THESIS

COMPARISON OF FIVE ORAL CANNABIDIOL PREPARATIONS IN ADULT HUMANS: PHARMACOKINETICS, BODY COMPOSITION, AND HEART RATE VARIABILITY

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ABSTRACT

COMPARISON OF FIVE ORAL CANNABIDIOL PREPARATIONS IN ADULT HUMANS: PHARMACOKINETICS, BODY COMPOSITION, AND HEART RATE VARIABILITY

Data supporting the physiological effects of cannabidiol (CBD) ingestion in humans are conflicting. Differences between CBD preparations and bioavailability may contribute to these discrepancies. Further, an influence of body composition on CBD bioavailability is feasible, but currently undocumented. The aims of this study were to: (1) compare the pharmacokinetics of five oral CBD preparations over 4 hours; (2) examine the relationship between body composition and CBD pharmacokinetics; and, (3) explore the influence of CBD on heart rate variability. In total, five preparations of CBD, standardized to 30 mg, were administered orally to 15 healthy men and women (21–62 years) in a randomized, crossover design. Prior to and 60 min following CBD ingestion, heart rate variability was determined. Body composition was assessed using dual energy X-ray absorptiometry. Peak circulating CBD concentration, time to peak concentration, and area under the curve was superior in a preparation comprising 5% CBD concentration liquid. Fat free mass was a significant predictor ($R^2 = 0.365$, $p = 0.017$) of time to peak concentration for this preparation. Several heart rate variability parameters, including peak frequency of the high frequency band, were favorably, but modestly modified following CBD ingestion. These data confirm an influence of CBD preparation and body composition on CBD bioavailability, and suggest that acute CBD ingestion may have a modest influence on autonomic regulation of heart rate.

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Chapter 1. REVIEW OF LITERATURE

1.1. Cannabidiol: Sources and Structure

Cannabidiol is a natural component of *Cannabis* plants. *Cannabis* is a genus of dioecious flowering plants that belong to the family *Cannabaceae*. The genus originated in Central Asia, presumably somewhere in the foothills of the Himalayas (Merlin, 2003). It became widespread due to extensive cultivation for over 6000 years. Currently, three separate species are recognized: *Cannabis sativa* L.*, Cannabis indica* Lam., and *Cannabis ruderalis* Janisch., though some botanists consider the latter two as subspecies of *C. sativa* (Laursen, 2015; Turner, Elsohly, & Boeran, 1980). *C. sativa* and *C. indica* are more economically important and widely cultivated, while *C. ruderalis* is considered a hardier variety and is cultivated in harsher climatic regions (ElSohly, Radwan, Gul, Chandra, & Galal, 2017).

Since *C. sativa* is the main species purported for medicinal properties and economic potential, several different varieties arose from the original form, all possessing different physical and chemical qualities for which they were bred. *Cannabis* plants produce a number of phytochemicals that belong to three major groups: cannabinoids, flavones, and terpenes (Pellati et al., 2018). The most important of these is the unique family of phenolic compounds called cannabinoids. To date, about 120 cannabinoids have been isolated and identified. These can be classified into 11 general types. The most important types out of these 11 are tetrahydrocannabinol (THC-type) and cannabidiol (CBD-type) chemical classes (ElSohly et al., 2017). These two cannabis constituents are used in medications prescribed for treatment of epileptic seizures and as analgesics in advanced cancer. Cannabinoids accumulate mainly in the glandular trichomes that cover the leaves and buds of a plant (Kim & Mahlberg, 2003; Mahlberg & Kim, 2004). The concentration of THC and CBD in the dried inflorescence (leaves and buds)

is used to determine cannabinoid profile of a specific cultivar of *C. sativa*, which employs both quantitative and qualitative analysis (Calvi et al., 2018). Cultivation of *C. sativa* targets specific chemical profiles. Varieties cultivated for hemp production are described as "fiber type" and have low concentrations of cannabinoids, while varieties cultivated for medicinal and psychoactive purposes are enriched in cannabinoids, and specifically in the most desired components for drug production. Though the border between the "non-intoxicating" and "intoxicating" strains of Cannabis plants is somewhat blurred, some institutions established the guidelines according to the THC content, classifying plants with less than 0.3% THC as hemp and those with more than 0.3% THC as marijuana (Laursen, 2015). There is an even more precise definition of *C. sativa* phenotypes, based on THC and CBD content percentage, and their ratio, which describes three phenotypes. Phenotype I (drug-type): THC>0.5%, CBD<0.5%, ratio of THC/CBD >>1. Phenotype II (intermediate type): THC and CBD are present in various concentrations, THC/CBD ~1. Phenotype III (fiber-type): low THC contents, THC/CBD<<1. Though environmental conditions can influence cannabinoid concentrations at different stages of growth and in different parts of the plants, the THC/CBD ratio is considered to be genetically determined (ElSohly et al., 2017).

Returning to the 11 general types of cannabinoids, one class is called cannabidiol, or the CBD-type group of cannabinoids. In 1940, cannabidiol was isolated from marijuana extract and its structure described (Adams, Hunt, & Clark, 1940). Since then, more components of this group were identified and described. Currently, this chemical class includes seven distinct constituents (Hanus, Meyer, Munoz, Taglialatela-Scafati, & Appendino, 2016; Morales, Reggio, & Jagerovic, 2017). The CBD-type compounds have the same basic configuration, "50-methyl-20-(prop-1-en-2-yl)-10,20,30,40-tetrahydro-[1,10-biphenyl]-2,6-dioles retaining the trans-

(1R,6R) configuration" (Morales et al., 2017, p. 3); they differ in number and type of functional groups, and in the length of their side chains. The most active form of the CBD-type cannabidiol group, cannabidiol, exists in two optical isomers (Fig.1.1) (Li et al., 2020). Only (-)-CBD occurs naturally in cannabis plants; (+)-CBD is chemically synthesized (Burstein, 2015).

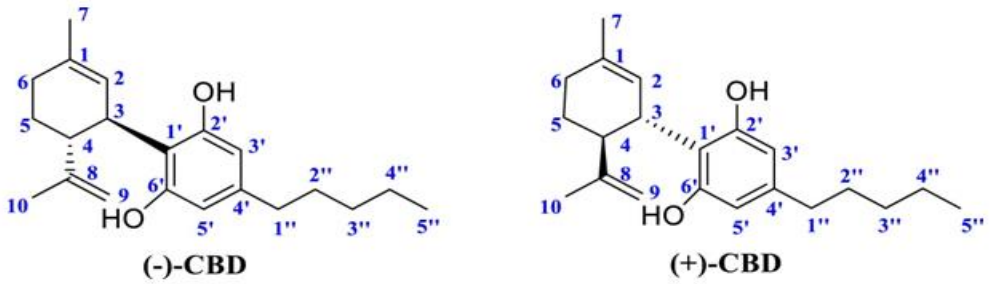

Figure 1.1. Optical isomers of CBD (Li et al. "Overview of cannabidiol (CBD) and its analogues: Structures, biological activities..." *European Journal of Medicinal Chemistry*, 2020).

Natural CBD compounds undergo biotransformation in the human body, and multiple natural metabolites are identified and described. The most recent review presents a list of 23 natural CBD metabolites and their chemical structures (Li et al., 2020). The very first and major active metabolite of CBD, 7-hydroxy-cannabidiol, 7-OH-CBD, is produced in the liver by hydroxylation at the C-7 position, followed by hydroxylation at C-6, which produces 6-OH-CBD and 6,7-di-OH-CBD. The CYP450 enzymes involved in these reactions are described in the following sections. Another metabolite, 7-carboxy-cannabidiol, 7-COOH-CBD, shows the highest plasma concentration after oral CBD administration (Taylor, Gidal, Blakey, Tayo, & Morrison, 2018). A number of other hydroxylated and carboxylated CBD metabolites is subsequently produced in the liver; and it is hypothesized that many observed biological effects may be due to active metabolites rather than CBD itself, but their modes of action are not yet elucidated (Li et al., 2020). Some of the natural metabolites of CBD and its synthetic analogs are presented in Fig. 1.2.

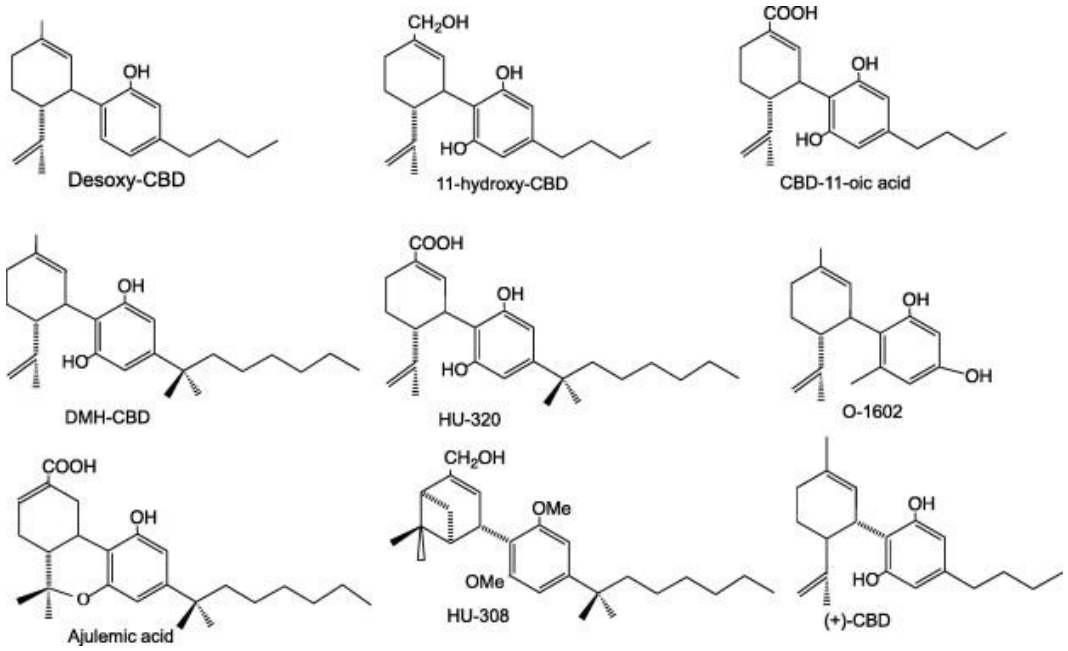

Figure 1.2. Phytocannabinoid CBD analogs (Morales et al. "An overview on medicinal chemistry of synthetic and natural derivatives of cannabidiol." *Frontiers in Pharmacology*, 2017).

1.2. Overview of Pharmacokinetic Parameters

Cannabidiol is a substance that is currently approved for specific therapeutic purposes, in contrast to THC or CBD+THC combinations that may be used for either therapeutic purposes, psychedelic effects, or both. Introduction of any substance into the human body with expectations of a particular effect is subject to an input-response relationship which consists of two parts: pharmacokinetic phase and pharmacodynamic phase (Tozer & Rowland, 2006). The pharmacokinetic phase describes the dose, frequency, route of administration, and concentrations of the substance in blood and tissues that are achieved with time. The pharmacodynamic phase describes the relationship between concentrations of the substance and its effects on the organism.

Pharmacokinetic parameters measure the systemic exposure-time profile as a function of dose and rate of input, distribution, and elimination. Different body fluids can be used for exposure assessment, such as, whole blood, plasma, serum, urine, and oral fluid (saliva). In

several studies, where CBD was administered by smoking cannabis cigarettes, oral fluid was collected for assessment (D. Lee et al., 2012; Newmeyer et al., 2014; Swortwood et al., 2017). One of the CBD studies used whole blood for pharmacokinetic assessments (Schwope, Karschner, Gorelick, & Huestis, 2011). However, the most commonly sampled fluid is blood plasma. It is easily obtained by centrifugation of the whole blood immediately following blood collection and can be stored in the freezer for long periods of time before samples are analyzed. Plasma analysis is considered an industry standard in pharmacokinetics. The rest of this section presents general information on the most common pharmacokinetic parameters: their meaning, measurement methods, and calculation algorithms. These descriptions are mostly based on the relevant information from two textbooks: *Introduction to Pharmacokinetics and Pharmacodynamics: The Quantitative Basis of Drug Therapy* (Tozer & Rowland, 2006) and *Concepts of Clinical Pharmacokinetics* (DiPrio, Spruill, & Blouin, 2010). In some places we also referred to the symposium notes from *Pharmacokinetics and Pharmacodynamics: Research Design and Analysis* (Smith, 1986).

For a drug to achieve its therapeutic effects, a specific concentration in the body is required. The most common approach is to examine the pharmacokinetics of the drug after a single dose administration. The drug is administered after collection of a baseline blood sample. After that, blood sampling continues at specific time intervals for the required number of hours. The plasma drug concentration data are plotted against time, creating a concentration-time curve (Fig. 1.3). The most important pharmacokinetic parameters are C_{max}, T_{max}, and AUC. The maximum concentration, C_{max}, also called maximum systemic exposure, is the highest concentration of the drug in the plasma. The time of maximum concentration, T_{max}, also called time of maximum exposure, is the time of C_{max} occurrence. The area under the concentration-

time curve, abbreviated AUC, is the measure of total systemic exposure. After a single oral dose, the plasma concentration continues to rise as long as the rate of absorption exceeds the rate of elimination, and eventually reaches C_{max} at T_{max}. This is the point at which the rate of absorption is equal to the rate of elimination. After that point, the rate of elimination exceeds the rate of absorption, and plasma concentration starts to decline.

