THE UNIVERSITY OF CHICAGO

EFFECT OF MENSTRUAL CYCLE PHASE AND CIRCULATING OVARIAN HORMONE LEVELS ON ACUTE RESPONSE TO ORAL DELTA-9-TETRAHYDROCANNABINOL (THC)

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List of Abbreviations

A	Amphetamine-like scale
AEA	Anandamide
AMPH	d-Amphetamine
ANS	Autonomic nervous system
ANOVA	Analysis of Variance
APA	American Psychiatric Association
ARCI	Addiction Research Center Inventory
BG	Benzedrine-like scale
BP	Blood pressure
CB_1R	Cannabinoid Type I Receptor
CB_2R	Cannabinoid Type II Receptor
CNS	Central Nervous System
CUD	*Cannabis* Use Disorder
CYP	Cytochrome P450
DA	Dopamine
DEQ	Drug Effects Questionnaire
DSM-V	Diagnostic and Statistical Manual, 5^{th} Edition
E	Estrogen/Estradiol
ECG	Electrocardiograph
EF	Early Follicular
ELISA	Enzyme-Linked Immunosorbent Assay

FSH	Follicle Stimulating Hormone
GABA	Gamma-aminobutyric acid
GPCR	G protein-coupled receptor
HR	Heart Rate
HF HRV	High frequency heart rate variability
i.v.	Intravenous
i.p.	Intraperitoneal
LF	Late Follicular
LH	Luteinizing Hormone
LSD	Lysergic acid diethylamide
MBG	Morphine and benzedrine-like scale
OVX	Ovariectomized
P	Progesterone
PCAG	Pentobarbital-chlorpromazine and alcohol-like scale
P:E	Ratio of circulating progesterone levels to estradiol levels
PET	Positron Emission Tomography
PL	Placebo
PNS	Peripheral Nervous System
POMS	Profile of Mood States
SE	Standard error
SEM	Standard error of the mean
THC	Delta-9-tetrahydrocannabinol
THCCOOH	11-nor-9-carboxy-delta-9-tetrahydrocannabinol

VTA	Ventral Tegmental Area
2-AG	2-Arachidonyl Glycerol
11-OH-THC	11-hydroxy-delta-9-tetrahydrocannabinol

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Abstract

Recent widespread policy changes have legalized or decriminalized *Cannabis* for medicinal and/or nonmedicinal use in over half of the United States. These policy changes will decrease the perception of harms, increase the availability and use of the drug, and thus bring concomitant increases in negative outcomes. This increased risk can be mitigated by a better understanding of its acute effects. *Cannabis* and its main psychoactive constituent, Δ^9-tetrahydrocannabinol (THC), can produce serious unwanted effects including anxiety, especially in women. Yet, because women have been historically underrepresented in *Cannabis* research, relatively little is known about sources of variability in women. One potential source is menstrual cycle phase and circulating ovarian hormones. In rodents, responses to THC differ in males and females, and sensitivity to THC depends on circulating estradiol levels. In humans, women are more susceptible than men to adverse responses to THC, but little is known about how cycle phase or hormone levels affect drug effects. Here, we compare responses to oral THC between the early and late follicular phase of the menstrual cycle. Then we analyze significant drug effects as a function of circulating ovarian hormone levels. The primary outcome measures were cardiovascular (heart rate, blood pressure, temperature, heart rate variability), biochemical, (salivary cortisol) and subjective (i.e., ratings of feeling drug, liking the drug, and anxiety) drug effects. Sixty women were randomly assigned to two groups, who were tested either the early follicular phase (days 1 – 5) when estrogen levels are low, or late follicular phase (days 9 – 14) when estrogen levels are higher. After recording baseline measurements and drawing blood for ovarian hormone analysis, oral

THC (7.5 mg and 15 mg or 15 mg only) and placebo were administered in a double-blind counter-balanced order. Drug effects were monitored continuously and recorded six times across the four-hour long experimental sessions. We hypothesized women would experience greater stress-related responses to THC during the late follicular compared to the early follicular phase. THC dose dependently increased HR and salivary cortisol. These effects were similar between the early and late follicular groups. THC also increased ratings of “feeling” a drug effect, anxiety, and confusion. Faster onset of subjective effects occurred during the early follicular phase compared to the late follicular phase. To determine whether circulating hormone levels mediated these phase differences, or otherwise independently modulated the acute effects of oral THC, we incorporated a continuous repeated measure of circulating hormone levels. We hypothesized estradiol would significantly affect drug effects across time whereas progesterone would not. The acute effects of THC were highly robust. Hormone levels did not significantly affect baseline cardiovascular, biochemical, or subjective measures, nor any drug effect across time. These findings suggest ovarian hormone levels do not i) underlie the previously reported menstrual cycle phase differences in response to *Cannabis*, or ii) modulate acute responses to THC in naturally cycling women. This study deepens our understanding of estrogen-cannabinoid interactions and their potential downstream biological effects, providing critical information regarding hormonal mechanisms underlying female-specific individual differences in responses to acute THC.

Chapter 1: Introduction

1.1 Summary

Despite its Schedule I status, since 1996, thirty-six states and the District of Columbia (D.C.) have legalized *Cannabis* for medicinal use; eighteen of these states and D.C. have also legalized *Cannabis* for adult recreational use. These policy changes are likely to decrease the perception of harms, increase the availability and use of the drug, and thus bring concomitant increases in negative outcomes. This increased risk may be mitigated by a better understanding of its acute cardiovascular, biochemical, and subjective effects. In addition to its known euphoriant effect or "high", *Cannabis* and its main psychoactive constituent, Δ^9-tetrahydrocannabinol (THC), can produce serious unwanted effects including tachycardia, anxiety, and paranoia, especially in women (Sholler et al., 2020; Williamson and Evans, 2000). Women have also been shown to report greater subjective effects related to abuse liability in response to 5 mg of THC, when compared to men (Cooper and Haney, 2014) and progress more rapidly from initial *Cannabis* use to the development of *Cannabis* use disorder or continued use despite negative consequences (CUD; Khan et al., 2013). Yet, because women have been historically underrepresented in clinical *Cannabis* research, relatively little is known about the specific mechanisms or sources of risk for these outcomes (Greenfield et al., 2010). One potential source of variability in responses to THC is ovarian hormones, such as estrogen (E) and progesterone (P; Struik et al., 2018; Moran-Santa Maria et al., 2014). Although preclinical studies suggest that responses to cannabinoids

are greater in the presence of higher circulating levels of E, little is known about the role of menstrual cycle phase or circulating ovarian hormones in women's responses to THC. Therefore, the focus of my graduate work has been to determine whether acute responses to THC vary with menstrual cycle phase and if individual differences in responses to THC are associated with circulating ovarian hormone levels. Not only does this research have clinical applications, but it also deepens our understanding of potential ovarian hormone-cannabinoid neurobiological interactions.

The experimental aims were designed to assess responses to THC in relation to both menstrual cycle phase and circulating ovarian hormone levels. First, we compare physiological and subjective responses to acute oral THC between two distinct phases of the menstrual cycle, the early (EF) and late follicular (LF) phase. Then, from measuring quantitative ovarian hormone levels, I determined whether circulating E levels, while controlling for menstrual cycle phase, were related to cardiovascular, biochemical, and subjective responses to THC. This research extends preclinical research on the interaction between estrous cycle phase, circulating E, and responses to cannabinoids to a human behavioral paradigm.

Within the following introduction I will a) explain the neurobiological mechanism of THC, b) describe its acute effects in animals and humans, c) provide an overview of the rodent estrous cycle and human menstrual cycle, d) review preclinical evidence of estrous cycle and ovarian hormone related differences in responses to cannabinoids and e) summarize my recent efforts to elucidate whether menstrual cycle phase and circulating E are related to responses to THC in humans.

1.2 Neurobiological Mechanism of THC

Since the first isolation of THC, nearly 65 years ago (Gaoni and Mechoulam 1964; Mechoulam and Gaoni, 1965), numerous studies have established that many of the psychoactive effects of *Cannabis* are mediated by THC and its activation of distinct endogenous cannabinoid receptors (Adams and Martin 1996; Wachtel et al. 2002). THC acts as a partial agonist on both the endogenous cannabinoid type 1 receptor (CB_1R) and the endogenous cannabinoid type 2 receptor (CB_2R) (Felder et al., 1992). CB_1Rs are more heavily concentrated in the Central Nervous System (CNS) but are also distributed throughout the Peripheral Nervous System (PNS) and peripheral tissues (Pertwee et al., 2006). Within the CNS, endogenous cannabinoids, anandamide (AEA) and 2-arachidonyl glycerol (2-AG), are produced 'on demand' by postsynaptic neurons, following large membrane depolarizing events such as action potentials or large influxes of calcium ions, and bind presynaptic CB_1Rs to inhibit neurotransmitter release (Freund et al., 2003). Although CB_1Rs are among the most abundant G protein-coupled receptor (GPCRs) in the brain, a relatively limited number of neurons express very high levels of CB_1Rs (Zou and Kumar, 2018). CB_1Rs are densely expressed in the neocortex, hippocampus, basal ganglia, amygdala, striatum, cerebellum, and hypothalamus (Alger, 2013; Mackie, 2005; Herkenham et al., 1991; Glass et al., 1997; Mato et al., 2003). Thus, endocannabinoid signaling is involved in a variety of high-order behavioral functions, including learning and memory, executive function, sensory and motor responsiveness, and emotional reactions, as well as eating and other homeostatic processes (Bossong et al., 2014; Curran et al., 2016; Pacher et al., 2006).

Preclinical studies report sex and hormone-related differences in overall expression of CB_1Rs throughout the rodent brain. A recent study found CB_1R mRNA expression differed between male and female mice in the orbital, insular, cingulate, piriform cortices, as well as the striatum and hippocampus (Liu et al., 2020). Another study found CB_1Rs expression was significantly lower in the prefrontal cortex and amygdala of female rats than in males (Castelli et al., 2014). Taken together, these findings suggest CB_1R expression within the CNS could be dependent on the activational effects of circulating hormones.

Conversely, CB_2R expression within the CNS is restricted mainly to the brainstem and hippocampus (Stempel et al., 2016; Van Sickle et al., 2005). CB_2Rs are primarily distributed throughout peripheral tissues and occur mainly on immune cells to modulate cytokine release and inflammation (Gong et al., 2006; Howlett et al., 2002). Activation of the CB_2R system results in inhibition of neuroinflammatory signaling pathways (Bie et al., 2019). The lack of CB_2Rs expressed within the CNS likely explain why they are not directly related to undesired psychotropic drug effects or addiction liability.

Although CB_1Rs and CB_2Rs serve different purposes within the CNS and PNS, they are both coupled through $G_{i/o}$ proteins, negatively to adenylyl cyclase and positively to mitogen-activated protein kinase (Pertwee et al., 2006; Howlett et al., 2002). CB_1R receptors can also be negatively coupled to voltage-dependent calcium channels necessary for neurotransmitter release (Pertwee et al., 2006; Mackie and Hille, 1992; Sullivan, 1999; Hoffman and Lupica, 2000), or positively to potassium channels, which shortens action potential duration and decreases the amount of neurotransmitter

release per action potential (Mu et al., 1999; Schweitzer, 2000; Robbe et al., 2001). It was not until 2001, when Huestis et al. blocked the acute effects of smoked *Cannabis* in humans using the CB_1R antagonist SR141716, also known as rimonabant, that the direct interaction between THC and CB_1Rs was identified as mediating the psychological and physiological effects of *Cannabis* (Huestis et al., 2001).

