Examining the Effects of Sustained Cannabis Abstinence on Affective Symptoms in Adults with Cannabis Use Disorder

Lyne Baaj, BSc

Integrated Program in Neuroscience, McGill University, Montreal

June 2024

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science (M.Sc.) in Neuroscience (Thesis)

TABLE OF CONTENTS

ABSTRACT

Introduction

Cannabis is often used to cope with affective symptoms, such as depression or anxiety. In Canada, 43% of people who used cannabis in 2023 perceived that cannabis use was beneficial to their mental health, while only 8% thought that it was harmful. Paradoxically, longitudinal studies suggest that cannabis use is associated with the development and maintenance of affective symptoms. It is therefore crucial to understand if cannabis abstinence benefits affective symptoms. Previous studies found that depressive and anxiety symptoms improved with 28 days of cannabis abstinence. However, most of these studies included participants with psychiatric/medical comorbidities or were conducted in adolescents. Studies that did include participants without comorbidities failed to include an adequate control group. Therefore, to determine if these findings extend to adults without comorbidities, we aimed to investigate the effects of 28 days of cannabis abstinence on depressive and anxiety symptoms in adults with cannabis use disorder (CUD) with no comorbidities using an appropriate control group. Given that previous research did not assess the effect of sex on changes in affective symptoms, our exploratory aim was to compare the trajectory of depressive and anxiety symptoms during 28 days of cannabis abstinence between males and females.

Methods

We recruited adults (N=25; 18-55 years old) with CUD, a positive cannabis urine toxicology, and no current DSM-5 disorders (other than CUD) or medical comorbidities. Participants were randomized using a 3:2 ratio to a cannabis abstinence arm (AB, n=16) or a non-abstinent (cannabis-as-usual control) arm (NA, n=9), respectively. Depressive symptoms were assessed weekly with the Hamilton-Depression Rating Scale. Anxiety was assessed weekly using the state

subscale of the State Trait Anxiety Inventory. Cannabis abstinence was determined with the Timeline Follow Back, a self-report interview, and was encouraged using contingency management and weekly behavioural support.

Results

Fourteen of the 16 participants (88%) in AB self-reported 28 days of cannabis abstinence. Relative to NA, depressive ($F(4,84)=1.83$, $p=.15$) and anxiety ($F(4, 84)=.79$, $p=.47$) symptoms did not significantly change during abstinence in AB. Further, the effect of sex on the trajectory of depressive ($F(4, 36)=0.22$, $p=.93$) and anxiety ($F(4, 48=.46$, $p=.60$) symptoms was not significant. Due to the study being underpowered, we also outlined the general pattern observed in the data. Among AB depressive symptoms increased from baseline to day 7, peaked at day 7, and then returned to baseline levels by day 28. Additionally, when parsed according to sex, females experienced a greater increase in depressive symptoms from baseline to day 7 than males. Conversely, anxiety symptoms decreased from baseline to day 28 in both AB and NA, and no sex differences were observed in anxiety symptoms.

Conclusion

In this preliminary study, severity of depressive, but not anxiety, symptoms increased from baseline to 7 days before returning to baseline levels by day 28 in people with CUD who underwent 28 days of cannabis abstinence. The peak in depressive symptoms at day 7 may reflect transient cannabis withdrawal effects. Further, females experienced a greater increase in depressive symptoms than males during the first week of cannabis abstinence, suggesting that females may be more vulnerable to relapse during the first week of cannabis abstinence. Importantly, our findings indicate that affective symptoms do not get worse after 28 days of cannabis abstinence which provides evidence that cannabis use does not benefit or improve affective symptoms. Future

studies should biochemically verify self-reported cannabis abstinence and include larger samples.

RÉSUMÉ

Introduction

Le cannabis est souvent consommé afin de faire face aux symptômes affectifs tels que la dépression ou l'anxiété. Paradoxalement, des études longitudinales suggèrent que la consommation de cannabis est associée au développement de symptômes affectifs. Il est donc crucial de comprendre si l'abstinence de cannabis est bénéfique aux symptômes affectifs. Des études ont montré que les symptômes affectifs s'améliorent avec 28 jours d'abstinence au cannabis. Cependant, ces études ont inclus des participants avec des comorbidités psychiatriques/médicales ou des adolescents. Les études chez les adultes sans comorbidités n'ont pas inclus un groupe témoin adéquat. Donc, nous avons étudié les effets de 28 jours d'abstinence de cannabis sur les symptômes dépressifs et anxieux chez les adultes avec un trouble lié à la consommation de cannabis (TLCC) sans comorbidités avec un groupe témoin approprié. Comme les recherches précédentes n'ont pas évalué l'effet du sexe sur les symptômes affectifs, notre objectif exploratoire était de comparer la trajectoire des symptômes affectifs pendant 28 jours d'abstinence entre les personnes du sexe masculin et féminin.

Méthodes

Nous avons recruté des adultes (N = 25 ; 18-55 ans) avec un TLCC, une toxicologie urinaire de cannabis positive et sans troubles de l'axe 1 du DSM-5 ni comorbidités médicales. Les participants ont été randomisés selon un rapport de 3:2 dans un groupe d'abstinence au cannabis (AB, n=16) ou un groupe témoin utilisant le cannabis comme d'habitude (NA, n=9). Les symptômes dépressifs et anxieux ont été évalués chaque semaine avec le Hamilton Depression Rating Scale et le State

Trait Anxiety Inventory. L'abstinence a été déterminée avec un entretien d'auto-évaluation et a été encouragée avec gestion de contingence et d'un soutien comportemental hebdomadaire.

Résultats

Quatorze participants (88 %) AB ont déclaré 28 jours d'abstinence de cannabis. Par rapport au NA, les symptômes dépressifs ($F(4,84)=1,83$, $p=0,15$) et anxieux ($F(4, 84)=0,79$, $p=0,47$) n'ont pas changé significativement durant l'abstinence dans l'AB. Nous n'avons pas observé d'effet significatif du sexe sur la trajectoire des symptômes dépressifs ($F(4, 36)=0,22$, $p=0,93$) et anxieux ($F(4, 48=0,46$, $p=0,60$). Comme notre étude était de faible puissance, nous décrivons aussi la tendance des données. Une tendance est apparue dans l'AB démontrant que les symptômes dépressifs ont augmenté du début au jour 7, ont culminé au jour 7, puis sont revenus aux niveaux de base au jour 28. Nous avons observé que bien que les personnes de sexe masculin et féminin aient suivi une trajectoire similaire de symptômes dépressifs, celles du sexe féminin ont ressenti une plus grande augmentation des symptômes dépressifs entre le départ et le jour 7 que celles du sexe masculin. Pour les symptômes d'anxiété, nous avons observé une diminution dans l'AB et l'NA et aucune différence entre les sexes n'a été observée.

Conclusions

Dans cette étude pilote, nous avons observé une tendance dans les symptômes dépressifs telle que la gravité de ces symptômes a augmenté fortement 7 jours après l'abstinence avant de revenir aux niveaux de base au jour 28. Cette augmentation des symptômes dépressifs peut refléter les effets transitoires du sevrage du cannabis. De plus, nous avons observé que les personnes du sexe féminin avaient une plus grande augmentation des symptômes dépressifs que celles du sexe masculin durant la première semaine d'abstinence. Cela suggère que les personnes du sexe féminin pourraient être plus vulnérables aux rechutes durant la première semaine d'abstinence.

Notamment, nos résultats indiquent que les symptômes affectifs ne s'aggravent pas après 28 jours d'abstinence, ce qui montre que la consommation de cannabis ne bénéficie les symptômes affectifs. Les études futures devraient vérifier biochimiquement l'abstinence autodéclarée et inclure des plus grands échantillons.

ACKNOWLEDGEMENTS

I would first like to extend my sincere and profound gratitude to my master's supervisor, Dr. Rachel Rabin for being the best graduate supervisor one could wish for. I am eternally thankful for her continuous support, efforts, and mentorship throughout my master's. She taught me to be a critical thinker, an optimistic researcher, and independent learner. Thanks to her guidance, I am a better researcher and a better person. I am lucky to be her student, and I look forward to all the future projects we will work on together.

I would also like to express my immense gratitude towards my friends and lab-mates at the Addiction, Imaging, and Mental Health Lab - Gabriella Malamud, Sophia Hanna, Renee He, and Zac Yeap. I would like to thank Gabriella who has shown nothing short of hard work, perseverance, and collaboration; I am thankful for her friendship and support as we work alongside each other on our projects. I would also like to thank Sophia who is the powerhouse of our lab; our team is forever indebted to her hard work with participant recruitment and data collection. I would also like to thank Renee for her dedication to our lab, I am thankful for her support with our data collection as we work on this project together. Lastly, since I joined the lab, Zac has been a tremendous source of support with my project, and I am sincerely appreciative of his time and efforts. All in all, I am incredibly thankful to have been able to see our lab grow and flourish with the efforts of this team. I also want to extend my gratitude to my friend at the Douglas Research Centre, Jessica, who has made my master's easier by being an incredible and supportive friend.

I also would like to thank my parents, Hassan and Hala, my brother, Sami, and my best friend/dog Oscar, for their unwavering support. They continued to believe in me when I doubted myself and

were the first to celebrate my successes. I owe my strength, my resilience, and my perseverance to them.

I would also like to thank my dear friends, Emma, Jennifer, and Nakita, for a decade of valuable support and friendship. They were by my side through the highs and lows, and I am eternally grateful for them.

Lastly, I would like to extend an immense thank you to our study participants. Our lab owes our research to their commitment and participation. I am particularly grateful for allowing me to learn from their insights about the ways cannabis use impacts their lives, which has inspired my research.

LIST OF FIGURES AND TABLES

List of Tables

List of Figures

LIST OF ABBREVIATIONS

2-arachidonoylglycerol (2-AG)

11-hydroxy-Δ9- tetrahydrocannabinol (11-OH-THC)

Abstinent arm (AB)

Addiction, Imaging, and Mental Health Lab (AIMH Lab)

Alcohol Use Identification Test (AUDIT)

Anandamide, AEA

Beck Anxiety Inventory (BAI)

Beck's Depression Inventory (BDI)

Brief Symptom Inventory (BSI)

Calgary Depression Scale for Schizophrenia (CDSS)

Cannabidiol (CBD)

Cannabinoid 1 receptors (CB1r)

Cannabinoid 2 receptors (CB2r)

Cannabis as usual control arm (NA)

Cannabis Use Disorder (CUD)

Cannabis Use Disorder Identification Test (CUDIT)

Cannabis Withdrawal Scale (CWS)

Cannabis Withdrawal Syndrome Criteria (CWSC)

Centre Intégré Universitaire de Santé et de Services Sociaux de l'Ouest-de-l'Île-de Montréal (CIUSS-ODIM)

Cytochrome P450 3A (CYP3A)

Cytochrome P450 2C (CYP2C)

Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)

Difficulties in Emotion Regulation Scale (DERS)

Endocannabinoid (eCB)

Fagerstrom Test for Nicotine Dependence (FTND)

Fatty acid amide hydrolase (FAAH)

G protein-coupled receptors (GPCRs)

Hamilton Anxiety Rating Scale (HARS)

Hamilton Depression Rating Scale (HDRS)

Healthy Control Group with Cannabis Use and no Comorbid Disorders (HCL)

Hospital Anxiety and Depression Scale (HADS)

Inventory of Depression and Anxiety Symptom (IDAS)
Magnetic resonance imaging (MRI)
Marijuana Motives Measure (MMM)
Mini International Neuropsychiatric Interview (MINI)
Monoacylglyceride lipase (MAGL)
Mood and Anxiety Symptom Questionnaire (MASQ)
Not Enough Information (NEI)
Participants with Cannabis Use Disorder (CB+)
Profile of Mood States (POMS)
Positive and Negative Affect Scale (PANAS)
Repeated measures analysis of variance (RM-ANOVA)
Schizophrenia (SCZ)
Snaith-Hamilton Pleasure Scale (SHAPS)
Société Québécoise du Cannabis (SQDC)
Standard Deviation (SD)
State Subscale of the State Trait Anxiety Inventory (STAI-state)
State Trait Anxiety Inventory (STAI)
Tetrahydrocannabinol (THC)
Timeline Follow-Back (TLFB)
Visual Analogue Scale (VAS)

1. INTRODUCTION AND STATEMENT OF PROBLEM

Since non-medical cannabis use was legalized in Canada in 2018, cannabis use rates have been on the rise. In people aged 16 and older rates have increased by 4% (from 22% in 2018 to 26% in 2023) (Canada, 2024b). Cannabis consumption frequencies have also increased; approximately 6% of Canadians use cannabis daily or almost daily (Canada, 2024b). These rates are concerning given that 25% to 50% of those who use cannabis daily or almost daily will develop a cannabis use disorder (CUD) (Canada, 2023b), which describes problematic cannabis use patterns that lead to significant distress or impairment (American Psychiatric Association, 2013).

People often report using cannabis to cope with or alleviate affective symptoms, such as depression and anxiety. In Canada, 43% of people who used cannabis in 2023 reported that cannabis use was beneficial to their mental health, while only 8% thought that it was harmful to their mental health (Canada, 2024a). Similarly, in an American sample of young adults, ~82% of people with frequent cannabis use endorsed using cannabis to self-medicate symptoms of anxiety, and ~60% for symptoms of depression (Wallis et al., 2022). In contrast, a meta-analysis compiled from cross-sectional studies showed that chronic (frequent and prolonged) cannabis use was associated with elevated rates of depressive and anxiety symptoms and their related disorders (Onaemo et al., 2021). While cross-sectional studies support an association between cannabis use and affective symptoms, these studies do not enable temporal inferences on the relationship between cannabis use and affective symptoms/disorders. Longitudinal studies overcome this limitation and can determine if affective symptoms precede cannabis use or are a consequence of cannabis use. Indeed, longitudinal studies have found support for a temporal association, whereby chronic cannabis use has been shown to contribute to the development and maintenance of both depressive (Gobbi et al., 2019; Hayatbakhsh et al., 2007; Patton et al., 2002) and anxiety (Duperrouzel et al.,

2018; Hayatbakhsh et al., 2007; Patton et al., 2002) symptoms and disorders. This suggests a causal role for cannabis use in the development of affective symptoms and disorders. Therefore, it is clinically relevant to determine if cannabis abstinence leads to improvements in affective symptoms.

Studies that assess participants before and after a period of abstinence are critical to better understand the impact of cannabis use and cannabis cessation on affective symptoms. Abstinence paradigms employ a prospective approach to assess within-subject changes over time and notably provide greater statistical power than cross-sectional designs. A growing body of literature has examined the relationship between cannabis abstinence and affective symptoms and studies report improvements in affective symptoms following 16-45 days of cannabis abstinence (Bonnet et al., 2015; Budney et al., 2003; Cooke et al., 2021; Feinstein et al., 2021; Galang et al., 2015; Jacobus et al., 2017; Lee et al., 2014; Lucatch et al., 2020; Milin et al., 2008; Rabin et al., 2018a). This provides evidence that cannabis use negatively affects affective symptoms and that people may benefit from quitting cannabis. However, these studies were limited by various factors. For one, samples were comprised of individuals with psychiatric/medical comorbidities (Feinstein et al., 2021; Galang et al., 2015; Jacobus et al., 2017; Lucatch et al., 2020; Milin et al., 2008; Rabin et al., 2018a), while limits the generalizability of results to people with cannabis use and no co-occurring psychiatric/medical disorders. Furthermore, other studies employed samples that were comprised of adolescents (Cooke et al., 2021; Jacobus et al., 2017; Milin et al., 2008; Sullivan et al., 2022), which limits the generalizability of these results to otherwise healthy adults using cannabis. Additionally, one study in non-psychiatric samples was comprised of adults who were inpatients in treatment for cannabis use; this presented a confounding factor as patients were prescribed medication to treat cannabis withdrawal symptoms, which may have influenced

affective symptoms (Bonnet et al., 2015). Lastly, not all studies included an appropriate control group, which prevents accounting for factors such as time spent with researchers on affective symptoms (Bonnet et al., 2015; Budney et al., 2003; Galang et al., 2015; Jacobus et al., 2017; Lee et al., 2014; Lucatch et al., 2020; Milin et al., 2008; Sullivan et al., 2022). Therefore, how cannabis abstinence affects the trajectory of affective symptoms in otherwise healthy adults with CUD without psychiatric and medical comorbidities is unknown.

To address this gap in the literature, this pilot study will assess the effects of 28 days of cannabis abstinence on affective outcomes in non-psychiatric adults with a CUD and with no severe medical comorbidities. Our study design will include the appropriate control group to account for the effects of study procedures on affective symptoms. As such, participants will be randomized either to a cannabis abstinence arm or to a cannabis as usual arm where they will continue to use cannabis. Furthermore, given that previous studies did not explore sex differences in the trajectory of affective symptoms during cannabis abstinence, we will further disaggregate the sample into males and females to study the effects of sex on affective symptoms during 28 days of cannabis abstinence.

Findings from this study will help elucidate the impacts of cannabis abstinence on affective symptoms in adults, and the effects of sex on the trajectory of affective symptoms during 28 days of cannabis abstinence. With the recent legalization of cannabis in Canada and the observable increases in cannabis use rates, it is imperative that Canadians be well informed of the risks and consequences of prolonged cannabis use on their mental health.

2. BACKGROUND INFORMATION

2.1 Cannabis Use Trends

2.1.1 Canadian Cannabis Policies

Cannabis use was legalized in Canada for medicinal purposes in 2001, and for non-medical use in 2018 under Bill C-45, the Cannabis Act (Canada, 2018a). This Act outlines the rules, regulations, and restrictions placed on the production, sale, and possession of cannabis in Canada. The Cannabis Act has the objectives of 1) protecting young Canadians from accessing cannabis, 2) protecting people who use cannabis through strict regulations on product quality and safety, and 3) deterring people from engaging in criminal activities through stricter penalties.

Provinces and Territories across Canada have the power to enact stricter rules and regulations. The cannabis laws in the province of Quebec, where this research takes place, are the strictest in Canada. In Quebec, adults over the age of 21 can legally buy and possess cannabis. Within the other Canadian provinces, it is legal to buy and possess cannabis at the age of 19 in all provinces but Alberta, where the legal age is 18. Additionally, the only legal seller of cannabis within Quebec is the government operated Société Québécoise du Cannabis (SQDC), while several other provinces across Canada allow for the purchase of cannabis from private sellers (Alberta, British Columbia, Manitoba, Newfoundland and Labrador, Nunavut, Ontario, Saskatchewan, and Yukon.)

In 2019, Quebec passed Bill 2, which increased the legal age of cannabis use purchase and possession from 18 to 21 years (Quebec, 2019). Bill 2 also led to tighter restrictions on cannabis use policies, including limits on the use of cannabis in public spaces (Quebec, 2019). This change was passed with the goal of reducing cannabis use within the 18-20 year old group. Nguyen and Mital (2022) assessed the effects of Bill 2 on cannabis use rates in Quebec and found significant decreases in cannabis use within the 18-20 year old age group, providing support for the efficacy

of Bill 2. This is further supported by data from the 2022 Quebec Cannabis Survey, which highlights a 1.3% decrease in past year cannabis use rates in the 18-20 year old age group in Quebec, going from 33% to 31.7% (Institut de la statistique du Québec, 2023).

2.1.2 Cannabis Use Rates

Current Rates: Canada has one of the highest rates of non-medical cannabis use in the world. In 2023, 26% of Canadians aged 16 and older reported using cannabis for non-medical purposes (Canada, 2024a). It is estimated that 25% to 50% of those who use cannabis daily or almost daily will develop a CUD (Canada, 2023b). Additionally, CUD has been noted to be one of the most prevalent substance use disorder in the world (Alcohol & Drug Use, 2018). For almost two decades, Canada has maintained the highest rate of Disability-Adjusted Life Years and Years Lived with Disability in the Americas due to CUD (data available from 2000 to 2019) (Pan American Health Organization, 2021). This underscores that CUD is indeed associated with relevant consequences.

By province, cannabis use rates are highest in the Territories and Nova Scotia, where 39% and 34% of residents report past year use in 2023, respectively (Canada, 2024b). Quebec reports the lowest cannabis use rates in Canada, with 18-19.4% of residents reporting past year use in 2023 (Canada, 2024b; Institut de la statistique du Québec, 2023).

By age group, Canadians aged 20 to 24 use cannabis the most (compared to 16 to 19 year old adolescents and adults above 25); 48% endorse past year cannabis use in 2023 (Canada, 2024a). In Quebec, 40.3% of adults aged 21-24 reported past year cannabis use in 2022 (Institut de la statistique du Québec, 2023). Twenty-three percent of Canadians above 25 report using cannabis in 2023, while 43% of Canadians aged 16 to 19 report using cannabis in 2023 (Canada, 2024a).

In Canada, where statistics on cannabis use are reported by sex, males report higher rates of cannabis use than females, with past year cannabis use rates of 29% for males and 23% for females in 2023 (Canada, 2024a). In Quebec, where statistics are reported by gender, this trend is maintained; 23.3% of men report past year cannabis use in 2022, while 15.6% of women do so (Institut de la statistique du Québec, 2023).

Since cannabis legalization: In Canada, past year cannabis use rates have increased by 4%, growing from 22% in 2018 to 26% in 2023 in people above the age of 16 (Canada, 2024b). In Quebec, past year cannabis use rates have increased by 5.4%, from 14% in 2018 to 19.4% in 2022 (Institut de la statistique du Québec, 2023).

Past year cannabis use rates have increased in both adults and adolescents. Adults aged 20-24 and 25+ saw a 4% increase (from 44% in 2018 to 48% in 2023 and from 19% in 2018 to 23% in 2023, respectively) (Canada, 2024b). In Quebec however, adults aged 25-34 years old saw the greatest increase in past-year cannabis use rates, increasing by 10.7% (from 25.8% in 2018 to 36.5% in 2022) (Institut de la statistique du Québec, 2023). In adolescents (16 to 19 years old), past year cannabis use rates have increased by 7% (from 36% in 2018 to 43% in 2023) (Canada, 2024b).

Cannabis use frequencies have also increased following cannabis legalization in Canada. Namely, rates of daily or almost daily cannabis use increased by 2.5% in the years following legalization, rising from 5.4% in 2018 to 7.9% in 2020 (Rotermann, 2021). In adults aged 20 to 24 years old, rates of daily or almost daily cannabis use in people who report past year cannabis use increased from 23% in 2019 to 29% in 2022, before decreasing to 23% in 2023 (Canada, 2024a).

Taken together, these data demonstrate that in Canada past year cannabis use rates and daily or almost daily cannabis use rates have increased since legalization. These increases are most

prevalently seen in young adults aged 20 to 24 years old. This emphasizes the need for research that investigates the consequences of cannabis use in adult populations.

2.1.3 Cannabis Use Modes

Cannabis administration can take many different modes that include inhalation, ingestion, and topical absorption. Cannabis inhalation includes smoking (e.g. joints, blunts), vaping, and dabbing, while cannabis ingestion includes edibles, beverages, oils, tinctures, and capsules. Topical absorption includes lotion, bath salts, balms, and patches (Quebec, 2023). In 2022, smoking was the most prevalent mode of cannabis administration with 70% of Canadians reporting past year cannabis smoking (Canada, 2022). In that same year, 52% of Canadians reported past year cannabis ingestion through edibles, 31% reported using a vape pen or e-cigarette, 18% reported using cannabis oil, 16% reported drinking cannabis-infused drinks, 7% reported using topical absorption, and lastly 6% reported dabbing (Canada, 2022).