The other two very useful pharmacokinetic parameters are "volume of distribution" (V_d) and "elimination rate constant" (K_e). After administration, the drug must cross multiple membranes before arriving at its target tissue, and the distribution of the drug is closely connected to perfusion. Movement of the drug out of the vasculature continues until equilibrium is achieved between the blood plasma and the tissues. At this point the volume of distribution can be calculated, customarily recorded in liters:

V_d = amount of drug in the body/plasma drug concentration.

The elimination rate constant (K_e) represents the fraction of a drug removed per unit of time and is usually expressed in reciprocal units of time (1/h, or h^{-1}); or it can also be expressed as percentage per hour: if K_e = 5%, then this amount of volume is eliminated from the body each hour. Another common characteristic in pharmacokinetics is a "half-life" ($t_{½}$), which describes a span of time during which the concentration of the drug is reduced by one half. It can be calculated based on the elimination rate (K_e) and a constant of 0.693 according to the following equation:

$t_{½} = 0.693/K_e$

One of the most important pharmacokinetic parameters is AUC: the area under the plasma concentration - time curve. The AUC represents total systemic exposure to the drug and

is determined by the given dose and rate of clearance. It is usually expressed in (mg x h)/L. AUC can be calculated by computer modeling applying the "trapezoid rule." This gives the AUC value from the time zero of drug administration to the time of the final measurement of drug concentration and is expressed as $AUC_{0\text{-}t}$, where t is the time of the last blood sample. The other parameter, $AUC_{0\text{-}inf}$, includes the small terminal area under the curve during which there is no measurement, and which is estimated by dividing the last plasma concentration by the elimination rate constant (K_e).

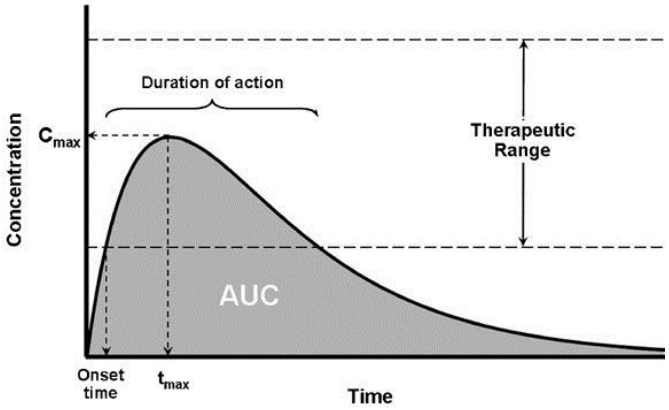

Figure 1.3. Pharmacokinetic parameters describing a typical plasma concentration time profile after an oral administration. (Mehrotra et al., "The role of pharmacokinetics and pharmacodynamics in phosphodiesterase-5 inhibitor therapy", *International Journal of Impotence Research,* 2007).

All the pharmacokinetic parameters described above are applicable to any mode of drug administration. An additional parameter, the absorption rate constant (K_a), is calculated when a drug is administered by extravascular mode, which is any mode except for intravenous injection. This value defines the fraction of a drug that is absorbed per unit of time and is expressed in 1/h units. It is hard to measure K_a directly because elimination can occur before absorption is complete. Therefore, it is usually calculated by the method of residuals, which estimates what the plasma drug concentration would be if absorption was instantaneous, and then uses the difference between the measured and estimated concentrations.

This covers all the essential pharmacokinetic parameters. However, an additional concept of "bioavailability" emerges for the drugs administered orally because absorption is always incomplete in the gastrointestinal tract. Bioavailability (F) estimates the fraction of the total given dose that reaches systemic circulation. The only way to specifically calculate F for a particular drug is to compare the AUC of intravenous administration, where the entire dose is injected into systemic circulation, to the AUC of oral administration, where only part of the dose reaches systemic circulation. The oral bioavailability of any drug is always less than 1, which means that entire administered dose never enters the system, and part of it is eliminated with feces. Alterations in formulations (for example, creating a water-based vs. an oil-based formula, or adding some active ingredients to different foods, etc.) can affect bioavailability and change absorption and distribution kinetics. Bioavailability can be calculated as following:

F = amount of drug reaching systemic circulation/total amount of administered drug

The main bioavailability study questions that are addressed in most pharmacokinetic studies are as follows: (a) Is the oral bioavailability of the drug in Formulation A different from that of Formulation B? (b) What factors contribute to bioavailability variations between the formulations? (c) What factors contribute to bioavailability variations between the subjects? Substantial inter-subject variability may exist in both pharmacokinetics and pharmacodynamics. Bioavailability is different for different drugs, and for the same drug it may reflect the type of formulation. It is possible to evaluate dose-exposure relationships more rigorously by comparing data across different studies. People differ in their responses due to a number of factors: age, genetics, body composition, state of health, behavioral habits, environmental exposures, etc. (Tozer & Rowland, 2006). Therefore, substantial inter-individual variability may be reflected in the findings of pharmacokinetic studies, especially if parallel-arm design is employed or if the

study focuses on a specific segment of the population. Analyzing all male groups *vs*. mixed male/female groups of subjects may affect concentration-time relationship due to a higher degree of clearance by the metabolically active and better perfused skeletal muscle tissue in males. Alternatively, greater body adiposity in females and/or older individuals may affect pharmacokinetics of lipophilic drugs also by altering the rate of clearance. Gastrointestinal conditions affect the rate of absorption and cardiovascular conditions affect the rate of distribution, thus influencing all pharmacokinetic parameters. Randomized cross-over study designs are the best way of minimizing the effects of inter-individual variability on PK parameters, however they cannot completely attend to the possible fluctuations of the state of individuals on separate visits. All available published pharmacokinetic information for oral CBD formulations is collated and analyzed in the following sections.

1.3. Pharmaceutical CBD Formulations and Routes of Administration

Cannabidiol formulations are based on either natural (extracted from the *Cannabis* plants) or synthetic (chemically synthesized in the laboratory) compounds. All natural *Cannabis* products contain several active compounds, with CBD/THC in different ratios. Depending on the purpose of the formulated drug, the preparation is specifically enriched in CBD, THC, or both. However, even the most purified preparation geared towards one of the compounds still contains some amount of the other. Natural CBD extract can be produced by several extraction techniques: carbon dioxide extraction, ethanol extraction, or solvent extraction with oil (Cather & Cather, 2020). The CBD extract can be categorized as full spectrum, broad spectrum, or isolate, with full spectrum containing most of the other *Cannabis* chemical compounds, and isolate being the most pure CBD (Cather & Cather, 2020).

CBD is used by consumers in a wide variety of formulations both in local and systemic applications. Popular CBD products with a local mode of administration are represented by creams, lotions, sprays, rubs, and other transdermal topicals. These are mostly effective for neuropathic pain relief (Eskander, Spall, Spall, Shah, & Kaye, 2020; Giacoppo et al., 2015; Xu, Cullen, Tang, & Fang, 2020).

Systemically, CBD can be introduced into the body either by intravascular or extravascular modes. Drugs are rarely given alone as a pure substance. Usually, they are formulated into products that are convenient for administration and are also supposed to optimize the drug's performance. The CBD/THC formulations can be designed for a fast or slow drug release depending on whether acute or a prolonged effect is desired. Delivery through the lungs facilitates fast absorption (Gould, 2015), and can be accomplished by smoking a cigarette or by using vaporizers (Arkell et al., 2019; Lanz, Mattsson, Soydaner, & Brenneisen, 2016; MacCallum & Russo, 2018; Spindle et al., 2020; van de Donk et al., 2019). Another method of fast delivery with direct absorption into the bloodstream is through the mucus membranes. Oral sprays, either sublingual, oropharyngeal, or buccal, accomplish that (Millar, Stone, Yates, & O'Sullivan, 2018). Sublingual drops are a way of combining trans-mucosal delivery with ingestion, and their mechanics are somewhat different from mucosal sprays (Guy & Flint, 2004). Intravenous formulations of cannabidiol are also available (Ohlsson et al., 1986). Although they are the most direct and complete route of drug delivery, they are also the most invasive and inconvenient for regular use. Ingestion is an administration route that involves compounds passing through the liver, which can destroy or modify cannabinoids (Gould, 2015). For the general consumer, some preferred products are commercial CBD-infused foods and drinks, oil capsules, and sometimes the addition of CBD oils or tinctures to home-made dishes. Ingestible

herbal preparations are considered hard to control therapeutically because they may contain many different compounds, some of which may be active and exert their own effect, or interfere with the effects of the main therapeutic ingredients (Tozer & Rowland, 2006).

Only two oral CBD formulations are currently licensed for medical use. The United States Food and Drug Administration and the European Medicine Agency approved two CBD formulations, Sativex® and Epidiolex®, both developed by GW Pharmaceuticals. Sativex® is an oromucosal spray, containing both CBD and THC (in 1:1ratio) suspended in ethanol anhydrous, propylene glycol and peppermint oil (Millar, Maguire, Yates, & O'Sullivan, 2020). It is produced by combining two standardized GW Pharmaceuticals' extracts (Tetranabinex®, which is high in THC, and Nabidiolex®, which is high in CBD) in almost equal amounts. THC and CBD represent approximately 70% of the product, and about 5% consists of other cannabinoids (Huestis, 2007). Epidiolex® is an oral solution that contains highly purified (98% pure) plant-derived CBD with less than 0.15% THC suspended in sesame oil at a concentration of 100 mg/ml (Leehey, 2020; Sekar & Pack, 2019).

Other ingestible CBD products have been developed by multiple pharmaceutical companies, and include liquid solutions, powders, soft-gel capsules, tablets, and more (Millar et al., 2020). In oromucosal administrations at least part of the administered dose is absorbed directly into the blood through mucus membranes, and the rest is swallowed. Ingested CBD has to be absorbed in the gastrointestinal (GI) tract, and the process of absorption has not been elucidated. As a lipophilic substance, in theory CBD should follow the mode of lipid absorption: micelle formation in the small intestine, passing through the watery brush border of enterocytes, entering lymphatic transport, and then delivered to the systemic circulation, bypassing the liver. Nevertheless, CBD is reported to be subject to extensive first-pass metabolism, which means

delivery to the liver through the portal vein. We can speculate that CBD enters GI epithelium by the "lipid route" but exits enterocytes into the capillaries rather than lacteals, and subsequently into the portal vein. It seems that the oromucosal route is somewhat superior to the ingestible route since it circumvents some of the problems associated with GI absorption and first-pass metabolism. However, it has been suggested that a substantial part of an oromucosal dose may be actually swallowed and processed through the GI route (Itin, Barasch, Domb, & Hoffman, 2020; Itin, Domb, & Hoffman, 2019). This idea is supported by a study in which cannabis-based medicine extract (CBME, formulation similar to Sativex ®) administered oromucosally *vs*. oral ingestible resulted in similar pharmacokinetic parameters (Guy & Robson, 2004). In the next section we compare the bioavailability and pharmacokinetics of oral CBD administrations.

1.4. CBD Bioavailability and Pharmacokinetics

The oral mode of CBD administration comprises oromucosal and ingestible formulations. As previously discussed, the swallowed dose takes the following route through the body: intestinal absorption in the gut, passage through the gastrointestinal epithelium into lacteals and/or capillaries and then into lymphatic circulation or the portal vein respectively. Therefore, systemic concentration, which is measured in venous blood, comes after all the possible losses in the prior sites have occurred. The term bioavailability could be applied to both the rate and the extent of drug input into systemic circulation (Tozer & Rowland, 2006). In a more narrow interpretation, bioavailability is defined as a fraction of the administered dose that became systemically available in the circulation and to the tissues where its effect is exerted.

First-pass metabolism refers to modifications of the substance by the liver, specifically those that change the chemical composition and/or the structure of the substance and thus alter plasma concentrations of the active form and its expected effect at the target tissues. In the liver,

CBD undergoes oxidation by cytochrome p450 (CYP450) enzymatic activity, which first converts it to 7-hydroxy-cannabidiol (7-OH-CBD), and then to several other hydroxylated metabolites. Studies on drug-drug interactions identified three major isoforms of CYP450 that are responsible for the biotransformation of active cannabidiol: CYP3A4, CYP2C19, and CYP2C8/9, out of which the first two are the most important (Brown & Winterstein, 2019). After this phase I metabolism, CBD and its metabolites undergo phase II glucuronidation by uridine 5'-diphosphoglucuronosyltransferase enzymes (UGT1A7, UGT1A9, and UGT2B7), which make molecules more water soluble and thus render them for easier excretion, therefore also affecting the bioavailability of active CBD forms (Brown & Winterstein, 2019).

