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EXPLORING THE EFFECT OF 8 WEEKS OF CANNABIDIOL ON SERUM BRAIN-DERIVED NEUROTROPHIC FACTOR

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EXPLORING THE EFFECT OF 8 WEEKS OF CANNABIDIOL ON SERUM BRAIN-DERIVED NEUROTROPHIC FACTOR

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ABSTRACT

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Studies have shown that cannabidiol (CBD) consumption may lead to neuroprotective effects. Brain-derived neurotrophic factor (BDNF) is a circulating biomarker of neural health and studies exploring the effects of CBD on BDNF concentrations are limited. **Purpose:** The purpose of this study is to determine whether a longer-term CBD intervention would significantly change BDNF concentrations in human serum. **Methods:** This study used a double-blind placebo-controlled design where thirty-five healthy adult participants were randomly assigned into two groups. Participants were 25.94 ± 6.5 years of age with a body weight of 72.35 ± 13.89 kg and a body fat percentage of 22.61 ± 7.95%. One group (CB, n=18) ingested a 50mg CBD gel capsule orally once a day for 8 weeks while the control group (PL, n=17) group consumed a calorie-matched placebo (PL) over the course of the same 8-week period. At pre intervention and post intervention time points, participant body size, body composition was obtained. Blood was also collected at both time points and serum was used to determine BDNF concentration using an enzyme-linked immunosorbent assay (ELISA). An ANOVA was used to determine whether there were differences at the pre intervention time point and the way CB and PL groups responded to the intervention. Significance was set at $p < 0.05$. **Results:** There were no differences between groups at the pre intervention time points or as a result of the intervention. Mean concentrations of BDNF at the pre intervention time point were 25.35 ± 7.3 ng/ml and

23.03 ± 5.2 ng/ml in the PL and CB groups, respectively. **Conclusion**: Eight weeks of daily, low dose CBD does not improve BDNF concentrations in healthy people. Future studies may want to explore a higher dose in individuals with impaired neural health.

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CHAPTER I

INTRODUCTION

As alternative medicine and herbal supplements have grown in popularity, compounds extracted from *Cannabis* are now more frequently studied for their potential health promoting benefits (Bridgeman & Abazia, 2017). Today, the cannabinoid cannabidiol (CBD) is one of the most widely recognized cannabis compounds. However, research with all cannabis related products was restricted until 2015 when the United States Drug Enforcement Administration eased the regulatory requirements for Federal Drug Administration (FDA)-approved clinical trials involving CBD (United States Drug Enforcement Administration, 2015). Prior to the lift in restrictions, research with CBD was difficult. For example, researchers were required to request further approval when their study called for changes in CBD delivery method or dose. *Epidiolex* is the only CBD-containing federally approved drug and is now prescribed for seizures associated with Lennox-Gastaut Syndrome, Dravet Syndrome, and tuberous sclerosis complex (Abu-Sawwa et al., 2020). Although there are many anecdotal claims which suggest that CBD may improve sleep, and reduce anxiety, pain, and depression, there are very few studies which consistently support its use in these situations.

Given these anecdotal claims, more recent studies have centered on the potential for CBD to improve brain health. In one study, 15 healthy participants were given one oral 600 mg dose of CBD or a placebo. This acute dose of CBD increased cerebral blood flow to the hippocampus, which was measured using arterial spin labeled magnetic resonance imaging. Next, the working memory of the participants was evaluated through 3 different tasks: Prose-recall task, N-back

task, and Digit Span task. Although improvements in memory after the CBD exposure were not significant; the increase in cerebral blood flow in the hippocampus suggests that other delivery routes or doses of CBD may have the potential to change hippocampal blood flow which, in turn, may lead to more measurable changes in memory (Bloomfield et al., 2020).

Other measures of brain health may include outcomes related to cognitive health, mental health, and coordination. Cognitive health is defined as the ability to think, learn, and use one's memory (U.S. Department of Health and Human Services, 2020). Neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease, and dementia, are associated with impairments in cognitive health. An older study showed that CBD has the potential to protect neurons. More specifically, when the brains of rats were injected with beta amyloid 42, which is a pathological biomarker for the presence of AD, and then treated with CBD at 10mg/kg in the presence or absence of either an antagonist PPAR-α or PPAR-γ, CBD appeared to interact with PPAR-γ to inhibit reactive gliosis. (Esposito et al., 2011).

Mental health is a state of mental well-being that enables people to cope with the stresses of life, realize their abilities, learn well and work well, and contribute to their community (World Health Organization, 2022). Mental health is also a complex concept with various influential factors including social, environmental, physical, and economic determinants. Stress related to these determinants may potentially lead to illnesses like anxiety and depression which can be debilitating to an individual (World Health Organization, 2022). In 2019, 1 in every 8 people, or 970 million people globally, were living with a mental disorder. Among those, 301 million and 280 million people were suffering from anxiety or depression, respectively (World Health Organization, 2022). Some research suggests that high dose of CBD (150-600 mg/day) may be a natural therapeutic treatment for anxiety (Zhornitsky & Potvin, 2012). When rats were injected

with a 30 mg/kg dose of CBD, there were improvements in swim time and reduced immobility time (Réus et al., 2011). The forced swim test for rodents is an established test for rodent mental health (Can et al., 2012). Although these findings are encouraging, results from other studies are not consistent.

Coordination in the brain is accomplished through motor control. The field of motor control explores how the central nervous system produces purposeful, coordinated movements in its interaction with the rest of the body and with the environment (Latash et al., 2010). Motor control deficits are observed in epileptic animal models. In 2019, researchers observed that seizures resulting from epilepsy decreased acutely in their mouse and rat models with a single 10 mg/kg dose of intravenous CBD (Patra et al., 2019).

Other measures of brain health include circulating biomarkers located in the blood or in the cerebrospinal fluid (CSF), which is located in the subarachnoid space around the brain and spinal cord. Beta-amyloid 42, which can be found in CSF, brain-derived neurotrophic factor (BDNF) in blood, and tau concentrations in CSF (Iqbal et al., 2020). BDNF has neurodevelopment and neuroprotective functions. Thus, it is one of the more commonly used biomarkers of brain health. Higher concentrations of BDNF are associated with slower cognitive decline in patients with neurodegenerative diseases (Laske et al., 2011). Additionally, BDNF can increase expression of the CB1 receptor, thus increasing sensitivity to endocannabinoids. This action may improve endocannabinoid system action, which might translate to improvements in learning, memory, and emotional processing (Maison et al., 2009). Currently, most studies exploring whether CBD might alter BDNF concentrations are limited to cellular and animal models (Mottarlini et al., 2022). These models suggest that the influence of CBD is highly dose-dependent and can impact BDNF expression.

