 days late, which is equivalent to 5% and 2% of total surveys completed, respectively. When completed late, participants were reminded to fill out the survey with the correct days in mind. Regarding seeking treatment for depression or perimenopausal symptoms, participants were permitted to stay in the study as long as they notified the research team regarding the medication or therapy they began. One participant reported starting antidepressant medication on week five of the weekly measures phase, but her weekly data was not included when examining weekly hormones and mood, as explained below.

Though 15 blood samples were collected for 26 participants, due to delays in analysing hormone levels related to a backorder of supplies, plasma levels of E2 were measured in 87 samples, P4 in 76 samples, and ALLO in 84 samples. This translated to partial data being available for 15 participants. All between-subjects analyses (e.g., examining the relationships between hormones) included all available data; however, hormonal sensitivity indicators were calculated only for the 10 participants having a minimum of six hormone-mood pairs. All 10 participants had between 6 and 8 hormone-mood pairs available.

3.2 Sample Characteristics

Of the 26 participants, the majority were located in Saskatoon (*n* = 18). Demographic characteristics are summarized in Table 1. All participants identified as women in the demographic survey except for one participant who did not answer. The age range for participants was 42 to 54, and there was an even distribution between the early and late perimenopausal stage as assessed by menstrual cycle patterns at baseline. The majority of participants were working full-time, married, heterosexual, and had children. The large majority of participants identified as white, but there were a number of ethnic origins that participants also identified with such as Indigenous, Other North American (e.g., Acadian, American, Canadian, New Brunswicker, Newfoundlander, Nova Scotian, Ontarians, Québécois), European, South America, and Oceanic. Regarding combined household income, participants reported earning a minimum of $40,000 annually. There was a high proportion of high-earning households, with 54% making $100,000 or more. The majority of participants did not live alone; 69% lived with a spouse or significant other, 73% lived with children, and 8% lived alone. For education, all participants had a minimum of a high school diploma, and the majority of participants had also completed a post-secondary certificate, diploma, or Bachelor’s degree.

The mean baseline CES-D score for the sample, measured in the eligibility survey, was 27.8 (SD = 6.0) out of a maximum of 60, with higher scores indicating more depressive symptomology. A score of this magnitude is well above the minimum

Table 1
Sample Characteristics (n = 26).

Mean Age (*SD*)	48.08 (3.69)
Mean Annual Personal Income (*n* = 22)	$60,000 to 69,999
Mean Annual Household Income (*n* = 22)	$90,000 to 99,999
Employment Status	-
Working Full-Time	77%
Working Part-Time	12%
Unemployed or laid off	8%
No Answer	4%
Sexual Orientation	-
Asexual	4%
Bisexual	8%
Heterosexual	85%
No Answer	4%
Marital Status	-
Single (Never Married or Common Law)	8%
Married	65%
Divorced or Separated	8%
Widowed	15%
No Answer	4%
Highest Education Level	-
High School Diploma or Equivalent	12%
Some Post-Secondary	4%
Post-Secondary Certificate or Diploma	39%
Post-secondary Bachelor’s Degree	27%
University Certificate/Diploma/Degree above Bachelor’s	15%
No Answer	4%
Ethnic Origin	-
Indigenous	12%

European	50%
Other North American	45%
Latin/Central/South American	4%
Oceanic	4%
No answer	4%
Racial Background	-
White	92%
Latin American	4%
No Answer	4%
Mean Number of Biological Children (*SD*)	2.15 (1.16)
Perimenopausal Status	-
Early	50%
Late	50%
Mean Baseline CES-D Score (*SD*)	27.81 (5.97)
Mean Baseline Stressful Life Events (*SD*)	4.88 (5.24)
Mean HFRDIS Score (*SD*), out of 10	1.85 (1.74)
HFRDIS Work (*SD*)	1.23 (1.90)
HFRDIS Social (*SD*)	1.08 (1.85)
HFRDIS Leisure (*SD*)	1.15 (1.76)
HFRDIS Sleep (*SD*)	4.77 (2.66)
HFRDIS Mood (*SD*)	2.58 (2.90)
HFRDIS Concentration (*SD*)	1.96 (2.75)
HFRDIS Relationships (*SD*)	1.27 (2.55)
HFRDIS Sexuality (*SD*)	1.69 (2.83)
HFRDIS Life Enjoyment (*SD*)	1.04 (1.61)
HFRDIS Overall Quality of Life (*SD*)	1.69 (1.95)
Mean Total PSQI Score (*SD*)	9.6 (3.25)
Subjective Sleep Quality Component Score (*SD*)	1.50 (0.65)
Sleep Latency Component Score (*SD*)	1.42 (0.95)
Sleep Duration Component Score (*SD*)	1.38 (0.98)

Sleep Efficiency Component Score (*SD*)	1.12 (1.07)
Sleep Disturbance Component Score (*SD*)	1.85 (0.46)
Use of Sleep Medication Component Score (*SD*)	0.50 (0.95)
Daytime Dysfunction Component Score (*SD*)	1.81 (0.63)
SCID Diagnoses	-
Current Depressive Disorder	12%
Current Anxiety Disorder	35%
Previous Depressive Disorder	42%
Previous Anxiety Disorder	42%

threshold of 16 that is indicative of clinically significant depressive symptoms and suggestive of potential major depressive disorder (16). Despite this, only 12% of the sample met criteria for a current depressive disorder at enrollment based on the SCID-5-RV. Interestingly, none of the late perimenopausal participants met current criteria for a depressive disorder. Despite the low proportion of participants meeting criteria for a current depressive disorder, nearly half of the sample had a history of depression. Most depressive disorders were major depressive disorder, but there were a few participants (n = 3) meeting criteria for persistent depressive disorder either currently or previously. Notably, 15% of participants met criteria for a previous major depressive episode with peripartum onset.

Over a third of the sample met criteria for a current anxiety disorder, and nearly half for a previous anxiety disorder. The most common anxiety disorders were panic disorder (23% current; 27% previous), social anxiety disorder (15% current; 12% previous), agoraphobia (12% current; 15% previous), and generalized anxiety disorder (8% current, 15% previous). At baseline, participants also endorsed experiencing a variety of stressful life events within the past 6 months on the modified Life Experiences Survey, and the range for this variable was high, ranging from 0 to 20 stressful life events. The average subjective stressfulness rating given to these events was 4.1 (SD = 0.9), corresponding to “very stressful” on the scale of “not stressful” to “extremely stressful.” None of the eligible participants indicated suicidal ideation, so none were prompted to complete the SBQ-R in the eligibility survey. Thus, there is no data to present for that measure.

Participants also reported some issues with sleep and vasomotor symptoms at baseline. The mean HFRDIS score was low, suggesting average mild levels of interference related to hot flashes in the current sample according to suggested cut-off scores of 0 to 3.9 for mild, 4 to 6.9 for moderate, and 7 to 10 for severe hot flashes related interference (Carpenter et al., 2017). However, the domain of highest rated interference in the moderate range was sleep. Rated interference for mood and concentration were also higher than the overall interference rating, though still in the mild range. This seems to correspond with the findings from the PSQI. The mean PSQI score was considerably higher than the cut-off score of 5 used to indicate poor sleep quality (Buysse et al., 1989). These results suggest that our sample experienced issues with sleep, perhaps in part due to bothersome vasomotor symptoms.

3.3 Weekly Depressive Symptoms, Vasomotor Symptoms, and Sleep Quality

Participants reported an average of 3.0 (*SD* = 2.1) hot flashes or night sweats per 24-hour rating period. Mild vasomotor symptoms were the most common (*M* = 1.7, *SD* = 1.2), followed by moderate (*M* = 0.9, SD = 0.8) and severe vasomotor symptoms (*M* = 0.5, *SD* = 0.8). The average sleep quality rating was 2.1 (*SD* = 0.3), corresponding to “fairly good.” The mean weekly CES-D score was considerably lower than the mean baseline CES-D score. Weekly CES-D scores ranged from 1 to 42 with an average of 15.3 (*SD* = 5.9). Another dimension of the CES-D measured was the number of instances of elevated scores (equal to or above the clinical cut-off score of 16). On average, participants experienced 6.8 instances (*SD* = 5.2) of clinically elevated CES-D scores with a range of 0 to 14. Over a third of participants displayed elevated scores for the majority of weeks; specifically, 19% had elevated scores for 0-2 weeks, 35% for 3-5 weeks, 12% for 6-8 weeks, 8% for 9-11 weeks, and 27% for 12-14 weeks.

3.4 Baseline Characteristics Predicting Weekly Sleep Quality, Vasomotor Symptoms and Depressive Symptoms

Several baseline variables predicted weekly vasomotor symptoms and sleep quality as revealed by multilevel modelling (see Table 2). In the univariate tests, older age, greater income, not currently meeting criteria for a current or previous depressive disorder, being in the late transition, reporting more stressful life events, and scoring higher on the HFRDIS at baseline each predicted more weekly moderate and severe vasomotor symptoms. When all these variables (minus HFRDIS) were included as fixed effects in the same model, only the effect of age and stressful life events remained significant, with depression history and household income approaching significance. In predicting weekly sleep quality, greater household income, currently meeting criteria for an anxiety disorder, not having a history of depressive disorders, and having a higher score on the PSQI at baseline predicted poorer sleep quality each on their own. Included in the same model (minus PSQI), only the effect of household income and current anxiety remained significant, with previous depression history approaching significance.

Multilevel modelling revealed several baseline variables that were associated with depressive symptoms during the weekly measures phase. In univariate tests, greater age, being in the late transition, having fewer biological children, a history of depressive disorders, a history of anxiety disorders, and a greater number of stressful life events were each associated with higher weekly CES-D scores (see Table 3). When these

Table 2

Baseline Characteristics and Weekly Vasomotor Symptoms and Sleep Quality

	Univariate, unadjusted		**Multivariate, adjusting for sleep or vasomotor symptoms**	
Variable	*B(SE)*	*p*	*B(SE)*	*p*
Moderate and Severe Vasomotor Symptoms				
Age	0.13 (0.04)	.002	0.12 (0.06)	.038
Household Income	0.17 (0.07)	.015	0.14 (0.07)	.060
Education level	0.21 (0.14)	.132	-	-
# Biological Children	-0.10 (0.14)	.474	-	-
Current Anxiety	-0.13 (0.33)	.699	-	-
Previous Anxiety	0.38 (0.32)	.235	-	-
Current Depression	-0.99 (0.49)	.047	-0.38 (0.57)	.501
Previous Depression	-0.64 (0.31)	.044	-0.66 (0.34)	.056
Late transition	0.92 (0.30)	.003	0.06 (0.43)	.890
Stressful Life Events	0.09 (0.03)	.003	0.08 (0.04)	.029
HFRDIS	0.35 (0.08)	<.001	NA	NA
PSQI	0.01 (0.05)	.810	-	-
Sleep Quality				
Age	-0.01(0.01)	.388	-	-
Household Income	0.05 (0.02)	.013	0.05 (0.02)	.022
Education level	0.02 (0.04)	.631	-	-
# Biological Children	0.04 (0.04)	.296	-	-
Current Anxiety	0.18 (0.09)	.047	0.25 (0.09)	.007
Previous Anxiety	0.13 (0.09)	.135	-	-
Current Depression	-0.26 (0.13)	.051	-	-
Previous Depression	-0.21 (0.09)	.017	-0.19 (0.10)	.052
Late transition	0.06 (0.09)	.501	-	-
Stressful Life Events	0.01 (0.01)	.117	-	-
HFRDIS	0.06 (0.02)	.027	0.01 (0.03)	.828
PSQI	0.05 (0.01)	<.001	NA	NA

Table 3
Baseline Characteristics and Weekly Depressive Symptoms

	Univariate, unadjusted		**Multivariate, adjusting for sleep and vasomotor symptoms**	
Variable	*B(SE)*	*p*	*B(SE)*	*p*
CES-D Score				
Age	0.50 (0.20)	.015	0.22 (0.23)	.339
Household Income	0.24 (0.34)	.486	-	-
Education level	0.57 (0.65)	.384	-	-
# Biological Children	-2.14 (0.61)	<.001	-1.51 (0.52)	.005
Current Anxiety	0.52 (1.59)	.745	-	-
Previous Anxiety	3.55 (1.48)	.019	0.46 (1.35)	.732
Current Depression	2.76 (2.35)	.245	-	-
Previous Depression	4.00 (1.44)	.007	3.67 (1.27)	.005
Late transition	3.91 (1.43)	.008	0.66 (1.70)	.699
Stressful Life Events	0.66 (0.12)	<.001	0.45 (0.13)	<.001
HFRDIS	<0.01 (0.45)	.999	-	-
PSQI	-0.06 (0.24)	.817	-	-
Instances of CES-D Elevations				
Age	0.03 (0.01)	.006	0.01 (0.01)	.263
Household Income	0.02 (0.02)	.335	-	-
Education level	0.04 (0.04)	.285	-	-
# Biological Children	-0.11 (0.03)	.002	-0.07 (0.03)	.021
Current Anxiety	-0.001 (0.09)	.989	-	-
Previous Anxiety	0.16 (0.08)	.056	-	-
Current Depression	-0.19 (0.13)	.148	-	-
Previous Depression	0.22 (0.08)	.008	0.19 (0.07)	.008
Late transition	0.23 (0.08)	.005	0.02 (0.10)	.806
Stressful Life Events	0.04 (0.01)	<.001	0.03 (0.01)	<.001
HFRDIS	0.001 (0.02)	.966	-	-
PSQI	-0.001 (0.01)	.935	-	-

variables were simultaneously included in the same model, only the effects of number of biological children, previous depression history, and stressful life events remained significant. Those with a history of depression scored nearly four CES-D points higher than those without such a history while each additional stressful life event was associated with an additional half point on the CES-D.

The same analyses were repeated with number of elevated CES-D scores as the dependent variable and adjusted for weekly sleep quality and moderate and severe vasomotor symptoms (see Table 3). The univariate results were similar, with age, menopausal stage, depression history, and stressful life events associated with a greater number of elevated CES-D scores. The only difference was that a history of anxiety disorders did not quite meet the significance threshold for predicting number of elevated scores. In the multivariate model predicting instances of high CES-D scores, again, only the effect of biological children, depression history and stressful life events remained significant. Notably, other demographic variables, baseline PSQI and HFRDIS, and current depressive or anxiety disorder status did not influence weekly depressive symptoms in any of these analyses.

3.5 Relationships Between Depressive Symptoms, Sleep, and Vasomotor Symptoms

Multilevel models examining the relationships between weekly sleep and vasomotor symptoms with depressive symptoms revealed that lower weekly sleep quality and moderate and severe vasomotor symptoms were associated with higher weekly CES-D scores on their own and when both were included in the same model (Table 4). Both poorer sleep quality and moderate and severe vasomotor symptoms also each significantly predicted the number of elevated CES-D scores on their own. However,

Table 4

Person-centered Weekly Vasomotor Symptoms (VMS) and Sleep Quality Predicting Depressive Symptoms

	Univariate		Multivariate	
Variable	*B(SE)*	*p*	*B(SE)*	*p*
CES-D Score				
Weekly Sleep Quality	2.39 (0.51)	<.001	2.08 (0.50)	.001
Weekly Mild VMS	0.47 (0.76)	.096	-	-
Weekly Moderate and Severe VMS	0.66 (0.20)	.002	0.62 (0.20)	.003
Instances of CES-D Elevations				
Weekly Sleep Quality	0.07 (0.03)	.049	0.03 (0.04)	.449
Weekly Mild VMS	0.01 (0.02)	.528	-	-
Weekly Moderate and Severe VMS	0.03 (0.01)	.023	0.03 (0.01)	.030

weekly sleep quality did not remain significant in the model including both of these variables predicting the number of elevated scores. Mild vasomotor symptoms were not associated with CES-D score nor number of elevated scores in any of the analyses.