2.1.4 Potency of Tetrahydrocannabinol (THC)

Tetrahydrocannabinol (THC) is the primary addictive and psychoactive ingredient in cannabis and is responsible for producing the feeling of the "high" (Haney, 2022; Sharma et al., 2012), while cannabidiol (CBD) does not possess addictive properties, it is psychoactive (Stella, 2023). Over the past decades, THC potency in cannabis has significantly increased from 3% in the 1980s to 16.1% on the legal market and 20.5% on the illegal market shortly after cannabis legalization (Canada, 2023a; Mahamad et al., 2020; Volkow et al., 2014). In 2022, 39% of Canadians indicated using cannabis with a predominantly higher THC potency than CBD, which is an increase from 2019 (36.5%) (data on potency unavailable prior to 2019) (Canada, 2019, 2022). Higher THC concentrations are concerning because they are associated with greater mental health consequences such as anxiety, depression, psychosis, and the development of CUD (Arterberry et al., 2019;

Petrilli et al., 2023; Volkow et al., 2014).

2.1.5 Cannabis-Related Legal Consequences

Although non-medical cannabis use was legalized in Canada in 2018, there remains strict legal regulations imposed on the manufacturing, sale, purchase, and consumption of cannabis products under the Cannabis Act. For example, throughout Canada, it is illegal to sell cannabis products that may be appealing to young persons, which includes colourful packaging and candies such as gummies and brownies (Canada, 2018a).

Regulations can vary from province to province. For example, growing cannabis at home for personal use is legal (with some restrictions) in all provinces but Quebec and Manitoba (Canadian Centre on Substance Use and Addiction, 2023). Importantly, Quebec has some of the strictest cannabis regulations. Namely, Quebec holds the highest legal age of cannabis purchase and consumption, prohibits cannabis use in both indoor and outdoor public spaces, imposes limits on the amount of cannabis possessed (150 grams per private residence), and prevents private sale of cannabis (Canadian Centre on Substance Use and Addiction, 2023).

Individuals who step outside of these legal regulations may face legal consequences. In Quebec, most rule-breaks will lead to a fine that may increase for subsequent offences (Canadian Centre on Substance Use and Addiction, 2023). For example, individuals stopped for consuming cannabis in public face a fine of $500-1,500 (Canada, 2018b; Canadian Centre on Substance Use and Addiction, 2023). For minors, the possession of <5grams of cannabis is punishable by a fine of $100 (Canada, 2018b; Canadian Centre on Substance Use and Addiction, 2023).

2.2 What is Cannabis?

2.2.1 Overview of Cannabis

Cannabis is made up of over 100 cannabinoids, with THC and CBD being the best characterized and the most abundant cannabinoids in cannabis (Haney, 2022; Ladha et al., 2020). Although both THC and CBD have the same molecular formula, their differing chemical structure leads to different properties.

Figure 1 Chemical Structure of Prominent Compounds in Cannabis

Figure 1.1

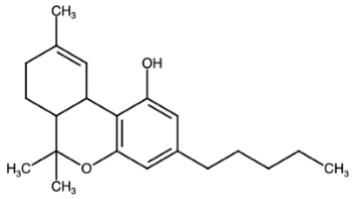


Figure 1.2

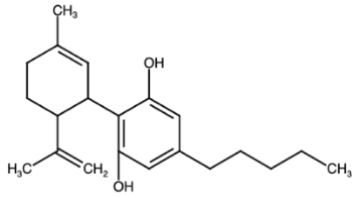


Figure 1.1: Chemical Structure of THC
Figure 1.2 Chemical Structure of CBD

The primary addictive ingredient in cannabis is THC, modulates the release of dopamine in the striatum, and thus plays an important role in reward, by inhibiting GABAergic neurotransmission (Bossong et al., 2009; Calakos et al., 2021) in the striatum. In contrast, CBD does not possess addictive properties (Haney, 2022; Sharma et al., 2012), given that its use is not associated with dopamine release (Navarrete et al., 2021). Lastly, both THC and CBD are psychoactive and thus affect mental processes (Haney, 2022; Sharma et al., 2012).

2.2.2 Cannabis Intoxication

Acute cannabis administration leads to a myriad of psychiatric, cognitive, motor, and physical symptoms. People under the influence of cannabis, particularly with preparations high in THC, report feeling pleasurable effects such as relaxation, euphoria, and reduced anxiety (Patel & Marwaha, 2023; Stella, 2023). People also often report feeling light-headedness, numbness, tingling, palpitation, sweating, and weakness (Stella, 2023). From a cognitive perspective, cannabis administration can induce mental confusion and impairment, altered time perception, reduced reaction time, impaired attention and memory, and reduced motor coordination (Crean et

al., 2011; D'Souza et al., 2004; Stella, 2023). Cannabis administration can also lead to reddened conjunctiva, dry mouth, and increased appetite (Patel & Marwaha, 2023). When used in higher doses, cannabis may produce undesirable effects such as increased anxiety, delusions, hallucination, and derealization (D'Souza et al., 2004; Patel & Marwaha, 2023). Importantly, CBD is thought to modulate the acute effects of THC, specifically by blunting the effects of THC on euphoria and psychiatric symptoms (Freeman et al., 2019). Using CBD also leads to fatigue and drowsiness, and has been shown to have anxiolytic and antipsychotic effects (Food and Drug Administration, 2020; Huestis et al., 2019).

2.2.3 Pharmacokinetics of Cannabis

Cannabis can be consumed in various manners, each with a distinct pharmacokinetic profile. When inhaled, cannabis is absorbed through the lungs and enters the bloodstream, where it is carried in the plasma and distributed throughout the body. When ingested, cannabis is absorbed in the gastrointestinal tract before entering the bloodstream. Importantly, certain modes of administration yield faster and stronger effects. For example, the bioavailability of THC is higher when cannabis is inhaled (ranging from 10% to 35%) than when cannabis is ingested (ranging from 4% to 12%) (Chayasirisobhon, 2020). This is also observed with CBD, where the bioavailability of CBD is higher when inhaled (ranging from 11% to 45%) than when ingested (6%) (Chayasirisobhon, 2020). The peak THC concentration in plasma is observed 3 to 10 minutes following inhalation and 1 to 2 hours following ingestion (Sharma et al., 2012). The difference in bioavailability following cannabis inhalation and ingestion is influenced by the incomplete absorption of cannabis in the gastrointestinal tract and its “first pass” metabolism by the liver (Chen & Rogers, 2019).

2.2.4 Cannabis Metabolism and Excretion

Following cannabis administration, THC is first metabolized to 11-hydroxy-Δ9-

tetrahydrocannabinol (11-OH-THC) in the liver by the enzymes cytochrome P450 3A and 2C (CYP3A and CYP2C) (Chayasirisobhon, 2020; Huestis & Cone, 1998; Sharma et al., 2012). Then, 11-OH-THC, which is psychoactive, is further metabolized in the liver to its primary inactive metabolite, 11-nor-9-carboxy-THC (11-THC-COOH) (Chayasirisobhon, 2020; Huestis & Cone, 1998; Sharma et al., 2012). Following cannabis metabolism, 11-THC-COOH is excreted in urine as a primary conjugate of glucuronic acid and 11-OH-THC is excreted predominantly in feces (Chayasirisobhon, 2020; Sharma et al., 2012).

Importantly, THC's lipophilic properties lead to its absorption into fatty tissue prior to metabolism (Thomas et al., 1990). THC is then slowly released into the blood stream, metabolized into THC-COOH, and then excreted (Goodwin et al., 2008; Lowe et al., 2009; Rabin et al., 2018b). In studies of monitored cannabis abstinence, THC-COOH concentrations peak in the first few days of abstinence before gradually decreasing over a period of up to four weeks (Goodwin et al., 2008; Lowe et al., 2009).

2.3 The Endocannabinoid System

2.3.1 Endocannabinoid System

The endocannabinoid (eCB) system is an important and widespread neuromodulatory network that is involved in various hemostatic functions such as emotion regulation, stress regulation, processing of fear and anxiety, and cognition (Lowe et al., 2021; Lutz et al., 2015). The eCB system is also involved in numerous physiological functions, including temperature regulation, pain sensation, appetite and metabolism, and fertility (Aizpurua-Olaizola et al., 2017; Battista et al., 2012; Cabral et al., 2008; Lowe et al., 2021).

The eCB system is comprised of endogenous lipid ligands [N-arachidonylethanolamide

(anandamide, AEA) and 2-arachidonoylglycerol (2-AG), their metabolic enzymes [fatty acid amide hydrolase (FAAH) and monoacylglyceride lipase (MAGL)], and cannabinoid receptors, cannabinoid 1 receptors (CB1r) and cannabinoid 2 receptors (CB2r) (Lutz et al., 2015; Volkow et al., 2017).

2.3.2 Cannabinoid Receptors

Endocannabinoids bind to both CB1r and CB2r, which are both G protein-coupled receptors (GPCRs). The CB1r is ubiquitously expressed in the central nervous system, in areas like the cerebral cortex, and limbic system, which includes the hypothalamus, amygdala, basal ganglia, insula, and the hippocampus (Connor et al., 2021; Zou & Kumar, 2018). Anandamide is a partial agonist at the CB1r, and binds to the receptor with high affinity (Meyer et al., 2018). On the other hand, 2-AG is a full agonist at the CB1r, with a lower binding affinity to the receptor (Baggelaar et al., 2018; Meyer et al., 2018). The CB1r is abundantly found on presynaptic terminals of GABAergic and glutamergic neurons, and thus modulates synaptic transmission (Lu & Mackie, 2021; Zou & Kumar, 2018). In contrast to CB1r, CB2r expression is limited in the central nervous system but abundant in peripheral tissues and immune cells (Galiegue et al., 1995).

2.3.3 Cannabis and the Endocannabinoid system

THC binds to CB1r with greater affinity than CBD, and acts as a partial agonist at the receptor (Volkow et al., 2017). When cannabis is administered, THC binds to CB1r and transiently inhibits the release of GABA and glutamate (Volkow et al., 2017). With chronic cannabis use, changes in the eCB system may occur. Researchers have observed that chronic cannabis use is associated with CB1r downregulation in the brain (Ceccarini et al., 2015; D'Souza et al., 2016; Hirvonen et al., 2012), which reverses and normalizes after 28 days of cannabis abstinence (D'Souza et al., 2016; Hirvonen et al., 2012). Chronic cannabis use has also been shown to affect circulating eCBs. In a

sample of people with current cannabis use (with greater than weekly cannabis use), Kearney-Ramos and colleagues found that more frequent cannabis use correlated with lower plasma 2-AG levels (Kearney-Ramos et al., 2022). In a sample of healthy volunteers with no current cannabis use, THC administration led to an increase followed by a significant decrease of eCB levels that returned to normal levels after 48 hours (Thieme et al., 2014). Therefore, chronic cannabis use may lead to transient changes in the eCB system, impacting both CB1r expression and circulating eCB levels.

2.4 Consequences of Chronic Cannabis Use

2.4.1 Cannabis Withdrawal

Cannabis withdrawal describes the negative or unpleasant symptoms that commonly occur following cessation (or reduction) of heavy or prolonged cannabis use. Cannabis withdrawal symptoms may include feelings of irritability, aggression, nervousness, anxiety, depression, sleep difficulties (e.g. insomnia), loss of appetite, and physical symptoms (e.g. tremors, headaches, abdominal pain, fever, or chills) (American Psychiatric Association, 2013; Bonnet & Preuss, 2017). The most common withdrawal symptoms are depression, irritability, anxiety, sleep difficulties, and loss of appetite (Connor et al., 2022). Craving for cannabis is also commonly experienced during cannabis withdrawal (Bonnet & Preuss, 2017; Lee et al., 2014; Levin et al., 2010), which is symptomatic of a CUD (American Psychiatric Association, 2013).

Cannabis withdrawal symptoms begin to appear within the first 24-48 hours following cannabis cessation, peak in the first three to seven days and can last up to four weeks or more (Connor et al., 2022). The prolonged time course of cannabis withdrawal may reflect THC's lipophilic properties given that it remains in the body for several weeks following cannabis cessation

(Goodwin et al., 2008; Lowe et al., 2009; Rabin et al., 2018b). The neurobiological basis of withdrawal may be attributable to eCB dysregulation as cannabis withdrawal symptoms have been shown to correlate with with CB1r density during abstinence (D'Souza et al., 2016).

The cannabis withdrawal syndrome is described in the the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) as the presence of three or more of six symptoms, which develop within one-week of cannabis cessation and cause clinically significant distress or impairment (American Psychiatric Association, 2013). Recent reports suggest that the cannabis withdrawal syndrome affects between 30-78% of outpatients with CUD (Bahji et al., 2020). Some people may be more vulnerable to experiencing more severe symptoms of cannabis withdrawal. For one, females report experiencing more cannabis withdrawal symptoms (Herrmann et al., 2015; Levin et al., 2010) and more severe symptoms (Herrmann et al., 2015), compared to males in retrospective studies. Furthermore, in their 16-day abstinence paradigm, Bonnet et al. (2014) found that females experienced more severe cannabis withdrawal symptoms throughout abstinence, relative to males. People who co-use tobacco with cannabis also report experiencing more severe cannabis withdrawal symptoms compared to those using only cannabis (Agrawal et al., 2008; Bahji et al., 2020; Ellingson et al., 2019; Hasin et al., 2008; Yeap et al., 2023). In line with this, work by our own group found that men with a CUD with heavy tobacco use experienced more severe cannabis withdrawal than those with low levels of tobacco use (Yeap et al., in press). Lastly, age may also play a role in cannabis withdrawal. Namely, young and middle-aged adults who consume cannabis (i.e. people aged 18-29 years and 30-49 years, respectively) report experiencing more severe symptoms of cannabis withdrawal relative to older cannabis consumers (i.e. people 50 years or older) (Sexton et al., 2019).

Importantly, cannabis withdrawal symptoms are clinically relevant because they predict cannabis

relapse (Allsop et al., 2012; Levin et al., 2010), though not all studies support this (Arendt et al., 2007). One reason for conflicting results may be that CUD severity may moderate this association (Allsop et al., 2012). Additionally, increases in depression and anxiety during cannabis abstinence may reinforce that cannabis use alleviates affective symptoms. Therefore, it is important for people with chronic cannabis use to recognize that increases in the severity of affective symptoms following cannabis cessation likely reflect cannabis withdrawal and are not due to cannabis remediating such symptoms.

2.4.2 Cannabis Tolerance

The downregulation of CB1r in the brain following prolonged cannabis use has been associated with tolerance to the acute psychoactive effects of THC (Ceccarini et al., 2015; D'Souza et al., 2016; D'Souza et al., 2008; Hirvonen et al., 2012). Cannabis tolerance refers to the blunted effect to cannabis following regular or prolonged use (D'Souza et al., 2008; Ramaekers et al., 2020). Namely, tolerance to cannabis use can occur in relation to cognitive function, mood, sleep, and psychomotor effects (Colizzi & Bhattacharyya, 2018; Sharma et al., 2012). Tolerance is evident either by: 1) experiencing a reduced effect by using the same amount of cannabis over time, or 2) experiencing the same effect with increased amounts of cannabis (American Psychiatric Association, 2013). Cannabis tolerance is clinically relevant given that it may result in using greater amounts of cannabis which can increase the risk of mental health related harms and CUD (Colizzi & Bhattacharyya, 2018).

2.4.3 Problematic Cannabis Use

The ingredient in cannabis responsible for its addictive properties is THC. Administration of THC increases dopamine release through GABAergic inhibition (Bossong et al., 2009; Calakos et al., 2021). Consequently, following cannabis use an increase in dopamine is observed in the striatum,

an important region for reinforcement and reward processing (Bossong et al., 2009; Calakos et al., 2021), which can lead to prolonged, heavy, and/or frequent cannabis use. Ultimately, these consumption patterns can lead to problematic cannabis use. Importantly, 25% to 50% of those who use cannabis daily or almost daily will develop a CUD (Canada, 2023b). In the DSM-5, CUD is characterized as a pattern of cannabis consumption that leads to clinically significant levels of impairment or distress to the user, manifested by at least two of the following 12 symptoms within a period of 12-months (American Psychiatric Association, 2013):

1. Cannabis is often taken in larger amounts or over a longer period than was intended.
2. There is a persistent desire or unsuccessful efforts to cut down or control cannabis use.
3. A great deal of time is spent in activities necessary to obtain cannabis, use cannabis, or recover from its effects.
4. Craving, or a strong desire or urge to use cannabis.
5. Recurrent cannabis use resulting in a failure to fulfill major role obligations at work, school, or home.
6. Continued cannabis use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of cannabis.
7. Important social, occupational, or recreational activities are given up or reduced because of cannabis use.
8. Recurrent cannabis use in situations in which it is physically hazardous.
9. Cannabis use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by cannabis.
10. Tolerance, as defined by either (a) a need for markedly increased amounts of cannabis to achieve intoxication or desired effect, or (b) a markedly diminished effect with continued

use of the same amount of cannabis.

11. Withdrawal, as manifested by either (a) the characteristic withdrawal syndrome for cannabis or (b) cannabis (or a closely related substance) is taken to relieve or avoid withdrawal symptoms.

All substance use disorder diagnoses are reported with a degree of severity. Namely, the presence of two to three symptoms defines a mild CUD, the presence of four to five symptoms defines a moderate CUD, and the presence of six or more symptoms defines a severe CUD.

2.5 Cannabis Use Motives

Cannabis has been used for centuries for therapeutic purposes and the notion that cannabis use is therapeutic and benefits mental health remains widely adopted in North America (Canada, 2024a; Wallis et al., 2022). Accordingly, people often use cannabis to self-medicate affective symptoms, which refers to the practice of using a substance to alleviate, cope with, or reduce psychological or physical symptoms (Wallis et al., 2022). In Canada, 43% of people who used cannabis in 2023 reported that cannabis use was beneficial to their mental health, while only 8% thought that it was harmful to their mental health (Canada, 2024a). Similarly, in an American sample of young adults, ~82% of frequent cannabis users endorsed using cannabis to self-medicate symptoms of anxiety, and ~60% for symptoms of depression (Wallis et al., 2022). Importantly, there is no evidence that cannabis use improves affective symptoms and moreover, there are currently no cannabinoid treatments approved for the treatment of psychiatric disorders (Mammen et al., 2018; Stanciu et al., 2021; Turna et al., 2017). Rather, chronic cannabis use has been shown to contribute to the development and maintenance of affective symptoms and their related disorders (Duperrouzel et al., 2018; Gobbi et al., 2019; Hayatbakhsh et al., 2007; Onaemo et al., 2021; Patton et al., 2002).

2.6 Associations between Cannabis Use and Affective Symptoms

2.6.1 Acute Effects of Cannabis Use on Affective Symptoms

In healthy adults with a history of nominal cannabis use, evidence suggests that THC exerts a dose dependent effect on anxiety. While low doses of cannabis produce anxiolytic effects, high doses are associated with anxiogenic effects (Lichenstein, 2022). High doses of cannabis can also produce symptoms of paranoia and psychosis (Ramaekers et al., 2021), and less frequent cannabis users may be more vulnerable to experiencing these adverse symptoms (Curran et al., 2019). Conversely, adults with frequent cannabis use show blunted anxiogenic reactions to cannabis (D'Souza et al., 2008) even at high doses (Curran et al., 2019), which may be due tolerance to cannabis that develops over time.

Studies have demonstrated that the relative proportions of the main cannabinoids, THC and CBD, in cannabis preparations may dictate its subjective effects. For example, pure THC consumption has been associated with aversive symptoms, while cannabis preparations that include CBD have been associated with less adverse clinical outcomes. This may be because CBD may offset or blunt some of the psychotropic effects of THC (Freeman et al., 2019; Karniol et al., 1974; Petrie et al., 2021). This highlights the importance of considering the proportions of THC and CBD when studying the effects of cannabis use on clinical outcomes.

2.6.2 Chronic Cannabis Use and Affective Symptoms

While people often use cannabis to self-medicate symptoms of depression and anxiety (Wallis et al., 2022), chronic cannabis use is, paradoxically, associated with negative effects on affective outcomes. In a systematic review and meta-analysis of epidemiological cross-sectional studies (N=8), Onaemo et al. (2021) reported that CUD commonly co-occurs with a major depressive

episode (OR=3.22) and generalized anxiety disorder (OR=2.99). Determining the temporal direction of this association is warranted because both a causal and reverse-causal hypothesis is plausible; while cannabis use may precipitate and worsen affective symptoms and disorders, people with worse affective symptoms and disorders may be more vulnerable to using cannabis.

A systematic review and meta-analysis of longitudinal studies by Gobbi et al. (2019) (N=11) found that cannabis use in adolescence significantly increased the risk of developing major depressive disorder (OR=1.37), but not anxiety symptoms. However, Gobbi et al. (2019) reported limitations to their findings. Namely, other substance use was not controlled for in all the studies included in the review, and the moderating effect of quantity of cannabis could not be assessed (Gobbi et al., 2019). Below, I describe four longitudinal studies that assessed depression and anxiety outcomes that were not included in their quantitative synthesis given that they were published after 2019 (Gobbi et al., 2019).

With respect to depression, a longitudinal study by Davis et al. (2023) provides evidence of a temporal relationship between cannabis use and subsequent increases in depressive symptoms in young adults (N=1534), where greater increases in cannabis use were associated with greater increases in depressive symptoms. Similarly, in their 30 year-long longitudinal study, Hengartner et al. (2020) found that cannabis use during adolescence was associated with major depressive disorder and suicidality in adulthood, but not with generalized anxiety disorder. The authors reported that young age of cannabis use and increased frequency of cannabis use further increased the risk of depression in adulthood (Hengartner et al., 2020). Conversely, Bolanis et al. (2020) found support for a reverse-causal relationship, where major depressive disorder at age 15 predicted weekly cannabis use at age 17 (AOR=2.30), while controlling for sex other substance use.

With respect to anxiety, Davis et al. (2022) found support for both a causal and reverse-causal relationship. Namely, they noted that greater cannabis use predicted greater anxiety symptoms and greater state anxiety predicted greater cannabis use. In contrast, greater trait anxiety predicted less cannabis use (Davis et al., 2022). Duperrouzel et al. (2018) found support for a causal relationship between cannabis and anxiety over a one-year period. Namely, higher cannabis use in adolescence predicted more persistent anxiety one year later, after controlling for sex, alcohol use, nicotine use, and concurrent depression. These studies suggest that cannabis use may contribute to the maintenance of anxiety over time (Duperrouzel et al., 2018).

Taken together, findings from cross-sectional and longitudinal studies provide evidence that cannabis use and CUD are significantly associated with affective symptoms and disorders (Onaemo et al., 2021). Further, longitudinal studies suggest that cannabis use during adolescence increases the risk of developing major depressive disorder in adulthood (Gobbi et al., 2019) and exacerbates the risk and severity of depressive symptoms (Davis et al., 2023; Hengartner et al., 2020). Lastly, chronic cannabis predicts and sustains symptoms of anxiety (Davis et al., 2022, 2023; Duperrouzel et al., 2018).