Given the lack of information related to the potential for CBD to alter BNDF in humans, the purpose of this study was to investigate whether a longer-term CBD intervention would significantly change BDNF concentrations in human serum. The hypothesis was that:

H1 8 weeks of daily CBD ingestion would significantly increase BDNF concentrations when compared to a placebo consuming control group.

CHAPTER II

LITERATURE REVIEW

This project is focused on exploring whether CBD alters BDNF, which is a biomarker of brain health. This literature review will provide an overview of the literature related to this project. These areas include: the effects of acute and chronic *Cannabis* use, as well as the effect of CBD on pain, anxiety, depression, and brain health biomarkers.

Cannabis

Cannabis is a flowering plant which is part of the family *Cannabaceae Martinov* (Natural Resources Conservation Service, 2022). Cannabis has been used in medicine as far back as 400 A.D. (Zlas et al., 1993). The plant, *Cannabis sativa*, has been an herbal treatment for various illnesses with history dating back to 400 A.D (Zlas et al., 1993). Chinese surgeons in 200 A.D. used cannabis combined with wine to form an anesthesia for patients during surgery (Li, 1973). In more recent times, cannabis has often been referred to as “marijuana”. However, this term is now considered derogatory due to its racist history as a drug affiliated with historical political strife between the US state of Texas and Mexico (Schlosser, 1994). This compound became taboo when the Marihuana Tax Act of 1937 was passed. During this time, the majority of users were people of color and because of the ongoing segregation of society, policy makers insisted that a tax should be placed on the compound to limit access for medicinal applications (Musto, 1972). In 1970, the federal government passed the Controlled Substances Act in which cannabis became a Schedule 1 compound. To this day, cannabis is still considered a Schedule 1 drug. This

means that cannabis has the high potential for abuse, and it is not accepted as medical use (Drug Enforcement Administration, 2020).

The popularity of cannabis has significantly risen over the course of the last century. Globally, about 147 million people (2.5% of the world population) consume cannabis. This equates to the identification of cannabis as the most trafficked and abused drug (World Health Organization). In contrast, cocaine and opiates are consumed annually by about 0.2% of the global population. In 1996, California was the first state to legalize the use of marijuana for medicinal purposes. Today, 39 states have legalized marijuana for medicinal purposes. Among the users of cannabis, currently only about 10% report using marijuana solely for medical purposes. About 1/3 (36%) of individuals report of using marijuana medically as well as recreationally, and ~90% report using marijuana solely for recreational purposes (Schauer et al., 2016).

Cannabis contains more than 400 chemical compounds; however, cannabidiol (CBD) and Δ^{9}-tetrahydrocannabinol (THC) are two of the most well studied. Both compounds were discovered in the mid 20th century (1963 and 1964, respectively) and research interest has grown since then due to their clinical relevance (Gaoni & Mechoulam, 1964; Mechoulam & Shvo, 1963). Recently, CBD has shown promise for its impact on patients who present with mental illness such as depression and anxiety. Additionally, scientists are exploring CBD and its effects on patients that present with COVID-19 (Malinowska et al., 2021). THC is the primary psychoactive compound (Schubart et al. 2011) in cannabis. THC acts on the CB1 and CB2 receptors which are located in the central nervous system and within the body, respectively (Hu & Mackie, 2015). The CB1 receptors can be found in the regions of the brain whose roles involve memory, coordination, emotion, and motivation. These CB1 receptors, when activated,

inhibit the release of neurotransmitters from axon terminals which can impact communication and signaling between neurons (Iversen, 2003). Ultimately, cannabis effectiveness of use in clinical settings would greatly benefit from further research. Other receptors such as a G-Protein coupled receptor, GPCR55, are binding sites for cannabinoids (Kano et al., 2009). However, expression of GPCR55 is much lower when compared to CB1 receptor expression. Therefore, more research needs explore CBD and its associated receptors.

Health Effects of Acute Cannabis Use

Acute cannabis consumption can cause impairments to cognitive function which includes working memory and executive function (Ranganathan & D'Souza, 2006). Acute cannabis use may lead to paranoia, cognitive impairment, and, due to its increase in legal availability, increases in drug-related motor vehicle accidents (Asbridge et al., 2012). In a report studying the trends of fatally injured drivers in the United States from 1999-2010, cannabis is the most common illicit drug linked to these deadly accidents (Brady & Li, 2014).

Not only can cannabis cause cognitive deficits, but it can also lead to bouts of paranoia. More specifically, researchers discovered that THC triggered thoughts of paranoia in individuals with history of paranoia. Some participants theorized that the increase in paranoia was due to the awareness that cannabis had the ability to affect their judgement ability (Freeman et al., 2015).

In addition to a decline of cognitive function, cannabis use may adversely affect the cardiovascular system. Acute cannabis use may increase blood pressure and heart rate (Pacher et al., 2008). Furthermore, acute cannabis use can lead to orthostatic complications perhaps due to decreased vascular resistance (Sidney, 2002). However, there are some acute benefits to cannabis use as well. In 2015, researchers studied whether cannabis helps with nausea and vomiting associated with chemotherapy as a cancer treatment. The results indicated that there was no

statistical difference between cannabis use and placebo for cancer treatment induced sickness (Smith et al., 2015). Conversely, a study done in 2022 on cannabis products and their effects on nausea intensity found that there is statistical significance in nausea relief during a short duration. The study included 886 participants completing self-administered sessions of cannabis to help with their respective levels of nausea. Results showed that 96% of the study sample experienced nausea relief within one-hour of cannabis ingestion. Using a visual analog scale of 0-10, researchers found that within one-hour of cannabis use, participants averaged a -3.85 reduction in nausea symptoms (Stith et al., 2022). Although there are preliminary studies on acute effects of cannabis, specifically THC, more research is needed in this area.