3.6 Baseline Characteristics Predicting Hormone Levels

The average level of reproductive hormones in our sample was 83.16 units (*SD* = 60.65) for E2 and 12262.19 units (*SD* = 13484.78) for P4. The relative average level of ALLO was calculated to be 1129.45 units (*SD* = 1554.67). Using multilevel modelling, person-centred P4 was significantly associated with person-centred ALLO levels ($\beta(SE) = 0.06$ (0.02), $p = .005$), but person-centred E2 was not ($\beta(SE) = 1.08$ (2.71), $p = .691$).

Examining the effect of all baseline characteristics listed in Table 2 for predicting levels of each neurosteroid, using all available hormone data, only two significant effects emerged. Education level was associated with lower levels of E2 ($\beta(SE) = -51.79$ (20.28), $p = .019$), greater age predicted lower levels of P4 ($\beta(SE) = -1978.43$ (856.09), $p = .026$), and no baseline variables significantly predicted ALLO levels ($p > .05$).

3.7 Hormones Predicting Depressive Symptoms

Using multilevel modelling, neither E2 ($\beta(SE) = -0.01$ (0.01), $p = .191$), P4 ($\beta(SE) < -0.01$ (<0.01), $p = .414$) nor ALLO ($\beta(SE) < 0.01$ (<0.01), $p = .746$) were predictive of weekly depressive symptoms across all 15 participants with available data. This remained true when the calculations for E2 ($\beta(SE) = -0.01$ (0.01), $p = .428$), P4 ($\beta(SE) < -0.01$ (<0.01), $p = .369$), and ALLO ($\beta(SE) < -0.01$ (<0.01), $p = .720$) were repeated using person-centred values. However, in calculating each individual's sensitivity to each hormone, all 10 participants with sufficient data (6+ data pairs) were found to exhibit increased sensitivity to at least one hormone (Table 5); 6/10 demonstrated mood

Table 5
Individual Profiles

Age	MT Phase	CES-D Score			E2 Sensitivity		P4 Sensitivity		ALLO Sensitivity	
		M (*SD*)	Min.	Max.	Category	Strength	Category	Strength	Category	Strength
44	Early	11.47	3	19	Withdrawal	.61	Change	.60	Increase	.57
53	Late	23.73	13	35	None	.21	Change	.42	Change	.32
51	Late	10.50	5	14	None	.09	Change	.79	Change	.96
46	Early	21.80	15	36	Change	.74	None	.22	None	.17
46	Early	13.07	5	24	Change	.58	None	.09	Change	.66
43	Early	9.60	2	32	Withdrawal	.71	Change	.42	Change	.45
49	Early	16.87	5	28	None	.28	Change	.31	Withdrawal	.41
43	Early	11.20	1	26	None	.28	None	.17	Change	.32
51	Late	19.53	12	32	Change	.50	Increase	.42	Increase	.34
52	Late	11.50	4	20	Withdrawal	.44	Change	.39	None	.29

sensitivity to E2, 7/10 to P4, and 8/10 to ALLO. Increased sensitivity to absolute changes was the most common profile across neurosteroids, and no participants displayed the same profile type for all three hormones. Several participants exhibiting a strong correlation between depressive mood and neurosteroid fluctuations were chosen for graphical representation. A variety of profile types were selected to demonstrate the high level of individual variability observed in this sample. Figure 2 represents the first participant in Table 5. This particular participant is characterized by three different sensitivity profiles for each hormone – withdrawal-sensitivity for E2, change-sensitivity for P4, and increase-sensitivity for ALLO. Figure 3 shows the third participant, who exhibits change-sensitivity to both P4 and ALLO and no sensitivity to E2, and Figure 4 shows the fifth participant who was characterized by change sensitivity to both E2 and ALLO but no sensitivity to P4.

3.8 Predictors of Hormone Sensitivity

A series of Pearson correlations were conducted to determine whether mean levels of hormones were related to hormone sensitivity indicators. Mean E2 was not associated with the correlation between E2 and CES-D score ($r(8) = -.08$, $p = .824$) nor the correlation between the absolute value of E2 and CES-D ($r(8) = -.31$, $p = .389$). Mean P4 was not associated with the correlation between the absolute value of P4 and CES-D ($r(8) = -.23$, $p = .516$) but was nearly significantly correlated with the correlation between P4 and CES-D ($r(8) = -.62$, $p = .054$). Mean ALLO was not associated with the correlation between ALLO and CES-D score ($r(8) = -.48$, $p = .161$) nor the correlation between the absolute value of ALLO and CES-D ($r(8) = -.42$, $p = .229$).

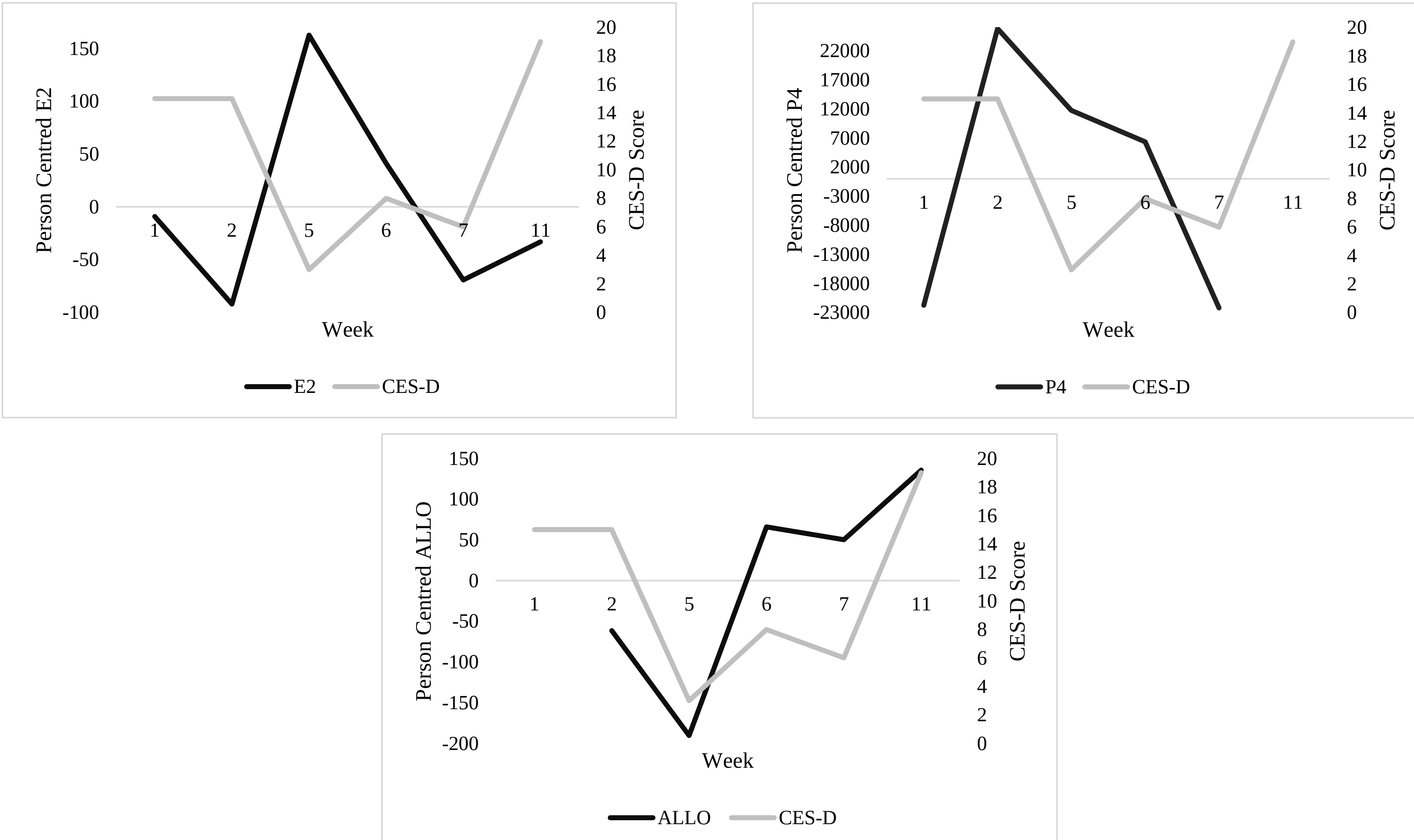


Figure 2. Hormones and CES-D Scores for the first participant in Table 5 (E2 withdrawal, P4 change, and ALLO increase sensitive).

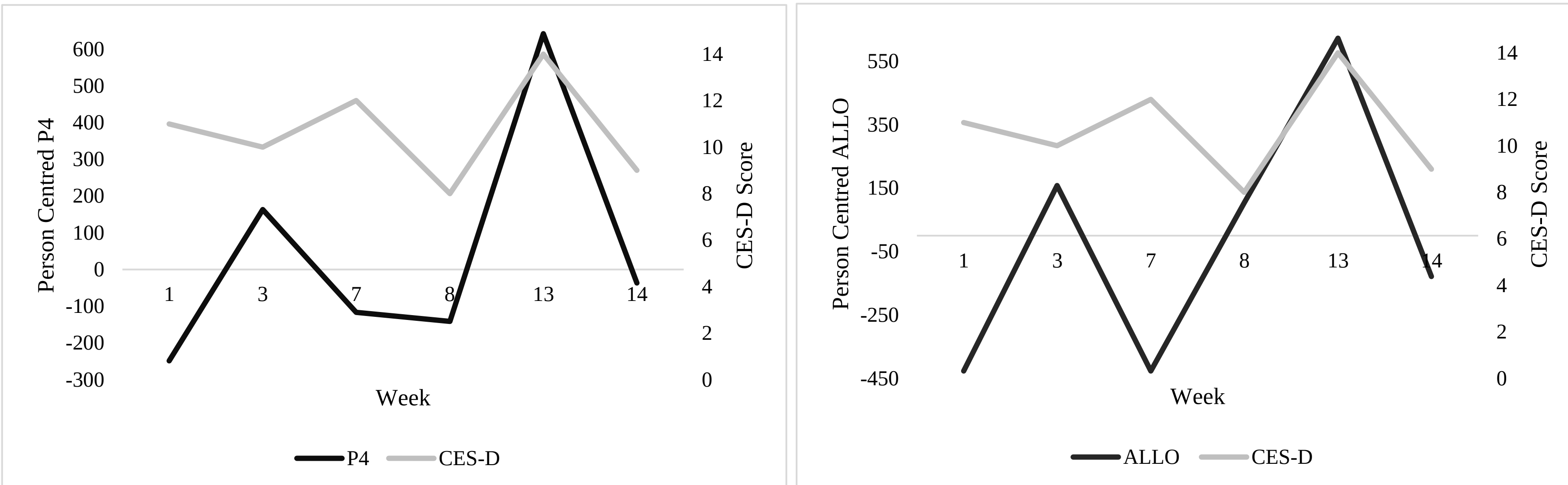
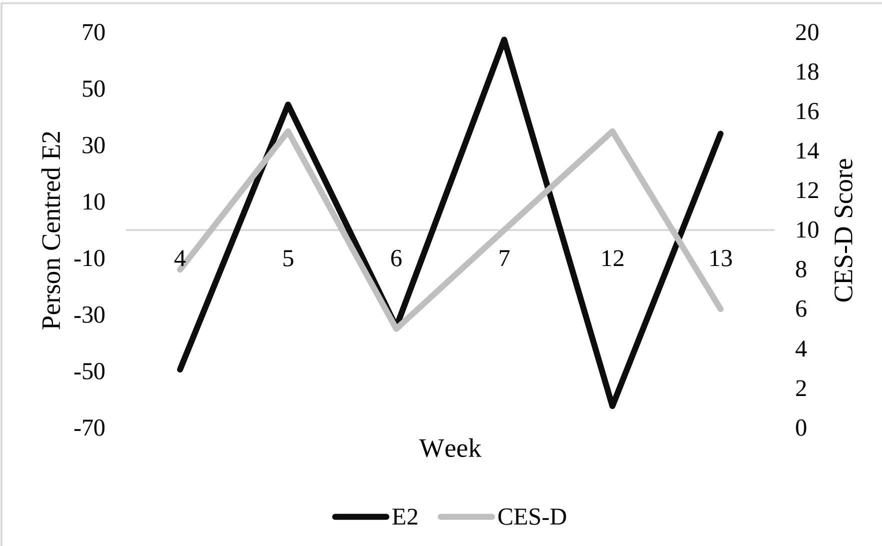


Figure 3. Person Centred Hormone Levels and CES-D Scores for the third participant from Table 2 (P4 change and ALLO change sensitive).



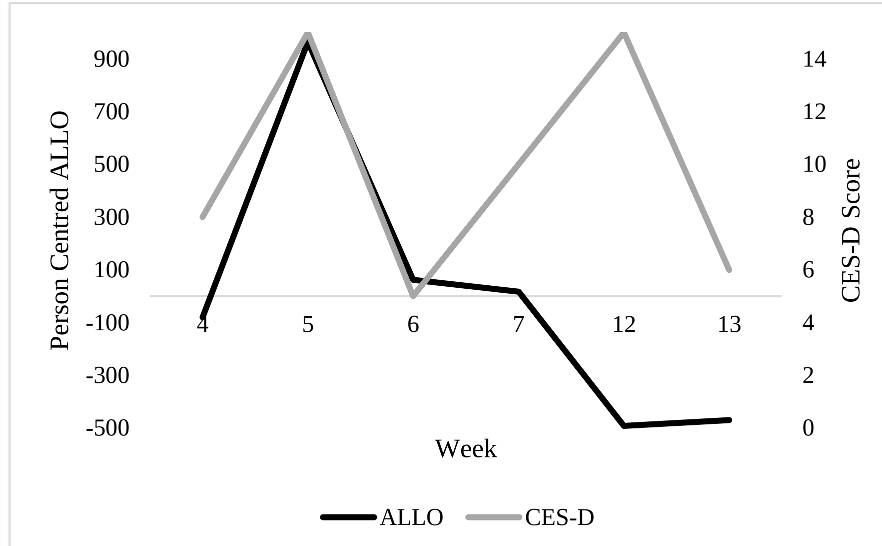


Figure 4. Person Centred Hormone Levels and CES-D Scores for the fifth participant from Table 2 (E2 change and ALLO change sensitive).

Very few baseline variables were found to predict the strength of hormone sensitivity indicators (see Table 6). Lower household income was found to predict P4 sensitivity strength. Notably, those with current depression had increased sensitivity strengths for E2 compared to those without current depression.

3.9 Correlations Between Hormone Sensitivity Indicators

Pearson correlations between sensitivity indicators (all calculated using comparing hormone levels and CES-D score for the same week) revealed no significant relationships (see Table 7). However, two correlations approached significant levels. First, the Pearson correlation between weekly CESD and weekly person-centred absolute hormone change for E2 and P4 (p = .09) were inversely related. Second, sensitivity strength for P4 was positively related to sensitivity strength for ALLO (p = .09).