2.6.3 Cannabis Abstinence and Affective Outcomes

Given that cannabis use may precipitate, exacerbate, and sustain affective symptoms (Davis et al., 2022, 2023; Duperrouzel et al., 2018; Gobbi et al., 2019), it is crucial to understand if sustained cannabis abstinence has a beneficial impact on affective symptoms. Cannabis abstinence paradigms enable a longitudinal exploration of the effects of prolonged cannabis abstinence, often over 28 days, on clinical outcomes. These paradigms are advantageous to cross-sectional designs as they offer greater statistical power by using within-subject approaches. Abstinence paradigms also help to determine the trajectory of symptom severity during cannabis abstinence, which

subsequently enables clinicians and healthcare workers to identify periods where patients may be most vulnerable to relapse or require interventions to address increases in affective symptoms.

There are several reasons why investigators employ a 28-day cannabis abstinence period to examine changes in affective symptoms. Evidence supports that withdrawal symptoms dissipate after 28 days of cannabis abstinence. Additionally, due to its lipophilic properties, THC is slowly eliminated over several weeks following cannabis cessation and the complete urinary elimination of THC in heavy users often coincides with the 28-day period (Goodwin et al., 2008; Lowe et al., 2009; Rabin et al., 2018b). Lastly, downregulation of CB1r associated with chronic cannabis use may normalize following 28 days of cannabis abstinence (D'Souza et al., 2016; Hirvonen et al., 2012). Taken together, given that cannabis' residual effects dissipate in 28 days justifies the use of a 28-day timeframe to examine affective symptom improvement associated with chronic cannabis use.

In these abstinence paradigms, contingency management is often employed to motivate participants to remain abstinent for four weeks (Budney et al., 2003; Cooke et al., 2021; Jacobus et al., 2017; Lucatch et al., 2020; Rabin et al., 2018a). Through this method, participants who successfully maintain abstinence for the duration of the study are incentivized, with rewards such as monetary bonus, at the end of the 28-day period (Rabin et al., 2018b; Schuster et al., 2017). Contingency management has shown great efficacy in sustaining cannabis abstinence over four weeks (Rabin et al., 2018b; Schuster et al., 2017). Rabin et al. (2018b) report an abstinence rate of 70% in their non-psychiatric control group and Schuster et al. (2016) report an 89.5% abstinence rate in non-psychiatric young adults. In addition to contingency management, providing participants with individual behavioral support sessions can also aid in maintaining abstinence and participant retention (Lucatch et al., 2020; Rabin et al., 2018a; Rabin et al., 2018b). In these

sessions, participants can discuss their challenges with cannabis cessation and receive support to identify strategies to better cope with abstinence (e.g., withdrawal and craving).

A growing body of research is investigating the effects of cannabis reduction and cannabis abstinence on affective symptoms in both adults and adolescents. Evidence supports significant improvements in affective symptoms following 16 days (Bonnet et al., 2015), 28 days (Cooke et al., 2021; Feinstein et al., 2021; Galang et al., 2015; Jacobus et al., 2017; Lee et al., 2014; Lucatch et al., 2020; Milin et al., 2008; Rabin et al., 2018a), and 45 days (Budney et al., 2003) of cannabis abstinence. **Table 1** summarizes the existing literature investigating the effects of cannabis use reduction and cannabis abstinence on depressive and anxiety symptoms. However, not all studies report improvements in either or both of these symptom (Budney et al., 2003; Cooke et al., 2021; Feinstein et al., 2021; Jacobus et al., 2017; Kouri & Pope, 2000; Lee et al., 2014; Lucatch et al., 2020; Milin et al., 2008; Rabin et al., 2018a; Sullivan et al., 2022).

Ten studies investigated the effects of 21 to 28 days of cannabis abstinence on affective symptoms in adolescent (n=4) and adult (n=6) samples, one study investigated the effects of 16 days of cannabis abstinence in adults, and one study investigated the effects of 45 days of cannabis abstinence in adults.

In studies that employed adolescent samples, researchers reported significant improvements in depression (Jacobus et al., 2017) and anxiety (Milin et al., 2008), while another study found that depression and anxiety only improved in adolescents who used cannabis to cope with negative emotions and experiences (Cooke et al., 2021). Sullivan et al. (2022) found no improvements in affective symptoms in their adolescent sample, suggesting that 21-days of abstinence may not have been long enough to observe notable improvements.

In adults, 8 studies investigated the effects of 16-45 days of cannabis abstinence. Depression was assessed in adults in six studies (Bonnet et al., 2015; Budney et al., 2003; Feinstein et al., 2021; Lee et al., 2014; Lucatch et al., 2020; Rabin et al., 2018a), and mood was assessed in one (Kouri & Pope, 2000). Significant improvements were reported in four studies (Bonnet et al., 2015; Feinstein et al., 2021; Lucatch et al., 2020; Rabin et al., 2018a), and four studies reported no change in depressive and mood symptoms (Budney et al., 2003; Kouri & Pope, 2000; Lee et al., 2014). Notably, Rabin et al. (2018a) studied two independent samples, a group of participants with schizophrenia who significantly improved and a group of non-psychiatric controls who demonstrated no change in depressive symptoms.

In adults, six studies assessed anxiety and one assessed anxiety-related panic. Of these, three studies found significant improvements in anxiety (Bonnet et al., 2015; Budney et al., 2003; Lee et al., 2014), and one found significant improvements in panic (Galang et al., 2015). Conversely, three studies noted no change in anxiety (Feinstein et al., 2021; Kouri & Pope, 2000; Lucatch et al., 2020).

Overall, the abovementioned studies, looking at depression and anxiety, have limitations. For one, six studies included samples with co-occurring psychiatric and medical disorders (Feinstein et al., 2021; Galang et al., 2015; Jacobus et al., 2017; Lucatch et al., 2020; Milin et al., 2008; Rabin et al., 2018a), or were conducted in adolescents (Cooke et al., 2021; Jacobus et al., 2017; Milin et al., 2008; Sullivan et al., 2022). Additionally, one study assessed adults who were inpatient receiving treatment for cannabis use (Bonnet et al., 2015), and the majority of participants were receiving medication to alleviate cannabis withdrawal (e.g. gabapentin), which likely impacted the severity of affective symptoms (Ahmed et al., 2019). Findings from these studies may not translate to otherwise healthy adults using cannabis.

Further, nine studies did not include an adequate control group in their study, which fails to control for the effects of confounding factors (i.e.as time spent with researchers) on outcomes (Bonnet et al., 2015; Budney et al., 2003; Galang et al., 2015; Jacobus et al., 2017; Lee et al., 2014; Lucatch et al., 2020; Milin et al., 2008; Rabin et al., 2018a; Sullivan et al., 2022). Taken together, these limitations highlight the importance of investigating the effects of cannabis abstinence on symptoms of depression and anxiety in adults with CUD and no co-occurring psychiatric or medical disorders while including an appropriate control group.

2.7 Sex and Gender Considerations

Males report higher cannabis use rates than females; in 2022, 30% of males reported using cannabis while 25% of females reported the same (Canada, 2024b). However, it is important to note that the sex gap in cannabis use rates is narrowing; Greater increases in rates of cannabis use are being documented in females relative to males since cannabis legalization. In fact, past year cannabis use rates have increased more in females (from 18% in 2018 to 25% in 2022) than in males (from 26% in 2018 to 30% in 2022) (Canada, 2024b). Nevertheless, Females (sex) and women (gender) are notoriously underrepresented in cannabis research. Importantly, research that investigates the effects of cannabis abstinence on symptoms of depression and anxiety has yet to prospectively assess sex differences in the trajectory of affective symptoms during cannabis abstinence.

From a biological perspective, evidence from preclinical and clinical studies suggest that females may be more sensitive to the effects of THC, which may be due to differences in CB1r expression and cannabis metabolism (Blanton et al., 2021). For one, researchers have found that females express CB1r at higher densities than males (Neumeister et al., 2013; Normandin et al., 2015),

though findings are equivocal and research is limited (Blanton et al., 2021; Van Laere et al., 2008). Furthermore, females express the Cytochrome P450 enzyme CYP3A4 at higher concentrations compared to males, generating the psychoactive 11-OH-THC metabolite at higher rates than males (Blanton et al., 2021; Nadulski et al., 2005). This is important given that 11-OH-THC is responsible for the psychological effects of cannabis (Sharma et al., 2012). Lastly, females experience more severe cannabis withdrawal symptoms than males, which may reflect sex differences in the endocannabinoid system (e.g. CB1R expression) (Schlienz et al., 2017).

In the context of gender differences, women and persons identifying as non-binary experience depression and anxiety at higher rates in both the general and cannabis using population compared to men (Cheung et al., 2020; Danielsson et al., 2016; Yang et al., 2021). Additionally, although men have higher rates of CUD, women develop CUD at a faster rate (Kerridge et al., 2018). This body of evidence highlights the importance of considering sex and gender in cannabis research and ensuring the recruitment of a representative sample that includes females and women.

Overall, research findings support the presence of important sex and gender differences in cannabis use and CUD rates, cannabis metabolism, and clinical symptoms associated with cannabis use. We theorize that these differences are largely driven by biological differences in the endocannabinoid system (e.g., downregulation of CB1R). Thus, the current study will explore sex differences (rather than gender differences) by disaggregating the sample into males and females to gain greater insights into the effects of sex on cannabis-associated clinical symptoms.

Table 1:
Summary of literature investigating the effects of cannabis reduction and abstinence on affective symptoms.

Adolescent Sample		
Sullivan et al. (2022)	Abstinence Length	21 days
	Sample	N=79; 45% Female
	Comorbidities	None
	Cannabis Use Frequency	≥ weekly use (mean = 424.7times//past year)
	Control Group	Healthy
	Affective Measured Outcomes	BDI, STAI
	Results	Anxiety decreased in both the cannabis-abstinent group and the healthy control group, which indicates no effect of abstinence on symptoms of anxiety. Depression symptoms were observed to increase in the cannabis-abstinent group.
	Conclusion	Depression ↑; Anxiety →
Cooke et al. (2021)	Abstinence Length	28 days
	Sample	N=179; 44% Female
	Comorbidities	None
	Cannabis Use Frequency	≥ weekly use (mean = 4.6 times/past year)
	Control Group	Non-abstinent
	Affective Measured Outcomes	MASQ, MMM
	Results	Depression and anxiety improved only in participants with problematic cannabis use (defined as CUDIT score ≥12) and those who used cannabis to cope.
	Conclusion	Depression ↓; Anxiety ↓ (no change in general sample)
Jacobus et al. (2017)	Abstinence Length	28 days
	Sample	N=56; 27% Female
	Comorbidities	Substance Use Disorder, in addition to CUD
	Cannabis Use Frequency	≥ month use (mean = 18.1 times /month)
	Control Group	Healthy

	Affective Measured Outcomes	BDI, STAI
	Results	Significant improvements in depressive symptoms, but not anxiety, after 28 days of cannabis abstinence.
	Conclusion	Depression ↓; Anxiety →
Milin et al. (2008)	Abstinence Length	28 days
	Sample	N=21; 33% Female
	Comorbidities	Psychotropic medication
	Cannabis Use Frequency	Daily or almost daily cannabis use
	Control Group	None
	Affective Measured Outcomes	CWS
	Results	Significant reductions in anxiety after 28 days of cannabis abstinence. No significant changes in depression over the 28 days of cannabis abstinence.
	Conclusion	Depression →; Anxiety ↓
Adult Sample		
Bonnet et al. (2015)	Abstinence Length	16 days
	Sample	N=35; 20% Female; Inpatients in treatment for cannabis use
	Comorbidities	None
	Cannabis Use Frequency	≥ weekly use (mean = 2.4grams/day)
	Control Group	None
	Affective Measured Outcomes	HDRS, HARS
	Results	Significant reductions in symptoms of depression and anxiety after 16 days of cannabis abstinence.
	Conclusion	Depression ↓; Anxiety ↓
Feinstein et al. (2021)	Abstinence Length	28 days
	Sample	N=40; 51% Female
	Comorbidities	Multiple Sclerosis with cognitive impairments

	Cannabis Use Frequency	Daily or almost daily cannabis use
	Control Group	Non-abstinent
	Affective Measured Outcomes	HADS, CWS
	Results	Significant improvements in depressive symptoms particularly in those who use cannabis to cope with depression. No change in anxiety symptoms.
	Conclusion	Depression ↓; Anxiety →
Lucatch et al. (2020)	Abstinence Length	28 days
	Sample	N=14; 64% Female
	Comorbidities	Major depressive disorder
	Cannabis Use Frequency	≥ weekly use (mean = 0.89grams/day)
	Control Group	None
	Affective Measured Outcomes	HDRS, BAI
	Results	Significant improvements in depression. No change in anxiety.
	Conclusion	Depression ↓; Anxiety →
Rabin et al. (2018a)	Abstinence Length	28 days
	Sample	N=39; 100% Males
	Comorbidities	SCZ
	Cannabis Use Frequency	≥ weekly use (mean = 0.89grams/day)
	Control Group	No SCZ
	Affective Measured Outcomes	CDSS, HDRS
	Results	Significant improvements in depression after cannabis abstinence in SCZ patients, measured with the CDSS. No significant improvements in depression in controls when measured with the HDRS.
	Conclusion	Depression ↓ in SCZ; Depression → in controls
Galang et al. (2015)	Abstinence Length	28 days
	Sample	N=104; Sex & Gender not reported; Veterans

	Comorbidities	Substance Use Disorder in addition to CUD, Panic, Anxiety
	Cannabis Use Frequency	daily use (mean = 39.8 times/month)
	Control Group	None
	Affective Measured Outcomes	IDAS
	Results	Significant reductions in panic symptoms after 28 days of cannabis abstinence.
	Conclusion	Panic ↓
Lee et al. (2014)	Abstinence Length	28 days
	Sample	N=29; 100% Males
	Comorbidities	None
	Cannabis Use Frequency	Daily or almost daily cannabis use
	Control Group	None
	Affective Measured Outcomes	VAS
	Results	Significant reductions in anxiety after 28 days of cannabis abstinence. No changes in depressive symptom.
	Conclusion	Depression →; Anxiety ↓
Kouri and Pope (2000)	Abstinence Length	28 days
	Sample	N=30; 13% Female
	Comorbidities	None
	Cannabis Use Frequency	Daily cannabis use
	Control Group	Ex-cannabis users and non-users
	Affective Measured Outcomes	HDRS, HARS
	Results	Depression and anxiety peaked on day 7 and returned to baseline.
	Conclusion	Depression →; Anxiety →
Budney et al. (2003)	Abstinence Length	45 days
	Sample	N=18; 39% Female

Comorbidities	None
Cannabis Use Frequency	Daily (mean = 3.5 use per day)
Control Group	Ex-cannabis users
Affective Measured Outcomes	BSI, POMS
Results	Significant reductions in nervousness/anxiety after 45 days of cannabis abstinence. Non-significant reductions in depression after cannabis abstinence.
Conclusion	Depression →; Anxiety ↓

Arrows indicate change in symptom: ↑, increase in symptom; ↓, decrease in symptom; → no change in symptom.
BAI, Beck Anxiety Inventory; BDI, Beck's Depression Inventory; BSI, Brief Symptom Inventory; CDSS, Calgary Depression Scale for Schizophrenia; CUD, Cannabis Use Disorder; CWS, Cannabis Withdrawal Scale; CWSC, Cannabis Withdrawal Syndrome Criteria; DERS, Difficulties in Emotion Regulation Scale; HADS, Hospital Anxiety and Depression Scale; HARS, Hamilton Anxiety Rating Scale; HDRS, Hamilton Depression Rating Scale; IDAS, Inventory of Depression and Anxiety Symptom; MASQ, Mood and Anxiety Symptom Questionnaire; MMM, Marijuana Motives Measure; PANAS, Positive and Negative Affect Scale; POMS, Profile of Mood States; SCZ, Schizophrenia; SHAPS, Snaith-Hamilton Pleasure Scale; STAI, State Trait Anxiety Inventory; VAS, Visual Analogue Scale.

3. AIM AND HYPOTHESIS

Primary Aim: The overarching aim of this study was to investigate the effects of 28 days of cannabis abstinence on depressive and anxiety symptoms in people with CUD and no co-occurring psychiatric/medical disorders.

Hypothesis: Depressive and anxiety symptoms will improve over time during 28 cannabis abstinence in non-psychiatric adults with CUD.

Exploratory Aim: To assess sex differences in the trajectory of depressive and anxiety symptoms during cannabis abstinence in people with CUD and no co-occurring psychiatric/medical disorders.

Hypothesis: Female participants will experience elevated severity of depression and anxiety during cannabis abstinence relative to male participants.

4. METHODS

4.1 Study Overview

This study was approved by the "Centre Intégré Universitaire de Santé et de Services Sociaux de l'Ouest-de-l'Île-de Montréal" (CIUSS-ODIM) Research Ethics Board (approval number 2021-312, IUSMD-21-11). This study was a secondary analysis of a larger study examining cannabis abstinence on brain outcomes using neuroimaging techniques.

4.1.1 Recruitment Approaches

Participants were recruited between April 2022 and February 2023 through online and community poster advertisements. Posters in English and in French were placed in cafes, store fronts, and street poster boards (see Appendix 8.1. for posters). Online, posters were shared on Facebook groups dedicated to sharing remunerated study opportunities (e.g. McGill Studies for Cash). Participants expressed interest in our study by completing an online form that gathered contact information and availability. Participants were followed up with an email outlining the details of a study and a phone call to ascertain basic eligibility information. Eligible participants from the phone screen were then invited for an in-person screen.

4.1.2 Phone Screen

The purpose of the phone screen was to assess general eligibility criteria related to medical and psychiatric history, CUD symptomology, and current substance use. For cannabis use, we asked about CUD symptomology based on DSM-5 CUD, treatment seeking status for cannabis. For other substance use, we gathered information on the frequency of use. We also assessed for the presence and timeline of psychiatric disorder symptoms and diagnoses (e.g. depressive disorder, psychotic disorder) and the current and past use of psychotropic medication. Lastly, we asked about

participants' current medical conditions that required regular monitoring (e.g. Crohn's disease), the current use of medication (e.g. thyroid hormone replacement), a history of a neurological incident that caused loss of consciousness for at least 5 minutes (e.g. stroke, concussion), and the implant of a device that may interfere with magnetic resonance imaging (MRI; assessed for a different project).

4.1.3 In-Person Screen

Participants who met eligibility criteria based on the phone screen were invited to the Addiction, Imaging, and Mental Health Lab (AIMH Lab) at the Douglas Research Centre in Montreal, Quebec to further establish study eligibility. We began the in-person screen by reviewing the study procedures in detail and answering any questions that participants. Following, we had the participant read the consent form and the re-reviewed it with the participant. Once consent was signed, the session proceeded, and data collection began.

We assessed demographic variables (e.g. sex, gender, educational attainment) through a self-report demographic questionnaire. We assessed substance use patterns through self-report questionnaires and interviews (CUDIT, AUDIT, FTND, and Drug Use Survey; see section 4.3 for more details on the measures used). We also assessed current and past psychiatric disorders using the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). Lastly, participants provided a urine sample for drug testing using a 8-panel dip-stick test (VeriCheck® 8-panel cup).

4.1.4 Randomization

Eligible participants were randomized using a 3:2 ratio to the abstinent arm (AB) or the cannabis as usual control arm (NA) respectively. The purpose of the NA group was to control for study effects that may be due to time and/or study participation. Assigning more participants to the

abstinence arm accounted for an expected relapse rate of 30% in AB, which has been previously established by our group (Rabin et al., 2018b). Eligible participants were informed of which arm they were randomized to following their in-person screen and prior to their baseline visit. Participants were sent a detailed e-mail outlining their study arm assignment and the date and time at which they needed to cease cannabis use prior to their baseline visit.

4.1.5 Overview of Study Design

Following randomization, participants were invited to come for a baseline visit and then weekly study visits, seven days apart, over 28 days. At each visit, depression and anxiety symptoms were assessed, along with cannabis withdrawal symptoms and substance use over the past week using self-report questionnaires.

Participants in both arms were asked to remain abstinent from cannabis for 12 hours before their baseline visit, and for 12 hours. Given that the onset of cannabis withdrawal is ~ 24 hours post-cessation, this ensures that participants were not experiencing symptoms of cannabis withdrawal at the time of their baseline visit. Participants in the AB arm were asked to abstain from cannabis for 28 days. For these participants, cannabis abstinence was encouraged using contingency management and weekly behavioural support session. However, to keep study procedures consistent across study groups, all participants received a weekly short behavioural support session (15-minutes; see Appendix 8.2). For AB participants, cannabis abstinence was verified using a self-report interview (the Timeline Follow-Back). Figure 2 and Table 4 summarize the study timeline and assessment schedule.

4.2 Participants

Table 2:
Inclusion and Exclusion Criteria

Inclusion Criteria
Male or female
Comprehension of English or French
Between 18-55 years old
Presence of DSM-5 CUD
Positive THC urine test at screen
Exclusion Criteria
Positive urine test for other psychoactive substances
Current DSM-5 disorders (other than a past depressive or anxiety disorder with at least one year of remission)
Treatment-seeking for cannabis use
Current or past year use of psychotropic medication
Current use of medication for medical disorder
Monitored medical condition
Neurological incident or disorder
Implant of device that interferes with MRI
Pregnant
Current suicidal or homicidal ideations

Note. Summary of study inclusion and exclusion criteria. The presence of all inclusion criteria and the absence of all exclusion criteria determines eligibility for the study.

4.2.1 Inclusion and Exclusion Criteria

Table 2 provides a detailed summary of our study's inclusion and exclusion criteria. Inclusion criteria was as follows: (i) between the ages of 18 and 55; (ii) DSM-5 CUD diagnosis (iii) positive urine toxicology for cannabis. Participants were excluded for the following: (i) currently seeking treatment for cannabis use; (ii) regular use of a psychotropic substance other than cannabis, alcohol, or nicotine. Participants were also excluded if they: (i) had a DSM-5 diagnosis with the following exceptions past major depressive episode or disorder or anxiety disorder; past alcohol/substance use disorder in remission for at least one year; (ii) had current or past year use of psychotropic medication to treat a psychiatric disorder; (iii) a current medical condition; (v) had a history of neurological incident; (vi) had an implant that interfered with MRI; (vii) were currently

pregnant; (iix) had current suicidal or homicidal ideations.

4.3 Measures

See Table 4 for the specific visit(s) that measures were administered.

4.3.1 Substance Use Measures

Cannabis Use Disorder Identification Test (CUDIT) Revised (Adamson et al., 2010)

The revised CUDIT is an 8-item self-report measure that identifies problematic cannabis use and its severity. Higher scores indicate more problematic cannabis use. Namely, scores from 1-7 indicate low-risk cannabis use, scores from 8-11 suggest hazardous cannabis use, and scores of or above 12 indicate the potential of a CUD.

This test was administered at screen to assess level of CUD severity.

Alcohol Use Identification Test (AUDIT) (Saunders et al., 1993)

The AUDIT is a 10-item self-report measure developed by the World Health Organization that identifies problematic alcohol use and its severity. Higher scores indicate more problematic alcohol use. Namely, scores from 1-7 indicate low-risk alcohol consumption, scores from 8-14 suggest hazardous alcohol consumption, and scores of or above 15 indicate the potential of alcohol dependence.

This test was administered at screen to identify problematic levels of alcohol consumption.