Health Effects of Chronic Cannabis Use

Although cannabis use may have some beneficial effects, chronic use can lead to addiction, decreased neural activity, and some have suggested is a gateway to illicit drugs (Williams, 2020). Cannabis Use Disorder is an illness that can arise with chronic cannabis use. This behavioral disorder can be defined as a pattern of cannabis use leading to clinically significant physiological damage (Patel & Marwaha, 2022). In another study done on a subset population of the Wave 1 of the National Epidemiological Survey on Alcohol and Related Conditions (NESARC), researchers found that 44.7% users who initially started using cannabis as opposed to other drugs, went on to use other illicit drugs (Secades-Villa et al., 2015). Interestingly, a more recent study found that states that had passed medical marijuana laws also had increases in illicit cannabis use and cannabis use disorder (Hasin et al., 2017).

Chronic cannabis use can impair neural connectivity. White matter regions are important due to their function of connecting axonal networks. When the axonal pathways of chronic cannabis users, who had been using the plant at least twice per month for the past 3 years, were

evaluated using diffusion MRI, white matter regions including the hippocampus, corpus callosum, and commissural fibers were significantly impaired when compared to non-cannabis using individuals (Zalesky et al., 2012). Additionally, chronic cannabis use can decrease gray matter volume in the brain. More specifically, in a study of 110 participants (62 non-users and 48 users), MRI scans showed less gray matter volume in the orbitofrontal cortex among cannabis users when compared with non-users. Interestingly, these researchers also discovered that cannabis users had a higher functional connectivity within the orbitofrontal cortex. The researchers hypothesized that this could be a neural adaptation technique for the brain to overcome the loss of gray matter (Filbey et al., 2014).

Cannabidiol (CBD)

The cannabinoid CBD has emerged as a potential health promoting compound; however, the consumption of CBD has vastly outpaced the research supporting many of its health-related claims (Hutchison et al., 2019). This lack of research was due mostly to the US government placing cannabis on the Controlled Substances Act in 1970 as a Schedule I Drug. The schedules range from 1-5 with 5 being the least addictive. Therefore, declaring cannabis as a Schedule I Drug indicated cannabis' high abuse potential. Fortunately, the 2018 Farm Bill outlined regulations for farmers to grow hemp by removing the plant from the Drug Enforcement Administration's schedule of controlled substances (Drug Enforcement Administration, Department of Justice, 2018). This change allowed companies, researchers, and the public to more easily access CBD. Today, CBD is on pace to become a 33-billion-dollar industry in the US by the end of 2022 (Morris, 2022).

Cannabidiol CBD is most well-known for its use in the treatment of Dravet syndrome, a childhood epilepsy disorder (Devinsky et al., 2017). Additionally, CBD has been used to reduce

seizures in individuals with Lennox-Gastaut Syndrome (Thiele et al., 2018). In fact, in 2018, the FDA approved the first CBD-derived drug, *Epidiolex*, for patients presenting with seizures and epilepsy.

Recently, commercially available CBD has gained popularity among the fitness community in the forms of drinks, topical lotions, and oils. In 2018, the World Anti-Doping Agency removed CBD from its list of prohibited substances. In turn, athletes have begun to use CBD as part of their training routines, with many athletes reporting the use of CBD or recovery. For example, 26% of a 517 sample of professional rugby players, indicated that they have or had been using CBD products for their recovery. Among this quarter of responses, 80% reported using CBD for muscle recovery and 78% reported using CBD for sleep. Of the 133 players that were currently or had previously taken CBD, 68% mentioned that they experienced benefits of CBD on their recovery routines (Kasper et al., 2020).

Today, most states have forms of CBD which are easily accessed by the general public. A recent survey of 2,409 Americans, which were balanced fairly evenly between female and male (50.87% and 47.4%, respectively) ranging from ages 24 to 75 (~40% respondents between 55 and 74 years of age), found that CBD was their preferred medication, for pain, anxiety, and depression (Corroon & Phillips, 2018).

Cannabidiol and Pain

Pain is defined as distressing sensory or emotional experience that can be the result of damage to tissue (Raja et al., 2020). Two common forms of pain are categorized as acute and chronic pain. Acute pain is defined as an immediate physiological response to avoid injury (Tighe et al., 2015). Acute pain studies with CBD are limited. However, there was a study done

on CBD's interaction with THC on acute pain that shows some potential use, but the effects were very small (Britch et al., 2017).

CBD has emerged as a potential treatment for chronic pain. Chronic pain is pain that lasts for more than 3 to 6 months (Treede et al., 2015). In a 2019 survey of adults in the US, 50.2 million people reported pain occurring every day, with most common locations to be in the back, hips, and knees (Yong et al., 2022). When 24 clinical patients with various illnesses (18 with multiple sclerosis, 4 with spinal cord injury, 1 with brachial plexus damage, and 1 with limb amputation caused by neurofibromatosis) were given a sublingual spray that included either THC, CBD, THC matched with CBD, or placebo spray to take daily during a two-week intervention period, patients using the THC-only and CBD-only sprays reported a reduction in pain symptoms compared with the placebo group (Wade et al., 2003). While this report was initially exciting, follow up studies using CBD for chronic pain have reported mixed results with respect to the effectiveness of CBD. This area of work is confounded by differences in dosing and method of treatment (Notcutt et al., 2004). Consequently, it is clear that the popularity of CBD is outpacing clinical trials with pharmaceutical grade products (Boyaji et al., 2020).

Cannabidiol and Anxiety

Anxiety is defined as the apprehension, tension, or uneasiness that stems from the anticipation of danger, which may be internal or external (Diagnostic and Statistical Manual of Mental Disorders, 1980). Studies within the past decade suggest that CBD may be helpful in the treatment of anxiety. In an early study, 24 participants with Social Anxiety Disorder and 12 healthy controls were tested to determine whether 600mg of CBD taken orally 1 hour before a public speaking event affected anxiety. Outcome measures included visual analog mood scales, a negative self-statement scale, and physiological measures (blood pressure, heart rate, and skin

conductance). Results revealed that 600 mg CBD significantly reduced anxiety during the speaking test after the ingestion of the 600 mg CBD capsule compared with the placebo group (Bergamaschi et al., 2011). A follow up study provided some support to these results. When researchers split 75 healthy men into four groups and had them complete a simulated public speaking event after consuming 150 mg, 300 mg, 600mg of CBD or a placebo pill one and a half hours before the event. Anxiety was measured using the Visual Analogue Mood Scale (VAMS) and blood pressure. Individuals in the 300mg of CBD group experienced significant reductions in anxiety during the speech while the other treatment groups experienced no significant change (Linares et al., 2019). These findings are intriguing and more exploration in the area of CBD and the control of anxiety is warranted.