As described in section 1.2, the bioavailability of a drug can be estimated by comparing the pharmacokinetic parameters of intravenous injection (I.V.) versus oral administration of the same dose. Data for CBD bioavailability in humans is very limited despite intravenous formulations being available. Intravenous and intraperitoneal administrations of CBD were used in multiple animal studies, but in human studies oral administration is the most common, followed by smoking (usually in combination with THC) or vaping. Only a couple of older studies (Johansson et al., 1987; Ohlsson et al., 1986) compared I.V. administration versus smoking in humans. They estimated CBD bioavailability from smoking at 31% (Ohlsson et al., 1986). Oral bioavailability of CBD was estimated based mostly on animal studies and was reported to be as low as 13-19% (Mechoulam, Parker, & Gallily, 2002). The most recent review gave an even lower estimate, 6%, which is based on data pulled together from most published pharmacokinetic information on oral CBD administration in humans (Millar et al., 2020).

The bioavailability of ingestible CBD formulations can potentially be increased in several ways. The easiest and the most applicable way of increasing CBD bioavailability is to enhance

its absorption in the GI tract. Since CBD is a lipophilic substance, its absorption depends on the presence of other lipids in the gut and on micelle formation. Fat presence in the small intestine stimulates bile secretion, emulsification of lipids, formation of smaller micelles, and more rapid transit through the brush border of the intestinal epithelium. The physiological logistics of greater absorption of lipophilic substances in combination with lipids in the gut is obvious. Subsequently, multiple studies have confirmed that CBD consumed with food or in a fed state has much higher bioavailability compared to that consumed in a fasted state. The Stott et al. study compared the pharmacokinetics of a single oromucosal dose of CBD (10 mg) administered to 12 adult males in a fasted *vs.* fed state (C. G. Stott, L. White, S. Wright, D. Wilbraham, & G. W. Guy, 2013). Even though time to peak plasma concentration (T_{max}) was delayed for about 2-2.5 hours in a fed state, the highest concentration and overall dose exposure were much higher (C_{max} = 3.66 ng/ml *vs*. 1.15 ng/ml, AUC_{0-t} = 20.21 h x ng/ml *vs*. 4.53 h x ng/ml in a fed state *vs*. fasted state respectively). As we can calculate from these numbers, a fed state increased CBD bioavailability about 3.5 - 4.5 times. Taylor et al. conducted a similar experiment, but with a significantly higher single dose of CBD oral solution (1500 mg) in 12 adult subjects, where CBD was administered following a high-fat breakfast *vs*. fasting state (Taylor et al., 2018). In this study, the T_{max} was not affected by the fed state, but the rest of the PK parameters were similarly much higher (C_{max} = 1628 ng/ml *vs*. 335.4 ng/ml, AUC_{0-t} = 8347 h x ng/ml *vs*. 1987 h x ng/ml in the fed state and the fasted state respectively). As stated by the authors, this constitutes about four- to fivefold increase in CBD bioavailability in a fed state. Crockett et al. compared the effect of four different meal compositions on CBD bioavailability (high-fat/calorie meal, low-fat/calorie meal, whole milk, and alcohol) relative to fasting state in healthy adults (about 15 subjects per group) consuming a single dose of Epidiolex® (750 mg) (Crockett, Critchley, Tayo,

Berwaerts, & Morrison, 2020). Compared to the fasting state, CBD and its metabolite exposures increased most with a high-fat/calorie meal, followed by a low-fat/calorie meal, whole milk, and to a lesser extent, alcohol. Another comparative study of a fed *vs*. fasted state with 99% pure CBD administration in an oral capsule was targeted specifically towards patients with refractory epilepsy (Birnbaum et al., 2019). Based on results, the authors recommended that CBD medications should be taken with a meal, and preferably a high-fat meal, to increase CBD bioavailability. Patients on a ketogenic diet (which is often used as means to control epileptic seizures) may have higher and more consistent bioavailability of CBD, as the fat and caloric content of their diet is better controlled (Birnbaum et al., 2019).

After absorption in the gut, the liver is the next place where CBD is processed. As discussed above, in phase I metabolism, CBD is modified by CYP450 enzymes, which are implicated in the primary metabolism and biotransformation of the majority of therapeutic agents and xenobiotics (Zanger & Schwab, 2013). Therefore, concurrent administration of drugs that are substrates for the same CYP450 enzymes creates a sort of "competition" for the active sites, and therefore, concurrently administered drugs exhibit higher bioavailability of their original forms since less of each is modified in phase I metabolism. Several studies confirmed higher CBD availability when it was administered parallel with other drugs. Dronabinol (synthetic THC) slightly increased the bioavailability of co-administered CBD (Eichler et al., 2012). In another study, subjects received four sprays of THC/CBD (10.8/10 mg) alongside single doses of the CYP3A and CYP2C19 inducer rifampicin (600 mg), the CYP3A inhibitor ketoconazole (400 mg) or the CYP2C19 inhibitor omeprazole (40 mg) (C. Stott, L. White, S. Wright, D. Wilbraham, & G. Guy, 2013). As expected, rifampicin reduced C_{max} and the AUC of THC/CBD, and ketoconazole increased these PK parameters, while omeprazole didn't have any significant

effect on THC/CBD plasma concentrations (possibly because of the low dose). A meta-analysis of four large randomized controlled trials (two in Lennox-Gastaut syndrome patients, and two in Dravet syndrome patients) that evaluated efficacy of cannabidiol with and without concomitant clobazam administration showed a synergistic effect associated with the combination of agents (Devinsky et al., 2020). A recent review lists several other studies of CBD interaction with co-administered drugs: tacrolimus, warfarin, and valprorate (Millar et al., 2020). Hepatic impairment, which can be associated with lower CYP450 enzymatic activity, also increases CBD bioavailability, and the severity of impairment positively correlates with C_{max} and AUC parameters compared to subjects with normal hepatic function (Taylor, Crockett, Tayo, & Morrison, 2019). Nevertheless, even in healthy individuals, and without concurrent drug administration, CBD bioavailability shows very high inter-subject variability, which can be attributed to variability in CYP450 enzymatic activity. In consideration, suppression of CYP450 by means of drugs or due to hepatic impairment is not a viable option for enhancing CBD bioavailability for general consumers. Interestingly, there are natural constituents of a healthy human diet that can compete with CBD in their affinity for CYP450, and thus divert its enzymatic activity from metabolizing CBD and subsequently increase its bioavailability. Grapefruit inhibits the CYP3A metabolism of cannabidiol, which increases its plasma concentration ("PubChem compound summary for CID 644019,Cannabidiol.," 2021). Therefore, taking CBD with a meal rich in fats and with grapefruit juice seems like the most efficient way to maximize exposure and get the maximum absorbed amount out of whatever CBD dose is consumed.

The most pharmaceutically viable ways of increasing CBD bioavailability can be based on developing novel medical products. The current CBD solid-state medications in pre-clinical

or early clinical stages include but are not limited to the following: self-emulsifying drug delivery systems, improved crystal formulations, and cocrystals (Millar et al., 2020). Self-emulsifying drug delivery systems (SEDDS) use a mixture of oils, surfactants, and solvents, which, upon coming into contact with the aqueous environment in the gut, get dispersed into micro and nano sized droplets (Knaub et al., 2019). New gelatin matrix-based pellets technology is also based on a self-emulsifying delivery system containing highly purified CBD embedded in seamless gelatin matrix beadlets (Atsmon, Cherniakov, et al., 2018). Improved crystal formulations create crystalline CBD that has a melting point significantly lower than regular CBD crystals, and thus may have increased aqueous solubility. Cocrystal technology combines CBD crystals with other crystallized plant-derived compounds, which may work synergistically with CBD to enhance desired outcomes. Other ways of improving CBD bioavailability are also currently under development (Millar et al., 2020).

Understanding the pharmacokinetics of a drug is essential to understanding its pharmacodynamics, and for maximizing therapeutic effects and minimizing adverse effects. The pharmacokinetics of cannabidiol in humans was studied in different subject populations, at different doses and formulations, heated or unheated, with or without concurrent drug administration, in a fasted or fed state, and with or without analysis of adverse effects and/or psychological states. To date, 38 studies can be identified that recorded the pharmacokinetics of CBD administration in human subjects, with a minimum of two reported PK parameters (C_{max} and T_{max}) and a maximum of nine PK parameters ($T_{max,}$ C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $t_{½}$, K_e, CL/F, V/F, and K_a). The range of administration mode is quite wide: intravenous injections, smoking cigarettes, aerosol, nebulizer, vaporizer, oromucosal spray (sublingual, buccal, oropharyngeal), oral capsules, oral fluid, oral solution, oral capsules with piperine pro-nanolipospheres, self-

emulsifying drug delivery system soft gel capsules, CBD encapsulating wafer matrix, cannabis containing brownies, and more. There is also a difference between the study subjects: habitual cannabis users *vs*. non-users, patients with epilepsy, patients with hepatic or renal impairment, children, and adults (either exclusively males or mixed male/female groups). The number of subjects was highly variable across the studies and ranged from 5 (Ohlsson, 1986) to 60 (Sellers, 2013). The number of research participants influences the statistical power, and therefore some studies may be insufficiently powered to report/detect significant observations. In some studies, PK data were recorded in unconventional units: C_{max} in [(ng/ml)/mg] or [pmol/ml] instead of the usual [ng/ml]. A single dose of CBD ranged from 5.4 mg to 6000 mg, and in some studies, it was concurrently administered with THC at various CBD/THC ratios. Though the sources of CBD extract were not always clearly identified in the studies, most commonly natural CBD extracts were used, and one study used synthetic CBD analog (Izgelov et al., 2020). The analyzed body fluids included whole blood, plasma, serum, and oral fluids (saliva). The many confounding elements in multiple study protocols make it difficult to compare the primary PK outcomes across studies. In our study, a single CBD dose was administered as an ingestible, in a fasted state, to healthy adults (males and females) in order to evaluate dose-exposure relationship of different CBD formulations. Accordingly, I narrowed the list of 38 down to 14 studies in which a single dose of CBD was administered as an ingestible, in a fasted state, and at least three PK parameters were recorded (C_{max}, T_{max}, and AUC_{0-t}) (with one exception). These studies give us 27 sets of pharmacokinetic data that have the greatest relevance to our study and are suitable for dose-exposure analysis (Table 1.1): studies published in 2005 – 2018 were listed in previously published review (Millar et al.,2018); I augmented their list with PK information published in 2019 -2020. All studies that used a single CBD dose of 200 mg or higher tested the products of

GW Pharmaceuticals: Epidiolex®, oral solution or oral capsules. The differences in dosage are significant between the studies; the rows in Table 1.1 are arranged from minimal to maximal single CBD dose.

Table 1.1. Pharmacokinetic parameters for oral CBD administration in comparable studies.

Study	Formulation, Administration, CBD Single Dose (mg)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-t} (hr x ng/mL)
(Nadulski, Pragst, et al., 2005)	Oral capsule (CBD+THC) 5.4 mg	0.99	0.93	4.35
(Nadulski, Sporkert, et al., 2005)	Oral capsule (CBD+THC) 5.4 mg	1.0	0.95	
(Guy & Robson, 2004)	GW oral capsule (CBD+THC) 10 mg	1.27	2.47	5.76
(Cherniakov et al., 2017)	Oral capsule (CBD+THC) 10 mg	1	2.1	6.9
(Atsmon, Heffetz, Deutsch, Deutsch, & Sacks, 2018)	PTL101* CBD oral capsule 10 mg	3	3.22	9.64
(Atsmon, Cherniakov, et al., 2018)	PTL401*(CBD+THC) oral capsule 10 mg	1.25	2.94	9.85
(Knaub et al., 2019)	Oral capsule MCT-CBD** 25 mg	3.0	3.05	9.51
(Hobbs et al., 2020)	Caliper CBD water soluble 30 mg	0.9	2.82	6.80
(Hobbs et al., 2020)	Caliper CBD lipid soluble 30 mg	1.5	0.65	1.51
(Atsmon, Heffetz, et al., 2018)	PTL101 CBD oral capsule 100 mg	3.5	47.44	150
(Taylor et al., 2019)	(Epidiolex®) 200 mg	2.3	148.0	449
(Tayo, Taylor, Sahebkar, & Morrison, 2020)	(Epidiolex®) 200 mg	2.5	200.0	671
(Tayo et al., 2020)	(Epidiolex®) 200 mg	2.0	172.0	530
(Tayo et al., 2020)	(Epidiolex®) 200 mg	2.5	155.0	532
(Tayo et al., 2020)	(Epidiolex®) 200 mg	2.5	153.0	464

(Manini et al., 2015)	GW oral CBD capsule 400 mg	3	181.2	704
(Manini et al., 2015)	GW oral CBD capsule 400 mg	1.5	114.2	482
(Crockett et al., 2020)	(Epidiolex®) 750 mg	4.0	187.0	1077
(Manini et al., 2015)	GW oral CBD capsule 800 mg	3	221.1	867
(Manini et al., 2015)	GW oral CBD capsule 800 mg	4	157.1	722
(Taylor et al., 2018)	GW oral solution 1500 mg	4	292.4	1517
(Taylor et al., 2018)	GW oral solution 1500 mg	3.5	335.4	1987
(Schoedel et al., 2018)	(Epidiolex®) 1500 mg	6.13	524.5	2650
(Taylor et al., 2018)	GW oral solution 3000 mg	5	533.0	2669
(Taylor et al., 2018)	GW oral solution 4500 mg	5	722.1	3215
(Schoedel et al., 2018)	(Epidiolex®) 4500 mg	4.07	426.9	2339
(Taylor et al., 2018)	GW oral solution 6000 mg	5	782	3696

* PTL- gelatin matrix pellets technology-based formulation; ** MCT-CBD – medium-chain triglycerides.