Fagerstrom Test for Nicotine Dependence (FTND) (Heatherton et al., 1991)

The FTND is a 6-item self-report measure that assesses the presence and severity of nicotine dependence. Scores of 1 or 2 indicate no nicotine dependence, scores of 3 or 4 indicate low dependence, scores between 5 to 7 indicate moderate dependence, and scores between 8 to 10

indicate high dependence.

This test was administered at screen to identify and assess nicotine dependence.

Drug Use Survey

The Drug Use Survey is an interview developed in-house to assess the presence of current and past cannabis, alcohol, nicotine, and other substance patterns of use. Specifically, it collects information about past 30-day substance use and lifetime substance use.

This interview was administered at screen to comprehensively assess current drug use and history of drug use.

Timeline Follow-Back (TLFB) (Sobell et al., 1996)

The TLFB is a short interview that assesses the quantity of daily cannabis, alcohol, nicotine, and other substance use over the previous 7 days. Cannabis use was assessed in grams per day and alcohol was assessed in drinks per day.

This interview was administered weekly to assess past week substance use.

Urine Toxicology

We used an 8-panel urine drug toxicology screen (VeriCheck® 8-pannel cup) to qualitatively assess the presence of cannabis, amphetamine, cocaine, methamphetamine, opioids, Phencyclidine3,4-Methylenedioxymethamphetamine, and oxycodone. This urine test indicates the presence of THC-COOH at a threshold of >50ng/mL.

This urine test was administered at screen to assess the eligibility of a participant based on the presence or absence of substances in their urine.

4.3.2 Clinical Measures

Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998)

The MINI is a neuropsychiatric clinical interview used to diagnose the presence of current and past psychiatric disorders based on DSM-5.

This interview was administered at screen to assess the eligibility of participant based on the presence of a current CUD and absence of any other current psychiatric disorder.

Hamilton Depression Rating Scale (HDRS) (Williams, 1988)

The HDRS is a 17-item clinical interview used to assess past week symptoms of major depression episode symptoms. This interview is founded on the criteria of a major depressive episode outlined in the DSM-5. Scores are summed; a total score between 8-16 indicates mild depression, 17-23 moderate depression, and 24 and above severe depression.

This interview was administered at baseline and weekly to assess for symptoms of depression. Scores correspond to symptoms of a major depressive episode in the past week.

State Trait Anxiety Inventory (STAI) (Spielberger et al., 1983)

The STAI is a 40-item clinical self-report measure used to assess state (transient) and trait (stable) anxiety symptoms. State anxiety symptoms will be used in this research as trait scores should not fluctuate with cannabis abstinence. Scores between 20-37 on the state subscale indicate no to low levels of anxiety, 38-44 moderate anxiety, and 45-80 high anxiety.

This interview was administered at baseline and weekly to assess state anxiety. Scores correspond to symptoms of state anxiety at the moment the survey was taken.

Marijuana Withdrawal Checklist (MWC) (Budney et al., 1999)

The MWC is a 15-item clinical self-report measure to assess cannabis withdrawal symptoms and severity. Participants rate their withdrawal symptoms on a scale from 0-3 (0=none, 1=mild, 2=moderate, 3=severe), and a higher total score indicates worse withdrawal symptoms. The MWC

measures shakiness/tremulousness, depressed mood, decreased appetite, nausea, irritability, sleep difficulty, sweating, craving to smoke marijuana, restlessness, nervousness/anxiety, increased aggression, headaches, stomach pains, strange dreams, increased anger, and provides space to list other symptoms.

This test was administered at baseline and weekly to assess for symptoms of cannabis withdrawal.

4.4 Cannabis Abstinence

4.4.1 Encouraging Abstinence

Contingency Management

Cannabis abstinence in AB participants was encouraged using contingency management. This method encourages participants to remain abstinent using incentives and has shown to be effective in initiating and sustaining 28 days of cannabis abstinence in non-treatment seeking participants (Rabin et al., 2018b; Schuster et al., 2016). In our study, participants who successfully maintained cannabis abstinence were rewarded with a $300 bonus on day 28, in line with our previous procedures (Rabin et al., 2018b).

Behavioural Support

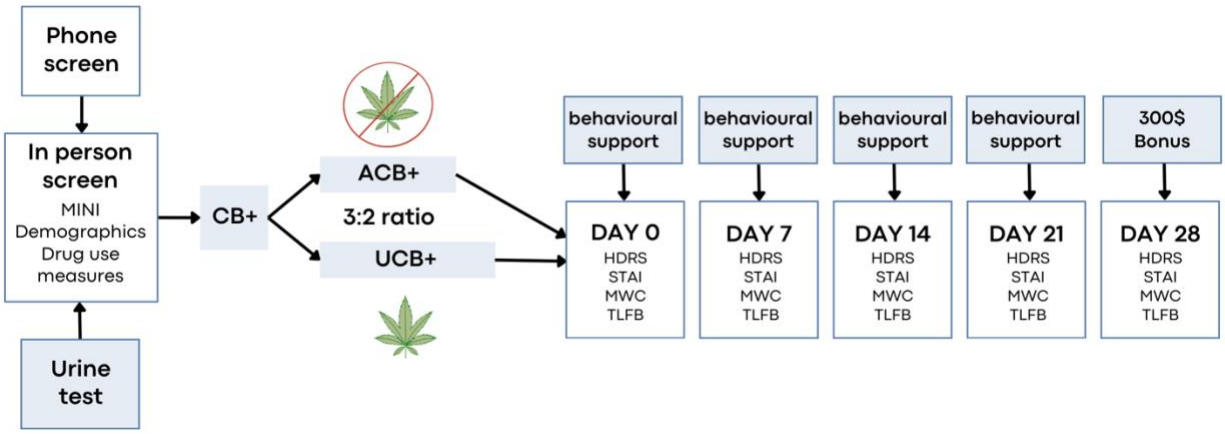
Participants received weekly one-on-one behavioural support (see Appendix 8.2). The main purpose of these sessions was to help participants manage abstinence (Rabin et al., 2018b). These sessions were conducted with trained graduate students and included a combination of motivational interviewing, psychoeducation, and coping skills. Participants in the NA arm also received weekly one-on-one behavioural support. Sessions were aimed to closely match the discussion material of the AB group. These weekly behavioural support sessions aimed to support abstinence in the AB arm and help encourage study retention in both AB and NA arms. On day

28, which is the final day of study participation, participants across both arms received psychoeducation about the risks of cannabis use on physical and mental health.

4.4.2 Abstinence Verification

Cannabis abstinence was determined using the TLFB self-report interview, where participants were prompted to indicate the amount of cannabis used (in grams) over the past week. Participants who self-reported zero grams of past week cannabis use at days 7, 14, 21, and 28 were considered abstinent.

Figure 2: *Study Timeline*



CB+, Participants with Cannabis Use Disorder; AB, Abstinent Arm; HDRS, Hamilton-Depression Rating Scale; MINI, Mini International Neuropsychiatric Interview; MWC, Marijuana Withdrawal Checklist; STAI, State Trait Anxiety Inventory; TLFB, Timeline Follow-Back; NA, As Usual Arm.

4.5 Data Analysis

4.5.1 Demographic, Clinical, and Substance Use Data

We used independent-sample t-tests to assess between-group differences in demographic and baseline characteristics between the AB and NA groups. Namely, we assessed group differences in age, years of education, cannabis use parameters, alcohol use, and baseline depressive and

anxiety symptoms. Differences in categorical data such as sex, gender, race, nicotine use, and percent of THC used were assessed using a chi square test.

Additionally, we used a one-way repeated measures analysis of variance (RM-ANOVA) to determine if the amount of cannabis used over time in the NA changed (days 0, 7, 14, 21, and 28).

4.5.2 Withdrawal Symptoms

A Repeated Measures ANOVA was used to assess between-group differences in cannabis withdrawal symptoms over time (days 0, 7, 14, 21, and 28). Assessing the trajectory of cannabis withdrawal symptoms allowed us to assess if abstinent participants underwent cannabis withdrawal according to the expected withdrawal trajectory, which would further confirm cannabis abstinence (or at least reduction) in the AB group.

4.5.3 Affective Symptoms

A RM-ANOVA was used to assess between-group differences in depressive symptoms (measured with HDRS) and anxiety (measured with STAI-state) symptoms over time (days 0, 7, 14, 21, and 28). Separate models were run for depression and anxiety.

For the exploratory sex differences analyses, a RM-ANOVA was conducted in AB participants, with sex as the independent variable and HDRS and STAI-state as the dependent variables, Separate models were run for depression and anxiety.

Lastly, we used Cohen's d to determine the effect size of change in affective symptoms from baseline (day 0) to the end of abstinence (day 28) in the AB group for each analysis. Given our small sample size, results were also interpreted from graphs to highlight emerging patterns.

Table 3:
Study Schedule of Assessments and Self-Report Measures.

	Screen	Day 0	MW 1	Day 7	MW 2	Day 14	MW 3	Day 21	MW 4	Day 28
Qualitative Urine Toxicology	•									
Demographics	•									
Drug Use Measures										
CUDIT	•									
AUDIT	•									
FTND	•									
Drug Use Survey*	•									
TLFB*		•		•		•		•		•
Clinical Measures										
MINI*	•									
HDRS*		•		•		•		•		•
STAI-state		•		•		•		•		•
MWC		•		•		•		•		•

AUDIT, Alcohol Use Disorder Identification Test; CUDIT, Cannabis Use Disorder Identification Test; FTND, Fagerstrom Test for Nicotine Dependence; HDRS, Hamilton-Depression Rating Scale; MINI, Mini International Neuropsychiatric Interview; MMM, Marijuana Motives Measure; MWC, Marijuana Withdrawal Checklist; PSS, Perceived Stress Scale; STAI, State Trait Anxiety Inventory; TLFB, Timeline Follow-Back. *Self-Reported Interview with Graduate Student

4.5.4 Power calculations

Power was calculated using the G*Power software for a RM-ANOVA. Cohen's f was calculated using results from previous studies that assessed the effects of cannabis abstinence on changes in depressive symptoms (f=0.21) (Bonnet et al., 2015; Feinstein et al., 2021; Jacobus et al., 2017; Rabin et al., 2018a; Sullivan et al., 2022) and anxiety symptoms (f=0.26) (Bonnet et al., 2015; Budney et al., 2003; Feinstein et al., 2021; Galang et al., 2015; Milin et al., 2008; Sullivan et al., 2022) (see table 3 for effect sizes used).

To power the study to detect a Cohen's f=0.21 in depressive symptoms, 80% power (1– β)], with an alpha error probability of p=0.05, N=30 participants (AB n=15; NA n=15) will need to complete the study. To power the study to detect a Cohen's f=0.26 in anxiety symptoms, 80% power (1– β)], with an alpha error probability of p=0.05, N=20 participants (AB n=10; NA n=10) will need to complete the study.

Given that our calculations to detect an effect in depression are more conservative, we will power our study with a Cohen's f=0.21. Therefore, N=30 participants need to complete the study to detect a Cohen's f=0.21. Accounting for a 30% expected relapse rate in the AB group and a 10% attrition rate for both groups based on our previous work (Rabin et al., 2018b), n=21 participants in the AB arm and n=17 participants in the NA arm will need to be recruited.

Table 4:
Effect sizes of change in affective symptoms during abstinence

	Effect Size (Cohen's f)
Depression	
Sullivan et al., 2022	-0.11
Cooke et al., 2021	NEI
Milin et al., 2008	NEI
Jacobus et al., 2017	0.18
Bonnet et al. 2015	0.42
Feinstein et al., 2021	0.22
Lucatch et al., 2020	NEI
Rabin et al., 2018 (SCZ)	0.33
Rabin et al., 2018 (HCL)	NEI
Lee et al., 2014	NEI
Kouri & Pope, 2003	NEI
Budney et al., 2000	NEI
Average	**0.21**
Anxiety	
Sullivan et al., 2022	0.15
Cooke et al., 2021	NEI
Milin et al., 2008	0.43
Jacobus et al., 2017	NEI
Bonnet et al. 2015	0.46
Feinstein et al., 2021	0.07
Lucatch et al., 2020	NEI
Galang et al., 2015	0.24
Lee et al., 2014	NEI
Kouri & Pope, 2003	NEI
Budney et al., 2000	0.23
Average	**0.26**

HCL, Healthy Control Group with Cannabis Use and no Comorbid Disorders; NEI, Not Enough Information. SCZ, Patients with Schizophrenia.
Note. Effect sizes were reported in the manuscript or calculated with the data reported.

5. RESEARCH FINDINGS

5.1 Participants

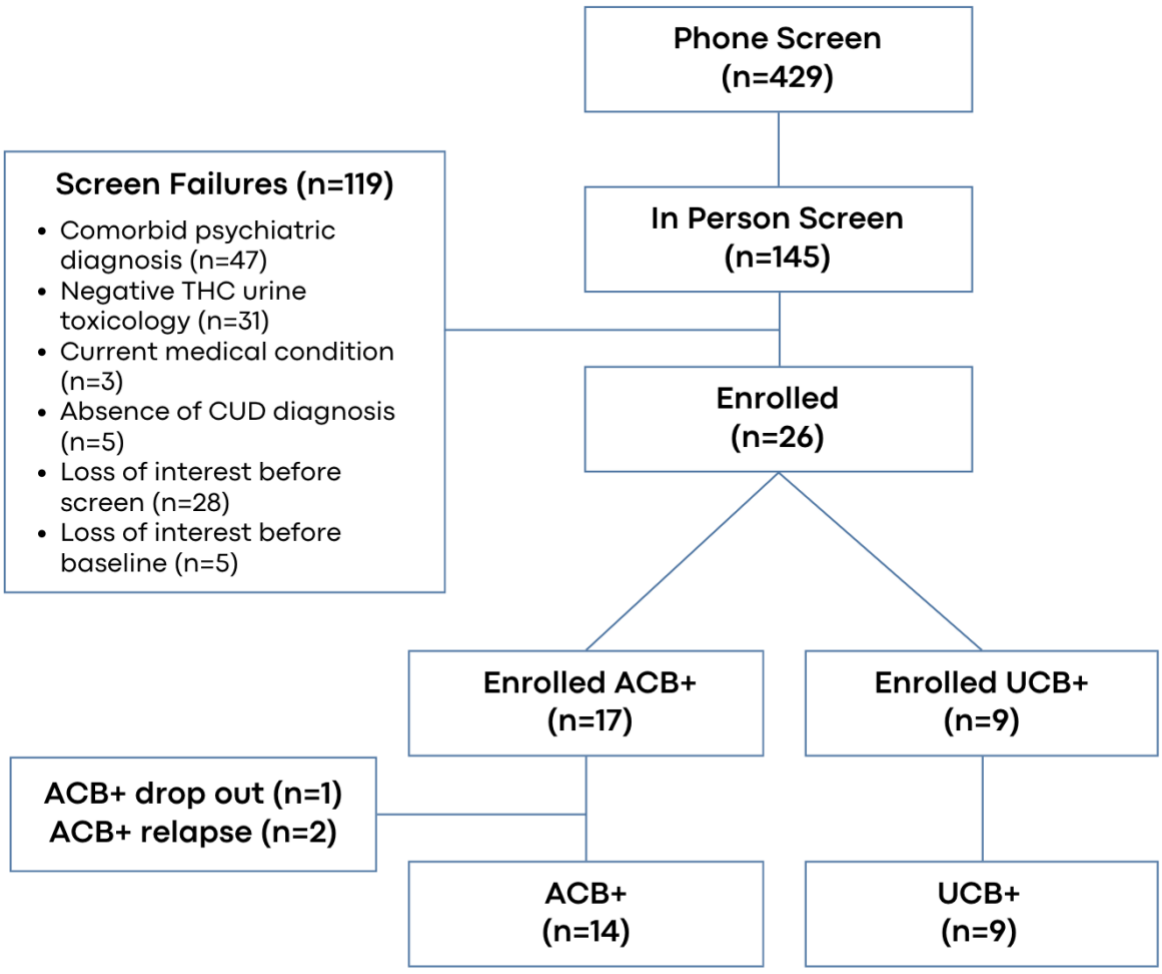
5.1.1 Participant Recruitment

We phone screened 429 participants. Participants were excluded for medical reasons (e.g. current psychotropic medication), psychiatric diagnoses, other substance use (e.g. current cocaine use), or the absence of a CUD. One-hundred-and-forty-five participants were eligible for an in-person screen, and 117 participants attended the in-person screen; 28 participants withdrew from the study before their in-person screen. Following the in-person screen, 91 participants were excluded from the study for the following reasons: the presence of a psychiatric diagnosis, other than CUD (n=47), a negative THC urine drug screen (n=31), current medical conditions (n=3), and the absence of a CUD (n=5). Five participants lost interest in the study and did not continue past the in-person screen (n=5). Twenty-six participants met eligibility criteria and were randomized to the AB arm (n= 17) or the NA arm (n=9). One participant in the AB arm dropped out after his baseline visit; all other participants completed the study (n=25). Participant recruitment numbers are summarized in Figure 3.

5.1.2 Abstinence Verification

Of the 16 participants assigned to the AB arm that completed the study, two participants relapsed: one participant self-reported relapse on day 10, and one participant self-reported relapse on day 5. Past week grams of cannabis used during the 28-day period in the AB and NA groups are summarized in Table 7 and Figure 4. Thus, 14 AB participants (88%) successfully maintained abstinence. Data from 23 participants (AB n=14, NA n=9) were included in our analyses (see Figure 3). Given that we did not have 15 participants complete the study in each arm, our study was underpowered. Thus, we also describe the overall pattern of the data.

Figure 3: *Consort Diagram*.



AB, Abstinent Arm; CUD, Cannabis Use Disorder; THC, Tetrahydrocannabinol; NA, As Usual Arm.

5.1.3 Participant Demographics

Participant characteristics are summarized in Tables 5. The AB arm included 7 female and 7 male participants and the NA arm included one female and 8 male participants. There were no significant group differences in demographic variables (see Table 5).

5.1.4 Substance Use Patterns

There were no between group differences in substance use measures assessed. See table 5. Past

week cannabis use measured as grams/day assessed at baseline did not differ between participants in the AB and NA arms. All participants reported a cannabis use initiation age of 18 or below, and mean cannabis initiation age did not differ between groups. Additionally, years of regular cannabis use did not differ between groups. CUDIT scores did not differ between groups. Scores on the CUDIT ranged from 6 to 22, and the mean scores were 13.21 (SD = 4.17) and 12.66 (SD = 3.32) in the AB and NA groups respectively, suggesting both groups were consuming cannabis at hazardous levels.

Six participants reported currently using nicotine daily (AB n=4, NA n=2), 4 additional participants reported using nicotine monthly (AB n=2, NA n=2), and 7 participants reported using nicotine daily in the past (AB n=4, NA n=3). Nicotine use, cigarettes per day, and FTND scores did not differ between groups. In participants who reported currently using nicotine, mean scores on the FTND were 1.25 (SD = 1.50) and 1.00 (SD = 1.41) in the AB and NA groups respectively. Scores between 1 and 2 on the FTND indicate a low nicotine dependence.

All participants reported using alcohol; past week alcohol consumption at baseline did not differ between groups. Additionally, AUDIT scores did not differ between groups. Mean scores on the ADUIT were 4.71 (SD = 2.40) and 4.67 (SD = 3.04) in the AB and NA groups respectively, indicating a low-risk alcohol consumption pattern.

Importantly, no participant reported illicit drug use (e.g., cocaine, opiates) during the study.

Table 5:
Baseline Demographics Data by abstinence arm.

	AB (n=14)	NA (n=9)	p value
Demographics			
Age	31.93 (9.43)	30.78 (9.38)	.78
Sex (male/female)	7/7	1/8	.06
Gender (man/woman)	7/7	1/8	.06
Race (White/mMiddle eastern/Black/Latin American/South Asian/Other)	9/2/1/0/1/1	3/1/2/2/1/0	.32
Years of education	15.21 (3.44)	15.11 (4.17)	.95
Employment status (employed/unemployed/student/in training)	9/4/1/0	5/2/1/1	.61
Substance Use Measures			
Cannabis			
CUDIT	13.21 (4.17)	12.66 (3.32)	.74
Past Week Cannabis Use/Day at Baseline (grams)	1.17 (1.13)	1.79 (1.23)	.23
Cannabis Initiation Age	16.36 (2.02)	15.78 (1.86)	.50
Years of Regular Use	9.68 (7.45)	11.78(9.73)	.56
Cannabis Use Modes (joint/vape/bong/pipe)[a]	13/1/0/0	7/0/1/1	.27
Average % THC in Cannabis (10-14%/15-19%/20-24%/25-30%/Don't know)[a]	0/0/8/3/3	1/2/3/2/1	.23
Nicotine			
Current Nicotine (use/no use)[a]	4/10	2/6	.74
Cigarettes per day[b]	6.78 (3.06)	4.50 (4.95)	.52
FTND[b]	1.25 (1.50)	1.00 (1.41)	.86
Alcohol			
Past Week Alcohol Consumption/Day at Baseline	.63 (.62)	.59 (.79)	.90
AUDIT	4.71 (2.40)	4.67 (3.04)	.97
Clinical Measures			
Past DSM-5 Diagnoses (presence/absence)[a]	4/10	3/6	.81
Baseline Affective Measures			
MWC	5.29 (4.56)	6.33 (4.47)	.59
HDRS	2.57 (3.74)	1.89 (2.80)	.64
STAI-state	29.71 (8.65)	28.11 (8.04)	.66

Values given in mean (standard deviation) unless otherwise stated, [a]Values are in numbers; [b]Among participants currently using nicotine.
AB, Abstinent; NA, As Usual. AUDIT, Alcohol Use Disorder Identification Test; CUDIT, Cannabis Use Disorder Identification Test; DSM-5; FTND, Fagerstrom Test for Nicotine Dependence; HDRS, Hamilton Depression Rating Scale; MWC, Marijuana Withdrawal Checklist; STAI, State Trait Anxiety Inventory.

Table 6:
Baseline Demographics Data by sex in the AB arm.

	Females (n=7)	Males (n=7)	p value
Demographics			
Age	29.71 (9.34)	34.14 (9.70)	.40
Substance Use Measures			
CUDIT	13.43 (4.54)	13.00 (4.12)	.86
Past week cannabis Use/Day at baseline (grams)	1.38 (1.34)	0.96 (0.94)	.51
Cannabis Initiation Age	15.86 (2.04)	16.86 (2.04)	.38
Years of Regular Cannabis Use	9.93 (9.22)	13.00 (7.21)	.50
AUDIT	4.00 (1.91)	5.43 (2.76)	.28
Baseline Affective Measures			
MWC	6.86 (5.46)	3.71 (3.09)	.21
HDRS	3.14 (4.41)	2.00 (3.16)	.59
STAI-state	29.71 (11.32)	29.71 (5.82)	1.00

Values given in mean(standard deviation). AB, Abstinent. AUDIT, Alcohol Use Disorder Identification Test; CUDIT, Cannabis Use Disorder Identification Test; HDRS, Hamilton Depression Rating Scale; MWC, Marijuana Withdrawal Checklist; STAI, State Trait Anxiety Inventory.

5.1.5 Psychiatric History

All participants met DSM-5 criteria for a current CUD. One participant met DSM-5 criteria for a past alcohol use disorder (AB n=1, NA n=0), three participants met DSM-5 criteria for a past major depressive episode (AB n=3, NA n=0), one participant met DSM-5 criteria for past generalized anxiety disorder (AB n=0, NA n=1), two participants met DSM-5 criteria for past social anxiety (AB n=1, NA n=1), and three participants met DSM-5 criteria for antisocial personality disorder (AB n=2, NA n=1). There were no significant group differences between groups in the number of past psychiatric disorders. DSM-5 diagnoses are summarized in Table 5.