THC produces its stereotypical acute effects by mimicking the actions of endogenous cannabinoids, activating CB_1Rs and inhibiting chemical transmission between neurons. CB_1Rs are present on various types of neurons, including pyramidal neurons, interneurons, and to a much lesser extent on glial cells, such as astrocytes (Navarrete and Araque, 2010; Katona and Freund, 2012). Within neuronal circuits, suppression of excitatory transmitter (i.e., Glutamate) release tends to dampen excitation, while suppression of inhibitory transmitter (i.e., gamma-aminobutyric acid (GABA)) release favors neuronal network excitation. Although the majority of CB_1Rs can be found on GABAergic neurons within the forebrain and midbrain, there is evidence for CB_1Rs expression on some glutamatergic neurons in the forebrain as well (Marsicano et al., 2003). Thus, acute THC exposure can directly dampen neural excitation in certain brain regions and increase neural excitation in others, depending on the localization of the CB_1Rs. In addition, CB_1R functionality may differ depending on the type of neuron. For example, using immunoreactivity assays, a preclinical study found, glutamatergic CB_1Rs were more efficiently coupled to G-protein signaling than GABAergic CB_1Rs (Steindel et al., 2013). As mentioned, CB_1Rs are expressed on various cell types within the neocortex, hippocampus, basal ganglia, amygdala, striatum, cerebellum, and hypothalamus (Alger, 2013; Mackie, 2005; Herkenham et al., 1991; Glass et al., 1997;

Mato et al., 2003). Further, these direct effects on excitatory or inhibitory transmission can also indirectly modulate other secondary neurotransmitter release. For example, although the euphoriant or rewarding effects of acute doses of THC are primarily mediated by dopaminergic signaling within the mesolimbic dopamine (DA) pathway (Bloomfield et al., 2016), this results from the activation of CB_1Rs on glutamatergic and GABAergic inputs into the ventral tegmental area (VTA; Lupica and Riegel, 2005, Mátyás et al., 2008, Melis et al., 2004). Preclinical findings have consistently shown acute THC evokes burst firing of the VTA dopaminergic neurons projecting to the nucleus accumbens, thereby increasing extracellular DA in striatal brain regions (Cheer et al., 2004, French et al., 1997, Riegel and Lupica, 2004, Tanda et al., 1997). Although findings in humans have been inconsistent (Volkow et al., 1996), using positron emission tomography (PET), a combined analysis of two previous studies did report acute THC increased DA release within the ventral striatum (Bossong et al., 2015). Inconsistent findings are likely due to the complex downstream effects of THC's partial agonism on CB_1Rs. Partial agonists can activate receptors, but do so with less efficacy (i.e., lesser maximal effect) than a pure agonist. Furthermore, partial agonists can also block activity of an endogenous ligand, thus inhibiting downstream signaling. Ultimately, THC acts on endogenous cannabinoid receptors to acutely modulate chemical transmission within and between certain brain regions resulting in its varied cardiovascular, biochemical, and psychological effects.

Lastly, it is important to note that if cannabinoid receptor expression or binding affinity is altered within the CNS, this not only affects endogenous signaling but could significantly alter the acute effects of THC. Thus, it is imperative we investigate the

underlying factors modulating cannabinoid receptor expression and functionality. This kind of research will deepen our understanding of the neurobiological underpinnings of individual differences in responses to acute THC.

1.3 Acute Effects of THC

Single doses of THC induce a broad range of transient and dose-dependent effects in both animals and humans. Although preclinical animal models have informed human clinical research for decades, they are limited in their ability to provide insight into subjective drug experience. The translation of preclinical animal research into human behavioral pharmacology research is imperative in furthering our understanding of the predictive validity of animal models, as well as measuring the subjective drug effects, such as self-reported changes in mood or feelings of intoxication, which cannot be measured in animals. Acute subjective effects of alcohol and stimulants predicted continued drug use and future drug taking behavior in several clinical human studies (Li et al., 2020; Murray et al., 2021; de Wit and Phillips, 2012; King et al., 2011; Ray et al., 2010). By investigating individual differences in the acute subjective effects of THC in humans, we can deepen our understanding of why individuals may choose to repeat or avoid THC use. Ultimately, human behavioral studies of the acute effects of THC not only have the capacity to translate preclinical findings, but also extend findings by adding self-reported measures of subjective drug experience, thus maximizing clinical applications.

In rodents, acute THC produces reinforcing effects, typical of other substances of abuse, as well as a characteristic tetrad of *in vivo* effects, suppression of spontaneous locomotor activity, antinociception, hypothermia, and catalepsy. Studies using the conditioned place preference assay and intracranial self-stimulation, two common methods used to establish the rewarding effects of drugs in rodents, found that low doses of acute THC induced conditioned place preference (Braida et al., 2004; Valjent and Maldonado, 2000; Lepore et al., 1995) and decreased the threshold for intracranial self-stimulation (Gardner et al., 1988). These findings demonstrated the positive rewarding effects of acute THC exposure on animals. However, at higher doses, THC can produce aversive effects and anxiety-like behavior (Schramm-Saptya et al., 2007; Onaivi et al., 1990). Additionally, at lower doses, THC increases locomotor activity of rats whereas high doses suppress locomotor activity (Sañudo-Peña et al., 2000). Across a range of moderate to high intravenous (i.v.) or intraperitoneal (i.p.) administered doses, acute THC exposure elicits reliable dose-dependent suppression of spontaneous locomotor activity, antinociception, hypothermia, and catalepsy in adult male mice (Smirnov and Kiyatkin, 2008; Compton et al., 1993; Martin et al., 1991). Furthermore, the magnitude of THC induced antinociception and hypothermia differs between sexes. Female rats exhibit greater sensitivity to the antinociceptive and hypothermic effects of THC (Craft et al., 2012; Tseng and Craft, 2001; Wiley et al., 2007; Borgen et al., 1973). Ultimately, preclinical findings indicate acute THC administration not only produces the prototypic reinforcing effects of other drugs, but also dose dependent effects on heart rate (HR), blood pressure (BP), locomotor activity, antinociception, body temperature, and anxiety-like behavior in rodents.

In humans, THC produces many of the same physiological and behavioral effects, but there are also various unique differences. Like in rodents, THC acutely increases HR (Ghasemiesfe et al., 2020) and produces anxiety (D'Souza et al., 2004, Bhattacharyya et al., 2017) in humans. Certain studies also report THC having an antinociceptive effect (Elikkottil et al., 2009; Wade et al., 2003; Campbell et al., 2001). Although findings are somewhat inconsistent on the antinociceptive effects in humans (Naef et al., 2003), there have been reports of analgesic effects in certain clinical populations (Elikkottil et al., 2009; Campbell et al., 2001). For example, Zeidenberg et al. (1973) reported orally administered THC capsules decreased discrimination of thermal stimulation in a sample of only men. More recently, Svendsen et al. (2004) reported orally administered dronabinol (10 mg), synthetic THC, significantly reduced pain intensity in a sample of multiple sclerosis patients when compared to placebo. Within the same year, another clinical study found whole plant *Cannabis* extract decreased self-reported pain scores in a sample of patients experiencing chronic neuropathic pain (Berman et al., 2004). Most of these clinical studies differ from rodent studies because they are testing the effects of sustained THC treatment, over a certain number of day or weeks, rather than just a single acute dose. Similar to the reinforcing effects observed in animals, oral THC also produces dose dependent positive subjective effects, such as 'liking', and 'wanting more drug', and is self-administered significantly more than placebo in humans (Chait and Zacny, 1992; Hart et al., 2002; 2005; Wachtel et al., 2002; Curran et al., 2002). Yet THC can also induce anxiogenic responses in humans (Hunault et al., 2009). During a laboratory study in which THC (0, 2.5 mg, and 5 mg) was administered intravenously the drug increased state anxiety

(D'Souza et al., 2004). Further, Bhattacharyya et al. (2017) reported that oral THC (10 mg) increased state anxiety and the severity of this anxiety was correlated with the baseline availability of CB_1 receptors in the amygdala, determined via PET scan. In contrast to preclinical findings, THC is associated with a dose-dependent increase in HR without significantly affecting blood pressure in humans (Pabon and de Wit, 2019; Ghasemiesfe et al., 2020; Hunault et al., 2008; Wachtel et al., 2002; Ashton et al., 2001; Lex et al., 1984; Kanakis et al., 1976; Beaconsfield et al., 1972; Kirk and de Wit, 1999; Karniol and Carlini, 1973; Zuurman et al., 2008). Differences between preclinical and human behavioral studies could be due to various factors, including route of administration and dosing. It is also important to note, clinical studies have found the acute effects of THC can vary between individuals and within individuals on different occasions of use (Green et al., 2003). For example, responses to THC vary with sex, BMI, age, personality, and frequency of *Cannabis* use (Fogel et al., 2017; D'Souza et al., 2008; Kleinloog et al., 2014; Pope and Yurgelun, 1996). To better understand how THC affects humans, it is imperative we identify any additional sources of variation in its acute effects. Although acute THC can produce rewarding and analgesic effects in humans, like those seen in animals, it can also produce tachycardia, anxiety, and effects that are dependent on various predisposing characteristics.

1.4 The Estrous Cycle & The Menstrual Cycle

The estrous cycle refers to the reproductive cycle or a cyclic pattern of physiological changes, associated with circulating ovarian hormones, in most non-

primate mammals, such as rats and mice. The estrous cycle has four phases, proestrus, estrus, metestrus and diestrus, and has an average length of 4-5 days (Ajayi and Akhigbe, 2020; Parkes, 1928; Long and Evans, 1922; Mandl, 1951). These phases are characterized by specific changes in circulating E and P levels (Cora et al., 2015). During proestrus, P levels decline, and E levels rise, indicating the development of the endometrium and ovarian follicle (Ajayi and Akhigbe, 2020). As a result, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) surge. The LH peak is associated with a decrease in E and ovulation occurs 24 to 48 hours later at the starts of the estrus phase (Caligioni, 2009). During the estrus phase, E levels are still elevated but declining and animals are in 'heat', during which they are the most sexually receptive. In the absence of conception, metestrus follows estrus (Byers et al., 2012). During this relatively brief phase, E levels remain low and P levels begin to rise, as the corpus luteum starts to form. Following metestrus is the final phase of the estrous cycle, diestrus, when P levels peak and the corpus luteum reaches maximum functionality and begins to regress (Ajayi and Akhigbe, 2020). By the end of diestrus, E begin rising again, immediately before the start of a new proestrus phase (Walmer et al., 1992).