Table 6
Baseline Variables and Hormone Sensitivity Indicators

Variable	*B(SE)*	*p*
E2 Sensitivity Strength		
Age	-0.02 (0.02)	.221
Household Income	-0.01 (0.03)	.854
Education level	0.05 (0.08)	.494
# Biological Children	-0.04 (0.07)	.548
Current Anxiety	0.07 (0.14)	.618
Previous Anxiety	0.13 (0.14)	.379
Current Depression	0.37 (0.14)	.022
Previous Depression	0.14 (0.14)	.334
Late transition (vs. early)	-0.21 (0.14)	.145
Stressful Life Events	-0.11 (0.02)	.533
HFRDIS	-0.03 (0.04)	.389
PSQI	0.01 (0.03)	.831
P4 Sensitivity Strength		
Age	<.01 (0.02)	.853
Household Income	-0.01 (0.02)	.010
Education level	0.04 (0.08)	.631
# Biological Children	-0.04 (0.07)	.524
Current Anxiety	0.11 (0.14)	.433
Previous Anxiety	0.11 (0.14)	.433
Current Depression	0.31 (0.14)	.055
Previous Depression	0.08 (0.14)	.587
Late transition (vs. early)	0.07 (0.16)	.647
Stressful Life Events	0.01 (0.02)	.749
HFRDIS	-0.07 (0.04)	.080
PSQI	0.01 (0.02)	.783
ALLO Sensitivity Strength		

Age	<0.01 (0.02)	.854
Household Income	-0.02 (0.03)	.520
Education level	0.05 (0.07)	.528
# Biological Children	-0.06 (0.06)	.404
Current Anxiety	0.01 (0.14)	.929
Previous Anxiety	0.01 (0.14)	.499
Current Depression	0.13 (0.17)	.450
Previous Depression	-0.18 (0.13)	.198
Late transition (vs. early)	0.01 (0.15)	.947
Stressful Life Events	0.01 (0.02)	.395
HFRDIS	0.03 (0.04)	.476
PSQI	-0.03 (0.02)	.260

Table 7

Correlations Between Hormone Sensitivity Indicators

		P4	ALLO
	CES-D Corr	$r = -.04, p = .903$	$r = .12, p = .738$
	Abs-CES-D Corr	$r = -.56, p = .094$	$r = .34, p = .338$
E2	CES-D Strength	$r = -.35, p = .316$	$r = -.36, p = .312$
	CES-D Corr	-	$r = .03, p = .942$
	Abs-CES-D Corr	-	$r = .15, p = .682$
P4	CES-D Strength	-	$r = .56, p = .091$

Note. CES-D Corr = the Pearson correlation between weekly CESD and weekly person-centred hormone change; Abs-CES-D Corr = the Pearson correlation between weekly CESD and weekly person-centred absolute hormone change; CES-D Strength = the largest of CES-D Corr or Abs-CES-D Corr.

CHAPTER 4: Discussion

4.1 Summary of Results

The current study followed 26 perimenopausal women reporting increased depressive mood since the onset of the menopause transition. Once weekly for 15 weeks, participants completed a blood draw and reported their depressive symptoms, vasomotor symptoms, and sleep quality. The within-person relationship between weekly depressive symptoms and plasma levels of E2, P4, and ALLO were analyzed in 10 of these participants. In line with the primary aim of this investigation, the correlation between mood sensitivity to E2 and mood sensitivity to ALLO were examined, testing the possibility that mood effects of E2 were driven by changes in ALLO. Several key observations were made. All 10 participants were mood-sensitive to at least one of the three hormones assessed. Over half of the participants (60%) exhibited increased mood sensitivity to weekly changes in E2 and nearly all participants (80%) exhibited increased sensitivity to ALLO. However, in examining the relationship between sensitivity to E2 versus ALLO, it appeared that the correlations between E2 and ALLO sensitivity indicators were small and not statistically significant. Four participants were deemed sensitive to both E2 and ALLO but only one participant fell within the same sensitivity category for both hormones. These findings therefore suggest that that ALLO is unlikely to be the mechanism by which E2 influences mood, but that both hormones play an important independent role in perimenopausal depressive mood.

4.2 E2 Sensitivity and Perimenopausal Depressive Mood

Increased E2 fluctuation has long been hypothesized to be implicated in the etiology of perimenopausal depressive mood (Gordon & Sander, 2021). Several lines of recent research have supported this hypothesis: not only has E2 variability been found to be correlated with perimenopausal depressive symptoms (Gordon et al., 2016; Joffe et al., 2020; Willi et al., 2021), but experimental manipulation causing E2 withdrawal has also been shown to cause negative mood symptoms in those with a history of perimenopausal depression successfully treated with hormone therapy (Schmidt et al., 2015). Furthermore, stabilizing E2 levels with hormone therapy has proven to be effective in reducing the risk of perimenopausal depressive symptoms (Gordon et al., 2018). In a recent study by our team known as the Fluctuating Estrogen and Menopausal Mood (FEMM) study including 101 euthymic perimenopausal women, 39% of the sample exhibited an increased sensitivity to E2 using the same calculation method as the current study (Gordon et al., 2021). The proportion of E2-sensitive women was found to be higher (60%) in the current study, which was expected given that participants were individuals specifically reporting that they had noticed an increase in depressive mood since entering the menopause transition. Similar to the FEMM study, though, the nature of E2 sensitivity varied from person to person, with some exhibiting an increased propensity to developing depressive symptoms following E2 withdrawal (30%) and others following a large change in either direction (30%). No participants in the current study exhibited mood sensitivity to E2 increases, but this may have been due to the limited small sample size. The current study's findings may be interpreted as further evidence implicating increased sensitivity to perimenopausal E2 fluctuations in the etiology of perimenopausal depressive mood; however, the fact that 4/10 participants were not sensitive to E2 changes suggests that not all cases of perimenopausal depressive symptoms are triggered by increased E2 fluctuation.

4.3 ALLO Sensitivity, E2 Sensitivity and Perimenopausal Depression

To my knowledge, the current study is the first to investigate the relationship between within-person changes in ALLO and depressive mood in the menopause transition. Somewhat surprisingly, 8/10 participants were deemed mood-sensitive to ALLO. Similar to E2, however, the nature of this sensitivity varied from person to person. Specifically, 2/10 participants fell in the ALLO increase sensitivity category, 1/10 fell in the withdrawal category, and 5/10 fell in the ALLO change sensitivity category. This finding that the relationship between ALLO and mood varies in its directionality is consistent with the finding that while ALLO is typically associated with antidepressant and anxiolytic properties (Bitran et al., 1999; Shirayama et al., 2011), and sensitivity to ALLO withdrawal is implicated in postpartum depression (Kanes et al., 2017; Meltzer-Brody et al., 2018), increases in ALLO have been found to trigger negative mood effects in those with PMDD (Bäckström et al., 2021; Bixo et al., 2017; Martinez et al., 2016). Thus, prior research is consistent with our finding that ALLO changes can have opposite effects depending on the individual.

The primary aim of this investigation was to test the hypothesis that changes in ALLO may be a mechanism by which changes in E2 may modulate perimenopausal mood. For the most part, this hypothesis appears to be not supported. Inconsistent with research finding that E2 likely positively modulates levels of ALLO by increasing levels of the enzymes involved in the conversion of P4 to ALLO (Pluchino et al., 2009), levels of E2 were not found to predict levels of ALLO. Though not possible in the current partial dataset, follow up analyses in the full dataset will be conducted examining potential lags between E2 and ALLO is warranted. For example, E2 levels at week 1 may significantly predict ALLO levels at weeks 2, 3, or 4.

Though mood sensitivity to E2 and mood sensitivity to ALLO were both highly prevalent in this sample, contrary to my hypothesis, the direction of the sensitivity was, more often than not, in different directions. One possible explanation for this finding is that while mood sensitivity to ALLO may be the mechanism by which E2 changes are linked to depressive mood, our study was not designed appropriately to detect this relationship. For example, the weekly measures used in the current study may not have been adequate to detect individual variability in the time lag between hormone changes and mood changes. Indeed, in PMDD, there is research to suggest that individuals vary in the delay between menstrual cycle hormonal triggers and PMDD symptoms (Eisenlohr-Moul et al., 2020). Moreover, if E2 is acting via ALLO to influence mood, there may be a longer time lag between E2 and mood change as compared to ALLO. Thus, the E2 and ALLO sensitivity indicators, which were based on weekly measurements, may appear to act in opposite directions due to these differential timing effects. Experimental research directly manipulating E2 levels in women and measuring the resulting effect on ALLO and mood is best suited to characterize the time that it takes for E2 changes to exert effects on ALLO and, in turn, ALLO changes to exert effects on mood in different individuals.

Though our failure to detect a relationship between E2 sensitivity and ALLO sensitivity may be a product of the observational nature of the current study, it is also possible that E2 and ALLO simply exert independent effects on perimenopausal depressive mood. Thus, the etiology of perimenopausal depressive mood may vary from individual to individual. It may also be that while ALLO and E2 influence mood independently, they do so by shared mechanisms. Although this thesis focused on the effect that ALLO has on the $GABA_A$ receptor, there is also some animal model research demonstrating that E2 may modulate the sensitivity of the receptor to GABA (Schumacher et al., 1989) and downregulate the number of receptors in synapses (Mukherjee et al., 2017). Thus, E2 and ALLO may affect GABAergic transmission in different ways to impact depressive mood. In any case, better characterising each individual's sensitivity to each hormonal trigger may facilitate the future investigation of etiological pathways by which perimenopausal depressive mood is triggered, including genetic pathways.

While E2 sensitivity did not seem to be related to ALLO sensitivity in a straightforward way, increased sensitivity to P4 and ALLO were correlated. Moreover, person-centred levels of P4 positively predicted person-centred levels of ALLO throughout the study. This is in line with prior research in PMDD in which the experimental administration of a drug that blocks the conversion of P4 to ALLO caused luteal phase symptom remission (Martinez et al., 2016). In other words, it appears that mood sensitivity to P4 in the menopause transition is largely driven by mood sensitivity to ALLO, as is the case with PMDD. As P4 has received relatively less attention in perimenopausal depression, more research is needed to clarify the role of P4 changes in perimenopausal depressed mood.

To date, there has been little investigation of ALLO in the menopause transition. Only one cross-sectional study including late perimenopausal and early postmenopausal women has examined the relationship between ALLO and perimenopausal mood

(Slopien et al., 2018), finding that lower postmenopausal ALLO levels were correlated with depressive symptoms. However, this study was not designed to look at within-person effects of ALLO on depressive mood and was therefore not able to detect individual differences in the effect of ALLO on mood (Slopien et al., 2018). Our findings suggest that fluctuations in ALLO play an important role in contributing to perimenopausal depressive symptoms. This finding is consistent with prior studies identifying PMDD, a mood disorder that is known to be caused by an increased sensitivity to menstrual cycle changes in ALLO, as a risk factor for perimenopausal depressive mood (Bromberger et al., 2003; Dennerstein et al., 2004; Freeman et al., 2006; Freeman et al., 2004; Morse et al., 1998; Stewart & Boydell, 1993). It is also consistent with a recent hypothesis paper I published suggesting that an important subset of perimenopausal depression cases may, in fact, be instances of PMDD exacerbated by the hormonal environment of the menopause transition (Sander & Gordon, 2021). Future longitudinal research tracking mood and reproductive hormones among reproductive-aged women with PMDD into the menopause transition will be best suited to further clarify the relationship between PMDD and depressive mood in the menopause transition.

The current study's findings raise the question: Why was such a high proportion of our participants sensitive to the mood effects of ALLO? Are there other hormonal changes associated with the menopause transition, apart from exposure to extreme E2 levels, that could result in increased sensitivity to ALLO changes? One possibility may relate to exposure to extended low levels of ovarian hormones, such as during an anovulatory cycle or a very long early follicular phase. Indeed, there is evidence from rodent research that extended periods of stable low levels of ALLO, maintained through low-dose oral contraceptive injections, contributes to the increased expression of certain $GABA_A$ receptor subunits in the brain (Follesa et al., 2002). Furthermore, as previously described, the effect of ALLO at the $GABA_A$ receptor has shown to be reversed by high hormonal flux (Griffiths & Lovick, 2005; Lovick et al., 2005; Shen et al., 2007). Indeed, although this is an area in need of further research, it has been hypothesised that changes in the $GABA_A$ receptor due to changes in the relative hormonal environment after childbirth may result in increased sensitivity of this receptor to ALLO in late onset postpartum depression (Burke et al., 2019). In other words, ALLO's psychological effects may be amplified or altered in some way following extended periods of low ALLO.

In light of the above research, it seems highly relevant that ovulation-induced increases in P4, and therefore ALLO, become less and less frequent as a woman progresses in the menopause transition. Anovulatory cycles are also often characterised by very low and stable levels of E2 (Burger et al., 2005; Santoro et al., 2017), presumably further contributing to abnormally low levels of ALLO for extended periods of time. It may therefore be that some instances of elevated depressive mood in the menopause transition are related to an increased sensitivity to ALLO increases following extended periods of low ALLO. Though the current study's small sample size and relatively short time frame limits the possibility of testing this hypothesis, future research in this area seems warranted given the apparent importance of ALLO in triggering depressive mood highlighted by this study.

4.4 The Transient Nature of Perimenopausal Depressive Symptoms

Notably, although many participants consistently scored highly on the CES-D (i.e., 38% scoring a 16 or above on 8 or more weeks), many participants exhibited more short-lived, transient bouts of clinically significant depressive symptoms. Furthermore, although all participants indicated worsening mood with onset of perimenopause at baseline, only a small proportion (12%) met criteria for a depressive disorder at baseline. Interestingly, during the baseline psychiatric interview, many participants did not meet criteria for a major depressive disorder because their symptoms had not been present for two weeks or more. Although many described significant clinical symptoms of depression, participants commonly described symptoms that “come and go” within a few days at a time. This is in line with the notion that depressive symptoms may fluctuate considerably due to rapidly changing hormone levels. In the FEMM study, similar patterns of transient increases in depressive symptoms were observed in the absence of satisfying criteria for major depressive disorder. In phase two of that study, participants completed 9 monthly CES-D assessments, and if they scored a 16 or above, they also completed the mood module of the SCID-5-RV over the phone. Although 59% of participants scored above the clinical threshold in the CES-D, only 17% of participants met criteria for at least one major depressive episode over that time (Gordon et al., 2021). Previous research also indicates that while the risk of experiencing clinically significant depressive symptoms increases in the general population during the menopause transition, the risk of major depressive disorder may only be elevated in women with a prior history of major depression (Bromberger et al., 2011; Bromberger et al., 2015). Thus, our findings support the conceptualization of perimenopausal depressive mood as a reproductive mood disorder that is similar to PMDD – characterized by symptoms that come and go according to menstrual cycle events.