5.1.6 Participant Demographic and Substance Use Patterns by Sex

Table 6 summarizes demographic characteristics of participants assigned to the AB arm parsed by sex. The AB arm included 7 female and 7 male participants. There were no significant differences

in demographic variables between male and female participants in the AB arm (see Table 6). Past week cannabis use in grams/day assessed at baseline did not differ between female and male participants in the AB arm. CUDIT scores did not differ between sex. The CUDIT mean scores were 13.32 (SD = 4.54) and 13.00 (SD = 4.12) for females and males respectively, suggesting both sexes were consuming cannabis at hazardous levels. We also did not observe significant differences in cannabis initiation age and years of regular use.

5.3 Cannabis Withdrawal Symptom Severity

5.3.1 Changes in Withdrawal Symptoms

At baseline, withdrawal symptoms following a minimum of 12hrs of abstinence did not differ between the two groups. A RM-ANOVA revealed a significant effect of time on cannabis withdrawal symptom severity ($F(4, 84)=3.76$, $p=.02$), but no significant time x group effect on cannabis withdrawal symptom severity ($F(4, 84)=2.34$, $p=.09$).

While there was no significant time x group effect, there was a trend towards significance. Withdrawal symptoms in participants in the AB group increased from baseline to day 7, peaked at day 7, and then decreased in severity back to baseline levels by day 28. In the NA arm, withdrawal symptoms remained stable between days 0, 7, and 14, then decreased on days 21 and 28. Withdrawal scores over 28 days are summarized in Table 8 and Figure 5.

5.4 Depressive Symptoms

5.4.1 Changes in Depressive Symptoms

At baseline, depressive symptoms, assessed with the HDRS, did not differ between participants in the AB and NA groups. Scores on the HDRS ranged from 0 to 12, with a mean of 2.57 (SD = 3.74)

and 1.89 (SD = 2.80) for participants in the AB and NA arms, respectively, indicating no levels of depressive symptoms in the week preceding the baseline visit. In the AB arm, 12 participants had scores on the HDRS below 8, indicating no depression, and two participants had scores between 8 and 16, indicating symptoms of mild depression. In the NA arm, 8 participants had HDRS scores below 8, suggesting no depression, while one participant had a score of 8, suggesting symptoms of mild depression in the week preceding their baseline visit.

There were no significant effect of time on depressive symptoms ($F(4, 84)=1.48$, $p=.23$). We also observed no significant time x group effect on depressive symptoms ($F(4, 84)=1.83$, $p=.15$). In the AB arm, the effect size between days 0 and 28 was negative and small ($d=-0.07$). In the NA arm, the effect size between days 0 and 28 was small ($d=0.25$).

While no significant change occurred over time among participants, a pattern emerged among AB participants demonstrating that depressive symptoms increased from baseline to day 7, peaked at 7-days post-abstinence, and then returned to baseline levels by day 28 (see figure 6.1). In the NA arm, depression symptoms remained relatively stable, with a slight decrease in symptom severity on day 21 which then remained stable until day 28. Depressive symptoms over the 28 days of cannabis abstinence are summarized in Table 8.

5.4.2 Sex Differences in Depressive Symptoms in Abstinent Participants

At baseline, depressive symptoms did not differ between male and female participants in the AB group. There was no significant effect of time on depressive symptoms ($F(4, 48)=1.43$, $p=.24$). We also observed no significant effect of time x sex effect on depressive symptoms ($F(4, 48)=0.22$, $p=.93$). In females, the effect size between days 0 and 28 was negative and small ($d=-0.04$). In males, the effect size between days 0 and 28 was negative and small ($d=-0.10$).

From Figure 6.2, we can see that both abstinent male and female participants experienced an increase in depressive symptoms from baseline to day 7, symptoms peaked at day 7 and then. returned to baseline levels by day 28. Notably, female participants experienced a greater increase in depressive symptoms at day 7 compared to males. Depressive symptoms in female and male participants in the AB group over the 28 days cannabis abstinence period are summarized in Table 9 and Figure 6.2.

5.5 Anxiety Symptoms

5.5.1 Changes in Anxiety Symptoms

At baseline, anxiety symptoms (STAI-state) did not differ between participants in the AB and NA arms. Scores on the STAI-state ranged from 20 to 53, 29.71 (SD = 8.65) and 28.11 (SD = 8.04) for participants in the AB and NA arms, respectively, indicating no to low levels of state anxiety at baseline. In the AB arm, 12 participants had scores on the STAI-state between 20 and 37 indicating no to low levels of anxiety, one participant had a score of 38 indicating symptoms of moderate anxiety at the time of the survey, and one participant had a score of 53, indicating high anxiety at the time of the survey. In the NA arm, 7 participants had STAI-state scores between 20 and 37 indicating no to low levels of anxiety, two participants had a score of between 38 and 44 indicating symptoms of moderate anxiety at the time of the survey.

There was no significant effect of time on anxiety symptoms ($F(4, 84)=1.90$, $p=.16$). We also observed no significant effect of time x group on anxiety symptoms ($F(4, 84)=.79$, $p=.47$). In the AB arm, the effect size between days 0 and 28 was small to moderate ($d=0.41$). In the NA arm, the effect size between days 0 and 28 was small ($d=0.14$).

From Figure 7, we can see that in the AB arm, anxiety symptoms were stable up until day 7 before

decreasing below baseline levels and remining stable until days 28. Participants in the NA group demonstrated that anxiety symptoms remained stable from day 0 through day 14 and slightly decreased on day 21 before stabilizing again on day 28, below baseline levels. Anxiety symptoms over the 28 days of cannabis abstinence are summarized in Table 8 and Figure 7.1.

5.5.2 Sex Differences in Anxiety Symptoms in Abstinent Participants

At baseline, anxiety symptoms did not differ between male and female participants in the AB group. There was no significant effect of time on anxiety symptoms ($F(4, 48)=2.16$, $p=.15$). We also observed no significant effect of time x sex on anxiety symptoms ($F(4, 48=.46$, $p=.60$). In females, the effect size between days 0 and 28 was moderate ($d=0.51$). In males, the effect size between days 0 and 28 was small ($d=0.26$).

According to Figure 7.2, both male and female participants experienced a similar trajectory with respect to changes in anxiety symptoms over time. In males, anxiety symptoms increased at day 7, then decreased below baseline levels on days 14 and 21, before increasing on day 28. In females, anxiety symptoms increased at day 7, then decreased below baseline levels on day 14, then increased on day 21, before decreasing again on day 28. Anxiety symptoms over the 28 days of cannabis abstinence are summarized in Table 9 and Figure 7.2.

Table 7:
Changes in Cannabis Use During 28 Days of Cannabis Abstinence.

	Day 0	Day 7	Day 14	Day 21	Day 28
Past Week Cannabis Use in Grams					
AB (n=14)	1.17 (1.13)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
NA (n=9)	1.79 (1.23)	1.27 (0.90)	1.04 (0.92)	1.23 (0.82)	0.91 (0.70)

Values given in mean(std). AB, Abstinent; NA, As Usual. Past week cannabis use in grams was measured with the Timeline Follow back.

Table 8:
Changes in Withdrawal and Affective Symptoms During 28 Days of Cannabis Abstinence.

	Day 0	Day 7	Day 14	Day 21	Day 28
Withdrawal					
AB (n=14)	5.29 (4.56)	9.28 (6.04)	5.71 (3.27)	5.43 (4.94)	4.50 (3.23)
NA (n=9)	6.33 (4.47)	5.22 (3.27)	6.22 (4.27)	3.22 (2.44)	3.57 (4.06)
Depression					
AB (n=14)	2.57 (3.74)	4.71 (3.40)	3.07 (2.56)	3.43 (3.30)	2.79 (2.91)
NA (n=9)	1.89 (2.80)	1.78 (2.05)	3.33 (4.27)	0.67 (0.87)	1.33 (1.58)
State Anxiety					
AB (n=14)	29.71 (8.65)	31.07 (9.58)	26.86 (6.24)	27.36 (7.11)	26.50 (7.11)
NA (n=9)	28.11 (8.04)	28.78 (8.07)	28.89 (11.46)	27.22 (6.48)	26.78 (10.99)

Values given in mean(std). AB, Abstinent; NA, As Usual. Withdrawal was measured with the marijuana withdrawal checklist. Depression was measured with the Hamilton Depression Rating Scale. State anxiety was measured with the State Trait Anxiety Inventory.

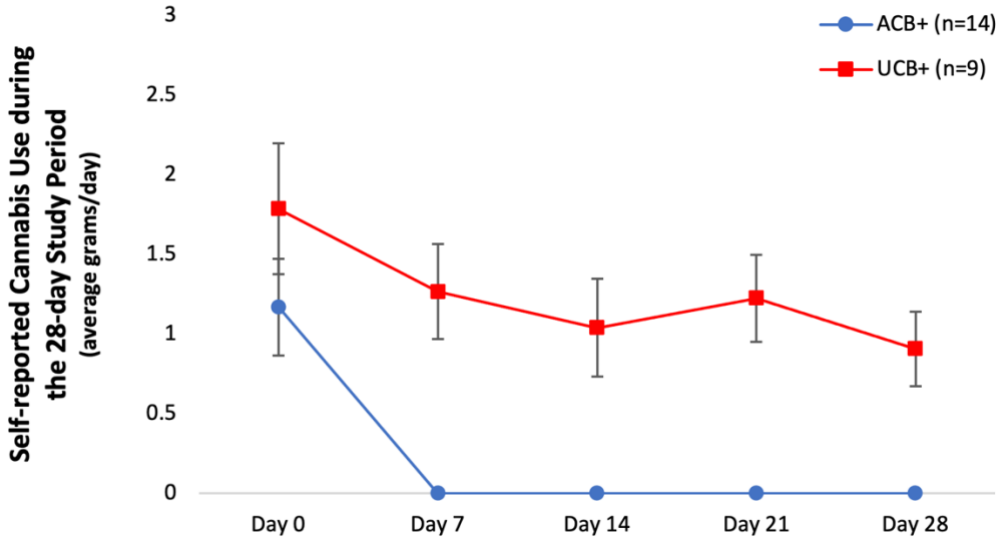
Table 9:
Sex Differences in Changes in Affective Symptoms During 28 Days of Cannabis Abstinence.

	Day 0	Day 7	Day 14	Day 21	Day 28
Depression					
Females (n=7)	3.14 (4.41)	6.00 (3.27)	3.57 (2.37)	4.14 (4.06)	3.29 (3.04)
Males (n=7)	2.00 (3.16)	3.43 (3.26)	2.57 (2.82)	2.71 (2.43)	2.29 (2.93)
State Anxiety					
Females (n=7)	29.71 (11.32)	31.29 (10.69)	26.00 (5.66)	28.57 (6.21)	25.43 (3.21)
Males (n=7)	29.71 (5.82)	30.86 (9.19)	27.71 (7.11)	26.14 (8.21)	27.57 (9.83)

Values given in mean(std). Depression was measured with the Hamilton Depression Rating Scale. State anxiety was measured with the State Trait Anxiety Inventory.

Figure 4

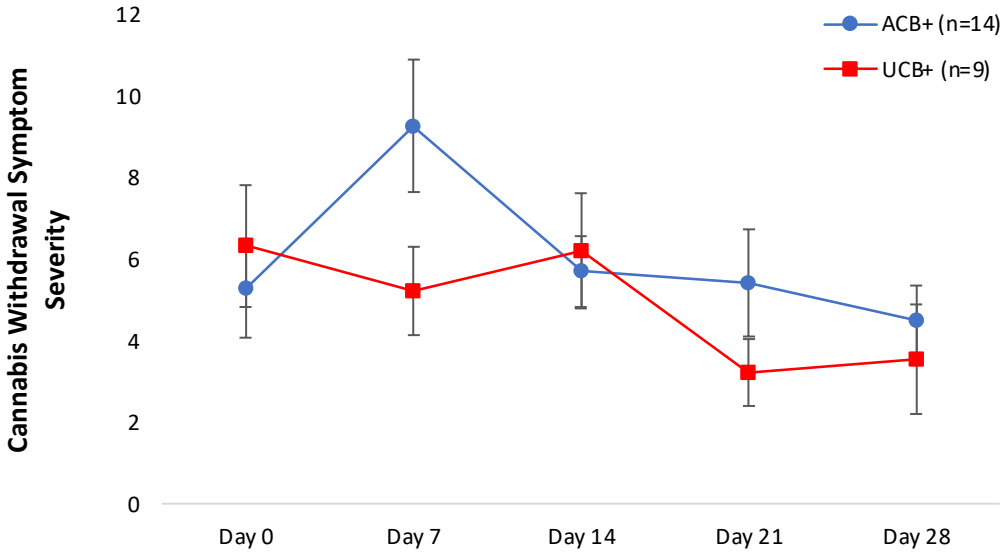
Cannabis Use During the 28-day study period.



Note. Values represent means and error bars represent standard error of the mean. Measured with the Timeline Follow Back. There were no significant effect of time on past week grams of cannabis used (F(4, 32)=2.79, p=.10).

Figure 5

Withdrawal Symptom Severity.

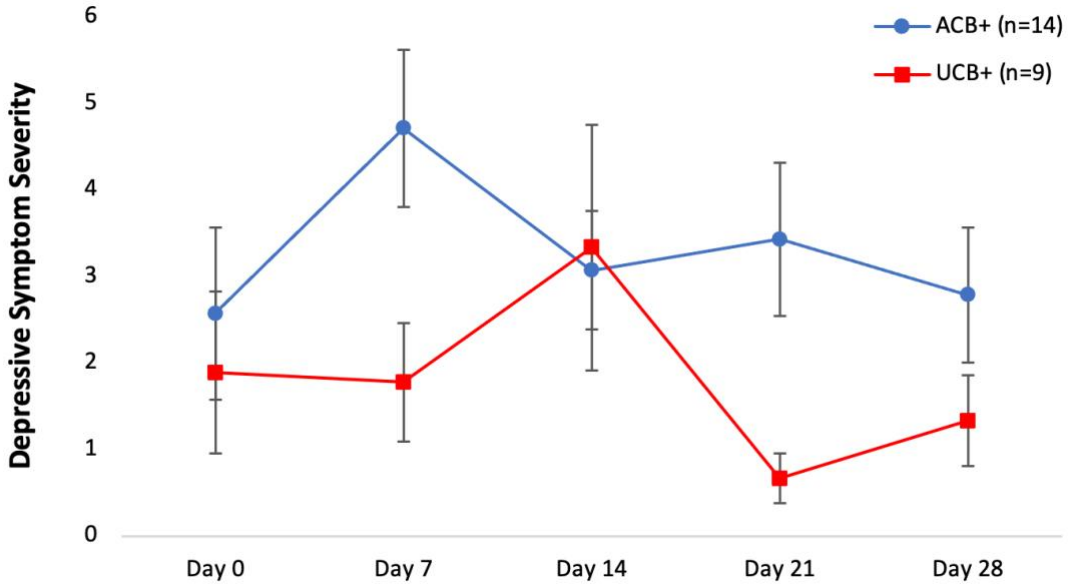


Note. Values represent means and error bars represent standard error of the mean. Measured with the Marijuana Withdrawal Checklist. There were no significant effect of time and arm on cannabis withdrawal symptom severity (F(4, 84)=2.34, p=.09).

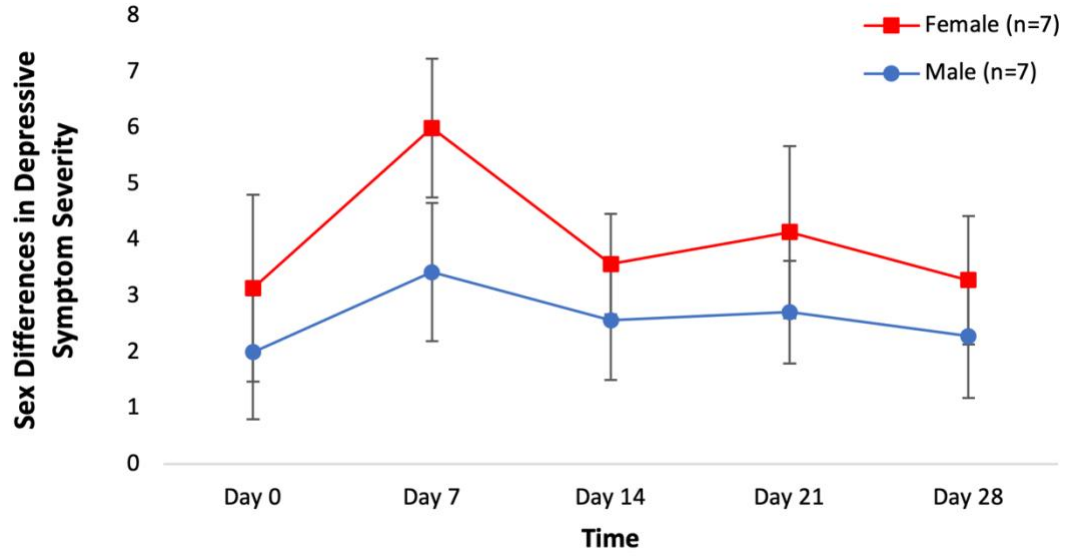
Figure 6

6.1. Depressive Symptom Severity. 6.2. Sex Differences in Depressive Symptoms.

6.1



6.2

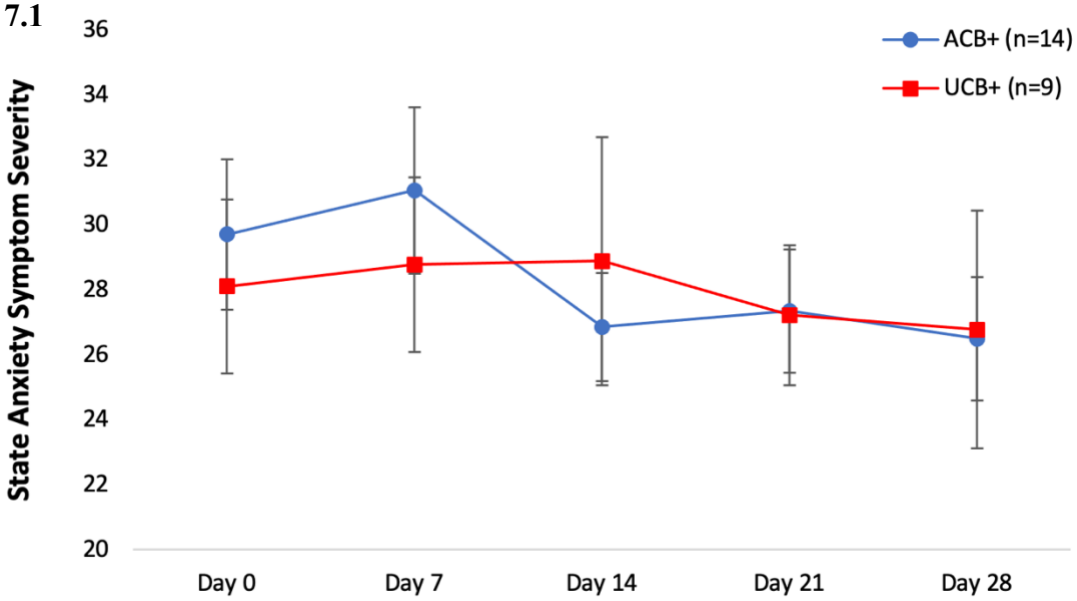


Notes. Values represent means and error bars represent standard error of the mean. Measured with the Marijuana Withdrawal Checklist. There were no significant effects of time and arm on depressive symptoms in the full sample ($F(4, 84)=1.83$, $p=.15$). There were no significant effect of time and sex on depressive symptoms in the AB arm ($F(4, 36)=0.22$, $p=.93$).

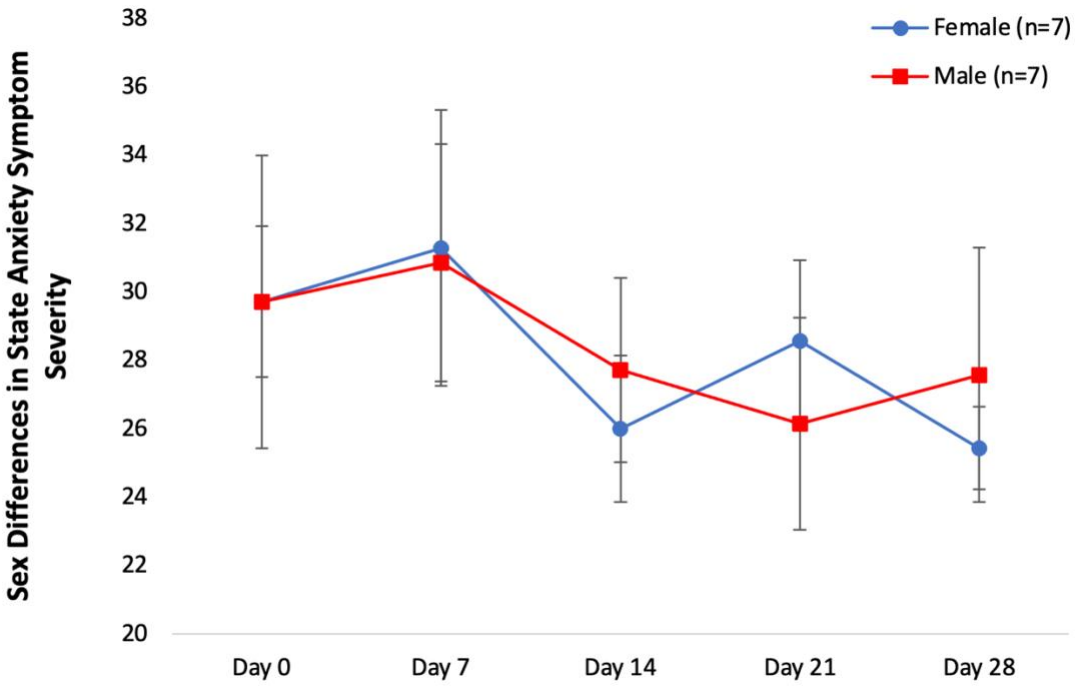
Figure 7

7.1. State Anxiety Symptom Severity. 7.2. Sex Differences in State Anxiety Symptoms.

7.1



7.2



Notes. Values represent means and error bars represent standard error of the mean. Measured with the State Trait Anxiety Inventory. There were no significant effects of time and arm on anxiety symptoms in the full sample (F(4, 84)=.79, p=.47). There were no significant effect of time and sex on anxiety symptoms in the AB arm (F(4, 48=.46, p=.60).