Several studies have examined responses to psychoactive drugs at different phases of the estrous cycle (Lacy et al., 2016; Craft and Leitl, 2008). In rats, release of DA after d-amphetamine (AMPH) varied with estrous cycle phase: The greatest DA release occurring during phases when E levels were elevated (Becker and Ramirez, 1981; Becker and Cha, 1989). These increases in DA release corresponded with increased behavioral responses to AMPH, such as locomotor activity, rotational behavior, and stereotypy (Becker et al. 1982; Joyce and Van Hartesveldt, 1984; Becker

and Cha, 1989). P levels also influence responses to stimulant drugs. One study found P levels had an inhibitory effect on AMPH-induced stereotype activity (Michanek and Meyerson, 1982). Another study found intermittent infusion of P potentiated AMPH-induced DA release *in vitro* (Dluzen and Ramirez, 1987).

In humans, the reproductive cycle in women is the menstrual cycle, also known as the uterine or ovarian cycle. Like the estrous cycle, the menstrual cycle is characterized by changes in E and P levels, as well as changes in physiology, mood, and behavior. The median length of a human menstrual cycle is approximately 28 days, but can vary between individuals (Treloar et al., 1967; Vollman, 1977). Ovulation occurs approximately halfway through the menstrual cycle around day 14. The phase before ovulation is the follicular phase (days 1-14) and after ovulation is the luteal phase (days 15-28) (Vollman, 1977). These phases can be further subdivided into early (EF; days 1-8) and late follicular phases (LF; days 9-14), as well as early, mid-, and late luteal phases. The follicular phase, also known as the proliferative phase, begins the first day of menstruation and ends with ovulation, closely resembling the proestrus phase of the estrous cycle. The maturation of ovarian follicles occurs during this phase. P levels remain low throughout the entire follicular phase, whereas E levels begin low during the EF phase and then rise as the follicle develops, peaking in the LF phase (Schmalenberger et al., 2021; Hampson, 2020). At the end of the follicular phase, there is a surge in LH, which is a result of the E peak, and ovulation occurs. The luteal phase, also known as the secretory phase, begins the day after ovulation until the end of the cycle and the begin of menstruation or the next follicular phase. This phase is characterized by the transformation of a dominant follicle into the corpus luteum. During

the early luteal phase, both P and E levels begin low but gradually rise as the corpus luteum produces them (Schmalenberger et al., 2021; Hampson, 2020). P levels peak during the mid-luteal phase, while there is also a secondary peak in E levels. If no fertilization of the oocyte occurs, the corpus luteum begins regressing, and both P and E levels decline during the late luteal phase. Eventually the endometrium begins to shed, marking the start of menstruation and the onset of another menstrual cycle.

It is important to note, the human menstrual cycle is also associated with changes in basal mood state, physiology, and other hormones such as prolactin, growth hormone, thyroid-related hormones, and melatonin, that can either precede or follow changes in levels of E and P (Leibenluft et al, 1994). Moreover, menstruation during the EF is also associated with increases in negative mood, anxiety, and dysmenorrhea (Agarwal and Agarwal, 2010). Any of these factors, outside of ovarian hormones, could also influence how naturally cycling women respond to psychoactive drugs, both physiologically and subjectively.

Several studies have reported effects of menstrual cycle phase or ovarian hormones on responses to stimulant drugs (Schiller et al., 2016; Terner and de Wit, 2006; Justice and de Wit, 2000a; 2000b; 1999). For example, healthy naturally cycling women not only reported greater subjective responses to AMPH during the follicular phase, compared to the luteal phase, but responses to AMPH during the follicular phase were related to E levels (Justice and de Wit, 1999). Another study comparing responses to AMPH during the EF and LF phase found most acute subjective and physiological effects were the same across phases. However, subjects reported greater Unpleasant Stimulation after AMPH and less Unpleasant Sedation during the LF phase when E

levels were higher, although this was not related to E levels (Justice and de Wit, 2000b). In another study (Justice and de Wit, 2000a) E was administered exogenously to healthy naturally cycling women in the early follicular phase, in combination with AMPH or placebo. Most of the subjective and physiological effects of AMPH were not affected by the supplemented E. Nevertheless, E pretreatment increased the magnitude of the effects of AMPH on subjective ratings of 'pleasant stimulation' and decreased ratings of 'want more'.

Several other studies have examined responses to cocaine in relation to menstrual cycle phase. Although some studies did not find the effects of cocaine to vary across the menstrual cycle (Kaufman et al., 2001; Mendelson et al., 2001), most studies found women report greater positive subjective effects of cocaine during the follicular compared to the luteal phase (Evans et al., 2002; Lukas et al., 1996; Sofuoglu et al., 1999). More specifically, Evans et al. (2002) revealed women who smoked cocaine reported higher ratings of "good drug effect," "high", and "stimulated" during the follicular compared to the luteal phase.

To our knowledge, only two studies, from the 1980s, have attempted to determine whether menstrual cycle phase caused differences in cannabinoid response. One of the studies recorded self-reported *Cannabis* use across the menstrual cycle and found no effect of cycle phase on *Cannabis* use (Griffin et al., 1986). The other study examined the effect of smoked *Cannabis* on pulse rate and mood in females during the follicular, ovulatory, and luteal phases of the cycle. Again, no significant menstrual phase difference in HR or changes in mood was detected (Lex et al., 1984). Although these results would suggest menstrual phase has little effect on response to *Cannabis*,

the first study only recorded self-reported *Cannabis* use, not physiological or subjective effects, and the second did not directly measure hormone levels to verify cycle phase. Furthermore, these studies focused on smoked whole plant *Cannabis*, rather than its primary psychoactive ingredient, THC. Whole plant *Cannabis* varies widely in THC content, and it is difficult to standardize the amount of smoked inhaled by each subject. Thus, these early studies did not establish whether cycle phase affects response to the drug. By investigating whether response to acute THC differs within the follicular phase, between early and late phases, we can explore how E fluctuations may modulate sensitivity to THC.

1.5 Ovarian Hormone-related Differences in Rodent Cannabinoid Responses

There is growing evidence that responses to cannabinoids depend on ovarian hormones and estrous cycle phase in rodents. Naturally cycling female rats, as well as ovariectomized (OVX) female rats who were given supplemental E, self-administered greater amounts of CB_1 receptor agonists, developed greater levels of dependence and exhibited more severe withdrawal symptoms when repeatedly administered THC (Fattore et al., 2007; Marusich et al., 2015). In a follow up study, gonadally intact females were more sensitive to both drug- and cue-induced reinstatement of cannabinoid-seeking behavior than OVX females (Fattore et al., 2010). Further, supplemental E enhanced THC-induced antinociception (Craft and Leitl, 2008) and self-administration of a CB_1R agonist (Fattore et al., 2010) in OVX female rats. When comparing intact female animals between different phases of the estrous cycle, rodents

were the most sensitive to the antinociceptive effects of THC in late proestrus to estrus, when E is the highest (Craft and Leitl, 2008; Wakley and Craft, 2011). These studies provide compelling evidence of the activational effects of ovarian hormones on cannabinoid-induced behavioral responses in rodents. This evidence that circulating levels of E modulate responses to cannabinoids in rodents, suggest that similar relationships may occur in humans. To evaluate the predictive validity of these preclinical models and determine the translatability of their findings, my graduate work investigated whether menstrual cycle phase and circulating E levels in humans influence responses to THC.

1.6 Estrogen-Cannabinoid Interactions

Interactions between circulating gonadal hormones, specifically E, and the endogenous cannabinoid system may underlie the hormone related differences in responses to exogenous cannabinoids. In preclinical rodent models, the endocannabinoid system is strongly influenced by hormonal factors, particularly circulating levels of E (López, 2010). For example, several studies have shown E modulates the synthesis and metabolism of endocannabinoids in the periphery (MacCarrone et al., 2000; 2002; 2004; Xiao et al., 2002; Guo et al., 2005; Grimaldi et al., 2009; Ribeiro et al., 2009; El-Talatini et al., 2010). Within the brain, fluctuations in E also affect endocannabinoid signaling activity (Rodríguez de Fonseca et al., 2005; González et al., 2000; Mize and Alper, 2000; Corchero et al., 2001; Bradshaw et al., 2006; Nguyen and Wagner, 2006; Hill et al., 2007). Some of the most compelling

evidence comes from studies investigating estrogenic modulation of cannabinoid receptor expression and density within various brain regions (Rodríguez de Fonseca et al., 1994; González et al., 2000; Riebe et al., 2010). When comparing OVX to E-treated OVX female rats, E-treated animals not only showed decreased CB_1R binding site density in the hypothalamus, but also the hippocampus, a brain region known to have a high density of CB_1R (Riebe et al., 2010). In addition, E-treated animals also had higher CB_1R binding site density in the amygdala compared to OVX females. These findings suggest E modulates CB_1R expression within the rodent brain, which may in turn explain the differences in behavioral responses seen in other preclinical studies.

In addition to estrogenic modulation of endogenous cannabinoid signaling, the metabolism of exogenous cannabinoids is also hormone dependent. THC is metabolized in the liver by microsomal hydroxylation and oxidation catalyzed by enzymes of the cytochrome P450 (CYP) complex (Sharma et al., 2012). More than 100 THC metabolites have been identified, with 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) being the major active metabolite and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) being the major inactive metabolite (Sharma et al., 2012; Huestis, 2005; 2007; Grotenhermen, 2003; Burstein et al., 1972). 11-OH-THC is as potent and psychoactive as THC, in both rodents (Ford et al., 1977; Tseng and Craft, 2001) and humans (Lemberger et al., 1972). Following THC administration in rodents, brain and/or blood levels of the major active metabolite 11-OH-THC have been reported to be greater in gonadally intact female rats compared to males (Tseng et al., 2004; Wiley and Burston, 2014; Britch et al., 2017) and in liver microsome preparations from female rats compared to male (Narimatsu et al., 1991). Although an early clinical study

reported no sex differences in THC metabolism after oral or i.v. THC administration (Wall et al., 1983), a more recent study with a larger sample size, found women had a significantly greater maximum concentration of 11-OH-THC after oral THC administration when compared to men (Nadulski et al., 2005). These results suggest the metabolism of THC is influenced by ovarian hormones, which is not surprising, given E can influence liver production of CYP2C7 (Bandiera and Dworschak, 1992). Furthermore, supplemental E also significantly increased serum levels of 11-OH-THC, four hours after THC administration, in OVX rodents (Craft et al., 2017). Thus, circulating E affects the metabolism of THC, increasing the production of the equally psychoactive 11-OH-THC. This mechanism may also play a role in hormone related differences in responses to exogenous cannabinoids.

1.7 Outline of Experimental Aims

The research presented in this thesis represents one of the first attempts to examine menstrual cycle phase and ovarian hormone-related variability in acute responses to oral THC in humans. The main goal of this research was to compare acute responses to THC in women at the early and late phases of the follicular menstrual phase, which differ mainly in circulating levels of E. We designed a mixed within- and between-subject study to examine effects of oral THC (0, 7.5 mg and 15 mg) in two groups of female occasional *Cannabis* users: females tested in the early follicular phase of their menstrual cycle (EF; N=30) and females tested in the late follicular phase (LF; N=30). Occasional *Cannabis* users were defined as individuals who have used

Cannabis ten or fewer occasions in the past thirty days. Females were randomly assigned EF or LF groups. EF females will be tested 1 to 5 days since the first day of menstruation and LF females between days 10 and 14. Subjects attended two or three 4-hour experimental sessions during which they received THC (7.5 or 15 mg) or placebo in counterbalanced order under double blind conditions. With this study we aimed to (1) examine and compare cardiovascular, biochemical, and subjective effects of THC between the EF and LF groups and (2) determine whether any effects of THC are related to circulating ovarian hormone levels, while controlling for menstrual cycle phase. This research will contribute significantly to the small literature on individual differences in response to THC. It will lay foundation for future female-specific *Cannabis* use guidelines and new female-specific *Cannabis* use disorder treatment options.