In an attempt to evaluate dose-exposure relationship, I calculated the Pearson correlation coefficients for dose *vs*. three PK parameters. There are only nine studies that used a low single oral CBD dose between 5.4 mg and 30 mg, and the PK data are highly variable. Dose-exposure response is inconsistent for low doses. No correlation is observed between the CBD dose and C_{max}, T_{max} and AUC_{0-t} at low dose administration (Fig. 1.4 – 1.6; the correlation, r, values for each parameter are presented under the figures), which could be reflective of insufficient number of observations. If all the doses (5.4 to 6000 mg) are included in calculations, the results are more consistent, and positive linear correlation is observed between the dose and each of the PK parameters. Therefore, data are very limited on ingestible low CBD doses (both oral capsules and oral solution), and currently do not allow for the development of normative PK values for a single oral dose under 100 mg. Since most of the CBD products available without prescription contain 5 to 30 mg of CBD per serving, more studies should be warranted to explore dose – exposure relationship at these doses.

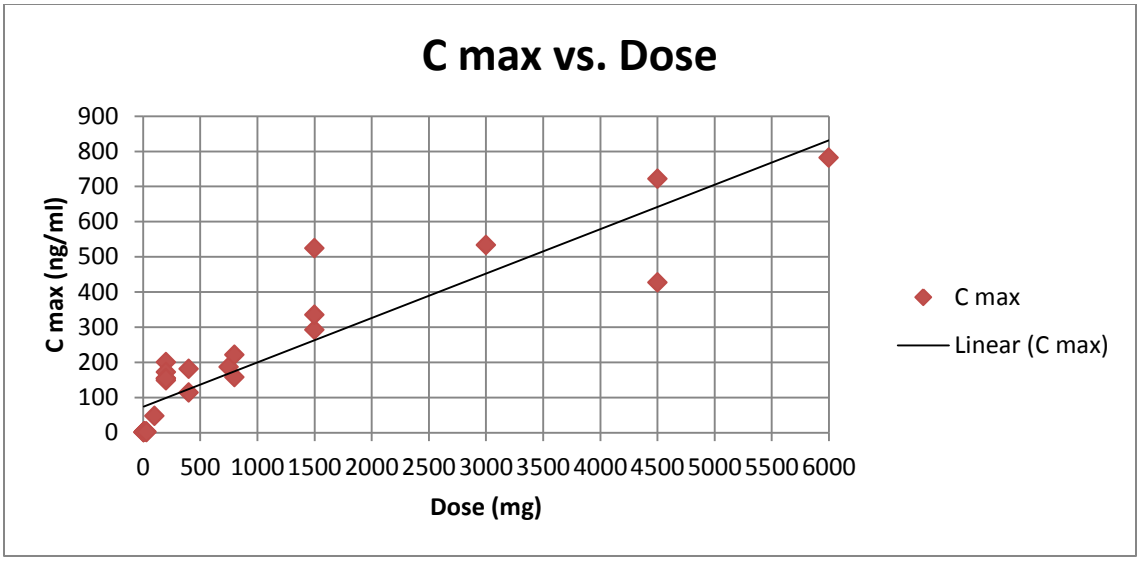

Figure 1.4. C_{max} values plotted against administered single oral CBD dose. r = 0.913 (p-value <0.01) for dose 5.4 to 6000 mg; r = 0.110 (p-value = 0.77) for dose 5.4 to 30 mg. Based on data presented in Table 1: pooled from pharmacokinetic studies published in 2004 – 2020.

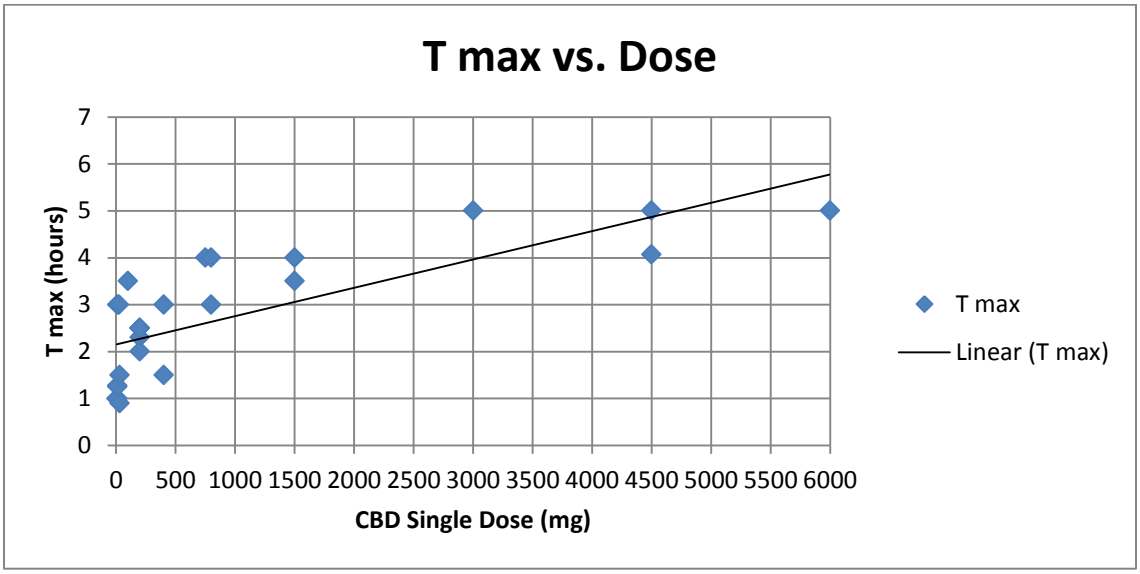

Figure 1.5. T_{max} values plotted against administered single oral CBD dose. r = 0.689 (p-value <0.01) for dose 5.4 to 6000 mg; r = 0.189 (p-value = 0.63) for dose 5.4 to 30 mg. Based on data presented in Table 1: pooled from pharmacokinetic studies published in 2004 – 2020.

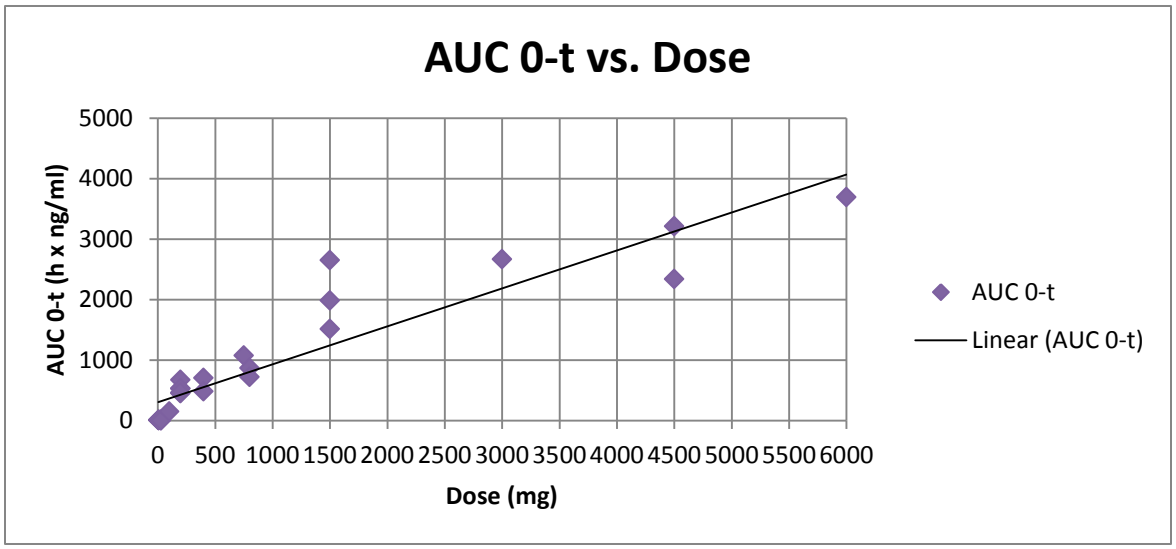

Figure 1.6. $AUC_{0\text{-}t}$ values plotted against administered single oral CBD dose. r = 0.922 (p-value <0.01) for dose 5.4 to 6000 mg; r = -0.488 (p-value = 0.22) for dose 5.4 to 30 mg. Based on data presented in Table 1: pooled from pharmacokinetic studies published in 2004 – 2020.

This dose – exposure analysis serves as a future rationale for our study of comparing pharmacokinetics of several CBD formulation since development of formulations that provide higher consistency of dose – exposure response could possibly secure better therapeutic effect of CBD products offered over-the-counter and in the free market.

A much more sophisticated approach in evaluating the dose-exposure relationship and bioavailability of oral CBD administration was employed in a recent study: to describe CBD disposition and absorption kinetics the authors used a three-compartment model with a Weibull or zero-order absorption model (Lim, Sharan, & Woo, 2020). The algorithms were quite complex and based on computer modeling, but the assessed PK data were the same: CBD dose, C_{max}, T_{max}, and AUC. The authors extracted pharmacokinetic data from 15 published studies where CBD was administered orally. The inclusion criteria were as follows: CBD single-dose 5–6000 mg, oral application (Epidiolex®, Sativex®, or oral capsule), healthy adults, either fed or

fasted state. These PK data of oral administration were compared to the Ohlsson's et al. older data of intravenous administration, which is the only CBD PK data of I.V. administration available up to date (Ohlsson et al., 1986). The analysis reveals that the CBD dose, dosage form, and feeding status affect CBD bioavailability and rate of absorption by various degrees. For oral capsule formulations, variability in the bioavailability ranged between 3.4% and 11.1%, but was not significantly associated with food. On the contrary, the bioavailability of oromucosal spray was significantly increased by the presence of food. The bioavailability of oral capsule (5.6%) and fed-state oromucosal spray (6.2%) were similar, but it was much lower in fasted-state oromucosal spray (0.9%). CBD exposures increased less than proportionally with doses of 750 mg or greater. The lowest administered CBD oral solution dose of 750 mg had 16.3% bioavailability, whereas the highest dose of 6000 mg had 3.7% bioavailability.

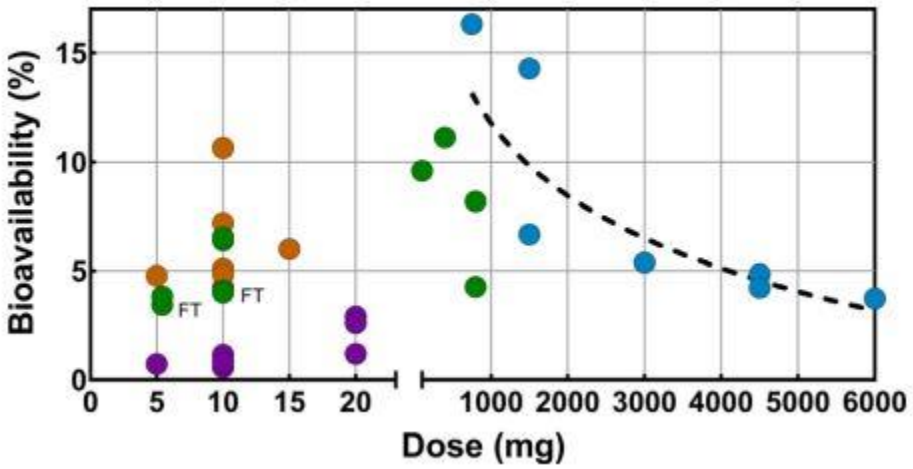

Figure 1.7. Cannabidiol bioavailability (F) and dose relationship for oral solution (blue), oral capsule (green), fed-state oromucosal spray/drop (brown), and fasted-state oromucosal spray/drop (purple). FT represents the two oral capsule studies conducted under fasting conditions. The dashed line denotes the predicted F across the dose range for oral solution. (Lim et al., "Model-Based Analysis of Cannabidiol Dose-Exposure Relationship and Bioavailability", *Pharmacotherapy*, 2020)

The PK analysis with oral solution suggests that CBD absorption saturates at around 4000 mg, where the amount absorbed into the body approaches its plateau. Fig. 1.7 presents the bioavailability % *vs*. dose graph modeled in the Lim et al. study (2020). Though not extensively discussed by the authors, in my view, the figure shows the "best value" for exposure at around a 750-1000 mg CBD dose, where the absorbed amount per unit of CBD administered is the highest. At lower doses (under 20 mg) bioavailability is higher or similar to super-high doses (above 3000 mg), but the amount of active CBD that reaches systemic circulation and target tissues at low dose administrations may be too low to exert any significant physiologic effects. At super-high doses, even though the blood concentration of CBD may be greater than at 750-1000 mg dose, also a much greater portion of administered CBD is unabsorbed and eliminated with feces. The limitations of the Lim et al. study listed by the authors addressed high intersubject variability that was impossible to evaluate in their model-based analysis due to the lack of individual subject data: sex, body weight, etc. Possibly, future studies may be structured in a way that allows to avoid this potential pitfall.