Interestingly, our results indicate that those who met criteria for a depressive disorder at baseline were more likely to have an increased sensitivity strength for E2, but there was no such relationship for ALLO or P4. Although this should be interpreted with caution given the very low number of participants who met criteria for a current major depressive disorder in the current sample, these results may suggest that E2-driven mechanisms for mood change, separate from changes in ALLO, may be particularly relevant in the etiology of longer-lasting, clinically significant, depressive mood in the menopause transition. More research is needed to investigate the mechanisms by which increased sensitivity to E2 changes causes an increased risk of major depressive episodes during perimenopause as opposed to transient increases in depressive mood. Studies measuring mood and related constructs in relation to the menstrual cycle, although not designed to tease apart the effects of E2 and ALLO, suggest that E2 may influence a variety of psychological processes relevant to depressive mood (Sander, 2020). Specifically, menstrual cycle fluctuations in E2 have been found to be related to changes in rumination after a laboratory sad mood induction, (Graham et al., 2018), the perception of emotions in others (Derntl et al., 2008; Derntl et al., 2013; Rubinow et al., 2007), memory of emotional stimuli (Pompili et al., 2016; Wegerer et al., 2014) and sensitivity to psychosocial rejection and stress (Sander, 2020). This is also in line with the effects that E2 has on emotion-centres of the brain; the limbic regions are known to be populated with E2 receptors (McEwen, 2001).

4.5 Other Possible Mechanisms Involved in Perimenopausal Depressive Mood

The current study's findings suggest that further research investigating the mechanisms by which E2 affects mood is warranted. For example, the modulation of serotonin by E2 may be a mechanism worthy of further investigation. Serotonin, a known target for many antidepressants, has long been thought to play a role in depression (Coppen, 1967). Moreover, there is a body of research that suggests that E2 has downstream effects on serotonin levels (Hernández-Hernández et al., 2019). Although the relationship between serotonin and mood is likely not direct nor straightforward (Jauhar et al., 2023), decreases in serotonin have been linked to depressive mood. One study that experimentally reduced E2 in reproductive-aged women found that E2 withdrawal resulted in increased binding of the serotonin transporter responsible for reuptake, indicating lower synaptic levels of serotonin, and resulting in increased depressive symptoms (Frokjaer et al., 2015).

Another potential mechanism concerning the effect of E2 withdrawal on mood involves the various neuroprotective effects of E2 (Garcia-Segura et al., 2001). Specifically, disruptions in brain-derived neurotrophic factor (BDNF), an important growth factor that promotes neuron growth and survival, has been linked to depression (Yu & Chen, 2011). Moreover, E2's neuroprotective effects may in part mediated by BDNF (Scharfman & MacLusky, 2006). However, research on both PMDD (Oral et al., 2015) and perimenopausal depression (Harder et al., 2022) have found higher BDNF to be related to mood symptoms, though this was hypothesized to reflect a possible compensatory process (Oral et al., 2015). Thus, more research is needed to clarify the role of BDNF in relation to reproductive hormones and negative mood symptoms.

E2's anti-inflammatory effects have also been investigated as a mediator in the relationship between E2 withdrawal and perimenopausal depressive symptoms. Granted, few studies have investigated this hypothesis, and two studies have found markers of inflammation to be unrelated to perimenopausal depressive symptoms (Karaoulanis et al., 2012; Matthews et al., 2007). One compelling study, on the other hand, found that an established mood-sensitivity to E2 withdrawal combined with a history of perimenopausal depression is associated with heightened expression of proinflammatory gene CXCL10 (Rudzinskas et al., 2021). More research is needed to uncover the role of inflammation in perimenopausal depression.

It is also possible that E2 interacts with other hormones changing throughout the menopause transition not assessed in the current study may be at play. A recent study by our team that assessed E2 and testosterone every 3 weeks in perimenopausal women over the course of 3 months found that the testosterone-to-estradiol ratio is positively associated with depressive symptoms (Sander et al., 2021). However, testosterone levels on their own were not found to be related to depressive symptoms. Other studies, which assessed hormones less frequently, have been mixed, with some finding higher testosterone to be related to perimenopausal depressive symptoms (Bromberger et al., 2010; Milman et al., 2015) and another finding testosterone to be unrelated (Woods et al., 2008). Another androgen that has been investigated for its potential role in perimenopausal depression is dehydroepiandrosterone sulfate (DHEA-S). However, findings regarding this hormone have been fairly mixed, with some studies finding negative mood in perimenopause to be related to decreases in DHEA-S (Barrett-Connor et al., 2015; Rudzinskas et al., 2021; Schmidt et al., 2002; Schmidt et al., 2005), others finding a significant relationship with DHEA-S increases (Morrison et al., 2011), and another finding no effect (Bromberger et al., 2010). Moreover, these studies used infrequent assessments, were often cross-sectional, and often found no relationship between E2 and mood when E2 was assessed (Barrett-Connor et al., 2015; Schmidt et al., 2002).

Genetics is a natural area of interest when investigating the mechanisms of hormone sensitivity in perimenopausal mood, as genetic differences could potentially explain the large degree of individual variability observed in the relationship between hormone changes and mood. There have been a few interesting studies in this area to date, with some studies finding depressive symptoms to be associated with differences in genotypes related to estradiol (Rebbeck et al., 2010; Różycka et al., 2016; Zhang et al., 2017) and neurotransmitter systems (Różycka et al., 2016) in perimenopausal women. Given our findings, the genetic contributions to particular profiles of ALLO and E2 mood sensitivity could be examined.

4.6 Non-Hormonal Contributors to Perimenopausal Depressive Symptoms

A secondary aim of this work was to examine other contributors to depressive mood in this population, including baseline characteristics such as lifetime stress and prior mental health as well as weekly changes in menopausal symptoms including sleep disruption and vasomotor symptoms. The findings pertaining to this investigation are discussed below.

4.6.1 Stressful Life Events

A greater number of recent stressful life events at baseline was a strong predictor of both continuous depressive symptoms as well as instances of clinical elevations in depressive symptoms. Specifically, every additional two stressful life events reported in the last six months was associated with a 1.2-point increase on the CES-D and one additional clinical elevation in depressive mood over the 15-week study. This is in line with previous reports that a greater number of recent upsetting life events is associated with a higher risk for a major depressive episode during the menopause transition (Bromberger et al., 2011). Of course, life stressors are known to be an important causal factor for depression generally (e.g., see Kendler et al., 1999).

In the current study, however, a greater number of stressful life events was not related to increased sensitivity to E2 or ALLO changes. This is not in line with several prior studies regarding the interaction between E2 changes and recent stress exposure on depressive mood in perimenopause. Specifically, a study testing the prophylactic effects of transdermal E2 and micronized P4 found that hormone therapy was more effective for those with a greater number of recent life stressors (Gordon et al., 2018). Another study assessing reproductive hormones and depressive symptoms four times over 14 months reported an that increased E2 variability was predictive of higher depressive symptoms at the end of the study only in women with greater numbers of baseline stressful life events (Gordon et al., 2016). Moreover, it has been hypothesized that the effects of E2 on mood are moderated by the downstream effects that ALLO has on biological stress systems (i.e., the hypothalamic-pituitary-adrenal or HPA axis) (Gordon et al., 2015). It is possible that the current study was merely underpowered to detect the interaction between stressful life events and neurosteroid sensitivity.

4.6.2 Prior History of Depression

A prior history of depression was predictive of greater continuous depressive symptoms such that those with a history of depression scored an average of 3.7 points higher on the CES-D relative to those without a history of depression. Those with a history of depression also experienced an additional 2.9 instances of elevated depressive symptoms relative to those without a history of depression. These findings are consistent with previous research that has found previous depression history to be a strong predictor of major depression during perimenopause (Bromberger et al., 2011; Bromberger et al., 2015). Given our participants all endorsed increased depressive symptoms at baseline, the relatively high prevalence of past depressive episodes (42%) is also consistent with this prior research that a history of depression is a strong risk factor for depressive symptoms in the menopause transition.

Though the current study was limited in its statistical power to detect statistically significant effects, those with a history of depression were not found to be more sensitive to hormone changes relative to those with no history of depression. These findings are consistent with the findings of the FEMM study described earlier in which 101 perimenopausal women were assessed weekly for 12 weeks (Gordon et al., 2021). Similar to the current study, FEMM study participants with a prior history of depression were not found to be more hormone sensitive than those without. However, the current finding that meeting criteria for a depressive disorder at baseline was related to E2 sensitivity strength may suggest that recent depression history may indeed play a role. Regardless, midlife women with a prior history of depression should be advised by their healthcare provider that the menopause transition represents a high-risk time for them.

4.6.3 Anxiety

Though a prior history of anxiety was not predictive of depressive symptoms or instances of elevated symptoms in multivariate analyses, both current and previous anxiety disorders were highly prevalent in our sample. Previous research indicates that anxiety may be a risk factor for experiencing depression during perimenopause (Bromberger et al., 2015; Kravitz et al., 2014). Although the focus of this thesis has been on the role of ALLO on depressive symptoms, ALLO levels are also thought to have important effects on anxiety levels as well (Bali & Jaggi, 2014). The connection between ALLO and anxiety symptoms has been studied in the other reproductive mood disorders; for instance, second trimester ALLO has been found to predict postpartum anxiety symptoms (Osborne et al., 2019).

Although the current study did not measure weekly levels of anxiety symptoms, this is an important topic to further explore. More research studies that frequently track anxiety symptoms across the reproductive lifespan are needed. There is some cross-sectional evidence of anxiety symptoms increasing during the menopause transition (Tangen & Mykletun, 2008), and in one longitudinal study, the risk for high anxiety symptoms was increased during perimenopause and postmenopause in those who reported low levels of anxiety at baseline. Those with high levels of baseline anxiety experienced similar higher rates of symptoms across reproductive phases (Bromberger et al., 2013). Additional research investigating hormonal sensitivity profiles in relation to repeatedly measured anxiety symptoms may shed light on the effect of the perimenopausal hormonal environment on anxiety.

4.6.4 Other Psychosocial Variables

Although the current study focused on biological mechanisms of perimenopausal depression, it should be noted that various social and psychological variables are likely at play in the development of perimenopausal depression. Indeed, major etiological theories of depression in general emphasize a biopsychosocial model in which a combination of personality traits, genetic predispositions, cognitions, disruptions in brain chemistry, emotions, and life events may all play a role in the development of any major depressive episode (e.g., see Garcia-Toro & Aguirre, 2007). For example, recent stressors may combine with biological vulnerabilities to result in depressed mood, as described already in this work. This is commonly known as the diathesis stress model – a major conceptual framework in the etiology of mental disorders originating from early work in schizophrenia research (Meehl, 1962). There are several psychosocial factors that have been found to increase one's risk for perimenopausal depression. For example, a history of childhood adversity, having less education, being single, and experiencing health problems or physical symptoms, interpersonal problems, stressful events, and financial difficulties are all associated with depressive symptoms during perimenopause (Bromberger& Epperson, 2018). Psychological traits or tendencies such as neuroticism (a greater vulnerability to experiencing negative emotions), rumination, and pessimism may also increase one's vulnerability to depression in midlife (Bromberger & Epperson, 2018). A recent study, which also found E2 variability to be a predictor of perimenopausal depressive symptoms, also found that poor body image and self-esteem predict depressive symptoms in the perimenopause (Willi et al., 2021).

Although psychosocial factors inevitably play an important role in depressive symptoms during perimenopase as with any other phase of life, social aspects of the transition itself, previously believed to account for the increased depression risk during this time, do not appear to fully account for these symptoms. While early research in this area primarily focused social factors conceptually associated with midlife, including "empty nest syndrome" as women experienced their grown children moving out of the family home (Raup & Myers, 1989), most women actually experience an increase in wellbeing during this time (Harkins, 1978; Mitchell & Lovegreen, 2009; Perrig-Chiello & Sturzenegger, 2001; Radloff, 1980). The empty nest theory is also flawed because many perimenopausal women continue to have children live in the home; 73% of our sample lived with children. Interestingly, in the current study, having more biological children was predictive of lower weekly depressive symptoms. Although one may hypothesize that a greater number of previous pregnancies (and exposure to their associated hormonal changes) may prepare women for the hormonal changes of the menopause transition, a greater number of biological children was not significantly related to reduced sensitivity to any of the neurosteroids in this sample. More negative and outdated notions of menopause hold that menopause is a time of loss of fertility, libido, and femininity (Deutsch, 1984). Yet, there is little-to-no evidence that mourning one's fertility or lossed sense of femininity explains the increased risk for depression during the menopausal transition. In fact, most women have neutral or positive attitudes towards menopause (Avis & McKinlay, 1991).

4.7 Vasomotor Symptoms and Sleep Quality

Another important dimension of perimenopause is the bothersome physical symptoms that many individuals experience, such as vasomotor symptoms and sleep difficulties. These symptoms were present in our sample at both baseline and in the weekly measures phase. At baseline, women reported significant issues with sleep on the PSQI but only mild levels of interference in their lives overall due to hot flashes. Hot flashes related interference was rated as moderate, however, for sleep. Regarding weekly symptoms, the daily vasomotor symptoms reported by participants were mostly mild, and mean sleep quality throughout the weekly phase was fairly good. However, there was considerable variability in these symptoms and participants demonstrated a range of scores from week to week. Participants reported an average of three vasomotor symptoms per day, which is less than the 5.9 reported in a previous study using the same measure (Gordon et al., 2021). This may be explained by the higher proportion of women in the late compared to early perimenopausal stage in that previous study (87% versus 50% in the current study). Indeed, older age was a significant predictor of moderate and severe vasomotor symptoms in the current study. Interestingly, a greater number of recent life stressors also predicted more moderate and severe vasomotor symptoms throughout the study. This could be explained by the effect of stress on one's appraisal of these symptoms. One previous study reported that stress, anxiety, and one's tendency to experience somatic sensations intensely predicts greater vasomotor symptom problem ratings as moderated by negative beliefs about such symptoms (Hunter & Chilcot, 2013).

Although baseline scores on the PSQI and HFRDIS did not predict levels of weekly depressive symptoms, there was a significant relationship between concurrently measured weekly symptoms, suggesting that bothersome physical symptoms of perimenopause may contribute to within-person fluctuations in depressive symptoms. Mild vasomotor symptoms did not emerge as a significant predictor, which indicates that only more severe and bothersome symptoms had a significant impact on depressive mood. Regarding the effect on continuous CES-D score, both sleep and moderate and severe vasomotor symptoms appear to contribute to depressive mood on their own, as the effects of both variables remained significant when included in the same model. On the contrary, for number of clinically elevated CES-D scores, sleep was no longer a significant predictor when included in the same model as moderate and severe vasomotor symptoms.

Our finding that weekly levels of vasomotor and sleep symptoms were related to higher depressive symptoms reflects the reports of a considerable body of research reporting significant relationships between the uncomfortable physical symptoms of perimenopause and depressive symptoms (Avis et al., 2001; Bosworth et al., 2001; Bromberger et al., 2003; Bromberger et al., 2010; Bromberger et al., 2015; Freeman et al., 2006; Joffe et al., 2002). In the context of the aforementioned domino theory, which posits that perimenopausal vasomotor symptoms cause sleep disturbance, which then go on to cause depressive symptoms, our findings provide mixed support for this theory. Though there was a relationship between weekly levels of vasomotor symptoms, sleep disturbance, and depressive symptoms, and at baseline, participants rated moderate levels of sleep interference due to hot flashes, both sleep and hot flashes appeared to independently contribute to depressive symptom score when both variables were included in the same statistical model. The low levels of hot flash-related interference for both mood and quality of life at baseline furthermore suggest that these symptoms do not account for the high rate of depressive symptoms in this sample. Baseline scores on the PSQI and HFRDIS were also not found to predict sensitivity strength for any of the neurosteroids – thus it seems likely that sensitivity to hormone changes, sleep disturbance, and hot flashes, are all independent predictors of depressive mood in the menopause transition.