6. DISCUSSION

6.1 Overview

To our knowledge, this pilot study is the first to examine the effects of 28 days of cannabis abstinence on affective symptoms in adults with CUD and no co-occurring psychiatric or medical comorbidities using a non-abstinent cannabis control group. Previous research demonstrated improvements in affective symptoms following 16 to 45 days of cannabis abstinence in people with cannabis use (Bonnet et al., 2015; Budney et al., 2003; Feinstein et al., 2021; Galang et al., 2015; Jacobus et al., 2017; Kouri & Pope, 2000; Lee et al., 2014; Lucatch et al., 2020; Rabin et al., 2018a). However, findings from these studies were confounded with the presence of co-occurring psychiatric/medical comorbidities (Feinstein et al., 2021; Galang et al., 2015; Jacobus et al., 2017; Lucatch et al., 2020; Milin et al., 2008; Rabin et al., 2018a). Studies that included participants without comorbidities were conducted in adolescents (Cooke et al., 2021; Jacobus et al., 2017; Milin et al., 2008; Sullivan et al., 2022) or failed to include an appropriate control group. (Bonnet et al., 2015; Budney et al., 2003; Galang et al., 2015; Jacobus et al., 2017; Lee et al., 2014; Lucatch et al., 2020; Milin et al., 2008; Sullivan et al., 2022). Therefore, the primary aim of the current study was to investigate the effects of cannabis abstinence on affective symptoms in adults with CUD and no other co-occurring disorders. Furthermore, the abovementioned studies cited did not explore the impact of sex on their results, despite that females may be more sensitive to the clinical effects of THC (Blanton et al., 2021). Therefore, as an exploratory aim, we investigated potential sex differences in affective symptoms during cannabis abstinence.

According to our power analysis, we required 15 participants in each arm to complete the study. However, there was a high level of screen failures, so study enrolment occurred at a slower rate

than predicted. Despite the low self-reported relapse rate in the abstinent group (we estimated a 30% relapse rate, but observed a 14% relapse rate), only 14 participants in the abstinent arm and 9 participants in the as-usual arm completed the study. Given that our study was underpowered, we interpreted emerging patterns from graphs. In this respect, we found that, in our sample of adults with CUD and no co-occurring disorders, depressive symptoms changed over time in the abstinent group, relative to the as-usual control group. Interestingly, the general pattern observed in the data indicated a (non-significant) change in depressive symptoms during abstinence exhibited the same trajectory as the expected change in cannabis withdrawal symptoms during 28 days of cannabis abstinence (i.e. peaking after 7 days of cannabis abstinence then subsiding to baseline levels at day 28). In contrast, the trajectory of anxiety symptoms during the 28-day study period did not differ between participants in the abstinent and as-usual groups; both groups showed decreases in anxiety symptoms over the 28-day period. Lastly, the general pattern observed in the data with respect to sex differences revealed changes in depressive scores, but not anxiety. More specifically, our preliminary data suggests that, relative to their males, females experience elevated depressive symptoms across 28 days of cannabis abstinence with the most pronounced difference at day 7.

6.2 Efficacy of the Cannabis Abstinence Paradigm

Overall, we recruited 25 participants (abstinent, n=16, as-usual, n=9). In the abstinent group 88% (n=14) of participants achieved sustained cannabis abstinence, as confirmed by self-report. It is important to note, however, that we did not objectively assess abstinence using biochemical methods. As a result, it is possible that some participants did not self-report cannabis use (i.e., cannabis lapse/relapse).

Notably, the pattern of cannabis withdrawal symptoms observed in the abstinent group, relative to the as-usual group, provides some validation that cannabis abstinence was initiated and sustained throughout the 28-day period. In the abstinent group, withdrawal symptom severity followed the expected trajectory (Connor et al., 2022), increasing from baseline to day 7, peaking at day 7, decreasing in severity until day 28 to return to baseline levels. In the as-usual group, withdrawal symptoms remained relatively stable over the first two weeks of abstinence, then showed a decrease in the last two weeks. However, the emergence of cannabis withdrawal symptoms is also possible with reductions of cannabis use. Therefore, given the subjective assessment of cannabis abstinence, we cannot be certain that cannabis abstinence was sustained throughout the 28-day abstinence period in the abstinent group. Future studies should employ quantitative urine analyses to determine if a relapse episode occurred during the 28-day abstinence period.

6.3 Changes in Affective Symptoms

6.3.1 Depressive Symptoms

While we did not observe a statistically significant change in depressive symptoms during cannabis abstinence, a clear pattern in the abstinent group emerged. The severity of depressive symptoms increased from baseline to day 7, peaked at day 7, and then returned to baseline levels by day 28. The trajectory of depressive symptoms in the abstinent group paralleled that of the trajectory of cannabis withdrawal symptoms observed in this study. Thus, the observed trajectory in depressive symptoms in the abstinent group may reflect cannabis withdrawal. This is expected, as depression is a common symptom of cannabis withdrawal (Connor et al., 2022). Given that the severity level in depressive symptoms did not differ between day 0 and day 28, suggests that cannabis abstinence was not associated with overall improvements in depression.

While our pilot findings are not in line with our hypothesis that depressive symptoms would improve with 28 days of cannabis abstinence, results do align with research conducted in adult samples without psychiatric or medical comorbidities. Lee et al. (2014), Kouri and Pope (2000), and Budney et al. (2003) found no overall improvement in symptoms of depression during their cannabis abstinence period. Similarly, Rabin et al. (2018a) found that depressive symptoms did not improve in their non-psychiatric control group. Our findings further support that symptoms of depression do not improve following cannabis abstinence in non-psychiatric samples. Contrasting these findings, participants in the study by Bonnet et al. (2015) did show improvement in depressive symptoms. While their sample was comprised of adults with no psychiatric or medical comorbidities, participant were inpatients in treatment for cannabis use with the majority on medication for withdrawal symptoms which may have improved their depressive symptoms (Ahmed et al., 2019). We posit that the lack of improvement in depressive symptoms (i.e., days 28 symptom severity was not below baseline levels) after cannabis abstinence may be due to (1) the low severity of depressive symptoms at baseline or (2) the low amount of cannabis used on average by the participants in our sample.

First, it is possible that baseline depressive symptoms were too low (i.e., a floor effect) to detect a decrease following cannabis abstinence. Our study employed a sample with a CUD with no co-occurring psychiatric/medical disorders, and we assessed sub-clinical levels of depressive symptoms. Participants at baseline had, on average, minimal if any depressive symptoms. Studies that employed adolescent and adult samples with co-occurring psychiatric disorders and medical illnesses with cognitive impairments reported improvements in depressive symptoms following 28 days of cannabis abstinence (Feinstein et al., 2021; Jacobus et al., 2017; Lucatch et al., 2020; Rabin et al., 2018a). Notably, these studies had higher severity levels of depressive symptoms at baseline.

For example, Lucatch et al. (2020) examined adults with comorbid CUD and major depressive disorder and found that participants experienced significant improvements in depressive symptoms following 28 days of cannabis abstinence. Importantly, participants in their study had a mean depression severity score of 17.21 on the Hamilton Depression Rating Scale, indicative of moderate depression. On the other hand, participants in our current study had a mean depression severity score of 2.57 on the Hamilton Depression Rating Scale, for abstinent participants, indicative of no to low depression. Thus, depressive symptoms may need be at moderate to severe levels or part of a clinical diagnosis to see improvements following sustained cannabis abstinence. While not all the above-mentioned studies employed a sample with a diagnosis of depression, the psychiatric samples employed may have been more vulnerable to experiencing symptoms of depression than a non-psychiatric sample. For example, in the study by Rabin et al. (2018a), people with schizophrenia had symptoms of depression that were higher than the sample employed in the current study, with mean depression severity scores of 4.3 on the Hamilton Depression Rating Scale for abstinent participants (scores above 8 indicate mild depression). Similarly, people with multiple sclerosis with cognitive impairments also had higher levels of depressive symptoms relative to those in the current sample, with mean depression severity scores of 6.75 and 7.16 on the Hospital Anxiety and Depression Scale (depression subscale), where scores above 8 indicate considerable levels of depression (Feinstein et al., 2021). Lastly, in their sample of adults who were inpatients in treatment for cannabis use, Bonnet et al. (2015) found significant improvements in depression, where mean depression severity scores were 8.3 on the Hamilton Depression Rating Scale, indicative of mild depression. Thus, elevated levels of depressive symptoms at baseline may be needed for improvements to occur with 28 days of cannabis abstinence. Additionally, people with psychiatric and medical comorbidities may be more likely to experience changes in

depressive symptoms as they may be more vulnerable to the clinical effects of THC. Taken together, this evidence supports that significant improvements in symptoms of depression may be more likely to be observed in people with psychiatric/medical comorbidities compared to those with no psychiatric/medical comorbidities.

Second, our participants may not have used cannabis in amounts that were significant enough to elicit a notable change in their depressive symptoms following cannabis abstinence. In their 16-day abstinence paradigm, Bonnet et al. (2015) found significant improvements in depression in their sample of adults who were inpatient in treatment for cannabis use. The sample studied by Bonnet et al. (2015) used cannabis in larger amounts than the participants in our current study; while their participants used on average 2.4g of cannabis per day, our sample used on average 1.17g of cannabis per day in our abstinent group. Therefore, cannabis may need to be consumed in larger amounts for improvements in depressive symptoms to occur with 28 days of cannabis abstinence in adults without co-occurring psychiatric or medical disorders.

Overall, our findings are in line with previous literature conducted in adult samples without psychiatric or medical comorbidities (Budney et al., 2003; Kouri & Pope, 2000; Lee et al., 2014; Rabin et al., 2018a). We hypothesize that the low levels of depression at baseline created a floor effect from which depression levels could not have substantially improved. Furthermore, we posit that our sample consumed less cannabis, on average, than samples where significant improvements in depression were observed, which may have precluded changes in depressive symptoms following cannabis abstinence.

6.3.1 Anxiety Symptoms

In our pilot study, we found that the trajectory of anxiety severity did not differ between the abstinent and as usual groups during the 28-day study period. Notably, we observed a decline in anxiety symptoms in both groups. This suggest that 28 days of cannabis abstinence may not influence anxiety symptom severity in adults with CUD and no co-occurring disorders, which did not support our hypothesis.

Our pilot study findings align with research conducted by Feinstein et al. (2021), Jacobus et al. (2017), Kouri and Pope (2000), Lucatch et al. (2020), and Sullivan et al. (2022), which found no change in symptoms of anxiety during cannabis abstinence. Consistent with our findings, Sullivan et al. (2022) found decreases in anxiety in both the cannabis abstinent group and control group. Overall, our findings support that anxiety symptoms do not improve following 28 days of cannabis abstinence in adults with CUD and no co-occurring disorders.

Cannabis abstinence did not exert a change in anxiety symptoms in our sample of adults with CUD and no co-occurring disorders for two reasons: (1) the low amount of cannabis used on average by the participants in our sample, or (2) the lack of control group used in previous studies to control for confounding factors.

For one, it is possible that only in participants with very high levels of cannabis consumption would changes in anxiety symptoms be seen with 28 days of cannabis abstinence. Thus, participants in the current study may not have been using enough cannabis to elicit improvements in symptoms of anxiety following 28 days of cannabis abstinence. This is supported by findings from Bonnet et al. (2015), who reported significant improvements in anxiety in adult samples who used cannabis in higher amounts than the participants in the present study. Namely, participants in the current study used 1.17g of cannabis per day in our abstinent group. Conversely, participants

in Bonnet et al. (2015) used 2.4g of cannabis per day. While Budney et al. (2003), Galang et al. (2015), Lee et al. (2014), and Milin et al. (2008) also found significant improvements in anxiety, their reported data on the amount of cannabis used by their participants is limited to frequency (e.g. daily or almost daily). Although our participants all used cannabis daily or almost daily, differences in the amount of cannabis used per day are important considerations to make; we are therefore limited when comparing our participants' cannabis use amounts to their sample. Thus, improvements in anxiety symptoms may be more likely to occur in people with heavy cannabis use, such as over 2g of cannabis per day.

Furthermore, it is possible that the improvements observed in anxiety in previous studies were due to limitations in study methodology such as controlling for the effects of study procedures on outcomes (e.g., time spent with researchers), and not due to cannabis abstinence. We found that symptoms of anxiety improved in both our abstinent group and our non-abstinent control group, suggesting that factors unrelated to cannabis abstinence are affecting symptom severity, similar to Sullivan et al (2021). These results highlight the importance of employing an appropriate control group in the study. Thus, improvements in anxiety symptoms observed in previous work may have been interpreted incorrectly given that they did not include a control group in their paradigm (Bonnet et al., 2015; Galang et al., 2015; Lee et al., 2014; Milin et al., 2008). Another study (Budney et al., 2003) included a control group of adults who had not used cannabis in the past year, which may not be a representative well-matched group. Without an appropriate control group, we cannot definitively conclude that changes in affective scores were a result of cannabis abstinence. Taken together, the significant improvements in anxiety reported in previous studies may have been influenced by confounding factors.

Overall, our findings are in line with previous literature which showed no improvements in anxiety

symptoms following cannabis abstinence (Feinstein et al., 2021; Jacobus et al., 2017; Kouri & Pope, 2000; Lucatch et al., 2020; Sullivan et al., 2022). We posit that our sample used less cannabis, on average, than samples where significant improvements in anxiety were observed, which could have minimized changes in anxiety severity. It is possible that studies that found significant improvements in anxiety following cannabis abstinence may have been influenced by confounding factors, given that these studies did not include an adequate control group (Bonnet et al., 2015; Budney et al., 2003; Galang et al., 2015; Lee et al., 2014; Milin et al., 2008).

6.3.3 Sex Differences in Affective Symptoms

Our preliminary findings partially support our exploratory hypothesis given that changes in depression with cannabis abstinence may be sex dependent, but not changes in anxiety. We observed a similar trajectory in depressive symptoms in both male and female participants during cannabis abstinence. More specifically, males and females experienced an elevation in depressive symptoms from baseline to day 7, which then decreased and returned to baseline levels at day 14 and remained at this until day 28. Statistical analyses revealed no significant effect of sex on affective symptoms. Yet we observed a general pattern in the data indicating that females may experience a greater increase in depressive symptoms from baseline to day 7 than males, with the greatest difference in symptom severity during 28 days of cannabis abstinence occurring at day 7.

Depression is a common symptom of cannabis withdrawal (Connor et al., 2022). Peak cannabis withdrawal symptom severity is observed at 7 days post-abstinence (Connor et al., 2022). Thus, given that depression peaks in both abstinent males and females at day 7 suggests that increases in depression may reflect cannabis withdrawal. Previous studies report that females experience more severe cannabis withdrawal symptoms than their male counterparts (Herrmann et al., 2015; Levin et al., 2010). This further supports that the greater increase in depressive symptoms at day 7 in

females, relative to males, is an effect of cannabis withdrawal.

6.3.4 Clinical Significance

Our findings hold important clinical significance. While people commonly use cannabis to self-medicate symptoms of depression and anxiety (Canada, 2024a; Wallis et al., 2022), our findings indicate that cannabis use does not improve depressive and anxiety symptoms (Mammen et al., 2018; Stanciu et al., 2021; Turna et al., 2017). If cannabis use was beneficial to remedy affective symptoms, we would expect increases in affective symptoms that would remain elevated during cannabis abstinence (Cooke et al., 2021). However, we found that affective symptoms did not remain elevated, which provides support that cannabis is not beneficial to affective symptoms.

Our finding that female participants experienced greater increases in symptoms of depression in their first week of cannabis abstinence also hold important clinical significance. Namely, given that elevated cannabis withdrawal symptoms, such as depression, predict relapse (Allsop et al., 2012; Bonnet et al., 2014; Levin et al., 2010) suggests that females may be at an increased risk of cannabis relapse during their first week of quitting cannabis compared to males.

6.4 Study Strengths

Our study has many notable strengths. Our sample was comprised of adults with a CUD and no co-occurring comorbidities. This is a relevant population to study given that most adults with CUD do not have any co-occurring disorders (Onaemo et al., 2021). Furthermore, this population is important to investigate given that young adults (20 to 24 years old) have the highest rates of cannabis use compared to all other age groups. In addition, aging adults (45 years old and older) have had the greatest increase in cannabis use rates since cannabis legalization (Canada, 2024a; Statistics Canada, 2023). Importantly, our sample was diverse with respect to race, age, years of

education, and occupational status. This allows us to generalize our results to a broad population.

The naturalistic approach to our paradigm was also a significant strength to the study. Namely, we studied non-treatment seeking adults with CUD in an outpatient setting. This study design allows participants to experience abstinence in their natural environment. For example, they may encounter drug-related cues that may trigger cravings, similar to if they quit cannabis on their own, providing our study with ecological validity.

The prospective within and between subject design was also a significant strength of this study. Our designed allowed us to temporally assess affective symptoms weekly over a period of 28 days to observe symptom trajectories. Furthermore, not all studies that investigated the effects of cannabis abstinence on affective symptoms assessed affective symptoms on a weekly basis (Feinstein et al., 2021; Kouri & Pope, 2000). Conducting weekly assessments of affective symptoms enabled us to determine the trajectory of affective symptoms throughout abstinence. This was important in the context of our sex analyses, where we found that females experienced the same trajectory of depressive symptoms as males (i.e. peak in symptoms on day 7 followed by a return to baseline levels on days 14, 21, and 28), but experienced a greater increase in depressive symptoms from baseline to day 7. Thus, closely monitoring symptoms on a weekly basis is critical.

Lastly, we controlled for several confounding variables that may affect outcomes. Randomizing participants to an "as-usual" arm, where participants continued to use cannabis as usual for the 28-day study period allowed us to control for the effects of study procedures (e.g., time with researchers) on affective outcomes. Notably, the abstinent and as-usual groups were comparable on all demographic and clinical variables which ensured that any group differences that emerged could be attributable to the effects of cannabis abstinence. Similarly, in the abstinent group all

demographic and clinical variables were comparable between males and females allowing us to attribute any difference that emerged to sex effects associated with cannabis abstinence.

6.5 Study Limitations

This study has limitations in its design that are important to discuss. First, cannabis abstinence status was determined using self-report. Biochemical verification of cannabis abstinence is the gold standard for confirming sustained cannabis abstinence. However, for cannabis use this is complex. This is because with heavy cannabis use, THC can remain in the body for several weeks following cessation due to its absorption into fatty tissue and its slow rerelease back into the blood (Goodwin et al., 2008; Lowe et al., 2009; Rabin et al., 2018b). Determining sustained cannabis abstinence for a 28 day period requires frequent collection (e.g. twice weekly) of urine samples and the normalization of THC-COOH concentrations to creatinine to account for varying levels of hydration over time (Breindahl et al., 2021). Once these values are obtained, they can be inputted into specific mathematical models (Schwilke et al., 2011) to confirm sustained abstinence with a 99% degree of certainty. Therefore, we could not objectively verify if participants in the abstinent group used cannabis during the abstinence period. Notably, studies that use contingency management to encourage abstinence demonstrate a low agreement between self-reported cannabis abstinence and biochemically verified cannabis abstinence, because participants may have a higher incentive to self-report abstinence even after relapse (Baker et al., 2018). Therefore, our higher-than-expected abstinence rate of 88% (versus 70%) (Rabin et al., 2018b), may reflect that some participants may not have been accurate in their self-reported cannabis use. However, given that the trajectory of cannabis withdrawal symptoms of participants in the abstinent group was in line with the expected trajectory observed with 28 days of abstinence (Connor et al., 2022),

provides empirical evidence that participants did indeed maintain cannabis abstinence or at least substantially reduced their cannabis use during the 28-day period.

Despite our low relapse rates (we observed a relapse rate of 14%, but expected 30%), we did not meet our power requirements; this may have led to false negative findings. Of note, the majority of the papers included in our power analysis studied samples with psychiatric and medical comorbidities (Feinstein et al., 2021; Galang et al., 2015; Jacobus et al., 2017; Milin et al., 2008; Rabin et al., 2018a). As previously discussed, people with psychiatric/medical experienced greater levels of affective symptoms at baseline, which may contribute to a greater magnitude of change during cannabis abstinence. Conceivably, the magnitude of change following cannabis abstinence in non-psychiatric individuals may not be as large, and thus future studies should power the study using a smaller effect size (e.g. Cohen's f=-0.04). Furthermore, the studies that we included in our power analysis that studied adults without psychiatric and medical comorbidities studied samples with higher levels of cannabis use (i.e. 2.4g of cannabis per day, Bonnet et al., 2015) than the ones in our sample (i.e. 2.4g of cannabis per day 1.17g). As we previously discussed, changes in affective symptoms may be more pronounced in individuals with heavier cannabis use than the level of cannabis use in our sample. Thus, it is possible that employing a larger sample would enable the detection of more subtle effects of cannabis abstinence on affective symptoms that may be present in non-psychiatric individuals with CUD.

6.6 Conclusions and Future Directions

We investigated the effects of cannabis abstinence on depressive and anxiety symptoms in adults with CUD and no co-occurring psychiatric or medical disorders. We also aimed to investigate if sex moderated the effect of cannabis abstinence on affective symptoms. Overall, our findings

suggest that while depressive symptoms follow the classic cannabis withdrawal trajectory, both anxiety and depressive symptoms do not improve after 28 days of cannabis abstinence in adults with CUD and no co-occurring disorders. Additionally, we identified sex differences in changes in depression in abstinent participants such that female participants experienced a greater increase in depressive symptoms from baseline to day 7, relative to males.

Importantly, our finding indicate that affective symptoms do not get worse after 28 days of cannabis abstinence which provides evidence that cannabis use does not benefit or improve affective symptoms. If cannabis use did benefit affective symptoms, we would have observed increases in depression and anxiety that persisted throughout the 28-day period of cannabis abstinence (Cooke et al., 2021). Furthermore, our sex analyses suggest that females may experience elevated symptoms of depression after 7 days of cannabis abstinence, compared to males which may increase their risk of a relapse during the first week of a cannabis quit attempt.

Given our study limitations and the clinical implications of our findings, it is imperative that more research on this topic be conducted. First, it is important that this study is replicated in a larger sample. This would provide more robust insights into our findings. Second, future studies should utilize biochemical verification of cannabis abstinence to accurately determine the abstinence status of each participant. Lastly, future studies should extend their period of abstinence beyond 28 days to understand the effects of cannabis abstinence on a lengthier trajectory. Extending the abstinence period would be particularly useful to determine if depressive symptoms remain at baseline levels beyond 28 days of cannabis abstinence, or if they improve past a certain time.

A better understanding of the relationship between cannabis abstinence and affective symptoms is crucial given that rates of cannabis use in Canadian adults are rising. Namely, findings from our

study support that cannabis use does not benefit affective symptoms, given that depressive and anxiety symptoms did not persistently increase following 28 days of cannabis abstinence. Our study findings also further our understanding of sex differences in affective symptoms during cannabis abstinence. Findings from this study can be used to advise the public of the potential harms of using cannabis given that with the recent legalization of cannabis in Canada, it is imperative that Canadians be well informed of how their cannabis consumption may affect their mental health.