1.5. Mode of Action and Molecular Targets of Cannabidiol

The action of external chemical compounds introduced into the mammalian body can be based on their mimicking of internally produced chemical compounds that have their mode of action established through binding to certain receptors and activating specific pathways. The action of external cannabinoids is partially based on the presence of an endocannabinoid system, which is described as being composed of three major constituents: lipid signaling ligands, their G-protein coupled receptors, and the enzymes involved in ligand generation and metabolism (Silvestri, Ligresti, & Di Marzo, 2011). The two major endocannabinoids are anandamide (AEA), also known as N-arachidonoylethanolamine, and 2-arachidonoylglycerol (2-AG). The

other, less commonly measured, plasma endocannabinoids are oleoylethanolamine (OEA) and palmitoylethanolamine (PEA) (Jadoon, Tan, & O'Sullivan, 2017). The best studied receptors are CB1 and CB2, but some other receptors may also be a part of the endocannabinoid system. CB1 receptors are found throughout the body but mainly present in the central nervous system (CNS): the brain and spinal cord. CB2 receptors are also widely distributed and can be found in the CNS on basal nuclei, the hippocampus, microglia and activated astrocytes; in the peripheral nervous system (PNS); and on the immune cells, spleen, liver, and pancreas (Cather & Cather, 2020; Millan-Guerrero & Isais-Millan, 2019). Endocannabinoids are retrograde messengers derived from post-synaptic terminals; they bind to pre-synaptic cannabinoid receptors to modulate the effect of the neurotransmitter. Through CB1 receptors in the CNS, the endocannabinoid system modulates multiple aspects of central neural activities and disorders, including appetite, learning and memory, anxiety, depression, schizophrenia, multiple sclerosis, neurodegeneration, epilepsy, and addiction. Through peripheral CB2 receptors, the endocannabinoid system modulates pain, energy metabolism, cardiovascular and reproductive functions, inflammation, glaucoma, cancer, disorders of the liver, and musculoskeletal disorders (Zou & Kumar, 2018). External cannabinoids (CBD, THC, etc.), either directly by binding endocannabinoid receptors, or indirectly by upregulating or downregulating the internal endocannabinoid system, can to some extent affect all or some of the above listed physiological and pathological states.

It is well established that CBD has a very low affinity for CB1 and CB2 endocannabinoid receptors and cannot directly bind them. This explains the non-psychoactive nature of CBD when compared to THC, which has a very high affinity for CB1 and CB2. Nevertheless, even though CBD cannot directly bind CB-type receptors, there is evidence of its indirect agonism at these receptors. It can either increase CB1/CB2 constitutional activity or increase

endocannabinoid tone. The latter is accomplished by either increasing the levels of 2-AG or by inhibiting hydrolysis of AEA by fatty acid amide hydrolase (Cather & Cather, 2020; Leweke et al., 2012). Also, higher affinity of some CBD metabolites rather than CBD itself for CB-type receptors has been reported (Li et al., 2020). In addition, CBD itself and its metabolites may be interacting with some other less common and not well-understood receptors of the endocannabinoid system, or receptors that are not considered part of the endocannabinoid system but, nevertheless, participate to some extent in endocannabinoid activity (Li et al., 2020).

One example of such overlap is a group of ion channels known as "Transient Receptor Potential" (TRP) channels that are expressed throughout the body. The most extensively studied is the TRPV family: TRPV1, TRPV2, etc. They exhibit nonselective permeability to monovalent and divalent cations (Na^+, Ca^{2+}, Mg^{2+}), and their activation in neurons transduces chemical and physical stimuli from the periphery to the brain. TRPV1, also known as the capsaicin receptor, or vanilloid receptor 1, can be activated by heat, capsaicin, and, among other substances, by endocannabinoid ligand anandamide (AEA). CBD and one of its natural analogs have been shown to activate TRPV1 and TRPV2, which leads to desensitization of the neurons and possible reduction of sensory transmission (Iannotti et al., 2014; Ibeas Bih et al., 2015; Zou & Kumar, 2018). The TRP channels are involved in perception of temperature and thermal pain, modulation of noxious stimuli, and possibly involved in several biological functions, such as cell proliferation. It is hypothesized that through interaction with TRPV1 and/or TRPV2 receptors, CBD partially exerts its analgesic and anti-cancer effects (Pellati et al., 2018).

The effect of external cannabinoids in the body is not limited to interaction with the endocannabinoid system, but rather involves multitudes of other receptors and pathways that are located in almost all the systems including the nervous system, cardiovascular, gastrointestinal,

muscle, and also in adipose tissue. In fact, more than 65 discrete molecular targets have been reported in the literature for CBD and listed in a very thorough review (Fig. 1.8) (Ibeas Bih et al., 2015). Using information from the above mentioned review and some other sources I will examine the most plausible targets of CBD and the effects exerted on specific systems, organs, and tissues.

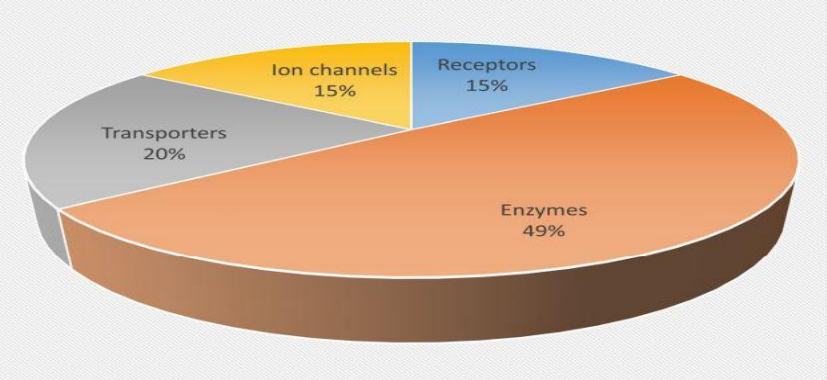

Figure 1.8. Pie chart showing the proportions of different molecular targets for cannabidiol described in the literature: percentage proportions from a total of 65 targets (Ibeas et al., "Molecular targets of cannabidiol in neurological disorders." *Neurotherapeutics*, 2015).

CBD can elicit effects within the central nervous system and within the cardiovascular system through its interactions with adenosine receptors A1 and A2, which have been implicated in regulating coronary blood flow and oxygen consumption by cardiac muscle and the brain. Also, CBD activation of A2 receptor-mediated signaling cascades have been reported to exert anti-inflammatory effects (Mecha et al., 2013). The other route of cannabidiol's anti-inflammatory and neuroprotective action is through the glycine receptors in the spinal cord. The effect of CBD on alpha-1 and alpha-1-beta glycine receptors (α1 and α1β GlyR) that play a role in development of chronic pain following inflammation or nerve injury has been reported first (Ahrens et al., 2009), with addition of alpha-3 glycine receptor (α3 GlyR) also as a CBD target in the following studies (Xiong et al., 2012). In the brain, opioid receptors that bind opioids in the

cerebral cortex are a major part of the CNS's pain control system. In one study, CBD acted as an allosteric modulator of two opioid receptor isoforms (μ and δ ORs) (Kathmann, Flau, Redmer, Trankle, & Schlicker, 2006), and thus its analgesic effect may be partially attributed to opioid system modulation. However, the most widespread effect of CBD in the nervous system might come through its agonism at 5-hydroxytryptamine (5-HT) serotonin receptors. 5-HT receptors, or serotonin receptors, are a group of G protein-coupled receptors widely distributed in the central and peripheral nervous systems, which have both excitatory and inhibitory effects. They modulate a variety of neurological and physiological functions, including, but not limited to, aggression, anxiety, appetite, cognition, learning, memory, mood, nausea, sleep, and thermoregulation. In a series of animal studies, CBD acted as an anti-stress and anti-panic agent attenuating behavioral and cardiovascular responses in acute stressful settings through activation of 5-HT1A receptors in rats (Gomes et al., 2013; Resstel et al., 2009; Soares Vde et al., 2010). In a couple of experiments with hippocampus preparations/slices, CBD showed positive interaction with the non-endocannabinoid G-protein-coupled receptor 55 (orphan GPCR 55) (Sylantyev, Jensen, Ross, & Rusakov, 2013), and with the nicotinic acetylcholine receptor (nAchR) (Mahgoub et al., 2013). Even though the significance of these interactions has not been evaluated in terms of *in-vivo* neural activity, the paramount role of the hippocampus in memory and learning, and its central position in the circuitry of the limbic system, suggests some possible routes of cannabidiol's influence in the cerebral cortex.

Outside of the nervous system, CBD targets have been studied in the cardiovascular, gastrointestinal, musculoskeletal, urinary, and reproductive systems, and in adipose tissue. Some anti-cancer effects of CBD are thought to be mediated through interaction with the peroxisome proliferator-activated receptor γ (PPARγ) (Ramer et al., 2013). PPARs, also known as glitazone

receptors, are a group of nuclear receptor proteins that function as transcription factors regulating gene expression. PPARs play essential roles in the regulation of lipid and glucose metabolism, cellular differentiation and development, and tumorigenesis. Different subtypes of PPARs are widely expressed throughout the entire body; and specifically, PPARγ subtype is expressed in the nervous system, heart and skeletal muscle, GI tract, kidney, pancreas, spleen, and adipose tissue. Effects of CBD - PPARγ interactions have been reported in a number of studies (Esposito et al., 2011; O'Sullivan, Sun, Bennett, Randall, & Kendall, 2009). Even though only 15% of CBD targets are different types of receptors (Fig. 1.8), it is worthy of mention that these interactions are the best studied up to date in comparison with other CBD – target interactions.

The largest portion of CBD targets is represented by different types of enzymes. Nineteen investigations of the effect of CBD upon 32 specific enzyme targets have been reported (Ibeas et al., 2015), which accounts for 49% of the known molecular targets of CBD (Fig. 1.8). These are mostly enzymes of xenobiotic metabolism and enzymes involved in lipid metabolism. Almost all the studies of CBD – enzyme interactions were conducted *in vitro* and with very high, non-physiologic molar concentrations of CBD, and, therefore, the significance of these interactions *in vivo* in terms of metabolic effects is yet to be investigated. The rest of the CBD targets are allocated to ion channels (15%) and membrane transporters (20%) (Ibeas Bih et al., 2015).

So far, our review of molecular targets and the mode of action of cannabidiol considered both *in vitro* and *in vivo* research, partially in disregard of its connection to any available evidence of CBD pharmacological efficacy in treatment of specific diseases and disorders, or in terms of general health enhancement. The next section will address these issues.

1.6. Clinical Applications and Therapeutic Potential of Cannabidiol

As stated in section 1.3, two CBD containing pharmaceutical products are clinically approved and available by prescription. Sativex® is indicated for the treatment of spasticity and neuropathic pain in multiple sclerosis, and as an adjunctive analgesic for moderate to severe pain in advanced cancer. It is an oromucosal spray that is administered buccally about 4-8 times per day (up to 12 times maximum). Each spray dose contains 2.7 mg THC and 2.5 mg CBD. Epidiolex® is prescribed for epileptic seizures, mostly associated with Lennox-Gastaut syndrome (LGS) and Dravet syndrome (DS). The dosage depends on the severity of the seizures and the age of the patient. It ranges from 2.5 mg/kg twice daily up to a maximum of 10 mg/kg twice daily (Brown & Winterstein, 2019). This means that clinical application of Epidiolex® is limited to much smaller doses than were used in some studies (Table 1.1). A 60 kg patient can receive a daily maximal dose of 1400 mg in two installments, while healthy subjects in the studies (Taylor et al., 2018) received up to a 6000 mg single dose of Epidiolex®. The major consideration here is possible drug-drug interactions, since LGS and DS patients are also commonly using other anti-epileptic drugs. The research that preceded approval of these two drugs was quite extensive and is omitted in our literature review. The main issue that must be addressed here is the safety and tolerability of CBD. At lower doses of oral administration, CBD was reported to be well tolerated and caused no observable adverse events (AE) (Guy & Flint, 2004; Sellers et al., 2013; C. G. Stott et al., 2013) or mild AE including somnolence, sedation and altered mood (Hosseini, McLachlan, & Lickliter, 2021). One study reported decreased appetite as an AE at oral CBD doses of 100 mg (Jadoon et al., 2016). At doses between 100 mg and 1500 mg, CBD was still well tolerated, but mild to moderate adverse effects were recorded in several studies (Bergamaschi, Queiroz, Zuardi, & Crippa, 2011; Crockett et al., 2020; Jadoon et al., 2017). The most comprehensive assessment of the safety and tolerability of CBD oral

administration in single or multiple doses between 750 mg and 6000 mg was undertaken by Taylor et al. (2018). The authors reported the following gastrointestinal disorders across all administered doses: diarrhea, abdominal discomfort, and nausea. Fewer AE were observed at doses of 750 mg and 1500 mg, while doses of 3000, 4500, and 6000 mg of CBD caused at least some of those AE in all subjects. The number of gastrointestinal AE was about the same across all three highest doses. The most common nervous system disorders resulting as side effects included somnolence, headache, and dizziness. The pattern was similar to the gastrointestinal AE with the number of cases increasing at higher doses. The other AE experienced by a limited number of subjects were flatulence, presyncope, fatigue, skin rash, and myalgia, all of which may or may not be attributed to CBD administration. The authors concluded that oral administration of CBD at doses up to 6000 mg was well tolerated, and the AE ranged from mild to moderate; no severe adverse events were observed at any doses.