Cannabidiol and Depression

Depression is defined as a mental disorder that can negatively impact the way one feels and acts (American Psychiatric Association, 2020). Globally, about 280 million people suffer from depression. Furthermore, depression can lead to suicide which is the fourth leading cause of death in 15–29-year-olds (World Health Organization, 2022). Adults suffering from depression can have a significant impact on the economy. From 2010-2018 adults with major depressive disorder increased by 12.9% which translates as an increase in economic burden from 236.6 billion dollars to 326.2 billion dollars during this time (Greenberg et al., 2021). A study examining CBD's anti-depressant effects in mice found that when male mice were injected 3,10,30, or 100 mg/kg of CBD and then placed in the swim tank to complete a forced swimming test 30 minutes later the mice injected with 30 mg/kg the CBD solution had reduced immobility time compared with the other groups (Zanelati et al., 2010). Although these studies are

intriguing, there is lack of consistency with respect to patient populations, CBD dose and administration routes. It is clear that more well-defined clinical trials are needed in this area.

Cannabidiol and Brain Health

While there are many ways to quantify one's health, preserving brain health can benefit memory, sensory, and motor systems of the central and peripheral nervous systems. The National Institute on Aging refers to brain health the ability for the brain to perform mental processes (U.S. Department of Health and Human Services, 2020). These cognitive functions include learning, intuition, judgement, language, and memory. Declines in brain health are often associated with various cognitive diseases such as dementia, Alzheimer's disease, strokes, and Parkinson's disease. In addition to diseases leading to a decline in brain health, other factors like education, nutrition, physical activity, smoking, obesity, and mental illnesses like depression can have an impact on brain health as well (Gorelick et al., 2011).

The acute effects of CBD Ingestion were most recently explored In healthy volunteers In a resting state with functional Magnetic Resonance imaging (fMRI). When 600mg of CBD taken orally 75 minutes before the fMRI, results showed improved frontal-striatal connectivity compared to the placebo group. The frontal-striatal region of the brain is important for executive function and decision making (Grimm et al., 2018). Other studies have shown that acute 600 mg CBD taken orally can help to reduce anxiety. Researchers studied 15 healthy men who had used cannabis less than 15 times in their lives. The participants took part in 3 trials looking at visuals of faces that would prompt various levels of anxiety all while using fMRI to record regional brain activation. Additionally, electrodermal activity and subjective anxiety levels were measured. After each trial, participants ingested 10 mg of THC, 600 mg of CBD, or a calorie-matched placebo. Researchers discovered that the 10 mg THC ingestion immediately following

the visual trial resulted in increased anxiety while the 600 mg CBD ingestion decreased the anxiety elicited by the visual faces trial (Fusar-Poli et al., 2009).

The effects of chronic CBD use on brain health is still under investigation. For instance, an early study found that 10 mg of orally ingested CBD for 21 days did not alter brain health, as measured by an electroencephalogram and electrocardiogram (Mincis et al., 1973). Later in the 20th century, a case study with one schizophrenic patient revealed that 4 weeks of 1500 mg/ day of CBD did not induce any negative side effects and improved schizophrenic symptoms (Zuardi et al., 1995). A more recent study found that chronic CBD injection (30 and 60mg/kg) in rats led to increased mitochondrial activity in the hippocampus. In this study, 40 adult male rats were split into two trials with 4 treatment groups each (n=5 per treatment group). The researchers injected CBD solutions of 15 mg/kg, 30mg/kg, 60 mg/kg, and control in rats for 14 days. Using spectrophotometry, the researchers found that acute and chronic injection of CBD increased mitochondrial complex activity in the hippocampus, striatum, and prefrontal cortex. Past literature has shown that decreased mitochondrial activity from baseline may lead to decreased neural plasticity and function, therefore, CBD may help to return these values back to baseline in rat models (Valvassori et al., 2013).

Cannabidiol and Measures of Cognitive Health

Cognitive health can be defined as a person's ability to think clearly, learn, and remember (U.S. Department for Health and Human Services). Various biomarkers have been used to evaluate cognitive health. Proteins such as beta-amyloid 42 and tau, are commonly used in Alzheimer's research (Vos et al., 2013) and tau is also used to determine progression of dementia (Hansson et al., 2006). Through methods such as magnetic resonance imaging (MRI) and fluorodeoxyglucose positron emission tomography (FDG-PET), researchers can determine

activity levels within the brain. Furthermore, FDG-PET provides information on metabolic activity occurring in the brain. Areas that have decreased metabolic activity correlate with decreased synaptic activity and thus, a decrease in cognitive health. These measures can assist researchers in determining whether there is neurodegeneration. Additionally, volumetric measurements taken with MRI can determine whether atrophy is occurring in certain areas of the brain.

Alzheimer's disease (AD) can be visualized as beta-amyloid deposits and tangles devised of hyperphosphorylated tau protein (Blennow et al., 2006). The potential neuroprotective actions of CBD may act to combat hyperphosphorylation of tau protein and thus, reduce the accumulation of these AD- causing proteins (Esposito et al., 2006). The preliminary research into CBD's effects on cognitive health are intriguing; however, more research is needed in order to provide any clear recommendations.

Another aspect of cognitive health includes working memory. A study on young healthy adults (age 18-30) sought to determine whether vaping CBD influenced verbal memory. The participants (n=39) were divided into two groups; a CBD group (12.5 mg of CBD) and a placebo group. All participants complete a verbal memory test consisting of 15 nouns, and then were asked to vape their treatment for 20 minutes. After the vaping session, participants completed another verbal memory test with the same 15 nouns. The group which vaped CBD had a better (10%) performance improvement on the working memory task (Hotz et al., 2021). In another study, 15 healthy participants were given either 600 mg oral CBD or placebo and 3 hours after ingestion, cerebral blood flow at rest was measured using fMRI. Then, working memory was evaluated using forward, backward, and n-back tasks while MRI was used to measure cerebral blood blow. Researchers concluded that 600 mg oral CBD significantly increases cerebral blood

flow to the hippocampus (Bloomfield et al., 2020). Although there were no differences between the groups with respect to memory tasks, this change in cerebral blood flow suggests that there may be a potential for CBD brain function.