We hypothesized women would be more sensitive to the acute effects of THC during the LF phase, mainly because of the relatively higher circulating levels of E. It is also important to note that the EF and LF phases of the menstrual cycle are associated with changes in mood, physiology, and behavior that are not directly related to the current hormonal state, but which could also influence acute responses to THC. That is, the female cycle is a dynamic process involving numerous physiological changes, some of which do not coincide temporally with momentary circulating levels of E and P (Leibenluft et al, 1994). For example, other hormones such as prolactin, growth hormone, thyroid-related hormones, cortisol, and melatonin, vary across the cycle and can either precede or follow changes in levels of E and P. These other hormones could contribute to observed differences in response to drugs at different cycle phases. For this reason, we analyzed the results of this study in two ways: first, comparing

responses to THC during the EF and LF phases, and second, examining responses to the drug in relation to quantitative circulating E and P levels, while controlling for cycle phase. For the first goal, we predicted women would display greater sensitivity to THC-induced changes in HR and ratings of subjective drug effects, specifically the anxiety-inducing effects, during the LF, compared to the EF phase.

To examine hormone-related individual variability in responses to THC, we examined whether circulating levels of E or P separately, or the ratio between E and P levels, were significant predictors of acute cardiovascular, biochemical, and subjective effects of oral THC. We hypothesized E levels would be significantly related to acute effects. Since P levels remain low and stable across the follicular phase, we hypothesized P levels would not be significantly related to acute effects.

Chapter 2: Responses to oral THC during early and late follicular phase of the menstrual cycle

2.1 Summary

To determine the potential relationship between menstrual cycle phase and response to cannabinoids in humans we tested the effects of oral THC in healthy naturally cycling female occasional *Cannabis* users at two hormonally distinct phases of the menstrual cycle, the EF and LF phases. As described earlier, the EF and LF phases are characterized by low and high circulating levels of E, respectively, while P levels remain low (Schmalenberger et al., 2021; Hampson, 2020). Sixty women who occasionally use *Cannabis* received oral THC (7.5 mg and 15 mg or just 15 mg) and placebo (0 mg THC) during either the EF phase (days 1 to 5 from first day of menstruation) or LF phase (days 9 to 14). Women were randomly assigned to the two groups, and the drug was administered in a double-blind and counterbalanced design. The primary outcome measures were cardiovascular and biochemical responses and subjective ratings of mood and drug effects. Blood serum E and P levels were measured at the start each session to confirm cycle phase. We hypothesized women would be more sensitive to the acute effects of THC during the LF phase, mainly because of the relatively higher circulating levels of E. As expected, E levels were higher in the LF group, and THC produced its expected physiological effects, including increased HR, decreased high frequency heart rate variability (HF HRV), and increased salivary cortisol. These physiological effects were similar in the EF and LF groups. THC also produced its

expected subjective effects, including increased ratings of “feeling” a drug effect, anxiety, confusion, and *Cannabis*-specific intoxication. These subjective effects occurred earlier in the session in the EF group compared to the LF group. The findings did not support the hypothesis that effects of THC would overall be greater during the LF phase. Instead, they provide novel findings that the time course of subjective drug effects varies across the follicular phase.

2.2 Introduction

As both medical and recreational *Cannabis* use rises, it is imperative we identify underlying sources of variability in responses to THC, its main psychoactive constituent. As reported earlier, preclinical evidence suggests estrous cycle phase and ovarian hormones modulate responses to THC in rodents, yet the effect of menstrual cycle phase on responses to THC in humans remains unstudied. In addition to hormone levels fluctuating across the menstrual cycle, women experience changes in mood (Farage et al., 2008), physiological responses to stress (Kajantie and Phillips, 2006), and autonomic nervous system activity (Uckuyu et al., 2013), all of which can influence responses to drugs, such as THC. Here we compare acute physiological and subjective effects of THC between the EF and LF phase of the menstrual cycle in healthy normally cycling women. Our cardiovascular and biochemical measures included HR, BP, body temperature, as well as two measures of autonomic nervous system activity, electrocardiography (ECG) and salivary cortisol. From the ECG recordings we isolated a measure of parasympathetic cardiac activity, HF HRV, which prior studies found

decreases after inhalation of smoked THC (Zuurman et al., 2008). From the salivary cortisol, we obtained a measure of stress-related biochemical effects of THC. Subjective measures included ratings of “feeling”, “liking”, and “disliking” the drug effect, “wanting more” of the drug, how “high” the participant felt, anxiety, elation, fatigue, depression, confusion, and *Cannabis*-specific intoxication. All measures were not only recorded continuously after drug administration, but were also taken at baseline, pre-drug, to check and account for baseline groups differences between the EF and LF phase. We hypothesized women would be more sensitive to the acute cardiovascular, biochemical, and subjective effects of THC during the LF phase compared to the EF phase, displaying greater increases in HR, salivary cortisol, and ratings of subjective drug effects and a larger decrease in HF HRV, mainly due to the relatively higher circulating levels of E.

2.3 Method

2.3.1 Overall Design

The study used a mixed within- and between-subject design to examine effects of acute oral THC (0, 7.5 mg and 15 mg) in two groups of female occasional *Cannabis* users: females tested in the EF phase of their menstrual cycle (N=30) and females tested in the LF phase (N=30). Subjects were randomly assigned EF or LF groups. The EF group was tested 1 to 5 days since the first day of menstruation and LF group between days 10 and 14. The first forty participants were tested with two doses of THC

(7.5 mg, 15 mg THC) and the final twenty participants were only tested with the higher dose (15 mg THC). Occasional *Cannabis* users were defined as individuals who have used *Cannabis* ten or fewer occasions in the past thirty days. Subjects attended three 4-hour experimental sessions during which they received THC (7.5 or 15 mg) or placebo in counterbalanced order under double-blind conditions. Blood serum and saliva samples were obtained before capsule consumption at the start of each session for baseline hormone and salivary cortisol levels. The outcome measures of interest included cardiovascular, biochemical, and subjective drug effects, which were measured 6 times throughout the session (-15, 30, 60, 90, 120, 180 minutes post capsule).

2.3.2 Participants

Healthy female recreational *Cannabis* users (18-35 years, >4 times lifetime use and <11 uses of *Cannabis* in past month) were recruited by posters, advertisements, and word-of-mouth referrals. Potential participants underwent a semi-structured clinical psychiatric interview (American Psychiatric Association, 2013) and provided information about current and lifetime history of drug use. Individuals taking any prescription medications, or with serious psychiatric disorders such as psychosis, generalized anxiety disorder, major depressive disorder, severe Post-Traumatic Stress Disorder or Obsessive-Compulsive Disorder were excluded. Other exclusionary criteria were moderate or severe Substance Use Disorder, BMI less than 19 or more than 26, abnormal resting-state ECG or HR, or pregnant or planning to be pregnant. To be

included in the study, participants were required to have regular menstrual cycles and not be using hormonal contraceptives. Participants were instructed to refrain from alcohol and over-the-counter drug use for 24 hours before and 12 hours after the session, from *Cannabis* use 7 days before and 24 hours after the session, and from all other recreational drugs 48 hours before and 24 hours after the session. Compliance was verified using breath (Alcosensor III, Intoximeters Inc., St. Louis, MO) and urine tests (ToxCup, Branan Medical Corporation, Irvine, CA). Participants were told that the purpose of the study was to investigate interactions between drugs and mood. For blinding purposes, participants were told that they might receive a stimulant (i.e., caffeine, amphetamine), a sedative/tranquilizer (i.e., diazepam, alprazolam), a cannabinoid-like drug (i.e., *Cannabis*, THC) or a placebo (sugar pill). The study was approved by the local institutional review board.

2.3.3 Procedure

Participants attended a pre-study orientation session followed by two or three, 4-hour experimental sessions. During the orientation session, participants provided informed consent and were familiarized with the study procedures and tasks and completed personality questionnaires. The experimental sessions were conducted during the early or late follicular phase of the menstrual cycle, in the early afternoon and separated by at least 3 days. Upon arrival, participants provided urine and breath tests to confirm drug abstinence and completed baseline mood and subjective drug questionnaires, and a blood sample was taken for hormone analyses. Then, electrodes

were placed on the participants in a standard lead II configuration (Berntson et al., 2008) for the measurement of the electrocardiogram (ECG) and in tetrapolar electrode configuration for impedance (Sherwood et al., 1990). Half an hour after arrival, participants ingested a capsule containing either THC (7.5 or 15 mg THC with dextrose filler, or only 15 mg for later subjects) or placebo (dextrose only). Drug was administered under double-blind conditions and drug order was counterbalanced. HR and BP were measured and recorded, with portable monitors (Omron 10 Plus, Omron Healthcare), 6 time throughout the experimental session, followed by the administration of the subjective drug effect questionnaires (-15, 30, 60, 90, 120, and 180 minutes post capsule). Saliva samples were also taken 5 times throughout the experimental session (-15, 60, 90, 120, and 180 minutes post capsule) for cortisol analyses. ECG signals were recorded continuously at a sampling rate of 1000Hz throughout the session.

2.3.4 Drug

THC (Marinol® [dronabinol]; Solvay Pharmaceuticals) was administered in doses of 7.5 mg and 15 mg, in opaque capsules with dextrose filler. Placebo capsules contained only dextrose. These doses of THC are known to impair performance and produce subjective intoxication with minimal adverse reactions in experienced occasional, but non-daily *Cannabis* users (Broyd et al, 2016; Hartman and Huestis, 2013).

2.3.5 Dependent Measures

2.3.5.1 Demographics and Drug Use History

Demographic information and past drug use were assessed during the screening interview. Questionnaires were used to record ethnicity and race as well as current use of caffeine, nicotine, alcohol, *Cannabis,* and lifetime use of nicotine, alcohol, *Cannabis*, sedatives, stimulants, opiates, hallucinogens, ecstasy and related drugs, and inhalants. Participants also completed the Trait-Anxiety inventory (Spielberger et al. 1971) to measure baseline anxiety. On this scale, responses for 20 anxiety symptom items are recorded on a 4-point scale from “almost never” (1) to “almost always” (4). Range of scores is 20–80, the higher score indicating greater anxiety.

2.3.5.2 Physiological Measures

2.3.5.2.1 Heart Rate, Blood Pressure and Body Temperature

HR, BP, and body temperature were monitored through the session with portable monitors (Omron 10 Plus, Omron Healthcare; Metene Infrared Digital Thermometer). Saliva samples were taken 5 times throughout the experimental session (-15, 60, 90, 120, and 180 minutes post capsule) for cortisol analyses.