For other therapeutic purposes, CBD has been marketed as a supplement and/or alternative medicine agent and is not regulated by FDA. A list of therapeutic applications of CBD, its natural and synthetic analogs, and their metabolites keeps expanding and currently includes, but is not limited to the following: anti-inflammatory (Fitzpatrick et al., 2020; Hobbs et al., 2020; Liu, Fowler, & Dalgleish, 2010; Muthumalage & Rahman, 2019), analgesic (Linher-Melville et al., 2020; Mlost, Bryk, & Starowicz, 2020), antibacterial (Appendino et al., 2008), anti-coagulant/anti-thrombotic (Coetzee, Levendal, van de Venter, & Frost, 2007), hypnotic/ sleep-inducing (Carlini & Cunha, 1981; Chagas et al., 2013; Monti, 1977), anti-anxiety/anti-depressant and anti-psychotic (Campos, Fogaca, Sonego, & Guimaraes, 2016), antioxidant (Hampson et al., 2000; Pellati et al., 2018), anti-nausea/anti-emetic (Rock et al., 2020), neuroprotective (Hampson et al., 2000; Karl, Garner, & Cheng, 2017), and anti-rheumatoid

(Lowin, Schneider, & Pongratz, 2019). The inhibitory effect of CBD on the development and spread of different cancers has been studied in human breast cancer cells (Murase et al., 2014), prostate carcinoma cells (De Petrocellis et al., 2013), and colon adenocarcinoma cells (Borrelli et al., 2014). CBD, its analogs, and metabolites acted via apoptotic mechanisms (De Petrocellis et al., 2013), by inhibiting cancer-induced inflammation (Pellati et al., 2018), and by stimulating the production of reactive oxygen species and reducing cancer cell growth (Borrelli et al., 2014).

The best studied in both animal models and human trials are anti-inflammatory properties of CBD. In a review that summarized numerous murine studies, CBD was reported to reduce inflammation in nephropathy, pancreatitis, Alzheimer's disease-related neuroinflammation, liver A-hepatitis, edema and hyperalgesia, inflammatory bowel disease, colitis, pneumococcal meningitis, hepatic ischemia-reperfusion injury, encephalitis, autoimmune encephalomyelitis, inflammatory lung diseases, and other conditions (Burstein, 2015). In human studies, *in vitro, in vivo*, as well as in clinical trials, the anti-inflammatory properties of cannabidiol have been investigated in relation to immune cells (monocytes and macrophages) (Fitzpatrick et al., 2020; Hobbs et al., 2020; Muthumalage & Rahman, 2019), gastrointestinal diseases (Couch et al., 2019), airway inflammation and fibrosis in asthma and other lung diseases (Muthumalage & Rahman, 2019; Vuolo et al., 2019), rheumatoid arthritis (Lowin et al., 2019), HIV (Costiniuk & Jenabian, 2019), Alzheimer's and Parkinson's diseases (Karl et al., 2017; Pellati et al., 2018), and inflammation of the sebaceous glands (acne) (Olah et al., 2016). This covers just a small portion of the recent publications. The molecular mechanisms of the anti-inflammatory effect of CBD in different systems, organs, and tissues have been extensively studied. In most general terms, acting through different receptors and pathways, CBD can cause a reduction in pro-inflammatory cytokines (IL-2, TNF-a, IFN-c, IL-6, IL-12 , IL-17, MCP-1, eotaxin-1, etc.) and

affect gene expression. Anti-inflammatory effect of CBD may be mediated by cannabinoid receptors (CBr), GPR55 receptors, adenosine A2A receptors, TRPV1 receptors, and CB2/5HT heterodimerization. In some studies, CBD and its metabolite cannabidiol dimethylheptyl (CBD-DMH) showed the ability to modulate production of reactive oxygen species and nitric oxide affecting production of TNF-α.(Burstein, 2015; Pellati et al., 2018). Overall, complete mechanisms of CBD anti-inflammatory actions are not elucidated yet.

Currently, most diseases and disorders (and even some non-disease states, like excessive weight, stress, and post-exercise recovery) are inevitably accompanied by inflammation in specific organs/tissues that are relevant to a particular condition. Many therapeutic effects of cannabidiol in multiple disorders can be at least partially attributed to its anti-inflammatory properties. Whether inflammation is acute or chronic, high- or low-grade, local or systemic, expressed in a patient's signs and symptoms or almost imperceptible, its effects cannot be underestimated in terms of a holistic approach to an organism. Therefore, as an anti-inflammatory agent, cannabidiol can possibly be used both in therapeutic applications for most human diseases and in general health maintenance.

1.7. Conclusion

Cannabidiol, a substance naturally produced by the *Cannabis* plants, has been a part of traditional medicine for thousands of years. In the last several decades, cannabidiol, its metabolites and synthetic analogs have been extensively studied in research laboratories all around the world. Currently, standardized CBD medications are approved for use in the treatment of epileptic seizures, spasticity, and neuropathic pain in multiple sclerosis, and as an analgesic for pain in advanced cancer. But this is just the tip of the iceberg. Cannabidiol has

tremendous therapeutic potential in almost all aspects of human health support. Current rapidly growing interest in its properties and effects is suggestive of cannabidiol possible extensive and successful use in medicine and in holistic health maintenance for hundreds of years to come.

Chapter 2. INTRODUCTION TO CANNABIDIOL STUDY

Cannabidiol has been purported to have a variety of beneficial physiological effects including but not limited to anti-inflammatory, analgesic, antibacterial, anti-coagulant/anti-thrombotic, hypnotic/sleep-inducing, anti-anxiety, anti-depressant, anti-psychotic, anti-oxidant, and neuroprotective (Appendino et al., 2008; Burstein, 2015; Campos et al., 2016; Carlini & Cunha, 1981; Chagas et al., 2013; Coetzee et al., 2007; Fitzpatrick et al., 2020; Hampson et al., 2000; Hobbs et al., 2020; Karl et al., 2017; Linher-Melville et al., 2020; Liu et al., 2010; Lowin et al., 2019; Mlost et al., 2020; Monti, 1977; Muthumalage & Rahman, 2019; Pellati et al., 2018; Rock et al., 2020). Subsequent to the legalization of marijuana in a majority of states, the popularity of different *Cannabis* components as therapeutic agents keeps growing among the general population, with the main focus on THC and CBD. The CBD industry is expanding quickly, its expansion matching that of the expanding market. Local and online markets for cannabidiol offer a very broad range of products at a very broad range of prices. The most widely offered CBD infused products are gummies, cookies, brownies, and drinks, including coffee and wine. There is also an option to add CBD cooking oil to homemade dishes. The CBD dose per serving for most products ranges from 5 mg to 30 mg. Of course, there are no listed limitations as to how many servings each consumer can eat, just recommendations, affordability, and personal preferences. A wide variety of CBD-containing edible and drinkable options combined with claims of numerous health benefits make these products quite appealing even for a skeptical consumer, and even in disregard of their cost.

Despite the enthusiasm of the CBD industry for all the health claims, the empirical evidence supporting favorable physiological responses at low CBD doses is inconsistent (Millan-Guerrero & Isais-Millan, 2019; VanDolah, Bauer, & Mauck, 2019), which is partially

influenced by CBD bioavailability. The bioavailability of oral CBD administrations has a very broad range of inter-individual variability, especially at lower doses of 5 to 30 mg, which are the most common per-serving doses of all the above listed marketed products, and at which the dose-exposure relationship is the most inconsistent and unpredictable. Differences in CBD formulations, co-administration with additional ingredients, preparation as a ready-made product *vs*. a powder to be mixed with liquid by the consumer before ingestion, personal preferences of taking CBD products in a fasting state *vs*. with a meal, CBD exposure to heat if used in home meal preparations – all can influence CBD bioavailability, and consequently alter the sought-after therapeutic effect.

One of the unexplored but potentially very important factors that influence CBD bioavailability is the body size and composition of the consumer. For example, lean mass is positively associated with total blood volume (Davy & Seals, 1994; Jones, Davy, DeSouza, van Pelt, & Seals, 1997). Thus, it is feasible that adults with a higher lean mass may demonstrate lower circulating CBD concentrations upon ingestion of the same dose under similar conditions as lower lean mass individuals on account of a larger blood volume in which to dilute the CBD. Alternatively, fat mass may influence CBD absorption as CBD is lipid soluble and can therefore potentially accumulate in adipose tissue in a manner similar to that previously reported for THC (Johansson, Noren, Sjovall, & Halldin, 1989; Wong et al., 2013). The purpose of the current study was to compare the pharmacokinetics of five oral CBD preparations standardized to 30 mg of CBD per single dose for a duration of 4 hours post-ingestion, and to examine the relationship between body composition and CBD pharmacokinetics.

One of the inconsistent physiological responses to CBD administration is the cardiovascular response (Sultan, Millar, England, & O'Sullivan, 2017). Some studies have

reported changes in heart rate and/or blood pressure following acute CBD consumption (Jadoon et al., 2017; Sultan, O'Sullivan, & England, 2020), while others have reported no measurable effect (de Faria et al., 2020; Hobbs et al., 2020). Changes in heart rate are almost always mediated by changes in the sympathovagal balance, this balance typically being described via heart rate variability. Heart rate variability refers to the inconsistency within the periods of time separating consecutive cardiac cycles. It is considered to have clinical relevance as it is able to predict future cardiac events and mortality (Hernandez-Vicente et al., 2020; Tsuji et al., 1996; Zbilut & Lawson, 1988). At the time of our study design, only one other study had examined the influence of CBD (specifically, a hemp-oil extract containing CBD) on heart rate variability in adult humans (Lopez et al., 2020), and this study reported on short-term CBD use (3 and 6 weeks) and not on acute response. Accordingly, an additional purpose of our study was to explore the acute influence of CBD ingestion on heart rate variability.

Chapter 3. MATERIALS AND METHODS

3.1 Study Subjects

Healthy adult men and women were invited to participate. Inclusion criteria included an age of 18 years and over, body mass greater than 50 kg, absence of any known gastrointestinal or metabolic disease, and willingness to abstain from all products containing CBD for 3 days prior to each study visit. Exclusion criteria included pregnancy, breast-feeding, known food allergies, autoimmune disorders, celiac disease, inflammatory bowel disease, gastrointestinal cancers, and a history of diabetes. In addition, anyone who reported experiencing a previous adverse reaction to ingesting products containing *Cannabis sativa* L. were excluded from participation. A total of 16 subjects were pre-screened for their eligibility and enrolled in the study.

3.2 Study Design

A randomized, double-blind, repeated measures cross-over study design was employed. The institutional review board at Colorado State University reviewed and approved all procedures in accordance with the principles established in the Declaration of Helsinki. Written informed consent was provided by all participants prior to commencement of any study activity.

The screening visit consisted of a review of medical history and an assessment of body composition. Then, participants reported to the laboratory on five separate mornings in a crossover design. In light of the considerable variability in CBD absorption and the resulting pharmacokinetic parameters following CBD ingestion (Hobbs et al., 2020; Izgelov et al., 2020; Millar et al., 2020; Millar et al., 2018), a crossover design was employed in an attempt to minimize the influence of inter-individual variability. Each morning began with a collection of baseline blood sample, and determination of blood pressure and heart rate variability.

Participants then ingested one of five CBD preparations (described below). Venous blood was sampled over the next 4 hours. Heart rate variability was reassessed one hour after CBD ingestion. Heart rate and blood pressure were recorded over the next four hours.

3.4 Study Procedures

Participant screening was comprised of completion of a detailed medical history questionnaire and an assessment of body size and composition using a physician's digital scale and dual energy X-ray absorptiometry (DEXA) technology (Hologic, Discovery W, QDR Series, Bedford, MA, USA). DEXA provides information on three compartments of body composition: fat mass, lean mass (or fat-free soft tissue), and bone mineral content. The three-compartment assessments are made for the whole body and also separate regional estimates of fat mass/fat-free mass are made for extremities and the trunk in accordance with a demarcation protocol. All measurements are analyzed by the software that gives estimate of body fat percent for the whole body and regional volumes. Body composition assessment implemented with DEXA technology ensures a high level of precision and is considered the most reliable method of evaluation for research purposes in clinical setting (Nana, Slater, Stewart, & Burke, 2015; Ryan et al., 2020).

The remaining five laboratory visits were identical in all aspects except for the CBD preparation that was administered. Participants reported to the laboratory on five separate mornings, each preceded by a 12-hour fast. The terminal elimination half-life of CBD administered to fasted humans varies between 6 and 32 h (Devinsky et al., 2020; Hosseini et al., 2021). To facilitate negligible baseline circulating CBD concentrations in each participant at the start of each study visit, every visit was preceded by a minimum 72 h abstention from all products containing CBD. The time of arrival was kept constant for each participant. On arrival,

participants were instrumented for measurement of heart rate (3-lead electrocardiogram (ECG)) and blood pressure (auscultation) using a physiological monitor (IntelliVue MP5 Patient Monitor, Philips Healthcare, Andover, MA, USA). Following this, a venous catheter was introduced into an antecubital vein.