Biomarkers of Brain Health

Researchers often use circulating biomarkers to evaluate the health of an individual. Brain-derived neurotrophic factor (BDNF) is an important biomarker for neuronal function and is a measure of brain health. BDNF was the second neuronal growth factor identified and was discovered after Nerve Growth Factor (NGF), which was found to benefit neuronal growth and survival (Levi-Montalcini & Hamburger, 1951). Although BDNF and NGF are different members of the neurotrophic factor family, they both serve the role of providing protection for the peripheral and central nervous systems (Nguyen et al., 2009).

BDNF promotes neurogenesis in the body. More specifically, BDNF binds to tyrosine kinase B (TyrkB) which leads to the activation of cAMP binding proteins (CREB, CBP) that activate pro-survival genes (Bathina & Das, 2015). In an adult mouse brain, BDNF is distributed in high concentrations in the hippocampus and cerebral cortex (Hofer et al., 1990). This distribution is similar to that of humans with the highest BDNF concentrations located in the hippocampus, amygdala, cerebellum, and cerebral cortex (Miranda et al., 2019). The hippocampus is a region of the brain in which memory and plasticity occur (Neves et al., 2008). As a result, BDNF is an important neurotrophic factor for neurogenesis and synaptic plasticity.

In patients with neurodegenerative diseases, serum BDNF concentrations are usually low. A study examining BDNF concentrations in 47 Parkinson's Disease patients revealed that BDNF concentrations in blood serum decreased significantly compared with the 20 control patients. Interestingly, researchers found that BDNF concentrations increase as Parkinson's Disease

progresses. Scientists theorized that this may be associated with a neuroplasticity-type mechanism (Scalzo et al., 2010). Alzheimer's Disease also affects BDNF concentrations. Researchers measured BDNF concentrations in preserved tissue samples from patients with AD and discovered that these patients had lower BDNF concentrations in the hippocampus and parietal cortex when compared to age matched controls (Hock et al., 2000). These regions of the brain are often areas where learning, memory, and somatosensory input occurs (Anand & Dhikav, 2012).

Although concentrations of BDNF are mostly studied in the brain, it can be found in the blood with detectable concentrations localized in the bladder, lungs, and colon. Mean values of BDNF in healthy individuals' plasma and serum are 92.5 pg/ml and 22.6 pg/ml respectively. (Lommatzsch et al., 2005). However, these values are not concrete and thus there is a significant range in BDNF concentrations in healthy individuals. For instance, one study reported mean serum BDNF of 32.69 ng/ml in a population of 259 healthy individuals (Naegelin et al., 2018). Another study reported mean BDNF serum values to be 16.3 ng/ml in a population of 118 healthy individuals (Lang et al., 2004). The range of BDNF in serum is quite large and thus, when considering BDNF concentrations, establishing an individual baseline is important.

Cannabidiol and Brain-Derived Neurotrophic Factor

While CBD has the potential to induce cognitive benefits, there is not much research exploring how the compound affects BDNF concentrations. Currently, studies similar to this present study will be detailed in the discussion section. More studies like this are underway.

CHAPTER III

METHODS

Participants

Healthy, physically active male and female individuals (18-50 years) were randomly assigned to placebo or treatment groups. Participants were excluded from the study if they had any diagnosed chronic disease (cancer, cardiovascular disease, type 2 diabetes, etc.) or uncontrolled moderate to severe depression or anxiety or a history or severe psychiatric disorders (schizophrenia, psychosis, etc.). These participants met the American College of Sports Medicine (ACSM) guidelines for physical activity, which recommends that adults participate in moderate intensity aerobic physical activity for 30 minutes at least 5 days a week (ACSM 2022). Additionally, participants were to have not used any CBD or tetrahydrocannabinol (THC) containing products within the 6 weeks prior to enrolling in the study. This project was approved by the University of Northern Colorado's Institutional Review Board.

Study Intervention

This was a double-blind, placebo-controlled clinical trial. Participants (N=35) were randomly placed into either CBD Treatment Group (CB) or a placebo Group (PL). The CBD group consumed capsules containing 50 mg of CBD (Six Degree Wellness, Boulder, Colorado) while the placebo group consumed a calorie-matched capsule which was filled with 225 mg of medium-chain triglyceride (MCT; Nutiva, Point Richmond, California). All participants were instructed to consume one of their assigned capsules nightly before bed for 8 weeks.

Study Visits

Participants visited the lab on two occasions. The first visit occurred just prior to the start of the intervention (Visit 1) and the second visit was conducted at the end of the last week of the intervention (Visit 2). Participants completed the informed consent at the beginning of visit 1 followed by body size and composition analysis. Then, fasted blood samples were collected. During the second visit, body size and composition were measured again, and another fasted blood sample was obtained. The details of the measure of body size and composition as well as the measure of neural/brain health are further explained below.

Body Size and Composition

Participants were instructed to dress in spandex before body size and composition testing. Participants were then asked their age and ethnicity for the calibration of the system. Next, participants' height (stadiometer, SECA 220, Chino, CA, USA) and weight (digital scale, Detecto, Webb City, MO, USA) were collected and entered into the system. Then, body composition measurements were collected using air displacement plethysmography and a predicted thoracic volume measurement that was provided by the BodPod (Cosmed Inc., Concord, CA, USA; Dempster & Aitkens, 1995) calibrated software. Measurements included: body mass (M), lean body mass (LM), body fat percentage (BF%).

Brain Health Biomarker Analysis

Blood samples were collected by a certified phlebotomist into serum separator tubes. Participants were fasted, which meant consuming nothing but water for 8 hours prior to blood collection. Participants also avoided moderate to strenuous exercise for 72 hours prior to blood collection. The tubes were placed in a tube rack and were left untouched for 30 minutes before

being spun at 2000 g for 15 minutes at room temperature in a centrifuge. Then the serum was pipetted into Eppendorf tubes (Eppendorf AG, Hamburg, Germany) and frozen at -80 degrees C.