2.3.5.2.2 ECG Cardiography: High Frequency Heart Rate Variability

ECG signals were processed using an integrated Mindware Bionex system (Mindware, Gahanna, OH). After visual inspection for artifacts, ECG waveforms were analyzed using Mindware Heart Rate Variability Analysis Software v3.1. From these traces we derived HF HRV. To calculate HF HRV, the software first prepared the IBI series for spectral analysis as follows: each IBI series was interpolated and sampled at 4 Hz to ensure adequate resolution of the appropriate frequencies and equal intervals between samples, and then de-trended with a quadratic function to ensure stationarity (full details of this procedure in Berntson et al., 1997). This signal was brought into the frequency domain using a fast Fourier transform and integrating the power over the respiratory frequency band (0.12 to 0.40 Hz) and then natural log transformed to provide the measurement we report as HF HRV. As previously described (Bernston et al., 1997), autonomic measures were scored minute-by-minute and then collapsed into 5-minute epochs every 30 minutes throughout the 4-hour session. The primary outcome measure, HF HRV, is a measure of parasympathetic cardiac activation.

2.3.5.3 Ovarian Hormones

Blood draws (5 ml) were obtained while participants were seated at the start of each experimental session. They were collected in BDVacutainer Gold Top Serum tubes and centrifuged 30 minutes after collection. The top layer of serum was collected and frozen at −80 ˚C until analyses for hormone levels occurred. Serum samples were assayed for E and P at the University of Chicago Endocrinology Laboratory and the

Medical College of Wisconsin Hillard Laboratory. Serum E and P levels were measured using Enzyme-Linked Immunosorbent Assays (ELISA).

2.3.5.4 Salivary Cortisol

Saliva samples for cortisol analysis were obtained pre-drug and 1, 1.5, 2, and 2.5 hours post-drug consumption by saliva swab. The participant placed a SalivaBio collection swab below their tongue as they filled out subjective questionnaires. These swabs were stored in Salimetrics saliva storage tubes at -80˚C until assayed. Levels of cortisol in saliva samples were measured using ELISA assays at the University of Chicago Clinical Research Center

2.3.5.5 Subjective Measures

1. The Drug Effects Questionnaire (DEQ, Johanson and Uhlenhuth, 1980; Morean et al., 2013): This questionnaire consisted of 5 visual analogue scales measuring subjective drug effects. Participants indicated their response on a scale of 0-100: “Do you feel any drug effect?” (rated from “none at all” to “a lot”), “Do you like the effects you are feeling now?” (rated from “not at all” to “very much”), “Do you dislike the effects you are feeling now?” (rated from “not at all” to “very much”), “Are you high?” (rated from “not at all” to “very much”), and “Would you like more of what you consumed, right now?” (rated from “not at all” to “very much”).

2. Addiction Research Center Inventory (ARCI, Haertzen et al., 1963): This scale contained 53 true-false statements commonly used to describe subjective effects of psychoactive drugs. It was comprised of subscales measuring Amphetamine-like effects (A scale), morphine and benzedrine like effects (MBG scale), lysergic acid-like (LSD scale), benzedrine-like (BG scale), pentobarbital-chlorpromazine and alcohol-like (PCAG scale), and *Cannabis*-like (Mar scale).

3. The Profile of Mood States (POMS, McNair et al., 1971): This scale consisted of 72 adjectives commonly used to describe momentary mood states. Participants indicated how they feel in relation to each of the 72 adjectives on a 5-point scale from “not at all” (0) to “extremely” (4). The questionnaire was comprised of eight subscales (Anxiety, Depression, Anger, Vigor, Fatigue, Confusion, Friendliness, Elation). The Anxiety scale of the POMS was used to examine changes in state anxiety pre- and post-drug consumption.

2.3.6 Statistical Data Analyses

2.3.6.1 Demographic Characteristics

Demographic characteristics, drug use history and trait anxiety scores were compared between the EF and LF using independent samples t-tests for continuous variables and Pearson’s chi-squared analysis for categorical variables.

2.3.6.2 Ovarian Hormones

Levels of E and P obtained at the beginning of each session were compared between the EF and LF groups. We averaged E and P levels across all sessions for each participant to calculate the average ovarian hormone levels during the time spent participating in the study. We then performed an independent sample t-test to determine whether E or P levels significantly differed between the EF and LF phases.

2.3.6.3 Drug Effect Measures

Effects of oral THC and menstrual cycle phase across the 4-hour session on cardiovascular and biochemical measures (HR, Systolic BP, Diastolic BP, body temperature, HF-HRV, salivary cortisol) and subjective measures (DEQ, POMS, ARCI) were analyzed using covariance pattern models with Toeplitz covariance structures. Time (linear and quadratic) effects were included to allow for analysis of linear or curvilinear trends across time and are reported if significant. All analyses were completed in IBM SPSS statistical software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). Mixed effects models offer significant advantages relative to traditional repeated-measures ANOVA in handling of missing data, relaxation of assumptions of homogeneity of variance, and increased statistical power for smaller sample sizes. All physiological and subjective effects models included the effect of drug, time (linear and quadratic effects), and menstrual cycle phase group (early follicular vs late follicular) as independent fixed

variables. The effect of drug in all models included contrasts between placebo and 7.5 mg THC and placebo and 15 mg THC. Effect sizes are reported as unstandardized coefficients (β) with standard errors (SE).

2.4 Results

2.4.1 Demographic Characteristics

Most participants were Caucasian, light drinkers, and occasional *Cannabis* users in their early twenties (Table 1). The EF group and LF group did not differ significantly on any demographic measure.

	Early Follicular (N = 30)	**Late Follicular (N = 30)**
Age	24.0 (4.2)	24.2 (4.3)
Race		
Caucasian	83.3%	53.3%
African American	16.7%	13.3%
Asian	13.3%	6.7%
Other	3.3%	26.6%
Body Mass Index (BMI)	23.2 (2.7)	23.7 (2.8)
Recent Substance Use		
Alcohol (occasions/week)	2.0 (1.3) [N = 28]	2.2 (1.5) [N = 27]
Nicotine (cigarettes/day)	6.3 (9.3) [N = 4]	3.8 (2.8) [N = 5]
***Cannabis* (occasions in past 30 days)**	4.1 (6.5) [N = 22]	3.1 (2.8) [N = 19]
Lifetime Substance Use		
***Cannabis* [Median, (SD)]**	25 (515.6)	15 (157.8)
Trait Anxiety Inventory	37.1 (7.9)	38.5 (12.2)

Table 1. Demographic characteristics of the participants in the EF and LF Groups. Data represents mean (SD), median (SD), or percent of participants in sample.

2.4.2 Serum Circulating Hormone Levels

On average, across all completed sessions, LF women had significantly higher levels of circulating E than EF women ($p < 0.001$; Figure 1). Average circulating levels of P did not significantly differ between the two groups (Figure 1).

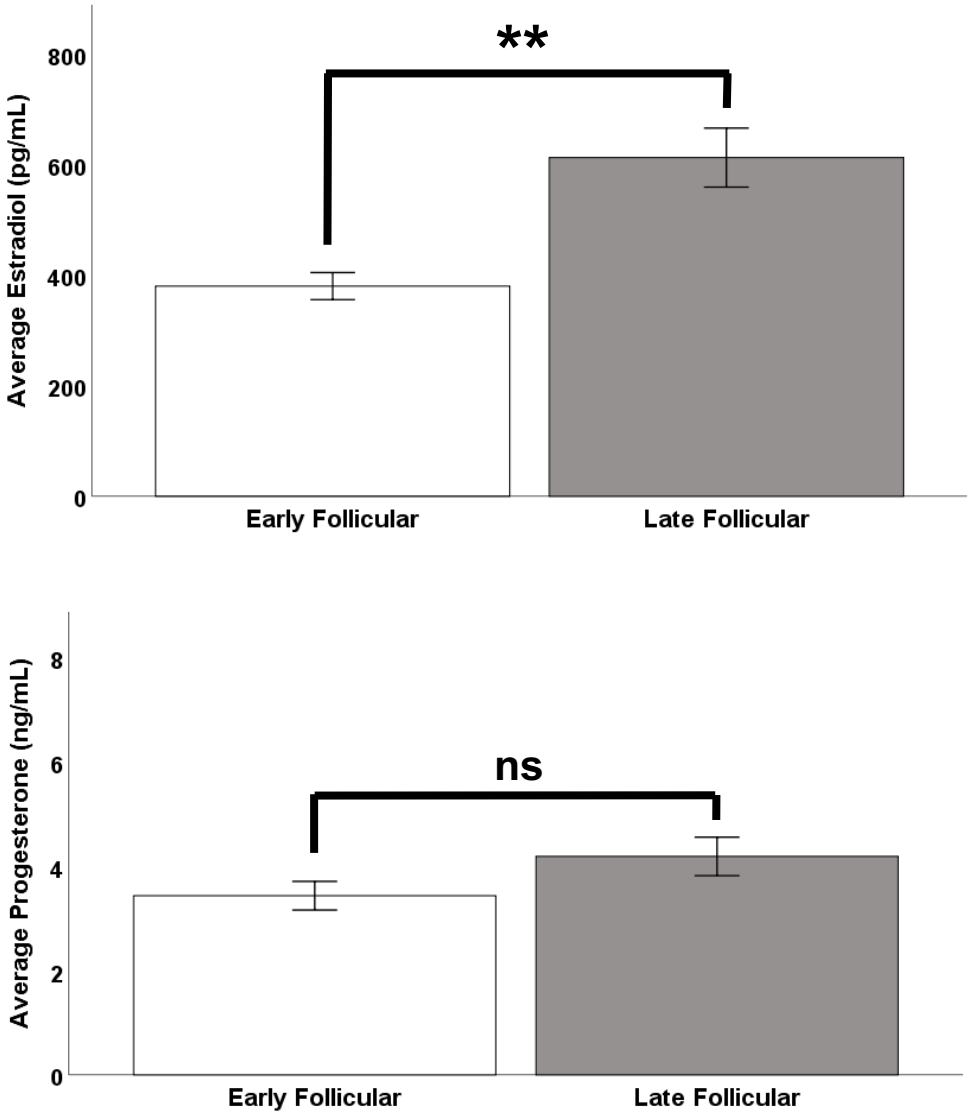

Figure 1. Serum ovarian hormone levels (A) estradiol and (B) progesterone by menstrual cycle phase. Error bars ± 1 SEM.

2.4.3 Drug Effects

2.4.3.1 Cardiovascular Measures

THC significantly increased HR and deceased HF HRV dose dependently (Table 2). At the 7.5 mg dose only, THC also decreased systolic BP. No significant effects due to menstrual cycle phase group (EF vs LF) were detected (Table 3).

2.4.3.2 Salivary Cortisol Levels

THC significantly increased salivary cortisol levels across time dose dependently (Table 2). No significant effects due to menstrual cycle phase group were detected (Table 3).