4.8 Strengths and Limitations

There are several important strengths of the current study. The longitudinal design consisting of frequent weekly assessments of mood and hormones allowed for a better depiction of the relationship between the studied variables compared to previous studies that assessed hormones cross-sectionally. Infrequent measures of hormones may not be well-suited to capture the considerable variability of fluctuations in hormones and mood in perimenopause. This design also allowed for the within-person analysis of the relationship between hormones and mood while taking into account the considerable amount of individual variability observed in the presence and directionality of the relationship between E2, ALLO, and depressive mood. Lastly, unlike our previous FEMM study that used similar methods minus the measure of ALLO (Gordon et al., 2021), the current study specifically recruited individuals who reported noticing an increase of depressive symptoms or mood swings since entering perimenopause, and all participants reported clinically significant levels of depressive symptoms at baseline as measured by the CES-D. This additional criterion allowed us to study individuals with clinical levels of depressive symptoms while potentially excluding those with normal, healthy mood fluctuations.

However, our results must be considered in the context of some notable limitations. Of course, the study was limited by a small sample size and a partial dataset of weekly hormones. Due to this limitation, the current analyses were also not corrected for multiple comparisons, which may have inflated the number of significant effects reported. Moreover, considering the magnitude of individual variability observed in the relationship between E2 and mood (Gordon et al., 2021), a larger sample size is important in capturing degrees of individual variability. Another weakness concerns the observational (as opposed to experimental) design, which does not allow for the inference of causation regarding the effects of neurosteroids on depressive mood. One important consideration is that participants may have been exaggerating their depressive symptoms at the beginning in order to meet eligibility criteria considering that the baseline mean CES-D score was much higher than the mean weekly CES-D score. Relatedly, conducting monthly psychiatric interviews (instead of just at baseline) would have allowed for the assessment of major depressive episodes instead of merely relying on the CES-D. Finally, a challenge encountered repeatedly during data collection was ensuring participants completed surveys on time. As a result, there were a number of late surveys included in the current analysis. Going forward for the larger study, more resources will be devoted to ensuring participants are completing surveys on time.

4.9 Future Directions

The limitations listed above represent planned improvements for future research. First of all, the current study represents partial data on a subgroup of participants from a larger project with a planned sample size of 100 participants. This larger sample size will be better equipped to capture the large degree of individual variability between hormone changes and mood. Another change going forward is that given the large proportion of participants with current and previous anxiety disorders in this subsample, participants will now complete an additional brief measure of anxiety symptoms each week. This will allow us to further characterize anxiety symptoms across the menopause transition, their relation to perimenopausal depressed mood, and their relation to fluctuating neurosteroid levels. Finally, as mentioned previously, there are other mechanisms of interest to be studied in the context of perimenopausal depression. With participants' permission, blood samples from the current study may be used for additional analyses on such mechanisms (e.g., genetics, other hormones, etc.). Beyond publication in a peer-reviewed journal, several forms of knowledge translation (e.g., social media posts, infographics, pamphlets, a brief video) will be pursued so that the results of this research may reach a greater public audience.

Ultimately, this research should be translated to an experimental design in order to determine whether changes in E2 cause changes in ALLO and mood symptoms. Moreover, in an experimental design that administers E2 in the absence of endogenous levels of E2 and P4, the timing of E2's effects (e.g., the E2-mood lag) can be clarified. Ideally, this research would include postmenopausal individuals with a confirmed history of E2 mood sensitivity during the menopause transition. Selected participants from the current study, who were asked permission to be contacted for follow-up research, may be such an ideal sample for this future experimental work.

4.10 Significance

Perimenopausal depression impacts a significant minority of women (Maki et al., 2019) and results in lower quality of life and increased disability (Wariso et al., 2017).

Though perimenopause is a very common experience that approximately half of adults will experience, little is known about how the hormonal changes of this transition influence mental health. The complex hormonal dynamics of the menopause transition represent a unique period of particularly increased risk for mood disturbance in those that are sensitive to hormonal changes. This research, albeit preliminary, is the first to suggest that a large proportion of women experiencing depressive mood in the menopause transition are mood-sensitive to changes in ALLO. Additional research investigating ALLO's role as well as the association between perimenopausal depression and other ALLO-related reproductive mood disorders is warranted. This line of research may help identify more targeted treatments for mental health problems in the menopause transition. ALLO-based medications are already demonstrating preliminary success in treating other disorders in which E2 is etiologically implicated, such as PPD (Meltzer-Brody et al., 2018) and PMDD (Bixo et al., 2017). Thus, although additional research with larger samples and experimental designs manipulating E2 levels will be needed to confirm our findings, this proposed work represents an important first step in understanding of the biopsychological correlates of perimenopausal depression, potentially identify treatment targets for this important disorder, and more broadly, extending current understanding of how hormones influence one's experiences and behaviour.

4.11 Conclusion

In summary, the current study is the first study implicating increased sensitivity to ALLO changes in the development of perimenopausal depressive mood. Though further analyses in a larger sample will be needed to confirm these findings, these preliminary findings suggest that additional research investigating ALLO in the menopause transition as well as further research investigating the link between perimenopausal depression and other ALLO-related mood disorders, namely premenstrual dysphoric disorder and postpartum depression, are warranted.

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Appendix A – Social Media Advertisement

The University of Regina's Women's Mental Health Research Unit is seeking individuals in the menopause transition experiencing mood changes to participate in a 15-week study aimed at understanding the biological basis of perimenopausal mood changes. Individuals in the age range of 42-55 with a uterus and at least one ovary living in the Saskatoon area may be eligible to participate. Participation will involve completing weekly questionnaires and hormone sampling via blood and urine collections. Participants will receive up to $400 in compensation for completion of the project. Please message us if you are interested!

Appendix B – Study Information for Women's Mid-Life Health Centre

Researchers at the University of Regina's Women's Mental Health Research Unit are conducting a research project on the role of hormones in perimenopausal mood changes.

Why am I being approached?

- This study is recruiting individuals in the menopause transition ages 42-55 living in Saskatoon who have been experiencing mood changes since entering the menopause transition

- Eligible participants must have a uterus and at least one ovary and not be taking any medications that affect hormones or mood

What does this study entail?

- Participation in this study will involve 15 weekly measurements of hormones via blood and urine samples as well as weekly mood surveys

- For completing the research project, participants will be compensated up to 400$

What is the purpose of the study?

- The purpose of this research is to understand the hormonal basis of perimenopausal mood changes

Interested participants will be given our email address to contact the research team at: wmh.research@uregina.ca

Appendix C – Eligibility Survey

Hello, thank you for your interest in our research study! This brief survey is intended to determine if you are eligible to participate in our research project. All responses are kept strictly confidential.

Please enter the participant ID given to you by the research team: ____

1. How old are you? _______________

2. Do you have a uterus and at least one ovary?
   ☐ Yes ☐ No

3. Do you live in Saskatoon?
   ☐ Yes ☐ No

4. Do you take any antidepressants, anti-anxiety medication, oral contraceptives, hormonal birth control, or hormone therapy?
   ☐ Yes ☐ No
   [If yes] Please specify what you take:
   ____

5. Are you currently pregnant or nursing?
   ☐ Yes ☐ No

6. Are you currently in therapy for depression?
   ☐ Yes ☐ No
   [If yes] Please describe the frequency and nature of the therapy you're receiving:
   ____
   ____

7. Have you ever been diagnosed with a psychotic or bipolar disorder?
   ☐ Yes ☐ No

8. When was the last time you had a menstrual period?
   ☐ Within last 12 months ☐ It's been more than 12 months

9. What was the start date of your most recent menstrual period?
   ____

10. Have you recently experienced <u>irregularity in your menstrual cycle</u>? (e.g., menstrual period beginning earlier or later than expected, missing menstrual periods entirely, or experiencing a long amount of time without a menstrual period)

☐ Yes ☐ No

11. Check all that apply:
    ☐ I have had regular menstrual cycles throughout my life (before entering the menopause transition)
    ☐ In the last year or two, I have had at least two consecutive menstrual cycles that are at least one week longer or one week shorter than what is typical for me. In other words, my period has come at least 7 days early or late at least twice.
    ☐ In the last year or two, I have experienced 60 days or more without a menstrual period

12. Have you noticed an increase in depressive symptoms or mood swings since you began experiencing changes in your menstrual cycle?
    ☐ Yes ☐ No
    [If yes] Please elaborate
    ____

13. What prescription medications do you take or have you taken in the last month?

    ____

14. Do you take any herbal supplements? If so, please list them.

____

15. Do you have any medical condition affecting hormone levels? (e.g., polycystic ovarian syndrome)
    ☐ Yes ☐ No

    [If yes] Please specify which condition you have:
    ____

16. Have you had thoughts of death or suicide or a suicide plan anytime within the last two weeks?

    ☐ Yes ☐ No

If the participant answers "Yes" to # 16, the participant will be prompted to complete the online ***Suicide Behaviours Questionnaire-Revised*** *in the same survey, below:*

1. Have you ever thought about or attempted to kill yourself? (check one only)
    □ 1. Never

□ 2. It was just a brief passing thought
□ 3a. I have had a plan at least once to kill myself but did not try to do it
□ 3b. I have had a plan at least once to kill myself and really wanted to die
□ 4a. I have attempted to kill myself, but did not want to die
□ 4b. I have attempted to kill myself, and really hoped to die

2. How often have you thought about killing yourself in the past year? (check only one)
□ 1. Never
□ 2. Rarely (1 time)
□ 3. Sometimes (2 times)
□ 4. Often (3-4 times)
□ 5. Very Often (5 or more times)

3. Have you ever told someone that you were going to commit suicide, or that you might do it? (check one only)
□ 1. No
□ 2a. Yes, at one time, but did not really want to die
□ 2b. Yes, at one time, and really wanted to die
□ 3a. Yes, more than once, but did not want to do it
□ 3b. Yes, more than once, and really wanted to do it

4. How likely is it that you will attempt suicide someday? (check one only)
□ 0. Never
□ 1. No chance at all
□ 2. Rather unlikely
□ 3. Unlikely
□ 4. Likely
□ 5. Rather Likely
□ 6. Very Likely

Ending message: Thanks so much for taking the time to answer our questions! A member of our research team will be in touch shortly to discuss your eligibility and next steps.

Appendix D – Zoom Enrollment Protocol

Hello __________*, my name is* _________*, and I will be giving you some more information about our research study today. Thank you so much for taking the time to complete our eligibility survey! We're just going to take a minute now to go over your answers to* our *survey, just to make sure we have all of the necessary information [review responses and request clarification, if necessary, especially regarding changes in mood and menstrual cycles].*

For today's meeting, I will start by giving you an overview of the study procedures and go over any other relevant information in the consent form. If you consent to participate, I will then ask you a series of questions about your mental health. Does that sound okay?

Before we begin, did you receive an email with the consent form and enrollment package attached? I invite you to open the consent form and enrollment package and follow along if you find that helpful as we go through it.

Great, and have you had the chance to read through the consent form?

If the participant responds no: *That is alright. I will be going over the consent form with you later in today's meeting, but if you need more time to read through the consent form and think about it before consenting to participate, that is okay! We can schedule a separate meeting for the mental health interview if you end up wanting to participate but need some time to decide.*

If the participant responds yes: *Great, I will be giving you the opportunity to ask any questions you may have once I review the research procedures and consent form.*

Study Procedure and Consent Form Overview

[Shares screen to PowerPoint Slide]

Research assistant presents each power point slide and reads the corresponding script for each slide.

*See file entitled Enrollment Presentation.pptx

Ask for Consent

Do you have questions regarding the procedures and goals of the study? Do you think you are interested in participating? If you do not wish to continue or if you need some time to think about it, that is okay.

If not interested or needs more time: Thank them for their time and end the call.

If interested: *By consenting to participate, you attest that you have read and understood the description provided, that you have had an opportunity to ask questions and have those questions answered, and that you consent to participate in the research project. A copy of this consent form has been given to you for your records.*

Do you consent to participate in the study?

[If yes] Great, now before we begin the mental health interview, I have a few questions.

(Research assistant will record answers in tracking log),

First, as indicated on the consent form, your biological samples may be kept indefinitely to investigate future research questions. You may still participate in the study if you do not wish to have these samples stored for future analysis. Please tell me if you agree or disagree for your samples to be stored for additional, future analysis.

Another section on the consent form addresses the follow-up study. It is not guaranteed that you will be eligible for the follow-up study or that you will be contacted, and if you are contacted in the future about the follow-up study, you are in no way obligated to participate. Please tell me if you agree or disagree to being contacted about possible participation in a follow-up study.

Can you provide me with your mailing address so that I can send you the necessary materials?

In order to receive compensation, we will need your SIN and DOB, which will be kept secure in a password-protected file. Are you comfortable providing me this information verbally now?

Next, do you wish to be emailed a summary of the results when the project is complete?

Finally, do you have a day of the week that would work best for the blood collection and mood surveys? Remember that this will be done on the same weekday each week for 15 weeks. If you need time to think about this or change your mind before we begin weekly measurements, that is alright. We will be confirming the day you choose once you have received all the materials.

*Proceed to SCID-5-RV Interview (see separate documents titled SCID Mood and SCID Anxiety)

Once SCID-5-RV is complete: *That concludes our meeting today! Thanks again for your time, and remember that you can contact us with the information provided on both the consent form and enrollment package if any questions come up.*

I will be emailing you the baseline survey to be completed whenever is convenient for you. We will also be sending a kit with all the study materials to your home.

We will follow-up with you by email within the next week to confirm whether you have finished the baseline survey and received the materials. We will talk then about when you *will begin the weekly measurement phase. If you receive the kit before we contact you, please refrain from collecting any urine samples until we tell you to do so.*

Appendix E – Consent Form

Participant Consent Form

Project Title: Neurosteroid Fluctuation in Perimenopausal Depression

Researcher(s):

Bethany Sander, MSc Student, Department of Psychology, University of Regina, sander2b@uregina.ca

Jennifer L. Gordon, Associate Professor, Department of Psychology, University of Regina, 306-585-4389, Jennifer.gordon@uregina.ca

Duncan Preston, Undergraduate Honours Student, Department of Psychology, University of Regina, wmh.research@uregina.ca

Sarah Gulash, Research Coordinator, Department of Psychology, University of Regina, wmh.research@uregina.ca

Purpose(s) and Objective(s) of the Research:

There is a significantly increased risk for experiencing depression during the menopause transition. Previous research suggests that increased fluctuations in sex hormones, such as estrogen, during the menopause transition, may play a role in causing this increased rate of depression. However, it is unclear what processes explain estrogen's effect on mood during the menopause transition. The purpose of this research study is therefore to better understand the processes that explain the link between estrogen changes and depression during the menopause transition with a focus on the hormone allopregnanolone. Finally, a secondary purpose of this study is identifying an eligible sample (i.e., those who display a relationship between estrogen levels and mood) for a separate follow-up study involving receiving hormone therapy.