Once all samples were collected, they were analyzed for BDNF using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, Minnesota, USA). The 96-well plates were pre-coated with anti-human BDNF antibody. A standard curve was prepared by combining the standard solution with the calibrator diluent (RD6P) and then titrating that sample seven times to create the standard curve. Next, 100 microliters of assay diluent (RD15) were separated into the wells and 50 microliters of either the standard, control, or sample at 1:50 dilution were added into each of their respective wells. After a 2-hour incubation process, human-free BDNF conjugate was added into each well and then incubated at room temperature for one hour. Next, the wells were aspirated and washed using the wash buffer. Then, 200 microliters of substrate solution were added into the wells after they had been blotted dry with a paper towel and the plates were incubated for another 30 minutes away from the light. After the incubation, 50 microliters of stop solution was added into each well. The wells were analyzed for BDNF at 450 nm in the microplate reader (Agilent; Santa Clara, California, USA) and analyzed using GraphPad (San Diego, California, USA). Then BDNF concentrations from GraphPad were multiplied by the dilution factor resulting in the adjusted BDNF concentrations.

CHAPTER IV

STATISTICAL ANALYSIS & RESULTS

Statistical Analysis

The purpose of this study was to investigate whether a longer-term CBD intervention would significantly change BDNF concentrations in human serum. The hypothesis was that:

H1 8 weeks of daily CBD ingestion would significantly increase BDNF concentrations when compared to a placebo consuming control group.

The outcome variables measured in this study were age, physical characteristics (height, weight, body fat percentage), and BDNF concentrations. Mean and standard deviations were determined for both groups at both time points. A t-test was used to determine whether there was significant difference between the CB group and PL group at the pre intervention time point. A 2 (group) x 2(time) ANOVA was used to determine whether the 8-week intervention period produced any significant changes in the outcome variables. Significance was set at $p<0.05$. All statistical analyses were performed using Microsoft Excel (Microsoft; Redmond, Washington, USA).

Participants

A total of 35 individuals participated in this study. Eighteen participants were placed in the CBD group (CB) and seventeen were in the placebo group (PL). Within the CB group, 7 were male and 11 were female. In the PL group, 11 were female and 6 were male. When groups were combined, the range of ages was from 18 to 42 years of age. The age range for CB and CN

was between 18 and 36 years and between 20 to 42 years, respectively. There were no significant differences between PL and CB with respect to age at the pre intervention time point (Table 1).

Body Size and Composition

Participant height and body mass are presented in Table 1. The ranges in height were 152-183 cm and 156-182.5 cm for CB and PL groups, respectively. Body mass ranges were 49.48-103.73 and 55.47-101.66 kg for CB and PL groups, respectively. There were no differences between the groups at the pre intervention time point for height (0.95) or for body mass (0.935). There was no time effect (p=0.87) or group x time interactions (p=0.82) for weight.

Table 1

Summary of Demographics and Body Composition Pre-Intervention

Pre-Intervention	CBD Group (CB)	Placebo Group (PL)	Overall
Age (years)	24.11	27.88	25.94 ± 6.5
Height (cm)	169.13	169.47	169.3 ± 10.3
Body Mass (kg)	71.92	72.81	72.35 ± 13.89

Note. There were no significant differences between groups.

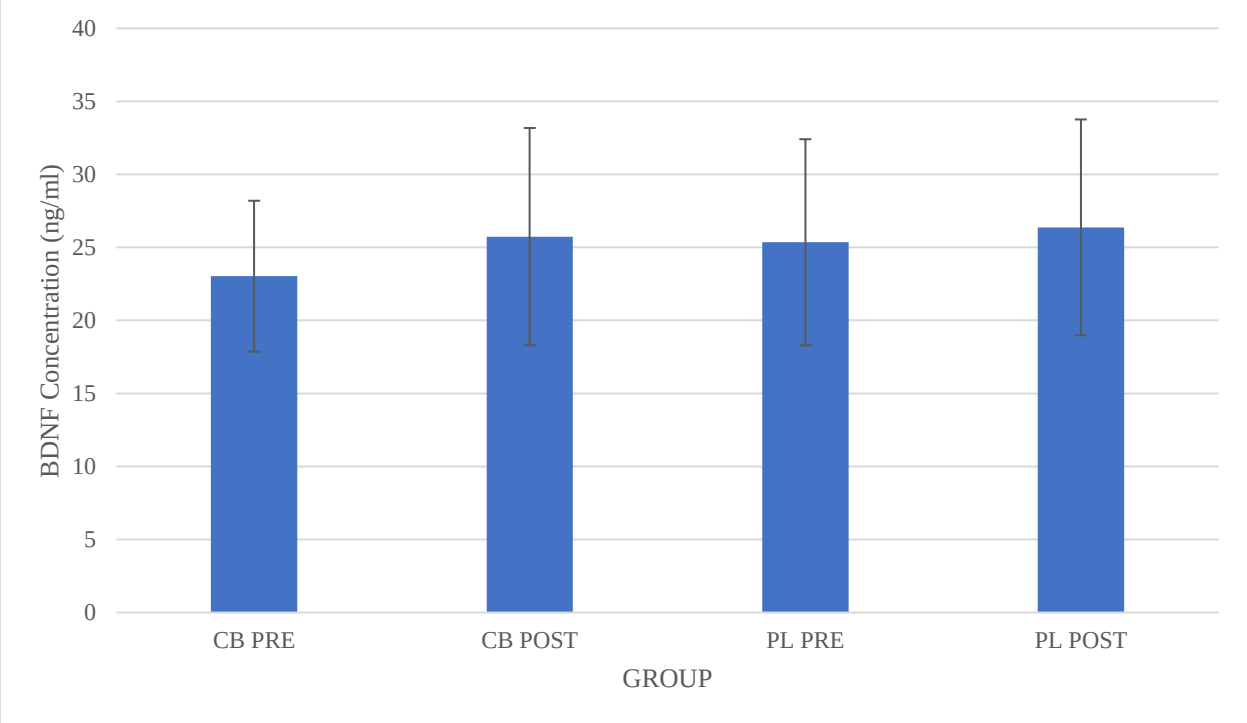
Brain-Derived Neurotrophic Factor Concentrations

BDNF concentrations remained consistent in both the pre-intervention and post-intervention measurements (Figure 1). When both groups were combined, the mean pre-intervention BDNF value was 24.19 ± 6.3 ng/ml, while the mean post-intervention value was 26.05 ± 7.4 ng/ml. The mean concentration of BDNF at the pre intervention time point were

25.35 ± 7.3 ng/ml and 23.03 ± 5.2 ng/ml in the PL and CB groups, respectively. The mean concentration of BDNF at the post intervention value were 26.37±7.62 ng/ml and 25.74±7.44 ng/ml in the PL and CB groups, respectively. There was no difference between PL and CB groups at the pre intervention time point (p= 0.26) and there was not, time effect (p=0.81), or interaction (p=0.91) when pre and post values were compared.