Physiological Measure	**7.5 mg THC v PLC [N_{EF} = 19] [N_{LF} = 21]**	**15 mg THC v PLC [N_{EF} = 19] [N_{LF} = 21]**
HR	1.2 (0.4); p = 0.002	2.5 (0.4); p < 0.0005
Systolic BP	- 0.7 (0.3); p = 0.024	ns
Diastolic BP	ns	ns
Body Temperature	ns	ns
HF HRV	- 0.2 (0.04); p < 0.0005	- 0.2 (0.04); p < 0.0001
Salivary Cortisol	0.02 (0.01); p = 0.015	0.04 (0.01); p < 0.0001

Table 2. Summary of overall physiological drug effects across session time by dose condition compared to placebo (PLC). Data represented as Drug*Time β (SE); p-value. Ns: non-significant

Physiological Measure	7.5 mg THC v PLC [N_{EF} = 19] [N_{LF} = 21]	15 mg THC v PLC [N_{EF} = 19] [N_{LF} = 21]
HR	ns	ns
Systolic BP	ns	ns
Diastolic BP	ns	ns
Body Temperature	ns	ns
HF HRV	ns	ns
Salivary Cortisol	ns	ns

Table 3. Summary of overall menstrual cycle phase differences across session time by dose condition compared to placebo (PLC). Ns: non-significant

2.4.3.3 Subjective Measures

THC significantly increased expected measures of subjective drug effects across session time, including DEQ ratings of "feeling", "liking", "disliking" and "wanting more", POMS anxiety, anger, fatigue, depression, confusion scores and ARCI M, A, LSD, BG, PCAG scores (Table 4). As shown in Figures 2, 3, and 4, EF women experienced marginally faster onset of subjective effects on several measures, although not all of these reached statistical significance. This trend toward faster onset was observed at both doses (Table 5). At the 7.5 mg dose, the EF reported higher ratings on DEQ "Feel" drug ($B_{Phase*Time*7.5\ mg}$ = -10.4, SE = 5.4, p = 0.053; $B_{Phase*Time*7.5\ mg}$ = 2.2, SE = 1.2, p = 0.07; Figure 2) during the 30 minutes (p = 0.08) and 60 minute (p = 0.07) time points, DEQ: "Want more" drug ($B_{Phase*Time*7.5\ mg}$ = -14.5, SE = 6.6, p = 0.028; $B_{Phase*Quadratic*7.5\ mg}$ = -0.2, SE = 1.3, p = 0.005; Figure 3) during 60 minute post-capsule time point, and ARCI M Scale ($B_{Phase*Quadratic*7.5\ mg}$ = 0.2, SE = 0.1, p = 0.078; Figure 4) during the 30 minute post-capsule time point (p = 0.02). At the 15 mg dose, the EF reported higher ratings on DEQ "Feel" drug ($B_{Phase*Time*15\ mg}$ = -9.5, SE = 4.9, p = 0.053; Figure 2) during the 90 minute (p = 0.04). The groups' responses on these measures did not differ significantly at other time points.

Subjective Measure	7.5 mg THC v PLC [N_{EF} = 19] [N_{LF} = 21]	15 mg THC v PLC [N_{EF} = 19] [N_{LF} = 21]
DEQ		
"Feel"	6.3 (0.8); p < 0.0001	10.6 (0.7); p < 0.0001
"Like"	4.5 (0.8); p < 0.0001	5.1 (0.8); p < 0.0001
"Dislike"	5.1 (0.8); p < 0.0001	7.4 (0.7); p < 0.0001
"High"	6.1 (0.8); p < 0.0001	11.0 (0.7); p < 0.0001
"Want More"	2.5 (0.8); p = 0.003	ns
POMS		
Anxiety	0.4 (0.1); p = 0.003	0.9 (0.1); p < 0.0001
Elation	- 0.3 (0.1); p = 0.04	- 0.3 (0.1); p = 0.027
Fatigue	ns	0.7 (0.2); p < 0.0001
Depression	ns	0.4 (0.1); p = 0.003
Confusion	0.7 (0.1); p < 0.0001	1.0 (0.1); p < 0.0001
ARCI		
Marijuana (M)	0.7 (0.07); p < 0.0001	0.9 (0.07); p < 0.0001
Lysergic Acid Diethylamide (LSD)	0.4 (0.08); p < 0.0001	0.6 (0.07); p < 0.0001
Benzedrine (BG)	- 0.4 (0.08); p < 0.0001	- 0.4 (0.07); p < 0.0001
Pentobarbital-chlorpromazine/Alcohol (PCAG)	0.7 (0.1); p < 0.0001	1.0 (0.1); p < 0.0001

Table 4. Summary of overall main subjective drug effects across session time by dose condition compared to placebo (PLC). Data represented as Drug*Time β (SE); p-value. Ns: non-significant

Subjective Measure	7.5 mg THC v PLC [N_{EF} = 19] [N_{LF} = 21]	15 mg THC v PLC [N_{EF} = 19] [N_{LF} = 21]
DEQ		
"Feel"	Trend toward faster onset in EF	Trend toward faster onset in EF
"Like"	ns	ns
"Dislike"	ns	ns
"High"	ns	ns
"Want More"	Faster onset in EF	ns
POMS		
Anxiety	ns	ns
Elation	ns	ns
Fatigue	ns	ns
Depression	ns	ns
Confusion	ns	ns
ARCI	ns	ns
Marijuana (M)	Trend toward faster onset in EF	ns
Lysergic Acid Diethylamide (LSD)	ns	ns
Benzedrine (BG)	ns	ns
Pentobarbital-chlorpromazine/Alcohol (PCAG)	ns	ns

Table 5. Summary of menstrual cycle phase group differences on subjective responses to THC by dose condition compared to placebo (PLC). Ns: non-significant

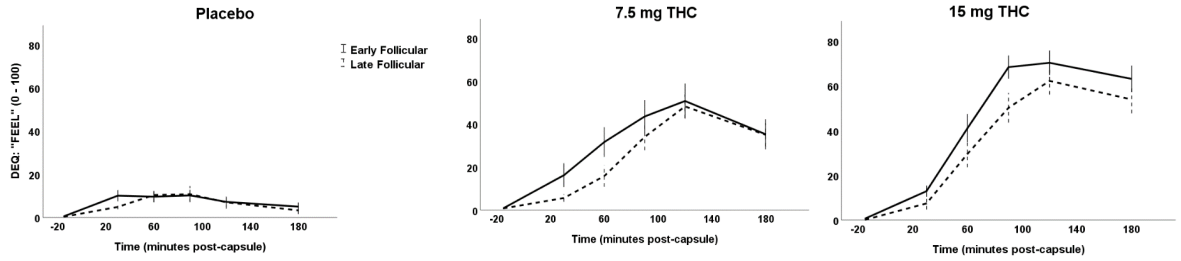

Figure 2. DEQ: "Feel" ratings for Placebo, 7.5 mg THC, and 15 mg THC conditions separated by menstrual cycle phase (EF: solid black line; LF: dashed black line). Error bars ± 1 SEM.

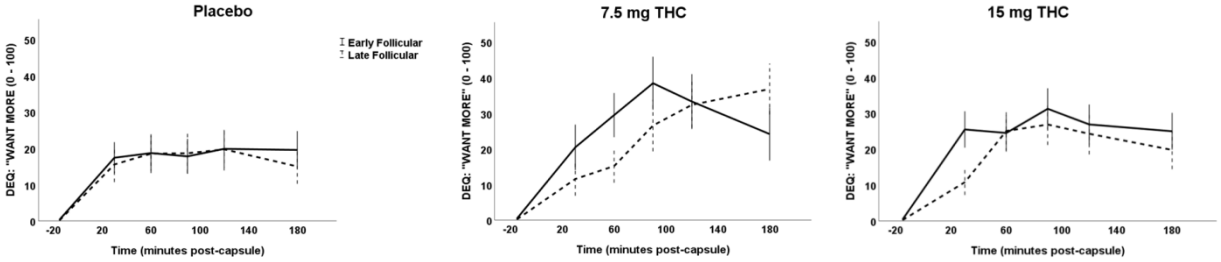

Figure 3. DEQ: "Want More" ratings for Placebo, 7.5 mg THC, and 15 mg THC conditions separated by menstrual cycle phase (EF: solid black line; LF: dashed black line). Error bars ± 1 SEM.

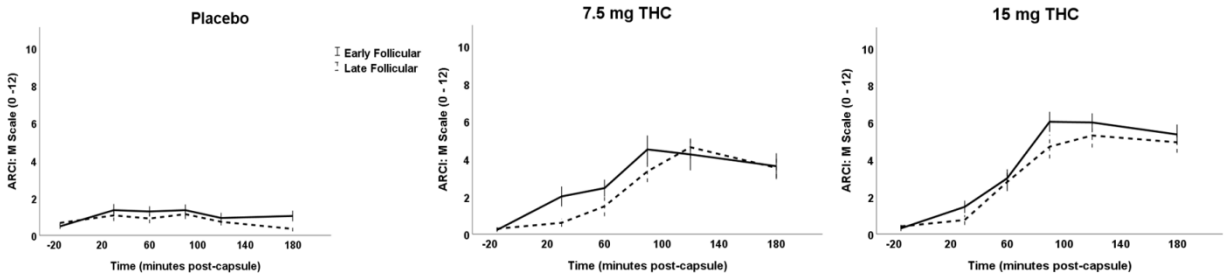

Figure 4. ARCI: M scale scores for Placebo, 7.5 mg THC, and 15 mg THC conditions separated by menstrual cycle phase (EF: solid black line; LF: dashed black line). Error bars ± 1 SEM.

2.5 Conclusion

The present study compared cardiovascular, biochemical, and subjective responses to oral THC (7.5 mg, 15 mg) in healthy women during the EF and LF phases of the menstrual cycle. As expected, on average across all sessions, LF women had significantly higher levels of circulating estradiol than EF women, while P levels did not differ between the groups. THC produced its prototypic acute effects, increased HR and salivary cortisol, decreased parasympathetic cardiac activity, and increased self-reported ratings of anxiety, fatigue, and confusion. Most measures did not differ between the two phases. However, ratings of "feel" drug effect, "want more" drug, and *Cannabis*-specific subjective intoxication symptoms occurred earlier in the EF group compared to the LF group, at both the 7.5 and 15 mg doses.

The finding that the EF group reported earlier effects than the LF group is novel. The fact that this earlier onset was detected with subjective ratings and not physiological responses indicates specificity of the cycle-related effects to certain measures, which argues against a pharmacokinetic explanation in terms of differences in rates of absorption. One possible explanation may be that participants tested during the EF were more sensitive to detecting subtle subjective effects, such as those during the onset of a drug effect. However, similar differences were not reported in previous studies conducted with AMPH (Justice and de Wit, 2000), suggesting this may be unique to *Cannabis*-like effects. This may be an interesting line of future research.

The findings did not support our hypothesis that responses to THC would be greater during the LF phase, compared to the EF phase. These two phases of the menstrual

cycle were selected mainly because of their different E levels (and low, stable P levels), and we discuss the role of these hormones in greater detail in the next section. However, the lack of difference in subjects' responses to the drug across these two cycle phases has some implications for both studies of cycle phase and use of cannabinoid drugs outside the laboratory. Although researchers are often (rightly) concerned that cycle phase might add unknown variability to responses to drugs in women, this concern is somewhat allayed by these findings. Moreover, the absence of differences across these two phases of the cycle also suggests that cycle phase, in this limited context of EF vs LF, also is unlikely to contribute to unexpected adverse responses in users outside the laboratory.