Procedures:

Participant involvement in this study is intended to last approximately 15 weeks, as outlined below:

1. **Enrollment:**

   a) **Zoom Enrollment Session:** You will be given a description of the study and the consent form will be explained. If you agree to participate, the researcher will ask you series of questions about your mental health history. The entire session will take approximately 45 minutes to complete.

b) **Baseline Surveys:** A link to the baseline questionnaires taking approximately 45 minutes to complete will be emailed to you. Questionnaires will collect data about your medical history, history of traumatic and stressful events, menopausal symptoms, mood, and pain history. You do not have to answer any questions that you do not want to.

c) **Delivery of Materials:** Following enrollment into the study, the following materials will be delivered to your home:

- A small cooler (used to store the samples on their way back to our research facilities at the end of the study)
- Fifteen plastic cups for collecting urine
- Fifteen disposable syringes for transferring urine from the cup to the plastic tubes
- Thirty small plastic tubes for storing urine samples
- A tray to store the urine samples
- A printed copy of the enrollment package and the hot flashes tracking logs

2. Mood Assessment and Biosample Collection:

a) **Mood Surveys:** Once a week for fifteen weeks, you will be asked to complete a brief (10-15 minute) survey in the evening asking you about your mood, current menstrual pain/interference (if applicable), as well as your sleep quality from the night before.

b) **Blood Collection/Pain Survey:** That same day you complete the mood survey each week, you will be asked to meet a certified phlebotomist to collect a sample of your blood (12 mls = a little less than a tablespoon) in a private and convenient location of your choice such as your workplace or home. This sample must be taken between 11:00 a.m. and 1:00 p.m. each week and will be used to measure your levels of allopregnanolone, a compound that is believed to play a role in depressive mood in the menopause transition. The phlebotomist will be fully vaccinated for COVID-19 (including booster), wear a mask and gloves, use appropriate sanitary methods, and come equipped with a biohazard sharps container. Following the blood collection, you will complete a brief survey regarding any pain and anxiety you felt during and after the blood collection.

c) **Hot Flashes Tracking:** Starting at 8:00 a.m. on the day of blood collection, you will be asked to begin tracking your hot flashes using a hot flash tracking sheet until 8:00 a.m. the following day. That following day (after completing a urine sample, outlined below), you will be sent another online survey asking you to report the number of hot flashes and night sweats you recorded.

d) **Urine collection:** The day after you complete the mood questionnaire and blood collection, you will be asked to collect a urine sample (4 mls) at home one day a week for fifteen weeks. The urine samples are to be collected first thing in the morning, stored in your home freezer, and will be used to measure your levels of estrogen.

e) **Ending Survey:** Once you have completed the weekly measures phase, you will be sent a brief online survey to assess for factors that may have affected your results and your current menopausal status at the time you finish the study. You will also have the option to provide answers to some open-ended questions if you wish.

f) **Biological Sample Pickup and Storage:** At the end of the study, we will retrieve your urine samples and deliver them to our facilities to be analysed. Urine samples will be shipped to the Endocrine Laboratory at the University of Saskatchewan while the blood samples taken by the phlebotomist will be shipped to the Women's Mental Health Research Unit at the University of Regina. Only the study's researchers and other key faculty and research personnel associated with this project are authorized to access these samples. At all times, your samples will only be identified by the name of the research study, your study identification code, and the time of collection. The electronic file that links your study code will be maintained separate from your samples on a secured, password protected computer drive and will only be accessible to the researchers listed on this consent form.

Future Sample Use:

After analysis, we wish to store any remainders of your samples for 10 years post publication of the current study so that Dr. Gordon may investigate as-of-yet undetermined research questions in relation to the broader aims of this study, which are to understand the causes and consequences of mental health problems in the menopause transition.

We would like to retain your samples for future research use that would look at biological processes that may influence mental health in perimenopause. Some examples of what we might be interested in measuring include markers of inflammation (e.g., C-reactive protein), proteins that influence brain health (e.g., brain-derived neurotrophic factor (BDNF), or other hormones like testosterone. We may also wish to examine portions of your DNA. DNA is a molecule that contains the genetic code that is unique to every individual. We are most interested in examining the portions of your DNA that are thought to contribute to individual differences in sensitivity to reproductive hormones like estrogen.

It is possible that your blood or urine would be analysed in a more specialized laboratory elsewhere in North America or Europe. However, your samples would be identified only by your participant ID number and the data would only be interpreted by the Women's Mental Health Research Unit team and our research partners. Your samples will not be shared with the larger research community and will only be used for the purposes outlined in this consent form.

We don't expect that our future analyses would reveal any unknown medical conditions as we are more interested in looking at normal human biological variations and how they can influence one's risk for perimenopausal depression. However, if we did identify a medical condition (e.g., we measure thyroid hormones and discover you have a thyroid condition), we would notify you by email, recommending that you follow up with a physician for official testing and diagnosis if you choose to be notified.

Future Data Use:

We would like to retain the data for this study for future research use on the menopause transition, you will be given the option to limit the use of your data to this study and a potential follow-up study on menopause transition and depression. If you agree to the future research use of the data it may be shared with other researchers studying the menopause transition.

Follow-up Study:

- Please note that if you participate in this study, we may later contact you to participate in a follow-up study involving taking hormone therapy for a short period of time.
- In consenting to participate in the current study, you are NOT required to participate in this follow-up study.
- It is not guaranteed that you will be eligible to participate in the follow-up study.

Funded by: The Natural Sciences and Engineering Research Council

Potential Risks:

- The questionnaires assessing your history of trauma and adverse events may be associated with some psychological distress. Usually, this distress will go away on its own relatively quickly.
- If at any time throughout your participation in the study, you feel you require the services of a mental health professional because of any psychological distress you might be experiencing, do not hesitate to contact the study personnel; they will provide you with a list of mental health resources in your area.
- Blood collection will involve insertion of a needle into your arm and may cause some temporary discomfort, syncope (light headedness), nausea, and bruising.

- While genetic research could have a potential for risks that are currently unknown, the future use of your urine and blood samples will only be used for the purposes specified in this consent form and the samples will be permanently destroyed using validated methods of sample destruction. Your samples will only be shared for the purpose of specialized testing that cannot be done locally and there will be agreements in place to assure that any leftover samples are permanently destroyed after the testing.

Potential Benefits: This research is designed to benefit society by obtaining new knowledge and may result in a better understanding of depression in the menopause transition. You will not benefit personally from being in this research study.

Compensation: You will be receiving up to $400 for completing the study in full compliance, prorated as follows:

- $25 for completing the Zoom enrollment session and baseline questionnaires
- $25 for each week (urine and blood collection and mood survey), for a total of $375

To be compensated $400 dollars as a research participant, we will require your date of birth and SIN as this payment is taxable and reported to the Canada Revenue Agency. Payment can be issued as a cheque or direct deposit; however direct deposit will require further banking information to be provided. Please note that your information will remain in the University's system but no information that can link you to this study will be retained.

Confidentiality:

- No subjects will be identified in any report or publication about this study.
- Your privacy and confidentiality will be protected by the use of ID numbers only and by securing all study files in a locked room and/or password-protected electronic file.
- A separate electronic, password protected file linking your study ID number to you name and other identifying information will be kept separate from any study data and will be destroyed after 10 years.
- We would like to retain the de-identified data for future research use on the menopause transition with your consent, it is possible we may share the data with other researchers internationally working on similar research but at no time will any identifying information about you be shared, you will be given the option to opt out of this future research use.
- Only the researchers will have access to your identifiable information.

Right to Withdraw:

- Your participation is voluntary and you can answer only those questions that you are comfortable with. You may withdraw from the research project for any reason, at any time, without explanation or penalty of any sort.
- Should you wish to withdraw, we will keep the data collected up until your withdrawal, unless you specify that you would like to have your data destroyed, in which case we will do so.
- Your right to withdraw data from the study will apply until one month after the end of your participation. After this date, it is possible that some form of research dissemination will have already occurred and it may not be possible to withdraw your data.
- If you decide to withdraw from the study and ask that your samples be permanently destroyed, we will comply with your wishes, however, any research findings from your sample analysis that have been disseminated already will not be able to be withdrawn.

Follow up:
- To obtain results from the study, please visit https://www.wmhresearch.ca/
- Upon request, we may also send you a summary of our research findings from this study

Questions or Concerns:
- Contact the researcher(s) using the information at the top of page 1;
- This project has been approved by the UofR Research Ethics Board on June 3rd, 2022. Any questions regarding your rights as a participant may be addressed to the committee at (585-4775 or research.ethics@uregina.ca). Out of town participants may call collect.

Please ask the researchers any questions regarding the procedures and goals of the study.

CONSENT TO PARTICIPATE

- I have read (or someone has read to me) the information in this consent form.
- I understand the purpose and procedures and the possible risks and benefits of the study.
- A copy of this consent form has been given to me for my records
- I was given sufficient time to think about it.
- I had the opportunity to ask questions and have received satisfactory answers.

- I am free to withdraw from this study at any time for any reason and request that my data and samples be permanently destroyed, I understand that once research results have been aggregated and published the data cannot be withdrawn.
- I understand that all the information that I provide will be kept confidential and that any research communication will not use my name or any other personal identifiers.
- I give permission for the use the use and disclosure of my de-identified personal information collected for the research purposes described in this form.
- I understand that by signing this document I do not waive any of my legal rights.
- I will be given a signed and dated copy of this consent form.
- I understand that the data from this study may be used for a future research follow up project by Dr. Gordon.

Participant

Do you consent to participate in the study?	☐ Yes	☐ No
Do we have permission to use the information collected as part of the eligibility survey that you completed for us previously?	☐ Yes	☐ No
Do you consent to the future research use of your de-identified data for future research purposes as stipulated in this consent form?	☐ Yes	☐ No
Do you agree for your urine and blood samples to be stored for the future analysis of other biological processes that may be related to perimenopausal depression?	☐ Yes	☐ No
Should our future research find any incidental findings that may affect your health or may indicate predispositions of health risks for your biological family members, would you like to be notified?	☐ Yes	☐ No
Do you authorize the study researcher to contact you for future research projects? You are not obligated to participate in any future research.	☐ Yes	☐ No

Name of Participant: Signature: Date:

_________________________ _________________________ ____________

Researcher Obtaining Consent

I have discussed this consent form with the research participant.

Name of Researcher:	Signature:	Date:
______________________________	______________________________	______________

Appendix F – Enrollment Package

Neurosteroid Fluctuation in Perimenopausal Mood
Project WMH Research Unit, University of Regina
Email: wmh.research@uregina.ca

Hello,
Thanks for agreeing to participate in our study! Below is an overview of the study components.

1. After completing the Zoom-facilitated enrollment session, you are asked to complete a series of online questionnaires that will be accessed via a link that is emailed to you.

2. Materials for urine collection will be delivered to your home. Please do not begin collecting samples until you are asked to.

3. The research team will instruct you on when to begin **weekly** measurements of mood surveys and collection of blood and urine, which will occur on a consistent weekday of your choosing.
   1) On a specified day each week, you will be asked to meet a trained phlebotomist in a convenient and private location for blood collection. You will receive an email reminder the evening before each scheduled appointment.
   2) Starting at 8:00 a.m. on that day, you will begin tracking your hot flashes using the hot flash tracking sheet until 8:00 a.m. the following day.
   3) Between 6:00 and 9:00 PM on this day, you will be asked to complete an online weekly mood survey, including questions about depressive symptoms, anxiety, and current menstrual pain (accessed via a link that is sent to you).
   4) The **morning after** your blood collection and mood survey, you are asked to collect a urine sample immediately upon awakening and store this in your home freezer until your participation is complete.
   5) By mid-morning, you will receive a brief survey asking you to confirm that your urine samples were collected and also asking you to report the total number of mild, moderate, and severe hot flashes you counted in the hot flash tracking sheet.
4. The research team will contact you once you are done the 15 weekly mood and biological sample collections to arrange a courier to retrieve your urine samples from your home. You will then be compensated for your participation.

Below are instructions pertaining to weekly surveys biological sample collection. Please do not hesitate to contact us wmh.research@uregina.ca if you have any questions.

Sincerely,

The Women's Mental Health Research Unit

Hot Flash Tracking

Starting at 8:00 a.m. on the morning of blood collection day and ending at 8:00 am the next morning, you will be asked to record any hot flashes you experience and any night sweats you recall experience during the night. A hot flash tracking log was included with the kit you received. Please keep this form with you throughout this 24-hour period and immediately track these symptoms in the mild, moderate, and severe categories as outlined in the log. We will ask you to report the total number of mild, moderate and severe hot flashes experienced in an online survey each week.

Weekly Surveys

You will be asked to complete weekly evening surveys once you have completed your enrollment session and baseline questionnaires. A survey link to be completed between 6:00 and 9:00 PM will be texted or emailed to you on the same weekday depending on your preference. After clicking the link, the survey should take 10-15 minutes to complete. If the link ever gives you any trouble, please contact us.

Blood Collection

Each week, the phlebotomist will contact you to confirm your meeting for blood collection. All blood collection meetings will be scheduled for, ideally at the same time each week. In a private location that is convenient for you, you will be seated in a stationary chair (i.e., no wheels). A needle will be inserted into your arm to collect blood. The phlebotomist will also ask you some questions regarding any pain and anxiety you felt during the blood collection.

Urine Collection

You have the following equipment for collecting urine samples:

- A tray containing 15 small plastic tubes. Each of the fifteen weeks of the study is associated with one tube. Thus, each tube has a number from 1-15 on the cap.
- Fifteen (15) plastic cups for collecting urine
- Fifteen (15) disposable syringes for transferring urine from the cup to the

plastic tubes Using this equipment, please follow these instructions:

1) First thing in the morning, go to the restroom and urinate in one of the plastic cups provided.
2) Using one of the syringes provided, fill the two small plastic tubes that have the number corresponding to the week (1-15) on the cap. When filling each tube, leave a little empty space at the top (the urine will expand when frozen).
3) Re-cap each of the tubes – push down on the cap until you hear a "snap".

4) Replace the tubes on the plastic tray provided, replace the plastic cover on the tray and keep the tray in your household freezer (not the fridge).
5) Keep the urine samples in the freezer until you've completed the entire study, at which time we will arrange for your urine samples to be retrieved from your house.