Figure 1

Brain-Derived Neurotrophic Factor (BDNF) Concentrations (ng/ml) in Cannabidiol (CB) and Control (PL) Groups at the Pre- Intervention (PRE)and Post Intervention (POST) Time Points



Note. Values presented are mean +/-SD. There are no significant differences between groups.

CHAPTER V

DISCUSSION

This study found that 50 mg of CBD per day for 8 weeks did not change serum BDNF concentrations in healthy volunteers. The participants in this study were considered healthy. Mean body fat percentage (BF%) pre-intervention was 22 ± 7.7 % for the CBD group and 22.6 ± 8.6% for the CON group. Post-intervention average BF% were 22 ± 7.5% and 23.5 ± 6.9 %, respectively. For healthy individuals, normal BF% for men is 17.6-25.3% and for women is 28.8%-35.7% (Branco et al., 2018). While there was no effect of the CBD intervention on body composition, this outcome was not expected because of the short study duration.

The effects of CBD on body composition are still relatively unexplored in humans. A study performed on adult patients with non-insulin treated type 2 diabetes demonstrated that 100 mg of CBD taken orally twice a day for 13 weeks resulted in no change in weight, waist circumference, and visceral body fat (Jadoon et al., 2016). There are animal studies exploring the CBD and body composition question, but even their results are inconsistent (Gáll et al., 2020; Ignatowska-Jankowska et al., 2011). One study explored the effects of 4 different diets on the development of liver disease in male mice. These diets included: a normal diet, a high-fat diet, a high-fat diet with CBD, and a high-fat diet with THC. The mice on the high-fat CBD diet experienced increased body weight gain and improved glucose tolerance compared to the normal diet (Gorelick et al., 2022). Given these results, future research exploring the relationship between CBD use and body composition is needed.

The ability of CBD to change body composition may be linked to proteins which are also involved in the regulation of glucose homeostasis and lipid metabolism. One such protein is peroxisome proliferator-activated receptor gamma (PPARγ), which upregulates genes involved in glucose uptake (Evans et al., 2004). Another train of thought has been that CBD ingestion activates CB1 receptors which increases food uptake in mammals (Koch, 2017). Over-activity of the endocannabinoid system, which contains these CB1 receptors, can lead to responses such as fat accumulation, increased appetite, and increased motivation to eat (Tibiriça, 2010). In fact, the endocannabinoid system is complex and different mechanisms can have a significant impact on food uptake. In a study examining CBD's impact on food intake in adult rats, researchers injected various drugs including THC, CBD, diazepam, and amphetamine at 0 mg/kg, 1mg/kg, 3 mg/kg, and 10 mg/kg, respectively. The rats were injected 1 hour prior to feeding and at the end of the hour, the rats were placed back into their cages. The rats that were injected with THC experienced increases in food intake and consumption, while the rats injected with diazepam and amphetamine experienced decreases in food consumption. Interestingly, CBD did not have any effect on food consumption (Wiley et al., 2005). In a later study, when rats underwent daily injections of CBD at doses of 2.5 and 5 mg/kg for 14 days, there was a significant decrease in body weight in the 5 mg/kg CBD dose compared to the 2.5 mg/kg CBD dose groups. (Ignatowska-Jankowska et al., 2011). An even later study injected CBD into rats at a dose of 3 mg/kg for 3 days while on either a standard diet, high fat diet, and a high fructose diet. Rats on the standard diet and high fructose diets failed to experience any change in body weight, while the rats on the high fat diet experienced significant increases in body mass and decreases in food consumption. (Wierucka-Rybak et al., 2014). It is important to note that the increase in body mass but decrease in food intake could be in part due to high fat diets being more calorically

dense than a standard diet. To summarize, although there were no CBD mediated changes in body composition observed in the present study, it is possible that a longer intervention with a higher dose of CBD may yield a different result. It is clear that more human clinical trials are necessary before a conclusion about CBD's effect on body composition can be made.

The BDNF concentrations obtained in this study are similar with the values reported in previous literature. Researchers examined the reproducibility of serum BDNF values among six commercial assays. They discovered that human serum BDNF concentrations ranged from 8 to 46 ng/ml (Polacchini et al., 2015). Another, more recent study, found that mean serum BDNF concentration in 259 volunteers was 32.97 ± 8.33 ng/ml (Naegelin et al., 2018). While both studies have demonstrated that mean concentrations in healthy individuals are similar to the BDNF concentrations reported in the present study, it is important to note that there is still uncertainty in the ranges of BDNF concentrations in serum in humans.

The potential role of BDNF as a biomarker of brain health has led to many recent discoveries about its neuroprotective effects. Earlier studies found that animal models with decreased circulating BDNF concentrations had more depressive-like symptoms (Duman & Monteggia, 2006; Dwivedi, 2009). This is an ideal model to explore the potential for CBD to change BDNF concentrations. For example, an older study explored the molecular and behavioral effects of rats treated with 14 days of saline, CBD (15mg/kg, 30mg/kg, or 60 mg/kg), or imipramine (30 mg/kg) using a forced-swimming test and an open field test. Treatments were administered 60 minutes prior to the tests. After the tests, the rat BDNF concentrations were measured in the hippocampus, prefrontal cortex, and the amygdala using an ELISA. CBD increased BDNF concentrations in the hippocampus (Réus et al., 2011). In another study, researchers explored CBD's role in mediating brain ischemia using mice. In this study, mice

received a short-term CBD treatment (10mg/kg) for 21 days after induced brain ischemia. After the treatment, it was clear that CBD increased hippocampal BDNF concentrations (Mori et al., 2017). Increased BDNF concentrations in this area of the brain may be linked to the capability of CBD to provide protection to neurons with a significant role in learning and memory.