7. REFERENCES

Adamson, S. J., Kay-Lambkin, F. J., Baker, A. L., Lewin, T. J., Thornton, L., Kelly, B. J., & Sellman, J. D. (2010). An improved brief measure of cannabis misuse: the Cannabis Use Disorders Identification Test-Revised (CUDIT-R). *Drug Alcohol Depend*, *110*(1-2), 137-143. https://doi.org/10.1016/j.drugalcdep.2010.02.017

Agrawal, A., Pergadia, M. L., & Lynskey, M. T. (2008). Is there evidence for symptoms of cannabis withdrawal in the national epidemiologic survey of alcohol and related conditions? *Am J Addict*, *17*(3), 199-208. https://doi.org/10.1080/10550490802019519

Ahmed, S., Bachu, R., Kotapati, P., Adnan, M., Ahmed, R., Farooq, U., Saeed, H., Khan, A. M., Zubair, A., Qamar, I., & Begum, G. (2019). Use of Gabapentin in the Treatment of Substance Use and Psychiatric Disorders: A Systematic Review. *Front Psychiatry*, *10*, 228. https://doi.org/10.3389/fpsyt.2019.00228

Aizpurua-Olaizola, O., Elezgarai, I., Rico-Barrio, I., Zarandona, I., Etxebarria, N., & Usobiaga, A. (2017). Targeting the endocannabinoid system: future therapeutic strategies. *Drug Discov Today*, *22*(1), 105-110. https://doi.org/10.1016/j.drudis.2016.08.005

Alcohol, G. B. D., & Drug Use, C. (2018). The global burden of disease attributable to alcohol and drug use in 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Psychiatry*, *5*(12), 987-1012. https://doi.org/10.1016/S2215-0366(18)30337-7

Allsop, D. J., Copeland, J., Norberg, M. M., Fu, S., Molnar, A., Lewis, J., & Budney, A. J. (2012). Quantifying the clinical significance of cannabis withdrawal. *PLoS One*, *7*(9), e44864. https://doi.org/10.1371/journal.pone.0044864

American Psychiatric Association (Ed.). (2013). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.).

Arendt, M., Rosenberg, R., Foldager, L., Sher, L., & Munk-Jorgensen, P. (2007). Withdrawal symptoms do not predict relapse among subjects treated for cannabis dependence. *Am J Addict*, *16*(6), 461-467. https://doi.org/10.1080/10550490701640985

Arterberry, B. J., Treloar Padovano, H., Foster, K. T., Zucker, R. A., & Hicks, B. M. (2019). Higher average potency across the United States is associated with progression to first cannabis use disorder symptom. *Drug Alcohol Depend*, *195*, 186-192. https://doi.org/10.1016/j.drugalcdep.2018.11.012

Baggelaar, M. P., Maccarrone, M., & van der Stelt, M. (2018). 2-Arachidonoylglycerol: A signaling lipid with manifold actions in the brain. *Prog Lipid Res*, *71*, 1-17. https://doi.org/10.1016/j.plipres.2018.05.002

Bahji, A., Stephenson, C., Tyo, R., Hawken, E. R., & Seitz, D. P. (2020). Prevalence of Cannabis Withdrawal Symptoms Among People With Regular or Dependent Use of Cannabinoids: A Systematic Review and Meta-analysis. *JAMA Netw Open*, *3*(4), e202370. https://doi.org/10.1001/jamanetworkopen.2020.2370

Baker, N. L., Gray, K. M., Sherman, B. J., Morella, K., Sahlem, G. L., Wagner, A. M., & McRae-Clark, A. L. (2018). Biological correlates of self-reported new and continued abstinence in cannabis cessation treatment clinical trials. *Drug Alcohol Depend*, *187*, 270-277. https://doi.org/10.1016/j.drugalcdep.2018.03.017

Battista, N., Di Tommaso, M., Bari, M., & Maccarrone, M. (2012). The endocannabinoid system: an overview. *Front Behav Neurosci*, *6*, 9. https://doi.org/10.3389/fnbeh.2012.00009

Blanton, H. L., Barnes, R. C., McHann, M. C., Bilbrey, J. A., Wilkerson, J. L., & Guindon, J. (2021). Sex differences and the endocannabinoid system in pain. *Pharmacol Biochem Behav*, *202*, 173107. https://doi.org/10.1016/j.pbb.2021.173107

Bolanis, D., Orri, M., Castellanos-Ryan, N., Renaud, J., Montreuil, T., Boivin, M., Vitaro, F., Tremblay, R. E., Turecki, G., Cote, S. M., Seguin, J. R., & Geoffroy, M. C. (2020). Cannabis use, depression and suicidal ideation in adolescence: direction of associations in a population based cohort. *J Affect Disord*, *274*, 1076-1083. https://doi.org/10.1016/j.jad.2020.05.136

Bonnet, U., Borda, T., Scherbaum, N., & Specka, M. (2015). Abstinence phenomena of chronic cannabis-addicts prospectively monitored during controlled inpatient detoxification (Part II): Psychiatric complaints and their relation to delta-9-tetrahydrocannabinol and its metabolites in serum. *Drug Alcohol Depend*, *155*, 302-306. https://doi.org/10.1016/j.drugalcdep.2015.08.003

Bonnet, U., & Preuss, U. W. (2017). The cannabis withdrawal syndrome: current insights. *Subst Abuse Rehabil*, *8*, 9-37. https://doi.org/10.2147/SAR.S109576

Bonnet, U., Specka, M., Stratmann, U., Ochwadt, R., & Scherbaum, N. (2014). Abstinence phenomena of chronic cannabis-addicts prospectively monitored during controlled inpatient detoxification: cannabis withdrawal syndrome and its correlation with delta-9-tetrahydrocannabinol and -metabolites in serum. *Drug Alcohol Depend*, *143*, 189-197. https://doi.org/10.1016/j.drugalcdep.2014.07.027

Bossong, M. G., van Berckel, B. N., Boellaard, R., Zuurman, L., Schuit, R. C., Windhorst, A. D., van Gerven, J. M., Ramsey, N. F., Lammertsma, A. A., & Kahn, R. S. (2009). Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology*, *34*(3), 759-766. https://doi.org/10.1038/npp.2008.138

Breindahl, T., Kimergard, A., Leutscher, P. D. C., & Hindersson, P. (2021). Implementation of Mathematical Models to Predict New Cannabis Use by Urine Drug Testing: It Is Time to Move Forward. *J Anal Toxicol*, *45*(6), e15-e19. https://doi.org/10.1093/jat/bkab037

Budney, A. J., Moore, B. A., Vandrey, R. G., & Hughes, J. R. (2003). The time course and significance of cannabis withdrawal. *J Abnorm Psychol*, *112*(3), 393-402. https://doi.org/10.1037/0021-843x.112.3.393

Budney, A. J., Novy, P. L., & Hughes, J. R. (1999). Marijuana withdrawal among adults seeking treatment for marijuana dependence. *Addiction*, *94*(9), 1311-1322. https://doi.org/10.1046/j.1360-0443.1999.94913114.x

Cabral, G. A., Raborn, E. S., Griffin, L., Dennis, J., & Marciano-Cabral, F. (2008). CB2 receptors in the brain: role in central immune function. *Br J Pharmacol*, *153*(2), 240-251. https://doi.org/10.1038/sj.bjp.0707584

Calakos, K. C., Liu, H., Lu, Y., Anderson, J. M., Matuskey, D., Nabulsi, N., Ye, Y., Skosnik, P. D., D'Souza, D. C., Morris, E. D., Cosgrove, K. P., & Hillmer, A. T. (2021). Assessment of transient dopamine responses to smoked cannabis. *Drug Alcohol Depend*, *227*, 108920. https://doi.org/10.1016/j.drugalcdep.2021.108920

Canada. (2018a). *Cannabis Act (S.C. 2018, c. 16)*.

Cannabis Regulation Act (C.5-3), (2018b).

Canada. (2019). 2019 Canadian Cannabis Survey. https://publications.gc.ca/collections/collection_2019/sc-hc/H21-312-2019-2-eng.pdf
Canada. (2022). *Canadian Cannabis Survey 2022: Summary*. https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/research-data/canadian-cannabis-survey-2022-summary.html#s3
Canada. (2023a). *About cannabis*. https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/about.html
Canada. (2023b). *Addiction to cannabis*. https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/health-effects/addiction.html
Canada. (2024a). *Canadian Cannabis Survey 2023: Summary*. https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/research-data/canadian-cannabis-survey-2023-summary.html
Canada. (2024b). *Cannabis use for non-medical purposes among Canadians (aged 16+)*. https://health-infobase.canada.ca/cannabis/
Canadian Centre on Substance Use and Addiction. (2023). *Policy and Regulations (Cannabis)*. https://www.ccsa.ca/policy-and-regulations-cannabis
Ceccarini, J., Kuepper, R., Kemels, D., van Os, J., Henquet, C., & Van Laere, K. (2015). [18F]MK-9470 PET measurement of cannabinoid CB1 receptor availability in chronic cannabis users. *Addict Biol*, *20*(2), 357-367. https://doi.org/10.1111/adb.12116
Chayasirisobhon, S. (2020). Mechanisms of Action and Pharmacokinetics of Cannabis. *Perm J*, *25*, 1-3. https://doi.org/10.7812/TPP/19.200
Chen, P. X., & Rogers, M. A. (2019). Opportunities and challenges in developing orally administered cannabis edibles. *Current Opinion in Food Science*, *28*, 7-13. https://doi.org/https://doi.org/10.1016/j.cofs.2019.02.005
Cheung, A. S., Leemaqz, S. Y., Wong, J. W. P., Chew, D., Ooi, O., Cundill, P., Silberstein, N., Locke, P., Zwickl, S., Grayson, R., Zajac, J. D., & Pang, K. C. (2020). Non-Binary and Binary Gender Identity in Australian Trans and Gender Diverse Individuals. *Arch Sex Behav*, *49*(7), 2673-2681. https://doi.org/10.1007/s10508-020-01689-9
Colizzi, M., & Bhattacharyya, S. (2018). Cannabis use and the development of tolerance: a systematic review of human evidence. *Neurosci Biobehav Rev*, *93*, 1-25. https://doi.org/10.1016/j.neubiorev.2018.07.014
Connor, J. P., Stjepanovic, D., Budney, A. J., Le Foll, B., & Hall, W. D. (2022). Clinical management of cannabis withdrawal. *Addiction*, *117*(7), 2075-2095. https://doi.org/10.1111/add.15743
Connor, J. P., Stjepanovic, D., Le Foll, B., Hoch, E., Budney, A. J., & Hall, W. D. (2021). Cannabis use and cannabis use disorder. *Nat Rev Dis Primers*, *7*(1), 16. https://doi.org/10.1038/s41572-021-00247-4
Cooke, M. E., Gilman, J. M., Lamberth, E., Rychik, N., Tervo-Clemmens, B., Evins, A. E., & Schuster, R. M. (2021). Assessing Changes in Symptoms of Depression and Anxiety During Four Weeks of Cannabis Abstinence Among Adolescents. *Front Psychiatry*, *12*, 689957. https://doi.org/10.3389/fpsyt.2021.689957
Crean, R. D., Crane, N. A., & Mason, B. J. (2011). An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. *J Addict Med*, *5*(1), 1-8. https://doi.org/10.1097/ADM.0b013e31820c23fa
Curran, H. V., Hindocha, C., Morgan, C. J. A., Shaban, N., Das, R. K., & Freeman, T. P. (2019). Which biological and self-report measures of cannabis use predict cannabis dependency

and acute psychotic-like effects? *Psychol Med*, *49*(9), 1574-1580. https://doi.org/10.1017/S003329171800226X

D'Souza, D. C., Cortes-Briones, J. A., Ranganathan, M., Thurnauer, H., Creatura, G., Surti, T., Planeta, B., Neumeister, A., Pittman, B., Normandin, M., Kapinos, M., Ropchan, J., Huang, Y., Carson, R. E., & Skosnik, P. D. (2016). Rapid Changes in CB1 Receptor Availability in Cannabis Dependent Males after Abstinence from Cannabis. *Biol Psychiatry Cogn Neurosci Neuroimaging*, *1*(1), 60-67. https://doi.org/10.1016/j.bpsc.2015.09.008

D'Souza, D. C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y. T., Braley, G., Gueorguieva, R., & Krystal, J. H. (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology*, *29*(8), 1558-1572. https://doi.org/10.1038/sj.npp.1300496

D'Souza, D. C., Ranganathan, M., Braley, G., Gueorguieva, R., Zimolo, Z., Cooper, T., Perry, E., & Krystal, J. (2008). Blunted psychotomimetic and amnestic effects of delta-9-tetrahydrocannabinol in frequent users of cannabis. *Neuropsychopharmacology*, *33*(10), 2505-2516. https://doi.org/10.1038/sj.npp.1301643

Danielsson, A. K., Lundin, A., Allebeck, P., & Agardh, E. (2016). Cannabis use and psychological distress: An 8-year prospective population-based study among Swedish men and women. *Addict Behav*, *59*, 18-23. https://doi.org/10.1016/j.addbeh.2016.03.005

Davis, J. P., Pedersen, E. R., Tucker, J. S., Prindle, J., Dunbar, M. S., Rodriguez, A., Seelam, R., & D'Amico, E. J. (2022). Directional associations between cannabis use and anxiety symptoms from late adolescence through young adulthood. *Drug Alcohol Depend*, *241*, 109704. https://doi.org/10.1016/j.drugalcdep.2022.109704

Davis, J. P., Pedersen, E. R., Tucker, J. S., Prindle, J., Dunbar, M. S., Rodriguez, A., Seelam, R., & D'Amico, E. J. (2023). Directional associations between cannabis use and depression from late adolescence to young adulthood: the role of adverse childhood experiences. *Addiction*. https://doi.org/10.1111/add.16130

Duperrouzel, J., Hawes, S. W., Lopez-Quintero, C., Pacheco-Colon, I., Comer, J., & Gonzalez, R. (2018). The association between adolescent cannabis use and anxiety: A parallel process analysis. *Addict Behav*, *78*, 107-113. https://doi.org/10.1016/j.addbeh.2017.11.005

Ellingson, J. M., Bidwell, L. C., Hopfer, C. J., Hutchison, K. E., & Bryan, A. D. (2019). Correlates and Potential Confounds of Cannabis Withdrawal Among High-Risk Adolescents. *J Stud Alcohol Drugs*, *80*(5), 557-562. https://doi.org/10.15288/jsad.2019.80.557

Feinstein, A., Meza, C., Stefan, C., & Staines, W. R. (2021). Discontinuing cannabis improves depression in people with multiple sclerosis: A short report. *Mult Scler*, *27*(4), 636-639. https://doi.org/10.1177/1352458520934070

Food and Drug Administration. (2020). *What you need to know (and what we're working to find out) about products containing cannabis or cannabis-derived compounds, including CBD.* https://www.fda.gov/consumers/consumer-updates/what-you-need-know-and-what-were-working-find-out-about-products-containing-cannabis-or-cannabis

Freeman, A. M., Petrilli, K., Lees, R., Hindocha, C., Mokrysz, C., Curran, H. V., Saunders, R., & Freeman, T. P. (2019). How does cannabidiol (CBD) influence the acute effects of delta-9-tetrahydrocannabinol (THC) in humans? A systematic review. *Neurosci Biobehav Rev*, *107*, 696-712. https://doi.org/10.1016/j.neubiorev.2019.09.036

Galang, J. N., Babson, K. A., Boden, M. T., & Bonn-Miller, M. O. (2015). Difficulties in emotion regulation are associated with panic symptom severity following a quit attempt among cannabis dependent veterans. *Anxiety Stress Coping*, *28*(2), 192-204. https://doi.org/10.1080/10615806.2014.934228

Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G., & Casellas, P. (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem*, *232*(1), 54-61. https://doi.org/10.1111/j.1432-1033.1995.tb20780.x

Gobbi, G., Atkin, T., Zytynski, T., Wang, S., Askari, S., Boruff, J., Ware, M., Marmorstein, N., Cipriani, A., Dendukuri, N., & Mayo, N. (2019). Association of Cannabis Use in Adolescence and Risk of Depression, Anxiety, and Suicidality in Young Adulthood: A Systematic Review and Meta-analysis. *JAMA Psychiatry*, *76*(4), 426-434. https://doi.org/10.1001/jamapsychiatry.2018.4500

Goodwin, R. S., Darwin, W. D., Chiang, C. N., Shih, M., Li, S. H., & Huestis, M. A. (2008). Urinary elimination of 11-nor-9-carboxy-delta9-tetrahydrocannnabinol in cannabis users during continuously monitored abstinence. *J Anal Toxicol*, *32*(8), 562-569. https://doi.org/10.1093/jat/32.8.562

Haney, M. (2022). Cannabis Use and the Endocannabinoid System: A Clinical Perspective. *Am J Psychiatry*, *179*(1), 21-25. https://doi.org/10.1176/appi.ajp.2021.21111138

Hasin, D. S., Keyes, K. M., Alderson, D., Wang, S., Aharonovich, E., & Grant, B. F. (2008). Cannabis withdrawal in the United States: results from NESARC. *J Clin Psychiatry*, *69*(9), 1354-1363. https://doi.org/10.4088/jcp.v69n0902

Hayatbakhsh, M. R., Najman, J. M., Jamrozik, K., Mamun, A. A., Alati, R., & Bor, W. (2007). Cannabis and anxiety and depression in young adults: a large prospective study. *J Am Acad Child Adolesc Psychiatry*, *46*(3), 408-417. https://doi.org/10.1097/chi.0b013e31802dc54d

Heatherton, T. F., Kozlowski, L. T., Frecker, R. C., & Fagerstrom, K. O. (1991). The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict*, *86*(9), 1119-1127. https://doi.org/10.1111/j.1360-0443.1991.tb01879.x

Hengartner, M. P., Angst, J., Ajdacic-Gross, V., & Rossler, W. (2020). Cannabis use during adolescence and the occurrence of depression, suicidality and anxiety disorder across adulthood: Findings from a longitudinal cohort study over 30 years. *J Affect Disord*, *272*, 98-103. https://doi.org/10.1016/j.jad.2020.03.126

Herrmann, E. S., Weerts, E. M., & Vandrey, R. (2015). Sex differences in cannabis withdrawal symptoms among treatment-seeking cannabis users. *Exp Clin Psychopharmacol*, *23*(6), 415-421. https://doi.org/10.1037/pha0000053

Hirvonen, J., Goodwin, R. S., Li, C. T., Terry, G. E., Zoghbi, S. S., Morse, C., Pike, V. W., Volkow, N. D., Huestis, M. A., & Innis, R. B. (2012). Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. *Mol Psychiatry*, *17*(6), 642-649. https://doi.org/10.1038/mp.2011.82

Huestis, M. A., & Cone, E. J. (1998). Urinary excretion half-life of 11-nor-9-carboxy-delta9-tetrahydrocannabinol in humans. *Ther Drug Monit*, *20*(5), 570-576. https://doi.org/10.1097/00007691-199810000-00021

Huestis, M. A., Solimini, R., Pichini, S., Pacifici, R., Carlier, J., & Busardò, F. P. (2019). Cannabidiol Adverse Effects and Toxicity. *Current Neuropharmacology*, *17*(10). https://doi.org/10.2174/1570159X17666190603171901

Institut de la statistique du Québec. (2023). *Québec Cannabis Survey 2022*. https://statistique.quebec.ca/en/document/quebec-cannabis-survey-2022

Jacobus, J., Squeglia, L. M., Escobar, S., McKenna, B. M., Hernandez, M. M., Bagot, K. S., Taylor, C. T., & Huestis, M. A. (2017). Changes in marijuana use symptoms and emotional functioning over 28-days of monitored abstinence in adolescent marijuana users. *Psychopharmacology (Berl)*, *234*(23-24), 3431-3442. https://doi.org/10.1007/s00213-017-4725-3

Karniol, I. G., Shirakawa, I., Kasinski, N., Pfeferman, A., & Carlini, E. A. (1974). Cannabidiol interferes with the effects of delta 9 - tetrahydrocannabinol in man. *Eur J Pharmacol*, *28*(1), 172-177. https://doi.org/10.1016/0014-2999(74)90129-0

Kearney-Ramos, T., Herrmann, E. S., Belluomo, I., Matias, I., Vallee, M., Monlezun, S., Piazza, P. V., & Haney, M. (2022). The Relationship Between Circulating Endogenous Cannabinoids and the Effects of Smoked Cannabis. *Cannabis Cannabinoid Res*. https://doi.org/10.1089/can.2021.0185

Kerridge, B. T., Pickering, R., Chou, P., Saha, T. D., & Hasin, D. S. (2018). DSM-5 cannabis use disorder in the National Epidemiologic Survey on Alcohol and Related Conditions-III: Gender-specific profiles. *Addict Behav*, *76*, 52-60. https://doi.org/10.1016/j.addbeh.2017.07.012

Kouri, E. M., & Pope, H. G., Jr. (2000). Abstinence symptoms during withdrawal from chronic marijuana use. *Experimental and clinical psychopharmacology*, *8*(4), 483-492. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med4&NEWS=N&AN=11127420

Ladha, K. S., Ajrawat, P., Yang, Y., & Clarke, H. (2020). Understanding the Medical Chemistry of the Cannabis Plant is Critical to Guiding Real World Clinical Evidence. *Molecules*, *25*(18). https://doi.org/10.3390/molecules25184042

Lee, D., Schroeder, J. R., Karschner, E. L., Goodwin, R. S., Hirvonen, J., Gorelick, D. A., & Huestis, M. A. (2014). Cannabis withdrawal in chronic, frequent cannabis smokers during sustained abstinence within a closed residential environment. *Am J Addict*, *23*(3), 234-242. https://doi.org/10.1111/j.1521-0391.2014.12088.x

Levin, K. H., Copersino, M. L., Heishman, S. J., Liu, F., Kelly, D. L., Boggs, D. L., & Gorelick, D. A. (2010). Cannabis withdrawal symptoms in non-treatment-seeking adult cannabis smokers. *Drug Alcohol Depend*, *111*(1-2), 120-127. https://doi.org/10.1016/j.drugalcdep.2010.04.010

Lichenstein, S. D. (2022). THC, CBD, and Anxiety: a Review of Recent Findings on the Anxiolytic and Anxiogenic Effects of Cannabis' Primary Cannabinoids. *Curr Addict Rep*, *9*, 473–485. https://doi.org/10.1007/s40429-022-00450-7

Lowe, H., Toyang, N., Steele, B., Bryant, J., & Ngwa, W. (2021). The Endocannabinoid System: A Potential Target for the Treatment of Various Diseases. *Int J Mol Sci*, *22*(17). https://doi.org/10.3390/ijms22179472

Lowe, R. H., Abraham, T. T., Darwin, W. D., Herning, R., Cadet, J. L., & Huestis, M. A. (2009). Extended urinary Delta9-tetrahydrocannabinol excretion in chronic cannabis users precludes use as a biomarker of new drug exposure. *Drug Alcohol Depend*, *105*(1-2), 24-32. https://doi.org/10.1016/j.drugalcdep.2009.05.027

Lu, H. C., & Mackie, K. (2021). Review of the Endocannabinoid System. *Biol Psychiatry Cogn Neurosci Neuroimaging*, *6*(6), 607-615. https://doi.org/10.1016/j.bpsc.2020.07.016

Lucatch, A. M., Kloiber, S. M., Meyer, J. H., Rizvi, S. J., & George, T. P. (2020). Effects of Extended Cannabis Abstinence in Major Depressive Disorder. *The Canadian Journal of Addiction*, *11*(3), 33-41.