Appendix G – Baseline Survey

Please enter your participant ID: ____

DEMOGRAPHIC INFORMATION

1. Date of birth: _____________
2. What gender do you identify with? __________

	Woman
	Man
	Non-binary
	Gender fluid
	Other
	Two-Sprit
	Trans man
	Trans woman
	Prefer not to answer

Not listed: ____________________

3. What is your ethnic origin? Select all that apply.

	North American Indigenous origins (e.g., First Nations, Inuit, Métis)
	Other North American origins (e.g., Acadian, American, Canadian, New Brunswicker, Newfoundlander, Nova Scotian, Ontarians, Québécois)
	Caribbean origins
	African origins
	Asian origins
	European origins
	Latin, Central, or South American origins
	Oceania origins
	Prefer not to answer

Not listed: ____________________

4. What is your racial background? Select all that apply.

	Arab
	Black
	Chinese
	Filipino
	Japanese
	Korean
	Latin American
	South Asian (e.g., East Indian, Pakistani, Sri Lankan)
	Southeast Asian (e.g., Vietnamese, Cambodian, Laotian, Thai)
	West Asian (e.g., Afghan, Iranian)
	White

	Prefer not to answer

Not listed: ____________________

4. What is your marital status?

	Never married/Never living common law
	Married
	Living common law
	Separated
	Divorced
	Widowed
	Prefer not to answer

Not listed: ____________________

5. Who do you live with? Please choose all that apply and indicate the number of people, if applicable.

	Alone		Spouse or significant other(s)		
			Children	If so, how many:	________
			Parents	If so, how many:	________
			Friends	If so, how many:	________
			Other	Please specify:	________
			Not listed		________
			Prefer not to answer		________

6. Select the sexual orientation that best describes how you currently think of yourself:

	Asexual
	Bisexual
	Gay
	Heterosexual
	Lesbian
	Pansexual
	Queer
	Two-spirit
	Prefer not to answer

7. What is the **highest** level of education you have attained?

	Grade 8 education or less
	Some high school education
	High school diploma or equivalent
	Some post-secondary education
	Post-secondary certificate or diploma
	Post-secondary bachelor’s degree
	University certificate, diploma, or degree above a bachelor’s degree

	Not listed
	Prefer not to answer

8. Which of the following best describes your current main daily activities and/or responsibilities?

	Working full time
	Working part time
	Retired
	Student
	Sick leave / maternity leave
	Keeping house or raising children full time
	Unemployed or laid off
	Disability
	Prefer not to answer

Not listed: ____________________

9. How much did **you** earn, before taxes and other deductions, **in the past year**? (The total earned on your last tax return)

	Less than $10,000
	$10,000 to $19,999
	$20,000 to $29,999
	$30,000 to $39,999
	$40,000 to $49,999
	$50,000 to $59,999
	$60,000 to $69,999
	$70,000 to $79,999
	$80,000 to $89,999
	$90,000 to $99,999
	$100,000 or more
	Don’t know
	Prefer not to answer

10. Which of these categories best describes your total **combined family income in the last year**? (Include income (before taxes) from all sources, wages, rent from properties, social security, disability and/or veteran’s benefits, unemployment benefits, workman’s compensation, help from relatives (including child payments and alimony), and so on)

Less than $10,000
$10,000 to $19,999
$20,000 to $29,999

$30,000 to $39,999
$40,000 to $49,999
$50,000 to $59,999
$60,000 to $69,999
$70,000 to $79,999
$80,000 to $89,999
$90,000 to $99,999
$100,000 or more
Don't know
Prefer not to answer

MEDICAL HISTORY

11. Do you have a history of the following health problems? For each problem you have, please indicate when you were diagnosed with this problem (month and year).

Health problem			Date of diagnosis
Chest pain or angina	☐ Yes	☐ No	
High blood pressure	☐ Yes	☐ No	
Any cardiovascular disease (e.g., coronary artery disease, arteriosclerosis, heart attack, or stroke; atrial fibrillation; frequent premature atrial or ventricular beats or other rhythm abnormalities; Stage 1 or 2 hypertension)	☐ Yes	☐ No	
Type I or Type II Diabetes	☐ Yes	☐ No	
High/marginally high cholesterol	☐ Yes	☐ No	
Kidney Disease (other than stones)	☐ Yes	☐ No	
Breast cancer	☐ Yes ☐ active ☐ in remission	☐ No	
Irradiation to the breast or chest wall prior to the age of 30 (such as for Hodgkin's disease)	☐ Yes	☐ No	
Any other cancer	☐ Yes ☐ active ☐ in remission	☐ No	

Breast biopsy with atypical hyperplasia	□ Yes	□ No	
Asthma	□ Yes	□ No	
COPD/emphysema/chronic bronchitis	□ Yes	□ No	
Allergies	□ Yes	□ No	
Thyroid problem	□ Yes	□ No	
Endometrial hyperplasia	□ Yes	□ No	
Abnormal uterine anatomy (e.g., fibroids)	□ Yes	□ No	
Thrombophlebitis or thromboembolic disorders	□ Yes	□ No	
Estrogen-dependent neoplasias	□ Yes	□ No	
Gall bladder disease	□ Yes	□ No	
Liver dysfunction	□ Yes	□ No	
Current migraine with aura or a history of migraine with aura within the last 5 years	□ Yes	□ No	
History of migraine with without aura	□ Yes	□ No	

12. Do you have any relatives who have been ever diagnosed with the following conditions?

a) Breast cancer

If yes to *a*:

How many first-degree relatives have had breast cancer? ___

At what age was each relative diagnosed (approximately is fine)? ___

How many second- or third-degree relatives have had breast cancer? ___

Have any of your relatives who have breast cancer had an additional diagnosis of ovarian cancer? □ Yes □ No □ I don't know

Were any of the relatives with breast cancer male? □ Yes □ No □ I don't know

b) Ovarian cancer

If yes to *b*:

Was the person a first-degree relative? □ Yes □ No □ I don't know

c) Any cancer before the age of 60

If yes to *c*:

How many first-degree relatives have had cancer before the age of 60? _____

d) A known BRCA genetic mutation

13. Are you of Ashkenazi Jewish descent?

□ Yes □ No □ I don't know

14. Please list any other health problems you have:

15. In the past 3 months, have you had any other major medical problems not mentioned?
□ Yes, **if yes**, please specify : ____
□ No

16. Please enter your weight (in pounds) and height.
Weight (lbs.): _____
Height: _____ ft ___ inches

□ Prefer not to answer

REPRODUCTIVE HEALTH HISTORY

25. Do you have any biological children?

□ Yes, If yes, how many children do you have? _______

	Child									
	1	2	3	4	5	6	7	8	9	10
When did you give birth? (year)										
Is the child male or female?										

□ No

Center for Epidemiological Studies-Depression (CES-D)

Below is a list of some ways you may have felt or behaved. Please indicate how often you have felt this way during the past week:

During the past week…	**Rarely or none of the time** (less than 1 day)	**Some or a little of the time** (1-2 days)	**Occasionally or a moderate amount of time** (3-4 days)	**All of the time** (5-7 days)
1. I was bothered by things that usually don't bother me.	0	1	2	3
2. I did not feel like eating; my appetite was poor.	0	1	2	3
3. I felt that I could not shake off the blues even with help from my family.	0	1	2	3

4. I felt that I was just as good as other people.	0	1	2	3
5. I had trouble keeping my mind on what I was doing.	0	1	2	3
6. I felt depressed.	0	1	2	3
7. I felt that everything I did was an effort.	0	1	2	3
8. I felt hopeful about the future.	0	1	2	3
9. I thought my life had been a failure.	0	1	2	3
10. I felt fearful.	0	1	2	3
11. My sleep was restless.	0	1	2	3
12. I was happy.	0	1	2	3
13. I talked less than usual.	0	1	2	3
14. I felt lonely.	0	1	2	3
15. People were unfriendly.	0	1	2	3
16. I enjoyed life.	0	1	2	3
17. I had crying spells.	0	1	2	3
18. I felt sad.	0	1	2	3
19. I felt that people disliked me.	0	1	2	3
20. I could not get "going".	0	1	2	3

Hot Flash Related Daily Interference Scale

Please select one number to the right of each phrase to describe how much **during the past WEEK** hot flashes have **interfered** with each aspect of your life. Higher numbers indicate more interference with your life. If you are not experiencing hot flashes or if hot flashes do not interfere with these aspects of your life, please select zero.

Do not interfere Completely interfere

Work (work outside 0 1 2 3 4 5 6 7 8 9 10
the home and housework)

Social activities (time spent with family, friends, etc.)	0	1	2	3	4	5	6	7	8	9	10
Leisure activities (time spent relaxing, doing hobbies, etc.)	0	1	2	3	4	5	6	7	8	9	10
Sleep	0	1	2	3	4	5	6	7	8	9	10
Mood	0	1	2	3	4	5	6	7	8	9	10
Concentration	0	1	2	3	4	5	6	7	8	9	10
Relations with others	0	1	2	3	4	5	6	7	8	9	10
Sexuality	0	1	2	3	4	5	6	7	8	9	10
Enjoyment of life	0	1	2	3	4	5	6	7	8	9	10
Overall quality of life	0	1	2	3	4	5	6	7	8	9	10

Pittsburgh Sleep Quality Index

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, what time have you usually gone to bed at night?
   Bed time: _________
2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?
   Number of minutes: _______
3. During the past month, what time have you usually gotten up in the morning?
   Getting up time: _______
4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.)

For each of these remaining questions, check the one best response. Please answer **all** questions.

5. During the past month, how often have you had trouble sleeping because you . . .

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
Cannot go to sleep within 30 minutes				
Wake up in the middle of the night or early morning				
Gave to get up to use the bathroom				
Cannot breathe comfortably				
Cough or snore loudly				
Feel too cold				
Feel too hot				
Had bad dreams				
Have pain				

6. Please describe if you have any other reason(s) for having trouble sleeping.

7. How often during the past month have you had trouble sleeping because of this? (*this question is only displayed if #6 is answered*)

□ Not during the past month □ Less than once a week

□ Once or twice a week □ Three or more times a week

8. During the past month, how would you rate your sleep quality overall?

□ Very good

□ Fairly good

□ Fairly bad

□ Very bad

9. During the past month, how often have you taken medicine to help you sleep (prescribes or "over the counter")?

□ Not during the past month □ Less than once a week

□ Once or twice a week □ Three or more times a week

10. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

□ Not during the past month □ Less than once a week

□ Once or twice a week □ Three or more times a week

11. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

□ No problem at all □ Only a very slight problem

□ Somewhat of a problem □ A very big problem

12. Do you have a bed partner or room mate?

□ No bed partner or room mate

□ Partner/room mate in other room

□ Partner in same room, but not same bed

□ Partner in same bed

Life Experiences Survey

A number of events that sometimes bring about change in people's lives will be described. Please answer yes if you have experienced this event in the **past six months**. Also indicate how stressful the event was for you when it occurred.

Relationships

1.a) Have you gotten married, engaged or made a formal commitment to a partner, including a ceremony?

□ Yes □ No

1.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

2.a) Have you separated, divorced, or had a break-up with a partner (mate, girlfriend, or boyfriend) due to conflict (must have been in the committed relationship at least 6 months)?

□ Yes □ No

2.b) How stressful was the event or difficulty <u>when it occurred</u>?

- Extremely stressful
- Very stressful
- Moderately stressful
- A little stressful
- Not stressful

3.a) Have you had an increase in serious arguments with a partner (mate, girlfriend or boyfriend) without separation or divorce (must have been in the committed relationship at least 6 months)?

 Yes No

3.b) How stressful was the event or difficulty <u>when it occurred</u>?

- Extremely stressful
- Very stressful
- Moderately stressful
- A little stressful
- Not stressful

4.a) Have you had a major change in closeness to a family member (estrangement from family, serious argument(s) with family members)?

 Yes No

4.b) How stressful was the event or difficulty <u>when it occurred</u>?

- Extremely stressful
- Very stressful
- Moderately stressful
- A little stressful
- Not stressful

Death or Illness Among Family and Close Friends

5.a) Have you experienced the death of a close family member?

 Yes No

5.b) Please indicate who.

- Husband/wife/partner
- Child
- Mother
- Father
- Brother
- Sister
- Grandparent
- Other relative that you are very close to

5.c) How stressful was the event or difficulty when it occurred?

- Extremely stressful
- Very stressful
- Moderately stressful
- A little stressful
- Not stressful

6.a) Have you experienced the death of a very close friend (do not include casual friends)?

 Yes No

6.b) How stressful was the event or difficulty when it occurred?

 Extremely stressful
 Very stressful
 Moderately stressful
 A little stressful
 Not stressful

7.a) Have you experienced a serious illness or injury of close family member (do not include those who have died)?

 Yes No

7.b) Please indicate who.

 Husband/wife/partner
 Child
 Mother
 Father
 Brother
 Sister
 Grandparent
 Other relative that you are very close to

7.c) How stressful was the event or difficulty when it occurred?

 Extremely stressful
 Very stressful
 Moderately stressful
 A little stressful
 Not stressful

8.a) Have you experienced the serious illness or injury of very close friend (do not include casual friends or those who have died)?

 Yes No

8.b) How stressful was the event or difficulty when it occurred?

 Extremely stressful
 Very stressful
 Moderately stressful
 A little stressful
 Not stressful

Work Problems/Finances

9.a) Have you had trouble with your employer (in danger of losing your job, being suspended or demoted, experiencing discrimination, other major problems with your job)?

 Yes No

9.b) How stressful was the event or difficulty when it occurred?

 Extremely stressful
 Very stressful
 Moderately stressful

□ A little stressful
□ Not stressful

10.a) Have you lost your job (fired, laid off, quit, retired)?

□ Yes □ No

10.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

11.a) Have you had trouble finding employment (you must be seriously looking AND out of work for at least 2 months)?

□ Yes □ No

11.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

12.a) Have you worked long hours (60 or more hours/week) for at least 1 month?

□ Yes □ No

12.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

13.a) Have you had a major worsening of your financial status (large drop in personal or family income) or had major chronic financial problems (e.g., months behind in bills, bill collectors, foreclosure on mortgage or loan, repossession of car, had to sell possessions, not enough money for basic necessities, such as clothing, housing, food, or health care)?

□ Yes □ No

13.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

Illness/Accidents/Injury/Safety

14.a) Have you had a major illness, chronic health problem, or injury?

□ Yes □ No

14.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful

□ A little stressful
□ Not stressful

15.a) Have you been in a motor vehicle accident (with some injury or financial loss, NOT fender bender)?

□ Yes □ No

15.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

16.a) Have you been hospitalized overnight for illness or injury?

□ Yes □ No

16.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

17.a) Have you been physically attacked or assaulted or had your life threatened?

□ Yes □ No

17.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

18.a) Have you been sexually abused or assaulted?

□ Yes □ No

18.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

19.a) Have you had problems feeling safe in your neighborhood?

□ Yes □ No

19.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

Crime or Legal Problems

20.a) Have you been arrested for a serious crime (e.g. driving under the influence of alcohol or drugs, robbery, drugs, crime involving more than just a fine)?

□ Yes □ No

20.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

21.a) Have you been convicted of a crime and went to jail or comparable institution?

□ Yes □ No

21.b) If yes, how many days: _____

21.c) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

22.a) Have you experienced a mate or close relative arrested for a serious crime (with likely jail sentence) or mate or close relative went to jail for at least one-month?

□ Yes □ No

22.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

23.a) Have you been robbed or your home was burglarized?

□ Yes □ No

23.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

Other Life Changes

24.a) Has your committed partner/wife become pregnant or had or adopted a baby?

□ Yes □ No

24.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

25.a) Have you became pregnant, had a baby, or adopted a baby?

□ Yes □ No

25.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

26.a) Have you come to the end of your formal schooling (e.g. graduation, flunking out of school)?

□ Yes □ No

26.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

27.a) Have you left home for the first time?

□ Yes □ No

27.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

28. a) Has your son or daughter moved away from your home for the first time (e.g. leaving to go to school, custody given to ex-mate)?

□ Yes □ No

28.b) How stressful was the event or difficulty when it occurred?

□ Extremely stressful
□ Very stressful
□ Moderately stressful
□ A little stressful
□ Not stressful

Appendix H – Weekly Mood Survey

Please enter the participant ID given to you by the research team: ____

1. What week of the study is this for you (1-15)? ____
2. Did you menstruate this week or are you currently experiencing a menstrual period?
   ☐ Yes ☐ No
3. What day did your menstrual period begin this week? *(this question is only displayed if they answer "Yes" to #2)* Month: __________ Day: ______
   I.