Chapter 3: Circulating ovarian hormone levels and response to oral THC

3.1 Summary

To determine whether circulating ovarian hormone levels affect acute responses to THC in humans, we measured circulating E and P levels from blood samples drawn before administering, acute doses of THC (7.5 mg and 15 mg or only 15 mg) to female occasional *Cannabis* users, as described in Chapter 2. The primary outcome measures were cardiovascular (HR, Systolic BP, Diastolic BP, Temperature, HF HRV) and biochemical (salivary cortisol) responses, as well as subjective ratings of mood and drug effects (DEQ, POMS, ARCI). The main drug effects across time on all outcome measures are reported in Chapter 2. Using the same dataset, while incorporating continuous repeated measures of quantitative E and P levels, for each experimental session, we analyzed whether ovarian hormone levels significantly modulated any of the drug effects across time. We hypothesized E levels would significantly impact cardiovascular, biochemical, and subjective effects. Since P levels remain low and stable across the follicular phase, we hypothesized P levels would not significantly impact responses to oral THC in our sample. Using mixed effects models, we found all acute effects of THC across time were highly robust. E or P levels did not significantly affect baseline cardiovascular, biochemical, or subjective measures, nor any effects of THC across time. These findings suggest circulating ovarian hormone levels do not significantly modulate acute responses to oral THC in naturally cycling women who

occasional use *Cannabis,* and thus do not underlie the previously reported menstrual cycle phase differences.

3.2 Introduction

Circulating ovarian hormone levels could mediate menstrual cycle phase differences in acute responses to THC in naturally cycling women. Preclinical studies have shown E can exert a positive effect on cannabinoid receptor expression throughout various limbic regions of the rodent brain (Riebe et al., 2010), and that ovarian hormones modulate behavioral responses to cannabinoids (Fattore et al., 2007; 2010; Marusich et al., 2015). However, these relationships remained unstudied in humans, until now. As stated in the literature, the EF and LF phases are characterized by low and high circulating levels of E, respectively, while P levels remain low (Schmalenberger et al., 2021; Hampson, 2020). The present data and Chapter 2 results confirmed, within our sample, EF women had significantly lower E levels than LF women on average across all sessions. By measuring blood serum levels of E and P at the start of each experimental session, we were first able to determine whether E or P levels, or the ratio between P and E levels (P:E) significantly predicted cardiovascular, biochemical, or subjective drug effects across time. Using a continuous repeated measure of hormone levels, one for each of the 2 or 3 experimental sessions, we examined whether E, P, or P:E were significant predictors of baseline cardiovascular (HR, Systolic BP, Diastolic BP, Temperature, HF HRV), biochemical (salivary cortisol), and subjective measures (DEQ, POMS, ARCI). Using placebo session data as a

reference to quantify effects of THC we also analyzed whether ovarian hormone levels on the day of the session, significantly influenced the acute effects of THC across time. We hypothesized E levels would significantly impact drug effects across time. Since P levels remain low and stable across the follicular phase, we hypothesized P levels would not significantly affect drug effects across time.

3.3 Method

Experimental and procedural methods are described in detail within Chapter 2. Using the same dataset as Chapter 2, we incorporated continuous repeated measures of circulating E and P levels separately, as well as the ratio of P to E for each session to analyze whether these ovarian hormone levels significantly impacted cardiovascular, biochemical, and subjective responses to acute oral THC.

3.3.1 Statistical Data Analysis

All analyses were completed in IBM SPSS statistical software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). First, one-way ANOVAs were conducted to determine whether E or P levels differed by session, across all participants, and within the EF and LF phases separately. Additional independent sample t-tests were conducted to compare E and P levels between the EF and LF groups within each experimental session.

Multilevel modeling was conducted, using Toeplitz covariance structures, to determine whether E or P levels separately, or the ratio between P and E levels significantly impacted cardiovascular, biochemical, and subjective effects of THC across time. Linear and quadratic effects were included to allow for analysis of linear or curvilinear trends across time. All cardiovascular (HR, Systolic BP, Diastolic BP, Temperature, HF HRV), biochemical (salivary cortisol) and subjective (DEQ, POMS, ARCI) effects models included the effects of drug and time (linear and quadratic) as independent fixed variables and circulating E, P levels or the ratio of E to P as a continuous repeated variable, one for each session. The effect of drug in all models were contrasts between placebo and 7.5 mg THC and placebo and 15 mg THC. Stepwise models were run first without the menstrual cycle phase variable (EF or LF), then run with the cycle phase variable to analyze whether circulating hormones alone were significantly related to the effects of THC and whether they potentially mediated the cycle phase differences seen in Chapter 2.

3.4 Results

Subjects were tested on 2 or 3 sessions within their assigned cycle phase, raising the question of whether their hormone levels were stable across the sessions. When data from all subjects were combined, mean circulating E and P levels did not significantly differ across the two or three experimental sessions (within subjects). When data were separated by EF and LF groups, mean circulating E and P levels also did not significantly differ across the two or three sessions (Figure 5). Across all sessions,

mean E levels were significantly greater in the LF phase, when compared to the EF phase (Figure 6). P levels did not differ between the phases (Figure 6).

The effect of drug across time on cardiovascular (HR, Systolic BP, Diastolic BP, HF HRV), biochemical (salivary cortisol) and subjective (DEQ, POMS, ARCI) measures was highly robust. In both the models omitting the cycle phase variable (EF v LF), as well as the models including cycle phase, levels of E, P, or P:E were not significantly related to any of the baseline cardiovascular, biochemical, or subjective measures or any of the acute effects of oral THC across time.

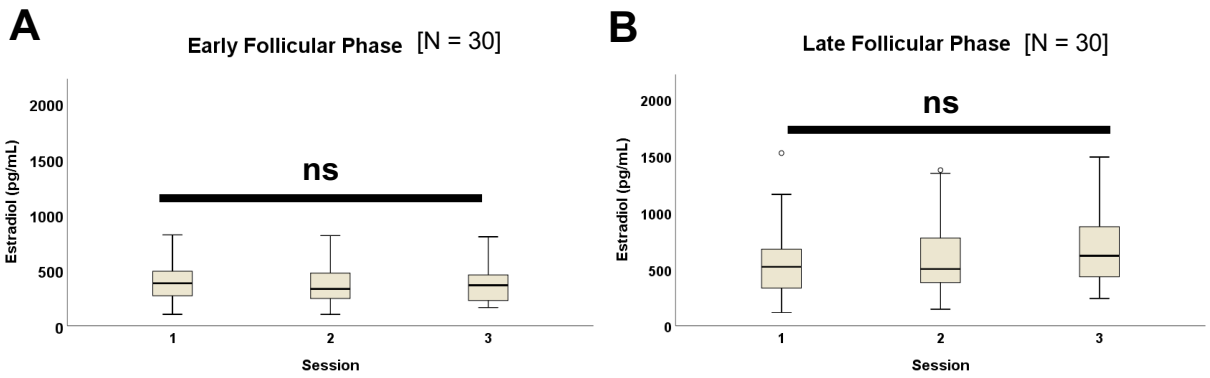

Figure 5. Circulating E levels by experimental session (1, 2, 3; chronological order), and separated by menstrual cycle phase: EF (A), LF (B). Error bars ± 1 SEM.

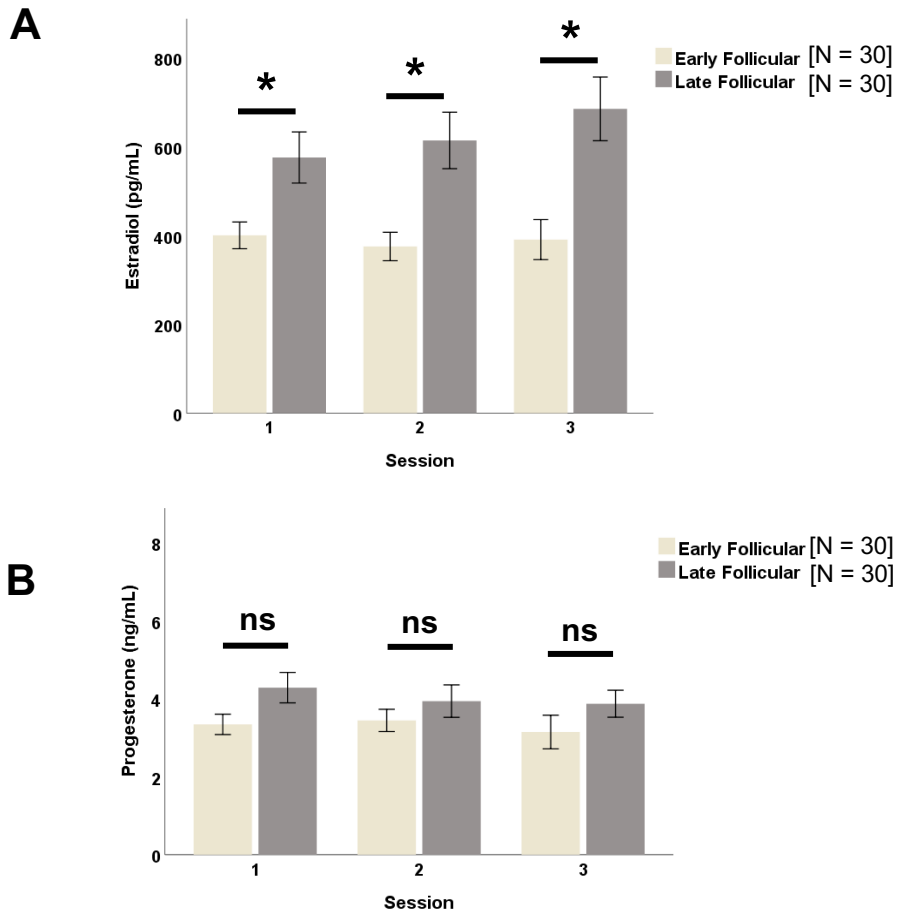

Figure 6. Ovarian hormones, E (A) and P (B), levels by experimental session (1, 2, 3; chronological order), separated by menstrual cycle phase. Error bars ± 1 SEM.

3.5 Conclusion

We found that circulating levels of ovarian hormones, E and P, did not significantly affect responses to oral THC in naturally cycling, healthy young women. Responses to the two doses of THC were similar, in the presence of different circulating plasma levels of E, P, or the ratio of P:E. Despite the differences in hormone levels, the cardiovascular, biochemical, and subjective responses were similar in the EF and LF groups.

Although we detected differences in responses to the drug when we analyzed the data by cycle phase (EF vs LF), as reported in Chapter 2, these differences could not be attributed to differences in E levels. That is, the faster onset of subjective effects in detected in the early follicular phase was not observed in relation to levels of E in the second analysis. This suggested that factors related to cycle phase but not to circulating levels of E and P contributed to differences in the onset of drug effects. Although this was unexpected, it was in line with findings from a previous study with another drug, amphetamine, where EF and LF groups differed in their subjective responses to AMPH, yet responses were not significantly associated with circulating E levels (Justice and de Wit, 2000). One potential explanation of why the cycle phase differences were not directly mediated by circulating E levels could be that responses to THC are more closely related to other factors that vary across the menstrual cycle. For example, basal mood state, physiology, and other hormones such as prolactin, growth hormone, thyroid-related hormones, and melatonin, vary across the cycle and can either precede or follow changes in levels of E and P (Leibenluft et al, 1994). Any of these other factors could contribute to observed differences in response to drugs at different cycle phases. We need additional research directly investigating the effects of these factors on responses to THC, to understand cycle phase differences in subjective drug responses. Ultimately, from our findings we can conclude E or P levels within our sample did not significantly affect responses to oral THC, and thus did not mediate the cycle phase differences reported in Chapter 2.