Heart rate variability was determined immediately prior to, and 60 min following CBD ingestion. During 11 min of paced-breathing (metronome: 6 breaths per minute) raw ECG signals were recorded using a personal computer and an analogue-to-digital convertor (WinDaq, Dataq Instruments Inc., Akron, OH, USA) (Paxton et al., 2011) and analyzed using commercially available software (Kubios HRV), as previously described (Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2014; Tarvainen, Ranta-Aho, & Karjalainen, 2002). The first minute was considered habituation and was discarded from the analysis. Additional measurements of heart rate and blood pressure were made prior to, and 30, 60, 120, 180 and 240 minutes after CBD ingestion. All measurements and recordings were completed in a temperature-controlled (20–22 °C), dimly lit room. During the measurements and recordings, participants were situated on a medical bed, in a comfortable, semi-recumbent posture.

Venous blood (approximately 10 mL) was collected prior to, and 10, 20, 30, 45, 60, 120, 180, and 240 min after CBD ingestion. Blood was immediately transferred to chilled tubes containing ethylenediaminetetraacetic acid (EDTA). Plasma aliquots (1 mL) were separated from each of the samples and stored at −70 °C for later analysis.

Then, 90 min following CBD ingestion, participants were provided with a standardized breakfast consisting of a bagel sandwich and a choice of a non-alcoholic beverage. The breakfast was different between participants but was constant throughout each individual's laboratory

visits (i.e., whatever a participant ate during the first visit, they ate for all of the subsequent visits).

3.5 Cannabidiol Preparations

Five different CBD preparations were provided by Caliper Foods (Commerce City, CO, USA). The investigators were supplied with exact instructions on how to administer each preparation to the subjects. Each of the preparations contained a standardized CBD dose of 30 mg. The characteristics of each of the preparations are described in Table 3.1. The CBD preparations differed in their solubility (i.e., water *vs*. lipid), the concentration of the CBD liquid or powder, and additional ingredients used in each specific formulation. All preparations were administered in 227 mL (8 oz) of water, chilled, and consumed within about 30 seconds of administration.

Table 3.1. Features of the cannabidiol (CBD) preparations.

Code	Preparation	Composition & Administration
178	CBD Tincture Base	MCT oil droplet containing CBD isolate; administered in 227 mL (8 oz) of water
203	CBD Powder in Water	CBD as powder, suspended in reverse osmosis water; administered in 227 mL (8 oz) of water
340	20% CBD Concentration Liquid	Reverse osmosis water, CBD, MCT oil, quillaja extract; administered in 227 mL (8 oz) of water
472	5% CBD Concentration Powder	Water soluble CBD, sorbitol, modified food starch, natural flavors, MCT oil; administered in 227 mL (8 oz) of water
707	5% CBD Concentration Liquid	Reverse osmosis water, gum arabic, CBD, MCT oil, citric acid; administered in 227 mL (8 oz) of water

All preparations standardized to 30 mg of CBD. MCT: Medium Chain Triglyceride

3.6 Plasma Cannabidiol Analysis

Reagents and supplies: CBD and CBD-D3 were purchased from Cerilliant (Round Rock, TX, USA). Water and acetonitrile (LC-MS grade) were obtained from Millipore (Burlington, MA, USA) and formic acid (LC-MS-grade) from Sigma-Aldrich (St. Louis, MO, USA). Bond Elut dSPE Universal Sorbent was purchased from Agilent Technologies (Santa Clara, CA, USA). Chromatography was performed with a Kinetex Phenyl Hexyl column (3.0 × 50 mm, 2.6 μm) purchased from Phenomenex Inc. (Torrance, CA, USA).

Plasma samples were prepared for LC-MS/MS analysis by protein precipitation and dispersive solid phase extraction. In total, 200 μL of sample was mixed with 400 μL of ice-cold acetonitrile containing 20 ng/mL CBD-D3 in a microcentrifuge tube and vortexed for 30 s to precipitate proteins. Next, 200 mg of dSPE sorbent was added, vortexed for 30 s, and centrifuged for at 14,000 rpm for 5 min. Sample supernatants were then transferred to an autosampler vial for LC-MS/MS analysis.

Samples were analyzed with an Agilent 1290 UHPLC coupled to an Agilent 6460 triple quadruple mass spectrometer equipped with an Agilent Jet Stream electrospray ionization source (Agilent, Santa Clara, CA, USA). Cannabinoids were first chromatographically separated on a Phenomenex Phenyl Hexyl column (3.0 × 50 mm, 2.6 μm) held at 40 °C. A sample volume of 10 μL was injected and a mixture of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) at a flow rate of 0.4 mL/min. The gradient elution used was 40% B for 0.5 min, increasing to 100% B at 2 min, and held at 100% B for 1.5 min. The ionization source conditions used were as follows: positive polarity, nebulizer 45 psi; gas flow of 10 L/min at 300 °C; sheath gas flow of 12 L/min at 375 °C. The ion transitions monitored for CBD were 315.2 → 193.1/123

m/z and 318.2 → 196.1/123.1 m/z for CBD-D3. CBD was confirmed by retention time and the product ion ratio correlation between the sample peaks and corresponding standards (±20%). The limit of detection and limit of quantitation for CBD in this analysis was 0.1 ng/mL and 0.25 ng/mL, respectively. The data collection and processing were performed by using Agilent MassHunter Quantitative software (v.B.08.01). Quantitative analysis was performed with linear regression using a 6-point calibration curves from 0.25 ng/mL to 50 ng/mL.

3.7. Pharmacokinetic Analysis

Pharmacokinetic analysis of the circulating concentrations of CBD for each of the preparations was completed using dedicated software (Phoenix WinNonlin v8.2, Certara, NJ, USA). Values below the limit of quantitation were classified as "missing". Areas under the CBD concentration curves were calculated using the trapezoidal method.

3.8. Statistical Analysis

All data, unless otherwise stated, are expressed as mean and standard deviation. Statistical calculations were performed using dedicated software (Prism v8.4.3, GraphPad Software, San Diego, CA, USA). Differences in the pharmacokinetic properties between the CBD preparations were examined using one-way analysis of variance mixed-effect models with Tukey tests employed to further explore identified main effects. Similarly, differences in the characteristics describing heart rate variability before/after CBD ingestion were also examined using one-way analysis of variance mixed-effect models, and Tukey test when appropriate. Relations between CBD pharmacokinetic parameters and body size and composition values were explored using Pearson correlations, and further examined with forward stepwise regression when pharmacokinetics parameters were correlated with multiple body composition variables

(SigmaStat 3.0, Systat Software Inc., San Jose, CA, USA). The level of statistical significance was set at $p < 0.05$. The boxplots were constructed using "R" free-domain statistical software (R x64 4.0.2).

Chapter 4. RESULTS

4.1 Subject Characteristics

The progress of all participants throughout the trial (from screening and enrollment through to completion) is presented in Figure 4.1. A total of 16 participants were enrolled in the study. Due to repeated scheduling difficulties, one participant was removed from the study and replaced with a new recruit; a total of 15 participants completed all procedures. Selected physiological characteristics are presented in Table 4.1. The demographics of the subjects and their baseline physiological characteristics are typical of a broad range of adults that are free from overt cardio-metabolic disease.

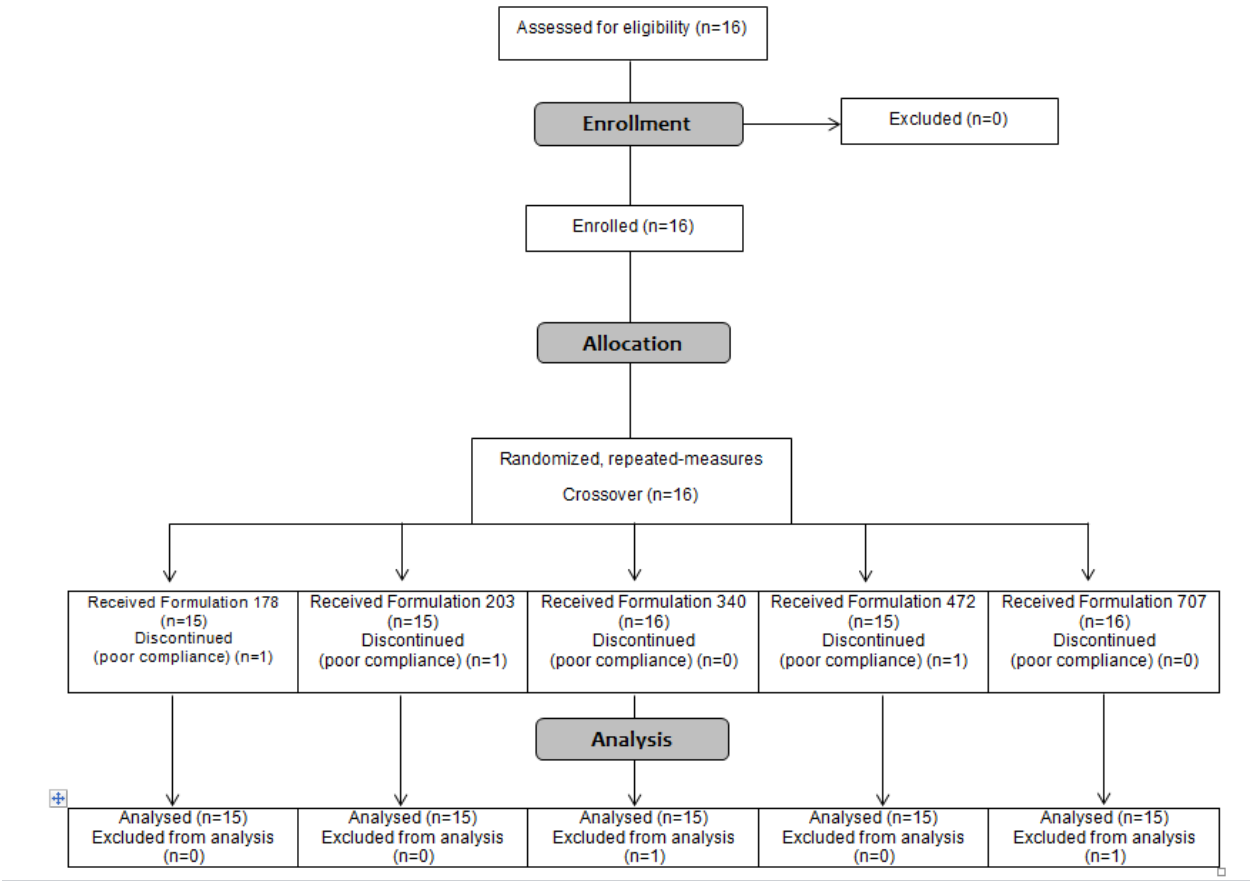

Figure 4.1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

Table 4.1. Selected physiological characteristics of study participants.

Characteristic	Mean ± SD	Range
Sex (M/F)	9 / 6	-
Age (years)	29 ± 11	21 - 62
Height (cm)	174 ± 9	155 – 191
Body Mass (kg)	75.5 ± 13.8	58.6 – 100.1
Body Mass Index (kg/m^2)	24.6 ± 3.5	18.8 – 31.4
Fat Mass (kg)	19.4 ± 4.8	12.0 – 30.1
Body Fat (%)	26.2 ± 6.6	18.0 – 40.7
Lean Mass (kg)	53.7 ± 12.3	33.3 – 70.8
Bone Mineral Content (kg)	2.4 ± 0.4	1.6 – 2.9

4.2 Pharmacokinetics of Plasma CBD Concentrations

Concentration of CBD in all baseline plasma samples for all preparations and all participants was below the limit of quantitation. Each ingested CBD preparation contained a standardized CBD dose of 30 mg. Circulating concentrations of CBD and the calculated pharmacokinetic parameters are presented in Figure 4.2 and Table 4.2, respectively. Plasma CBD concentrations rose rapidly for the 707 preparation, reaching peak concentration in 42 minutes (0.7 hours) (Table 4.2). For preparations 178 and 203, increase in plasma concentrations was slower, with the range of T_{max} 2 to 4 hours, and had the lowest inter-subject variability (coefficient of variation, CV=0.19 for both preparations). For the preparations 340 and 472, the timing for plasma concentration increase showed higher inter-subject variability than for 178 and 203 (CV=0.48 and 0.67 for 340 and 472 respectively). Informal visual inspection of Figure 4.2 suggested preparation 707 evoked the highest circulating CBD concentration, the shortest time to maximal concentration, and the greatest area under the curve during the blood collection period.

Formal statistical analysis, detailed in Table 4.2, supported several of these interpretations, although not all comparisons between preparation 707 and the other preparations attained statistical significance ($p < 0.05$). Preparation 707 also shows higher inter-subject variability for these PK parameters (Fig. 4.3). Visual examination of Figure 4.4 (Participants 2 and 16) offers some perspective on the high range of inter-subject variability.