Sleep Diary

Please answer the following questions pertaining to your sleep **LAST NIGHT**.

1. What time did you get into bed?

	Time (HH:MM)	AM/PM
Time	______________	☐ AM ☐ PM

2. What time did you try to go to sleep?

	Time (HH:MM)	AM/PM
Time	______________	☐ AM ☐ PM

3. How long did it take you to fall asleep?

Number of minutes: _________

4. Did you wake up throughout the night?

☐ Yes ☐ No

[If yes], How long did these awakenings last (in minutes)?

	Minutes
Awakening 1	
Awakening 2	
Awakening 3	
Awakening 4	
Awakening 5	
Awakening 6	
Awakening 7	

Awakening 8	
Awakening 9	
Awakening 10	

5. What time did you wake up?

	Time (HH:MM)	AM/PM
Time	_______________	☐ AM ☐ PM

7. Did you wake up earlier than planned?

☐ Yes ☐ No

[If yes] How much earlier?
Number of minutes: ________

8. In total, how long did you sleep?

Hour(s): _______
Minute(s): ______

9. How would you rate the quality of your sleep?
☐ Very Good
☐ Fairly Good
☐ Fairly Bad
☐ Very Bad

10. How many alcoholic drinks did you consume the day before?

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 or more

11. How many caffeinated drinks did you consumer the day before?

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 or more

12. What time did you have the last caffeinated drink before you fell asleep?

	Time (HH:MM)	AM/PM

Time	_______________	☐ AM ☐ PM

13. Did you take any over-the-counter or prescription medication(s) to help you sleep?
☐ Yes ☐ No

[If yes] Please name the medication(s) and dose.
Medication: ________
Dose: ________
Time taken: _________

14. How many times did you nap or doze?

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 or more

[If answer to 11 is >0] In total, how long did you nap or doze?
Number of minutes: _________________

Center for Epidemiological Studies-Depression (CES-D)

Below is a list of some ways you may have felt or behaved. Please indicate how often you have felt this way during the past week:

During the past week…	**Rarely or none of the time** (less than 1 day)	**Some or a little of the time** (1-2 days)	**Occasionally or a moderate amount of time** (3-4 days)	**All of the time** (5-7 days)
1. I was bothered by things that usually don't bother me.	0	1	2	3
2. I did not feel like eating; my appetite was poor.	0	1	2	3
3. I felt that I could not shake off the blues even with help from my family.	0	1	2	3
4. I felt that I was just as good as other people.	0	1	2	3
5. I had trouble keeping my mind on what I was doing.	0	1	2	3
6. I felt depressed.	0	1	2	3
7. I felt that everything I did was an effort.	0	1	2	3
8. I felt hopeful about the future.	0	1	2	3
9. I thought my life had been a failure.	0	1	2	3
10. I felt fearful.	0	1	2	3
11. My sleep was restless.	0	1	2	3
12. I was happy.	0	1	2	3
13. I talked less than usual.	0	1	2	3
14. I felt lonely.	0	1	2	3
15. People were unfriendly.	0	1	2	3
16. I enjoyed life.	0	1	2	3

17. I had crying spells.	0	1	2	3
18. I felt sad.	0	1	2	3
19. I felt that people disliked me.	0	1	2	3
20. I could not get "going".	0	1	2	3

Appendix I – Vasomotor Symptoms Survey

Please enter the participant ID given to you by the research team: _____

1. What week of the study is it (1-15)? _____

2. Did you collect your urine sample this morning?

☐ Yes ☐ No

3. Please indicate the times at which these samples were collected.

	Time (HH:MM)	AM/PM
Time	_______________	☐ AM ☐ PM

4. Did encounter any difficulties collecting your samples?

☐ Yes ☐ No

[If yes] What sort of difficulties did you encounter?

____

____

Hot Flashes & Night Sweats

1. Please indicate the extent to which you are bothered at the moment by hot flashes:

☐ Not at all ☐ A little ☐ Quite a bit ☐ Extremely

2. Please indicate the extent to which you are bothered at the moment by sweating at night:

☐ Not at all ☐ A little ☐ Quite a bit ☐ Extremely

3. Based on the hot flash diary that you've been keeping in the last 24 hours, how many mild, moderate, and severe hot flashes and/or night sweats have you had since 8:00 a.m. yesterday morning?

______________ mild hot flashes/ night sweats

______________ moderate hot flashes/ night sweats

______________ severe hot flashes/ night sweats

Appendix J – Blood Collection Protocol

Venipuncture Protocol

Procedure 1. **Check for history of previous blood draw problems (e.g., fainting). Gather quipment and wash hands.**
2. Prepare equipment so you are ready to obtain sample. a. Butterfly Vacutainer method: Attach butterfly needle to Vacutainer Holder and have proper blood specimen tube resting inside the Vacutainer. Do not puncture the rubber stopper yet.
3. Extend arm to form a straight line from shoulder to wrist.
5. Put on nonsterile gloves.
6. Apply tourniquet 3–4 inches above venipuncture site. NOTE: Tourniquet should be able to be removed by pulling the end with a single motion.
7. Check for distal pulse. If none, tourniquet is too tight and should be reapplied.
8. Have participant open and close fist several times, leaving fist clenched prior to venipuncture..
9. Maintain tourniquet only 1–2 minutes.
10. Identify best venipuncture site by palpation. Ideal site is straight, prominent vein that feels firm and slightly rebounds when palpated.
11. Select site. If tourniquet has been on too long release it, wait 1–2 minutes and reapply.
12. Cleanse site with approved solution per policy using circular motion at the site and extending 2 inches beyond the site. Allow to dry.
13. Remove needle cover and let participant know they will feel needle stick.
14. Place thumb or forefinger of nondominant hand 1 inch below site and pull skin taut.

15. Hold needle at 15°–30° angle from skin, bevel up.
16. Slowly insert needle.
17. After entering vein, slowly lower needle toward skin. Thread needle along path of vein.
18. Check for blood flow. Butterfly Vacutainer method. 1. Hold Vacutainer securely and advance specimen tube into needle of holder, being careful not to advance the needle further into the vein. Blood should flow into the collection tube. 2. After collection tube full, grasp Vacutainer firmly and remove tube; insert additional collection tubes as needed.
19. After specimen collection complete, remove tourniquet.
20. Apply 2 x 2 gauze over puncture site without applying pressure and quickly remove the needle from the vein.
21. Immediately apply pressure over venipuncture site with gauze for 2–3 minutes or until the bleeding has stopped. Hold arm straight.
22. Inspect the puncture site for bleeding. Reapply a clean tape and gauze if necessary.
23. Check tubes for external blood. If any, wipe away with alcohol.
24. Check tubes for proper labeling. Place tubes in appropriate bag or container along with laboratory requisition slips.
25. Dispose of equipment in a sealed sharps container.
26. Remove gloves. Wash hands.
27. Transport specimens to laboratory.

Appendix K – Hot Flashes Tracking Sheet

Hot Flash Record Sheet

For the next 24 hours, please fill in the appropriate circle immediately after experiencing a hot flash. Keep this sheet with you at all times, and record night hot flashes/night sweats in the morning. If a mistake is made, put an “X” through the circle. Tomorrow, we will ask you to report the total number of mild, moderate and severe hot flashes experienced in our online survey.

Mild: Sensation of heat without sweating/ dampness If at night, you do not wake up, but later notice damp clothes/sheets	OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO
Moderate: Sensation of heat with sweating/ dampness, but able to continue activity If at night, you wake up because you are hot and/or sweating, but no action is necessary	OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO
Severe: Sensation of intense heat with sweating causing disruption of current activity. If at night, you wake up hot and sweating and need to take action (remove clothes, open window, etc.)	OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO
Total Number experienced:	Mild:_____ Moderate:_____ Severe:_____

Appendix L – Variable Definitions

Sensitivity Coefficient Variables		
Name	**Statistical Definition**	**Meaning**
E2-CES-D correlation	Correlation between person-centered E2 and weekly CES-D score, range: -1.0 to +1.0	Degree to which mood is related to levels of E2
AbsE2-CES-D correlation	Correlation between the *absolute value* of person-centered E2 and weekly CES-D score, range: -1.0 to +1.0	Degree to which mood is related to E2 extremes in either direction
E2 mood sensitivity strength	Absolute value of the correlation coefficient (of the two described above) that was largest in magnitude for a given participant*, range: 0.0 to 1.0	An individual's maximum mood sensitivity to changes in E2
Sensitivity Profile Definitions		
E2-withdrawal sensitive	E2-CES-D correlation ≤ -0.3 that is larger in magnitude than that participant's absE2-CES-D correlation	Depressive symptoms increase in response to E2 decline
E2-increase sensitive	E2-CES-D correlation ≥ +0.3 that is larger in magnitude than that participant's absE2-CES-D correlation	Depressive symptoms increase in response to E2 increases
E2-change sensitive	absE2-CES-D correlation that is ≥ +0.3 and larger in magnitude than that participant's E1G-CES-D correlation	Depressive symptoms increase in response to extreme E2 levels in either direction
E2 insensitive	E2 sensitivity strength was found to be < 0.3	Depressive symptoms are unrelated to E2

*Note: For example, a participant with an E2-CES-D correlation of -0.20 and an absE2-CES-D correlation of +0.50 would have an E2 mood sensitivity strength value of 0.50. Conversely, a participant with an E2-CES-D correlation of +0.10 and an absE2-CES-D correlation of -0.70 would have an E1G mood sensitivity strength value of 0.7

Appendix M – Ethics

University of Regina

Research Ethics Board
Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	REB#
Jennifer Gordon	Psychology	2022-038

TITLE
Neurosteroid Fluctuation in Perimenopausal Depression

APPROVED ON	RENEWAL DATE
June 3, 2022	June 3, 2023

APPROVAL OF
Application for Biomedical Research Ethics Review
Participant Recruitment: Social Media Advertisement; Study Information; and Eligibility Survey
Enrollment: Zoom Enrollment Protocol; Consent Form; Enrollment Package; and Baseline Survey
Weekly Measurements: Weekly Mood Survey; Vasomotor Symptoms Survey; Blood Collection Protocol; and Hot Flashes Tracking Sheet
Email Templates and Resources: Email Correspondence Templates; and Mental Health Resources

Full Board Meeting ☐ Delegated Review ☒

The University of Regina Research Ethics Board has reviewed the above-named research project. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to the conditions outlined in the original protocol submitted for ethics review. This Certificate of Approval is valid for the above time period provided there is no change in experimental protocol, or related documents.

Any significant changes to your proposed method, procedures or related documents should be reported to the Chair for Research Ethics Board consideration in advance of implementation.

ONGOING REVIEW REQUIREMENTS
In order to receive annual renewal, a status report must be submitted to the REB Chair for Board consideration one month in advance of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for the renewal and closure forms:
https://www.uregina.ca/research/for-faculty-staff/ethics-compliance/human/ethicsforms.html

Kim Dorsch PhD
REB Chair
University of Regina

Please send all correspondence to:

Research Office
University of Regina
Centre for Kinesiology Building 227
Regina, SK S4S 0A2
Telephone: (306) 585-4775 Fax: (306) 585-4893
research.ethics@uregina.ca

Saskatchewan
Health Authority

July 4, 2022

Dr. Jennifer Gordon
Department of Psychology]

Study Title: Neurosteroid Fluctuation in Perimenopausal Depression
File Number: OA-UofR-2022-038

Authorization Granted By:

- Elaine Brecelj RN, BSN Mid-Life Coordinator
  Women's Mid-Life Health Program - Saskatoon City Hospital

RE: <u>LETTER OF AUTHORIZATION TO CONDUCT RESEARCH OA-UofR-2022-038</u>

Dear Dr. Jennifer Gordon and study team,

This letter is to notify you that the above-listed research study has been reviewed and meets all criteria for Operational Approval within the Saskatchewan Health Authority (SHA).

- Ability to access SHA resources, including facilities, is subject to all COVID–19 related restrictions that are in place now and in the future.
- Ability to access SHA resources is subject to all COVID–related restrictions that are in place now and in the future. This includes restrictions such as entry into SHA facilities/departments and requirements for physical distancing.
- Ability to access SHA patient records is subject to COVID restrictions regarding entry into SHA facilities and departments.

Please note that Operational Approval is conditional upon continued review and approval by the Research Ethics Board (SHA, U of R or U of S). Should Research Ethics approval lapse or be revoked, Operational Approval will also be suspended.

In addition, Operational Approval is issued based upon the details provided in the Operational Approval to Conduct Research Application Form.

Should the resource utilization deviate from what was requested in the initial application, Operational Approval may be revoked and an amendment must be submitted for review.

Any publications or presentations that result from this research should include a statement acknowledging the assistance of the Saskatchewan Health Authority.

This letter serves as your official authorization to conduct research; **study activities may now commence**.

If you have any questions, please contact the Research Approval Office at
ResearchApproval.Regina@saskhealthauthority.ca

Sincerely,

Dr. Elan Paluck
Director of Research
Saskatchewan Health Authority

University of Regina

Research Ethics Board
Certificate of Amendment Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	REB#
Jennifer Gordon	Psychology	2022-038

TITLE
Neurosteroid Fluctuation in Perimenopausal Depression

AMENDMENT APPROVAL OF:	NEXT RENEWAL DATE	AMENDMENT APPROVAL DATE
• Store and process the blood samples in the lab of Dr. Angela Baerwald, located on the University of Saskatchewan campus. • Add Dr. Angela Baerwald from the University of Saskatchewan as a co-investigator.	June 3, 2022	June 21, 2022

Full Board Meeting ☐ Delegated Review ☒

AMENDMENT CERTIFICATION
The University of Regina Research Ethics Board has reviewed the changes to the above-named research project as outlined in your memo dated June 16, 2022 and they are approved.

ONGOING REVIEW REQUIREMENTS
In order to receive annual renewal, a status report must be submitted to the REB Chair for Board consideration one month in advance of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for the renewal and closure forms:

https://www.uregina.ca/research/for-faculty-staff/ethics-compliance/human/ethicsforms.html

Ara Steininger
Research Ethics Board

Please send all correspondence to:
Research Office
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Regina, SK S4S 0A2
Telephone: (306) 585-4775
Fax: (306) 585-4893
research.ethics@uregina.ca

Research Ethics Board
Certificate of Amendment Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	REB#
Jennifer Gordon	Psychology	2022-038

TITLE
Neurosteroid Fluctuation in Perimenopausal Depression

AMENDMENT APPROVAL OF:	NEXT RENEWAL DATE	AMENDMENT APPROVAL DATE
• Expand recruitment to Regina • Hire Karen Mulatz, a registered nurse, to draw blood at participants' homes and bring the samples to the Institute of Environmental Change and Society to process and store. Assure that Karen Mulatz completes the U of R Biosafety Course and has an up to date Transport of Dangerous Goods certificate.	June 3, 2023	October 26, 2022

Full Board Meeting ☐ Delegated Review ☒

AMENDMENT CERTIFICATION
The University of Regina Research Ethics Board has reviewed the changes to the above-named research project as outlined in your memo dated October 17, 2022, and they are approved.

ONGOING REVIEW REQUIREMENTS
In order to receive annual renewal, a status report must be submitted to the REB Chair for Board consideration one month in advance of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for the renewal and closure forms:

https://www.uregina.ca/research/for-faculty-staff/ethics-compliance/human/ethicsforms.html

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