Chapter 4: Overall Discussion

The present research evaluated whether acute responses to oral THC varied by menstrual cycle phase or circulating ovarian hormone levels at two selected phases of the cycle, EF and LF. The first research aim was to identify cycle phase-related differences in cardiovascular, biochemical, and subjective responses to THC. As reported in Chapter 2, oral THC (7.5 mg, 15 mg) produced its prototypic acute effects in a sample of young healthy naturally cycling women – dose dependent increases in HR and salivary cortisol, decreases in parasympathetic cardiac activity, and increases in self-reported ratings of anxiety, fatigue, and confusion. None of the cardiovascular or biochemical drug effects differed between the EF and LF. However, increased self-reported ratings of "feel" drug effect, "want more" drug, and *Cannabis*-specific subjective intoxication symptoms occurred earlier in the EF group at the 7.5 and 15 mg doses. Although both phase groups experienced similar peak subjective effects, there was an earlier onset of effects during the EF than the LF. This pattern of effects on subjective ratings, but not cardiovascular or biochemical responses, suggests that cycle phase may specifically influence perception of acute drug effects. Notably, the lack of cycle phase differences in physiological measures suggests that the differences in subjective ratings were not directly related to pharmacokinetic factors. Thus, the time course of THC-induced self-reported subjective effects was more closely related to menstrual cycle phase, than acute physiological drug effects.

The second research aim was to evaluate whether circulating E and P levels significantly impacted acute responses to THC, while also controlling for menstrual

cycle phase. As reported in Chapter 3, quantitative levels of circulating E and P were determined using blood serum samples from the start of each experimental session. This repeated measure of ovarian hormone levels was incorporated into the Chapter 2 data set. Using mixed effects regression models, we included the fixed effects of time, drug, menstrual cycle phase, and either circulating E or P levels, or P:E. Responses to THC were not significantly impacted by circulating E or P, nor P:E. Circulating ovarian hormones did not mediate the cycle phase differences reported in Chapter 2. Taken together, the results suggest E and P levels did not significantly impact any of the acute physiological or subjective effects of THC.

The acute physiological effects of THC in the present study are consistent with numerous previous human studies. The dose-dependent effects on HR were consistent with past reports that found both THC and *Cannabis*, whether inhaled or ingested orally, acutely increased HR and could produce tachycardia (Ghasemiesfe et al., 2020; Pabon and de Wit, 2019; Hunault et al., 2008; Wachtel et al., 2002; Ashton et al., 2001; Kirk and de Wit, 1999; Lex et al., 1984; Kanakis et al., 1976; Beaconsfield et al., 1972; Johnson and Domino, 1971). Further, the decreased parasympathetic cardiac activity, measured by HF HRV, following acute oral THC was also consistent with previous findings using inhaled THC (Zuurman et al, 2008). The present study also replicated prior human studies reporting THC-induced increases in salivary cortisol levels (Klumpers et al., 2012; D'Souza et al., 2004; Ranganathan et al., 2009). Previous studies have primarily used inhaled THC or *Cannabis*, at a single dose. The present study not only extended prior research using two recreationally relevant doses of oral

THC (7.5 mg, 15 mg), but also tested the effects in a larger sample of naturally cycling women, while controlling for menstrual cycle phase,

There was a trend toward phase-dependent differences in subjective responses to the drug, but not the physiological effects. EF women reported faster onset of select measures of subjective drug experience, including self-reported ratings of “feeling” a drug effect, “wanting more” drug, and *Cannabis*-specific intoxication symptoms. The absence of differences on the physiological measures suggests the differences in subjective responses are not due to pharmacokinetic differences between the groups. Instead, the faster onset of subjective effects during the EF phase may be due to other cycle phase-related factors, including other hormones such as prolactin, growth hormone, thyroid-related hormones, and melatonin, or cognitive and psychological processes that may vary across the menstrual cycle.

Changes in perception of physiological drug effects may explain differences in subjective responses across the follicular phase. Interoception is the perception of physical sensations from inside the body (Vaitl, 1996; Cameron, 2001; Craig, 2002; Barrett et al., 2004). Perceptions of physical sensations from the body contribute to subjective emotions and feelings, particularly during intensely arousing or stimulating events, such as drug taking (Craig, 2002). Further, interoception serves an evolutionary purpose, it allows for the identification of and ability to respond to internal signals (Craig, 2015). Although historically interoception has been considered a static trait (Antony et al., 1994), more recent studies report it fluctuates in response to the state of the individual at a particular moment (Durlik et al., 2014; Ainley et al., 2012; Craig, 2002; Antony et al., 1995). Durlik et al. (2014) posited changes in interoception may be

reflective of a general strategy to optimize an individual's response to certain situations. Although there is little research investigating menstrual cycle phase-related changes in interoception, they could underlie the present findings. EF women when compared to LF women, reported earlier onset subjective drug effects, suggesting interoception could be elevated during EF when compared to LF or dampened during LF when compared to EF. During EF, women are E deficient and experience menstruation, which can be accompanied by increases in negative mood, anxiety, and dysmenorrhea (Agarwal and Agarwal, 2010). Interoceptive sensitivity during the EF phase could improve information processing and better direct emotional, behavioral, and cognitive processes during these symptoms. This may explain why the acute subjective effects of THC were perceived and reported earlier during the EF. Another potential explanation could be as E levels rise during the LF and the body prepares for ovulation, motivational drive shifts toward reproduction. This would potentially shift attention away from acute drug experience, thus resulting in a later onset of perception and reporting of subjective drug effects. Future studies investigating menstrual cycle and hormone-related differences in interoception are necessary to further our understanding of their directionality, and how they may relate to variability in acute drug responses.

The findings reported in Chapter 3 can be compared to previous preclinical and clinical studies of cycle phase and hormone-related variability in responses to other drugs. Circulating E or P levels did not mediate the menstrual cycle phase differences reported in Chapter 2, nor did they significantly influence any of the cardiovascular, biochemical, or subjective effects of oral THC across time. These results contrast prior preclinical studies reporting E-dependent cannabinoid responses in rodents (Maruisch

et al., 2015; Wakley and Craft, 2011; 2010; Craft and Leitl, 2008; Fattore et al., 2007; Craft, 2005). In rats, E modulates both DA and CB_1 receptor density (Vandegrift et al., 2017; Riebe et al., 2010; Lammers et al., 1999; Kelly and Wanger, 1999), either of which could influence responses to THC. Human PET studies have yet to study and detect changes in CB_1R density across the menstrual cycle or in relation to ovarian hormones in women. It is possible the present study did not replicate preclinical studies because we did not choose the most optimal phase of the menstrual cycle for our sample of naturally cycling women. E levels varied significantly between individuals, even within the same cycle phase; thus, downstream receptor expression effects could have varied between individuals as well. Another potential reason why preclinical studies detected E-dependent responses to THC, could be the doses administered or the route of administration. It is difficult to translate THC doses used in rodents to human clinical studies. Most preclinical studies use weight-dependent dosing (mg/kg) and i.p. or i.v. injections as the primary route of administration. In the present study we did not use weight-dependent dosing, we used two doses previously shown to produce acute intoxication effects in humans. These doses may have been either too high or too low for us to detect E-dependent responses. Future studies administering both smaller and larger doses of THC would add significantly to the present findings. Further, we administered THC orally, which results in a different time course of acute drug effects than i.v. administered drug, which also may contribute to the difference in results between rodents and humans. Although this research was the first acute administration study to directly compare responses to oral THC between women at these two different phases of the menstrual cycle, similar human behavioral studies have been carried out

with stimulant drugs (Justice and de Wit, 1999, 2000b). Like the present study, Justice and de Wit, reported cycle phase differences in responses to AMPH unrelated to circulating E levels. Although the cycle phase differences were not directly related to circulating ovarian hormone levels, they could be a result of other cycle-related physiological or behavioral changes. Future research should explore changes in interoceptive sensitivity across the menstrual cycle and other potential explanations for cycle phase differences in subjective drug experience, but not physiological drug effects. Additional research is needed to investigate other sources of menstrual cycle-related variability in responses to oral THC. Studies such as this would significantly add to the limited literature on individual differences in responses to cannabinoid drugs. Further, they would deepen our understanding of E-cannabinoid neurobiological interactions and their potential downstream effects.

The present research had limitations. First, the study was conducted in a controlled laboratory setting, where the cardiovascular, biochemical, and subjective responses to the drug may not have been reflective of responses in a natural setting. That is, the controlled setting may have dampened some of the responses that emerge only from external stimulation of a naturalistic setting (i.e., social context). In addition, only two moderate doses of oral THC were tested, and it is possible that these findings might not extend to other doses, to whole plant *Cannabis*, or THC or *Cannabis* administered by a different route of administration. THC levels in *Cannabis* have increased markedly in recent years (Elsohy et al., 2016), and it is likely users are exposed to higher concentrations of the drug than those attained in the present study. Finally, this study focused on women, within a specific age range, and only within the

follicular phase of their menstrual cycle. It will be important to determine if similar effects are detected in women during the luteal phase, in women taking exogenous hormones, and in men.

This research aimed to further investigate E-cannabinoid interactions, while understanding their potential clinical applications. THC produced stereotypical acute drug effects—dose-dependent increases in HR, salivary cortisol, and ratings of subjective intoxication and decreases in parasympathetic cardiac activity. Cardiovascular and biochemical responses to oral THC did not significantly differ between the EF and LF phase, while EF women experience marginally faster onset of select measures of subjective drug experience. When controlling for cycle phase and analyzing whether quantitative measurements of circulating E or P levels significantly impact drug response, neither E nor P significantly impacted any of the acute effects of THC. Thus, these findings suggest variability in subjective drug effects was related to menstrual cycle phase, but not circulating E or P levels. In addition, the results do not support the initial hypothesis of E levels increasing sensitivity to acute THC. This research not only contributes significantly to the literature on individual differences in response to THC, but also lays foundation for future female-specific *Cannabis* use guidelines. It also builds on prior preclinical studies of E-cannabinoid interactions, deepening our understanding of how these neurobiological mechanisms can have downstream behavioral effects in humans.

Additional research is needed to replicate and extend these findings. Future studies investigating related effects at higher doses, with other cannabinoids and combinations of cannabinoids as found in the plant, in heterogeneous populations

including men, drug users or individuals with psychiatric symptoms will extend the present findings. Studies of individual differences in response to acute THC will continue to build the foundation for safer *Cannabis* or THC use guidelines. Ultimately, this clinical cannabinoid research will aid in decreasing the public health risk associated with adverse responses to *Cannabis* or THC, such as tachycardia and anxiety, and maximize therapeutic applications.

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