In the present study, CBD did not affect BDNF concentrations. This could possibly be due to the dosage of CBD being significantly less proportionally to the body mass. Past studies exploring the CBD and BDNF question have some similarities to this present study. As mentioned previously, BDNF concentrations are altered in individuals with Parkinson's Disease (PD). A study exploring the influence of oral CBD capsules on patients with PD (n=21) placed participants into one of three groups: placebo (n=7), 75 mg CBD (n=7), and 300 mg CBD taken once a day for 6 weeks. Neurological assessments, psychiatric assessments, quality of life assessments and BDNF concentrations were reported. After the 6-week intervention, there was no statistical difference in neurological assessments, psychiatric assessments, and BDNF concentrations across the three groups. However, there was a statistically significant increase in quality of life for patients in the 300 mg CBD group (Chagas et al., 2014). In another study, researchers examined the impact of a single-dose of CBD on depression in male mice. Briefly, saline or CBD at doses of 7 mg/kg, 10 mg/kg, or 30 mg/kg were injected into the mice 30 minutes before a 6-minute forced swim test. After the test, their hippocampus and prefrontal cortex were removed and BDNF concentrations in these two regions were analyzed using an ELISA. BDNF concentrations were significantly higher in both the hippocampus and prefrontal cortex in the CBD treated mice compared to the control (Sales et al., 2019). A very recent study compared single and repeated CBD exposures in rats to better understand BDNF concentrations and signaling within the brain. Briefly, rats were separated into two experimental groups (single

and repeated) and then further divided into 4 subset groups which involved injections of saline, 5 mg/ kg CBD, 15 mg/kg CBD, or 30 mg/kg CBD for seven consecutive days. After the seven-day experiment, plasma and brain tissue were extracted and analyzed. A single CBD exposure of 30 mg/kg increased BDNF in the prefrontal cortex, but repeated exposures to CBD failed to induce any detectable changes in the area. Repeated exposures of 30 mg/kg CBD led to an increase of BDNF in the striatum and a slight decrease in the prefrontal cortex (Mottarlini et al., 2022).

In summary, eight weeks of daily, low dose CBD does not improve body composition or BDNF concentrations in healthy people. While improvements in body composition during the 8-week intervention period were not expected, there were no changes in BDNF. This lack of measurable response may be linked to the dose of CBD or the route of administration. It is also possible that CBD may be acting more quickly with the body adapting to the change over the course of a longer intervention.

Limitations

While the CBD capsules were considered a lower dose at 50 mg of CBD, it is possible that higher doses might induce measurable changes in circulating BDNF. For example, an early study explored the symptoms of psychosis one patient with schizophrenia. The patient took 1500 mg of CBD orally per day for four weeks. Using the Brief Psychiatric Rating Scale, researchers observed that the patient experienced significant reduction in psychotic symptoms (Zuardi et al., 1995). The same group of scientists examined 6 patients with Parkinson's Disease that had presented with psychosis symptoms for the 3 months prior to the start of the trial. Participants (4 male and 2 female) received oral CBD for 4 weeks with a mean dose between 150 mg and 400 mg depending on their current treatment regimen for Parkinson's Disease. CBD decreased symptoms of psychosis which were measured using the Brief Psychiatric Rating Scale and the

Parkinson Psychosis Questionnaire. Additionally, CBD did not worsen cognitive function and appeared to be well-tolerated by patients (Zuardi et al., 2009).

Another limitation of this study was that the intervention was done on healthy individuals. Thus, measurable CBD-related improvements in healthy individuals might not be as significant compared to a pool of participants that are unhealthy. Given these findings, another CBD intervention with daily doses in the 300mg range may have been more effective in the present study.

Future Directions

Although this study failed to demonstrate that 50 mg of CBD would induce significant changes in BDNF concentrations, higher CBD doses have potential to increase BDNF. Additionally, administering CBD to individuals with neurodegenerative diseases such as Alzheimer's and Parkinson's Disease might help further explore the CBD/BDNF question. Furthermore, BDNF concentrations have been shown to decrease as we age (Erickson et al., 2010). Therefore, exploring CBD's impact on older study populations may provide a better model to explore this question. As CBD becomes more accessible to the general population, it is important to increase the pace of research and ultimately, determine the benefits and consequences of CBD use.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL

UNIVERSITY OF
NORTHERN COLORADO

Institutional Review Board

Date:	06/23/2021
Principal Investigator:	Laura Stewart
Committee Action:	**APPROVED – Renewal with Amendment**
Action Date:	06/23/2021
Protocol Number:	2005001624R001
Protocol Title:	CBD, Inflammation, & Natural Killer Cell Study (CINS)
Expiration Date:	06/22/2022

Based on the information submitted, your study is currently: Active - Open to Enrollment. The University of Northern Colorado Institutional Review Board (IRB) for the protection of human subjects has reviewed and approved your renewal with amendment application.

As a reminder, all research must be conducted in accordance with the procedures outlined in your approved protocol.

This approval extends your expiration to the date listed above and approves the following amendments to your protocol:

We are requesting a renewal for this protocol. We have recruited 8 subjects into this project and have not had any serious adverse events. We have slightly adjusted the number of study visits (just to allow for equipment pick up and drop off). We have also changed the compensation. After consulting with Drs. Pullen and Haughian, they have agreed to list our study as an option (among others, as well as a not research opportunity) for extra credit in their courses. We have highlighted all changes to the consent form and study protocol in blue.

- Add/Modify Attachments
- Protocol Permissions

This project will continue to require renewal on an annual basis for as long as the research remains active. The investigator will need to submit a request for Continuing Review at least 30 days prior to the expiration date. If the study's approval expires, investigators must stop all research activities immediately (including data analysis) and contact the Office of Research and Sponsored Programs for guidance.

UNIVERSITY OF
NORTHERN COLORADO

Institutional Review Board

As principal investigator of this research project, you are responsible to:

- Conduct the research in a manner consistent with the requirements of the IRB and federal regulations 45 CFR 46.
- Obtain informed consent and research privacy authorizations using the currently approved forms and retain all original, signed forms, if applicable.
- Request approval from the IRB prior to implementing any/all modifications.
- Promptly report to the IRB any unanticipated problems involving risks to subjects or others and serious and unexpected adverse events.
- Maintain accurate and complete study records.
- Report all Non-Compliance issues or complaints regarding the project promptly to the IRB.

Please note that all research records must be retained for a minimum of three (3) years after the conclusion of the project. Once your project is complete, please submit the Closing Report Form.

If you have any questions, please contact Nicole Morse, Research Compliance Manager, at 970-351-1910 or nicole.morse@unco.edu. Please include your Protocol Number in all future correspondence. Best of luck with your research!

Sincerely,

Michael Aldridge
IRB Co-Chair, University of Northern Colorado: FWA00000784

Silvia Correa-Torres
IRB Co-Chair, University of Northern Colorado: FWA00000784