Lutz, B., Marsicano, G., Maldonado, R., & Hillard, C. J. (2015). The endocannabinoid system in guarding against fear, anxiety and stress. *Nat Rev Neurosci*, *16*(12), 705-718. https://doi.org/10.1038/nrn4036

Mahamad, S., Wadsworth, E., Rynard, V., Goodman, S., & Hammond, D. (2020). Availability, retail price and potency of legal and illegal cannabis in Canada after recreational cannabis legalisation. *Drug Alcohol Rev*, *39*(4), 337-346. https://doi.org/10.1111/dar.13069

Mammen, G., Rueda, S., Roerecke, M., Bonato, S., Lev-Ran, S., & Rehm, J. (2018). Association of Cannabis With Long-Term Clinical Symptoms in Anxiety and Mood Disorders: A Systematic Review of Prospective Studies. *J Clin Psychiatry*, *79*(4). https://doi.org/10.4088/JCP.17r11839

Meyer, H. C., Lee, F. S., & Gee, D. G. (2018). The Role of the Endocannabinoid System and Genetic Variation in Adolescent Brain Development. *Neuropsychopharmacology*, *43*(1), 21-33. https://doi.org/10.1038/npp.2017.143

Milin, R., Manion, I., Dare, G., & Walker, S. (2008). Prospective assessment of cannabis withdrawal in adolescents with cannabis dependence: a pilot study. *J Am Acad Child Adolesc Psychiatry*, *47*(2), 174-179. https://doi.org/10.1097/chi.0b013e31815cdd73

Nadulski, T., Pragst, F., Weinberg, G., Roser, P., Schnelle, M., Fronk, E. M., & Stadelmann, A. M. (2005). Randomized, double-blind, placebo-controlled study about the effects of cannabidiol (CBD) on the pharmacokinetics of Delta9-tetrahydrocannabinol (THC) after oral application of THC verses standardized cannabis extract. *Ther Drug Monit*, *27*(6), 799-810. https://doi.org/10.1097/01.ftd.0000177223.19294.5c

Navarrete, F., García-Gutiérrez, M. S., Gasparyan, A., & Amaya Austrich-Olivares, A. M., J. (2021). Role of Cannabidiol in the Therapeutic Intervention for Substance Use Disorders. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.626010

Neumeister, A., Normandin, M. D., Pietrzak, R. H., Piomelli, D., Zheng, M. Q., Gujarro-Anton, A., Potenza, M. N., Bailey, C. R., Lin, S. F., Najafzadeh, S., Ropchan, J., Henry, S., Corsi-Travali, S., Carson, R. E., & Huang, Y. (2013). Elevated brain cannabinoid CB1 receptor availability in post-traumatic stress disorder: a positron emission tomography study. *Mol Psychiatry*, *18*(9), 1034-1040. https://doi.org/10.1038/mp.2013.61

Nguyen, H. V., & Mital, S. (2022). Changes in Youth Cannabis Use After an Increase in Cannabis Minimum Legal Age in Quebec, Canada. *JAMA Netw Open*, *5*(6), e2217648. https://doi.org/10.1001/jamanetworkopen.2022.17648

Normandin, M. D., Zheng, M. Q., Lin, K. S., Mason, N. S., Lin, S. F., Ropchan, J., Labaree, D., Henry, S., Williams, W. A., Carson, R. E., Neumeister, A., & Huang, Y. (2015). Imaging the cannabinoid CB1 receptor in humans with [11C]OMAR: assessment of kinetic analysis methods, test-retest reproducibility, and gender differences. *J Cereb Blood Flow Metab*, *35*(8), 1313-1322. https://doi.org/10.1038/jcbfm.2015.46

Onaemo, V. N., Fawehinmi, T. O., & D'Arcy, C. (2021). Comorbid Cannabis Use Disorder with Major Depression and Generalized Anxiety Disorder: A Systematic Review with Meta-analysis of Nationally Representative Epidemiological Surveys. *J Affect Disord*, *281*, 467-475. https://doi.org/10.1016/j.jad.2020.12.043

Pan American Health Organization. (2021). *The burden of drug use disorders in the Region of the Americas*. Pan American Health Organization. https://www.paho.org/en/enlace/burden-drug-use-disorders

Patel, J., & Marwaha, R. (2023). Cannabis Use Disorder. In *StatPearls*. https://www.ncbi.nlm.nih.gov/pubmed/30844158

Patton, G. C., Coffey, C., Carlin, J. B., Degenhardt, L., Lynskey, M., & Hall, W. (2002). Cannabis use and mental health in young people: cohort study. *BMJ*, *325*(7374), 1195-1198. https://doi.org/10.1136/bmj.325.7374.1195

Petrie, G. N., Nastase, A. S., Aukema, R. J., & Hill, M. N. (2021). Endocannabinoids, cannabinoids and the regulation of anxiety. *Neuropharmacology*, *195*, 108626. https://doi.org/10.1016/j.neuropharm.2021.108626

Petrilli, K., Hines, L., Adams, S., Morgan, C. J., Curran, H. V., & Freeman, T. P. (2023). High potency cannabis use, mental health symptoms and cannabis dependence: Triangulating the evidence. *Addict Behav*, *144*, 107740. https://doi.org/10.1016/j.addbeh.2023.107740

Quebec. (2019). *Bill 2 An Act to Tighten the Regulation of Cannabis*. Quebec

Quebec. (2023). *Forms and methods of cannabis use*. https://www.quebec.ca/en/health/advice-and-prevention/alcohol-drugs-gambling/recognizing-drugs-and-their-effects/cannabis/description-effects-risks-cannabis/forms-methods-cannabis-use

Rabin, R. A., Kozak, K., Zakzanis, K. K., Remington, G., & George, T. P. (2018a). Effects of extended cannabis abstinence on clinical symptoms in cannabis dependent schizophrenia patients versus non-psychiatric controls. *Schizophr Res*, *194*, 55-61. https://doi.org/10.1016/j.schres.2017.03.010

Rabin, R. A., Kozak, K., Zakzanis, K. K., Remington, G., Stefan, C., Budney, A. J., & George, T. P. (2018b). A method to achieve extended cannabis abstinence in cannabis dependent patients with schizophrenia and non-psychiatric controls. *Schizophr Res*, *194*, 47-54. https://doi.org/10.1016/j.schres.2017.05.006

Ramaekers, J. G., Mason, N. L., Kloft, L., & Theunissen, E. L. (2021). The why behind the high: determinants of neurocognition during acute cannabis exposure. *Nat Rev Neurosci*, *22*(7), 439-454. https://doi.org/10.1038/s41583-021-00466-4

Ramaekers, J. G., Mason, N. L., & Theunissen, E. L. (2020). Blunted highs: Pharmacodynamic and behavioral models of cannabis tolerance. *Eur Neuropsychopharmacol*, *36*, 191-205. https://doi.org/10.1016/j.euroneuro.2020.01.006

Rotermann, M. (2021). Looking back from 2020, how cannabis use and related behaviours changed in Canada. *Health Rep*, *32*(4), 3-14. https://doi.org/10.25318/82-003-x202100400001-eng

Saunders, J. B., Aasland, O. G., Babor, T. F., de la Fuente, J. R., & Grant, M. (1993). Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II. *Addiction*, *88*(6), 791-804. https://doi.org/10.1111/j.1360-0443.1993.tb02093.x

Schlienz, N. J., Budney, A. J., Lee, D. C., & Vandrey, R. (2017). Cannabis Withdrawal: A Review of Neurobiological Mechanisms and Sex Differences. *Curr Addict Rep*, *4*(2), 75-81. https://doi.org/10.1007/s40429-017-0143-1

Schuster, R. M., Fontaine, M., Nip, E., Zhang, H., Hanly, A., & Evins, A. E. (2017). Prolonged cannabis withdrawal in young adults with lifetime psychiatric illness. *Prev Med*, *104*, 40-45. https://doi.org/10.1016/j.ypmed.2017.02.019

Schuster, R. M., Hanly, A., Gilman, J., Budney, A., Vandrey, R., & Evins, A. E. (2016). A contingency management method for 30-days abstinence in non-treatment seeking young adult cannabis users. *Drug Alcohol Depend*, *167*, 199-206. https://doi.org/10.1016/j.drugalcdep.2016.08.622

Schwilke, E. W., Gullberg, R. G., Darwin, W. D., Chiang, C. N., Cadet, J. L., Gorelick, D. A., Pope, H. G., & Huestis, M. A. (2011). Differentiating new cannabis use from residual urinary cannabinoid excretion in chronic, daily cannabis users. *Addiction*, *106*(3), 499-506. https://doi.org/10.1111/j.1360-0443.2010.03228.x

Sexton, M., Cuttler, C., & Mischley, L. K. (2019). A Survey of Cannabis Acute Effects and Withdrawal Symptoms: Differential Responses Across User Types and Age. *J Altern Complement Med*, *25*(3), 326-335. https://doi.org/10.1089/acm.2018.0319

Sharma, P., Murthy, P., & Bharath, M. M. (2012). Chemistry, metabolism, and toxicology of cannabis: clinical implications. *Iran J Psychiatry*, *7*(4), 149-156. https://www.ncbi.nlm.nih.gov/pubmed/23408483

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., & Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*, *59 Suppl 20*, 22-33;quiz 34-57. https://www.ncbi.nlm.nih.gov/pubmed/9881538

Sobell, L. C., Brown, J., Leo, G. I., & Sobell, M. B. (1996). The reliability of the Alcohol Timeline Followback when administered by telephone and by computer. *Drug Alcohol Depend*, *42*(1), 49-54. https://doi.org/10.1016/0376-8716(96)01263-x

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Consulting Psychologists Press.

Stanciu, C. N., Brunette, M. F., Teja, N., & Budney, A. J. (2021). Evidence for Use of Cannabinoids in Mood Disorders, Anxiety Disorders, and PTSD: A Systematic Review. *Psychiatr Serv*, *72*(4), 429-436. https://doi.org/10.1176/appi.ps.202000189

Statistics Canada. (2023). *Research to Insights: Cannabis in Canada*. https://www150.statcan.gc.ca/n1/pub/11-631-x/11-631-x2023006-eng.htm

Stella, N. (2023). THC and CBD: Similarities and differences between siblings. *Neuron*, *111*(3), 302-327. https://doi.org/10.1016/j.neuron.2022.12.022

Sullivan, R. M., Wallace, A. L., Stinson, E. A., Montoto, K. V., Kaiver, C. M., Wade, N. E., & Lisdahl, K. M. (2022). Assessment of Withdrawal, Mood, and Sleep Inventories After Monitored 3-Week Abstinence in Cannabis-Using Adolescents and Young Adults. *Cannabis Cannabinoid Res*, *7*(5), 690-699. https://doi.org/10.1089/can.2021.0074

Thieme, U., Schelling, G., Hauer, D., Greif, R., Dame, T., Laubender, R. P., Bernhard, W., Thieme, D., Campolongo, P., & Theiler, L. (2014). Quantification of anandamide and 2-arachidonoylglycerol plasma levels to examine potential influences of tetrahydrocannabinol application on the endocannabinoid system in humans. *Drug Test Anal*, *6*(1-2), 17-23. https://doi.org/10.1002/dta.1561

Thomas, B. F., Compton, D. R., & Martin, B. R. (1990). Characterization of the lipophilicity of natural and synthetic analogs of delta 9-tetrahydrocannabinol and its relationship to pharmacological potency. *J Pharmacol Exp Ther*, *255*(2), 624-630. https://www.ncbi.nlm.nih.gov/pubmed/2173751

Turna, J., Patterson, B., & Van Ameringen, M. (2017). Is cannabis treatment for anxiety, mood, and related disorders ready for prime time? *Depress Anxiety*, *34*(11), 1006-1017. https://doi.org/10.1002/da.22664

Van Laere, K., Goffin, K., Casteels, C., Dupont, P., Mortelmans, L., de Hoon, J., & Bormans, G. (2008). Gender-dependent increases with healthy aging of the human cerebral cannabinoid-type 1 receptor binding using [(18)F]MK-9470 PET. *Neuroimage*, *39*(4), 1533-1541. https://doi.org/10.1016/j.neuroimage.2007.10.053

Volkow, N. D., Baler, R. D., Compton, W. M., & Weiss, S. R. (2014). Adverse health effects of marijuana use. *N Engl J Med*, *370*(23), 2219-2227. https://doi.org/10.1056/NEJMra1402309

Volkow, N. D., Hampson, A. J., & Baler, R. D. (2017). Don't Worry, Be Happy: Endocannabinoids and Cannabis at the Intersection of Stress and Reward. *Annu Rev Pharmacol Toxicol*, *57*, 285-308. https://doi.org/10.1146/annurev-pharmtox-010716-104615

Wallis, D., Coatsworth, J. D., Mennis, J., Riggs, N. R., Zaharakis, N., Russell, M. A., Brown, A. R., Rayburn, S., Radford, A., Hale, C., & Mason, M. J. (2022). Predicting Self-Medication with Cannabis in Young Adults with Hazardous Cannabis Use. *Int J Environ Res Public Health*, *19*(3). https://doi.org/10.3390/ijerph19031850

Williams, J. B. (1988). A structured interview guide for the Hamilton Depression Rating Scale. *Arch Gen Psychiatry*, *45*(8), 742-747. https://doi.org/10.1001/archpsyc.1988.01800320058007

Yang, X., Fang, Y., Chen, H., Zhang, T., Yin, X., Man, J., Yang, L., & Lu, M. (2021). Global, regional and national burden of anxiety disorders from 1990 to 2019: results from the Global Burden of Disease Study 2019. *Epidemiol Psychiatr Sci*, *30*, e36. https://doi.org/10.1017/S2045796021000275

Yeap, Z. J. S., Baaj, L., George, T. P., Mizrahi, R., & Rabin, R. A. (in press). Characterizing the Cannabis Withdrawal Trajectory in Men with Cannabis and Tobacco Co-Use: A Preliminary Study. *Canadian Journal of Addiction*.

Yeap, Z. J. S., Marsault, J., George, T. P., Mizrahi, R., & Rabin, R. A. (2023). Does tobacco dependence worsen cannabis withdrawal in people with and without schizophrenia-spectrum disorders? *Am J Addict*, *32*(4), 367-375. https://doi.org/10.1111/ajad.13394

Zou, S., & Kumar, U. (2018). Cannabinoid Receptors and the Endocannabinoid System: Signaling and Function in the Central Nervous System. *Int J Mol Sci*, *19*(3). https://doi.org/10.3390/ijms19030833

8. APPENDICES

Appendix 8.1 *Recruitment Poster*

Appendix 8.2 *Cannabis Abstinence Behavioural Support Manual*

SESSION 1: DAY 0

Goals for session 1:

1. *Create a rapport/relationship with the participant*
2. *Identify participants' cannabis using patterns*
3. *Determine how the participant feels about being in the study*
4. *Discuss what the participant can expect while in the study*

INTRODUCTION

Hi, I'm _____ and I will be working with you during your participation in this research study. This is the last thing on the schedule for today. These sessions are meant to accompany you throughout the study and provide some support. I will be seeing you a total of five times: one session today and then one session per week over the course of the study. We are not collecting any data from these sessions, and all the information will remain confidential. I will write down some of your answers, this allows us to get an idea of participants' experiences with cannabis use as well as concerns about research participation, but as I mentioned, none of this will be used for any experiments or analyses.

Do you have any questions before we begin?

ELUCIDATION OF CANNABIS USE PATTERNS

- Let's briefly go over your cannabis use. Can you tell me a little bit about your cannabis use patterns or habits?
- When did you start using? How old were you?
- And how often do you use marijuana? (DAILY, WEEKLY, MONTHLY)
- Have you ever tried quitting before? (YES / NO)
- Ok, we will get back to this in more detail a little bit further in the session.

FEELINGS ABOUT THE STUDY

- Have you ever participated in a research study before? (YES / NO) How do you think this experience will be for you? *IF NEEDED*: difficult, stressful, helpful, positive/negative…
- Do you foresee any trouble making it to the twice weekly study visits? *IF NEEDED*: I would like to emphasize how helpful you are by coming to each visit, this allows us and the scientific community to learn more about how cannabis use affects the brain.
- Do you have any other concerns/comments about your participation in this research study?

→ *FOR PARTICIPANTS ASSIGNED TO CANNABIS ABSTINENCE ARM*

- As you know, this study involves being abstinent from cannabis use for 28 days. You (HAVE / HAVE NOT) tried to abstain from cannabis before. How do you feel about doing this (AGAIN)?

IF PARTICIPANT HAS ABSTAINED BEFORE: Let's talk a bit more about that period. How long did it last?

- Why did you start again?
- What symptoms did you experience during that period of abstinence?
- What strategies did you find useful to maintain abstinence?

- Do you have anything else to add about your cannabis use that you think is important?

IF PARTICIPANT HAS NEVER ABSTAINED: How do you feel about quitting? Do you think you can do it?
- Do you have any concerns about becoming abstinent?
- Do you have anything else to add about your cannabis use that you think is important?

→ *FOR PARTICIPANTS ASSIGNED TO <u>CANNABIS AS USUAL ARM</u>*
    - As you know, today's visit and the last visit involve being abstinent from cannabis for at least 12 hours. You (HAVE / HAVE NOT) tried to abstain from cannabis before. How do you feel about doing this (AGAIN)?

IF PARTICIPANT HAS ABSTAINED BEFORE: Let's talk a bit more about that period. How long did it last?
- Why did you start again?
- What symptoms did you experience during that period of abstinence?
- What strategies did you find useful to maintain abstinence?
- Do you have anything else to add about your cannabis use that you think is important?

IF PARTICIPANT HAS NEVER ABSTAINED: How did you feel about stopping for 12 hours? Did you have any problems doing this?
Do you have anything else to add about your cannabis use that you think is important?

<u>DISCUSS WHAT TO EXPECT DURING THE FIRST WEEK/12 HOURS OF ABSTINENCE</u>
When people stop using cannabis after prolonged or heavy use, some unconformable symptoms may emerge. These do not happen for everyone. For people who do experience these symptoms, some people have very mild/minimal symptoms and some people have more severe symptoms. These symptoms can be uncomfortable but are not a risk to your health.

<u>CRAVING COPING SKILLS AND MANAGEMENT</u>
Along with withdrawal symptoms, craving for cannabis is normal to experience. I will provide you with some coping skills that may assist you with these feelings.

1. *DISTRACTION TECHNIQUES*

- Keeping busy will really help keep your mind off cannabis and the cravings you may be experiencing. Try to distract yourself by focusing on other things you enjoy. Do you like to play any sports or have any hobbies you could use for this?
- *OTHER OPTIONS*: friends/family you like hanging out with, activities or volunteering you are involved in…

2. *AVOIDANCE OF CUES*

Another method to help you cope is to avoid drug-related cues. These can be people, places or things that remind you of or are associated with cannabis use. These events may trigger the urge to use and some people find it helpful to avoid such situations altogether, although I know this can be difficult. Sometimes, avoidance of these high-risk situations is not always possible. Let's

run through a possible scenario you may encounter and how to deal with it. (ASK IF PATIENT HAS AN EXAMPLE SCENARIO BEFORE PROVIDING EXAMPLE)
SCENARIO

- Imagine you are in a social situation, and you see your friend, someone who often smokes cannabis with you. He offers you a joint. How would you handle that?
- How would you feel about being honest with your friend about research participation and monetary gain?
- Are there any concerns you have about being honest with people about your abstinence goals?
- How would you feel about simply saying, 'No, thank you'?

SESSION WRAP-UP AND MOTIVATION
I know this sounds like a lot, but I really think you can achieve and maintain abstinence successfully! It was very nice to meet you and I look forward to meeting with you next week!
FOR PARTICIPANTS IN THE ABSTINENT ARM, YOU CAN REINFORCE THE CONTINGENT BONUS AT THE END OF 28 DAYS: Remember, remaining abstinent the whole time means you get the money reward at the end, so keep your mind on that!

SESSION 2: DAY 7 [*Same script for Session 3 (day 14), Session 4 (day 21)]*
Goals for sessions 2, 3, 4:

1. *Obtain patient abstinence status*
2. *Address how the presence of (withdrawal) symptoms has been experienced/dealt with*

CANNABIS USE CHECK-IN

- What have your cannabis use patterns been like in the last week?

REINFORCE POSITIVE BEHAVIOUR WITH POSITIVE FEEDBACK AND AFFIRMATION OF HARDWORK: Congratulations, I am very impressed! / Good job making it to your study visit on time! / Keep it up!

COPING STRATEGIES

- Did you try any of the coping strategies we talked about last time? What worked best for you?

MAINTAIN POSITIVITY IF LAPSES HAPPENED: Yes, that's okay, lapses happen. Can you walk me through what happened?

SYMPTOM MANAGEMENT

- Did you experience any symptoms in the last week? If so, can you please describe them?

IF NEEDED: Various symptoms can include mood changes, changes in sleep, physical symptoms…

→ *FOR PARTICIPANTS ASSIGNED TO CANNABIS ABSTINENCE ARM*

- What have been your most difficult symptoms?
- How have you avoided cannabis use?

- What could you do differently? (COPING MECHANISMS SUGGESTED IN SESSION 1 CAN ALSO BE DISCUSSED)

SESSION WRAP-UP AND MOTIVATION
Keep up the good work! I look forward to meeting with you next week!

SESSION 5: DAY 28
Goals for session 5:
1. *Cannabis use check-in*
2. *Symptom management*
3. *Psychoeducation*
4. *Therapy wrap-up*

CANNABIS USE CHECK-IN
- What have your cannabis use patterns been like in the last week?

REINFORCE POSITIVE BEHAVIOUR WITH POSITIVE FEEDBACK AND AFFIRMATION OF HARD WORK: Congratulations, I am very impressed! / Good job making it to your study visit on time! / Keep it up! / You're almost there! / Only one week left!

SYMPTOM MANAGEMENT
- Did you experience any symptoms in the last week? If so, can you please describe them?

IF NEEDED: Various symptoms can include mood changes, difficulty sleeping, headaches, irritability…

→ *FOR PARTICIPANTS ASSIGNED TO CANNABIS ABSTINENCE ARM*
- What have been your most difficult symptoms?
- How have you avoided cannabis use?
- What could you do differently? (COPING MECHANISMS SUGGESTED IN SESSION 1 CAN ALSO BE DISCUSSED)

PSYCHOEDUCATION (adapted from SAMHSA)
Cannabis can have some negative and long-term effects. We will go through and discuss some of these. This is purely from an informative standpoint and aims to provide you with as much information as possible to allow you to make informed decisions concerning your cannabis use. Let me know if you have any questions at any point.

BRAIN HEALTH: Marijuana can affect brain processes such as memory and decision-making. This can make doing well at school or in one's job difficult.

MENTAL HEALTH: Studies have linked marijuana use to depression, anxiety, suicide planning, and psychotic symptoms and episodes. It is not known, however, if marijuana use is the cause of these conditions.

ATHLETIC PERFORMANCE: Research shows that marijuana affects timing, movement, and coordination, which can harm athletic performance.

DRIVING: People who drive under the influence of marijuana can experience dangerous effects: slower reactions, lane weaving, decreased coordination, and difficulty reacting to signals and sounds on the road.

BABY'S HEALTH AND DEVELOPMENT: Marijuana use during pregnancy may cause fetal growth restriction, premature birth, stillbirth, and problems with brain development, resulting in hyperactivity and poor cognitive function. Tetrahydrocannabinol (THC) and other chemicals from marijuana can also be passed from a mother to her baby through breast milk, further impacting a child's healthy development.

DAILY LIFE: Using marijuana can affect performance and how well people do in life. Research shows that people who use marijuana are more likely to have relationship problems, worse educational outcomes, lower career achievement, and reduced life satisfaction.

<u>THERAPY WRAP-UP</u>

- Thank you for all your hard work in attending all the sessions and for your participation in the study.
- Do you have any comments regarding your experience over the last 4 weeks?
- Do you think you will remain abstinent?
- Do you have any plans on reducing or changing your cannabis use?