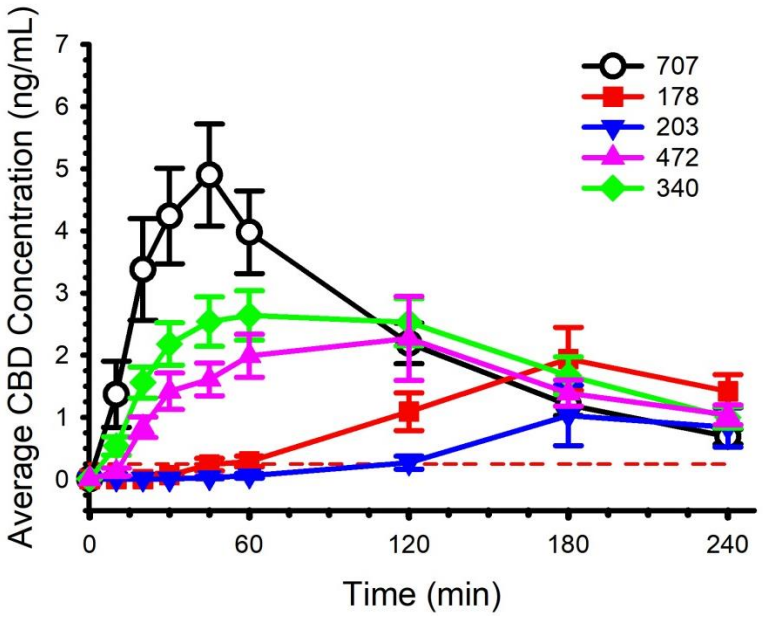

Figure 4.2. Circulating cannabidiol (CBD) concentration following ingestion of five different preparations. Dose was standardized to 30 mg. Limit of quantitation was 0.25 ng/mL and is represented by the red dashed line. Data are mean and standard error.

By the final blood collection (4 h), average concentration was above the limit of quantitation for each preparation, but not for each participant (1 participant below for 178, 2 participants below for 203, 0 participants below for 340, 1 participant below for 472, and 2 participants below for 707). The sets of the PK values are complete for preparations 340, 472, and 707. For preparations 178 and 203, it was not possible to calculate some pharmacokinetic parameters (i.e., $AUC_{0\text{-inf}}$, $t_{½}$, K_e and V_d) on account of insufficient values above the limit of quantitation during the initial 1–2 h of blood collection.

Table 4.2. Pharmacokinetic Parameters.

Parameter	178	203	340	472	707
T_{max} (h)	3.29 ± 0.61 [a,b] *n* = 14	3.39 ± 0.65 [c] *n* = 13	1.28 ± 0.62 [a] *n* = 15	1.53 ± 1.02 [b] *n* = 15	0.70 ± 0.23 [a,c] *n* = 15
C_{max} (ng/mL)	2.20 ± 1.88 [a] *n* = 14	1.29 ± 1.93 [b,c] *n* = 13	3.54 ± 1.65 [b] *n* = 15	2.88 ± 2.48 [d] *n* = 15	5.57 ± 3.32 [a,c,d] *n* = 15
$AUC_{0\text{-}4}$ (h × ng/mL)	4.58 ± 3.88 [a] *n* = 14	2.30 ± 2.77 [b,c] *n* = 13	7.81 ± 3.91 [a,b] *n* = 15	6.32 ± 4.57 *n* = 15	9.12 ± 5.21 [c] *n* = 15
$AUC_{0\text{-}inf}$ (h × ng/mL)	- *n* = 0	- *n* = 0	13.81 ± 8.2 *n* = 9	9.96 ± 8.11 *n* = 8	10.77 ± 5.71 *n* = 15
$t_{½}$ (h)	- *n* = 0	- *n* = 0	2.20 ± 1.14 *n* = 9	5.18 ± 7.07 *n* = 8	1.42 ± 0.52 *n* = 15
K_a (1/h)	0.32 ± 0.24 *n* = 5	0.24 ± 0.00 *n* = 2	1.19 ± 0.80 *n* = 15	1.87 ± 2.23 *n* = 14	1.43 ± 0.65 *n* = 12
K_e (1/h)	- *n* = 0	- *n* = 15	0.40 ± 0.21 *n* = 9	0.27 ± 0.16 [a] *n* = 8	0.56 ± 0.22 [a] *n* = 15
V_d (L)	- *n* = 0	- *n* = 0	7428 ± 2232 *n* = 9	20,178 ± 9989 [a] *n* = 8	8024 ± 6630 [a] *n* = 15

Data are mean and SD. Limit of quantitation: 0.25 ng/mL. Values below limit of quantitation were classed as "missing". n: number of observations used to calculate parameter. T_{max}: the time to maximum concentration. C_{max}: the maximum concentration. $AUC_{0\text{-}4}$: the area under the curve representing total cannabidiol exposure between 0 and 4 h. $AUC_{0\text{-}inf}$: an estimate of the total exposure to cannabidiol over time. $t_{½}$: the amount of time it takes to decrease the circulating concentration to half of its initial value. K_a: the rate at which the cannabidiol is absorbed into the body. K_e: the rate at which the cannabidiol is removed from the body. V_d: the volume of distribution, an estimate of the degree to which cannabidiol is distributed in the body tissue *vs*. the plasma. Values sharing the same superscript letter are different ($p < 0.05$).

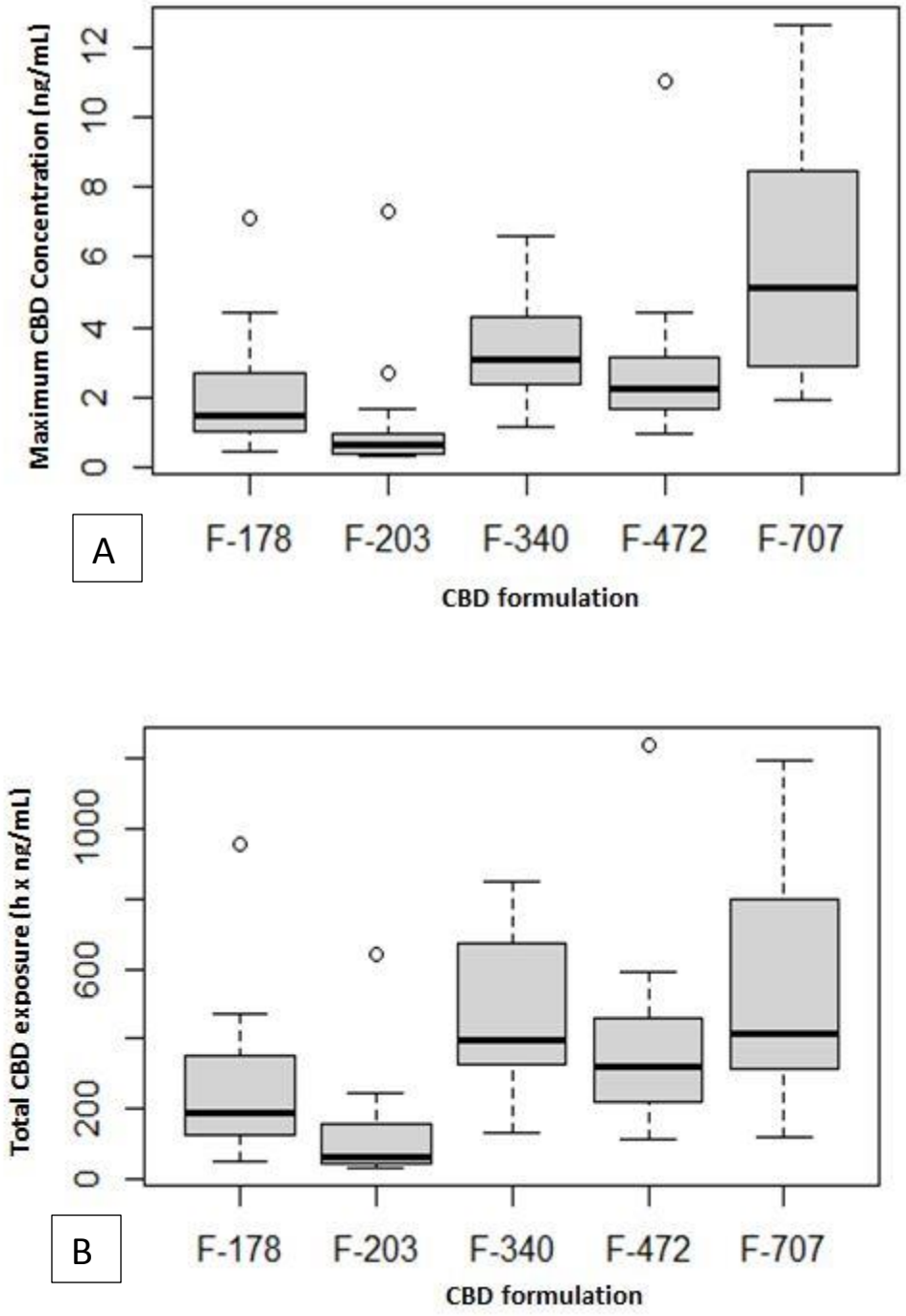

Figure 4.3. Maximum concentration of CBD in blood plasma (C_{max}) (A) and total drug exposure during four hours (AUC_{0-4}) (B) after administration of five CBD formulations/preparations.

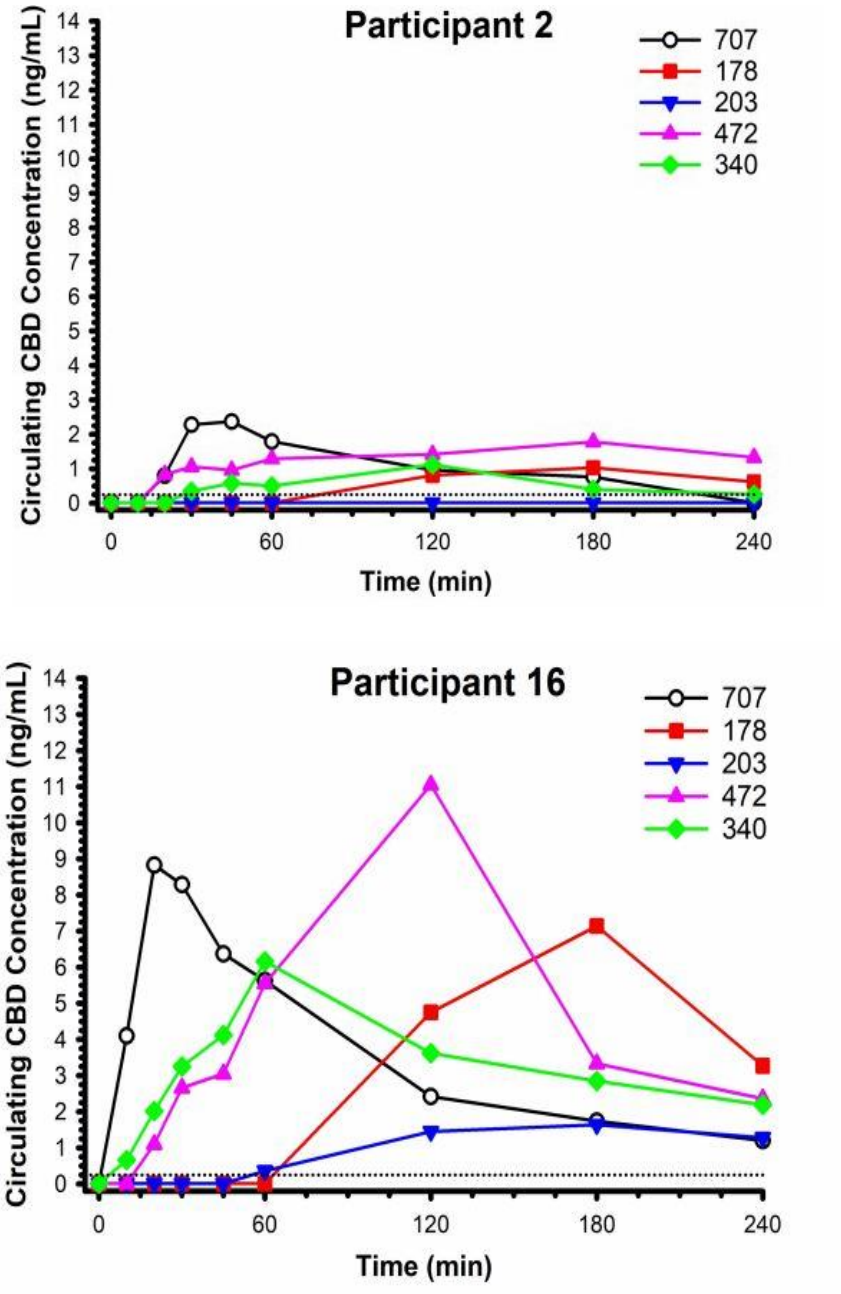

Figure 4.4. Individual data of circulating cannabidiol (CBD) concentration following ingestion of five different preparations for two participants. Limit of quantitation = 0.25 ng/mL.

4.3 Body Composition

Correlation analysis was carried out for pharmacokinetic parameters and selected physiological characteristics of the subjects. For 707, there was a correlation between T_{max} and height ($r = -0.44$, $p = 0.099$), bone mineral content ($r = -0.53$, $p = 0.04$), lean mass ($r = -0.60$, $p = 0.017$), fat free mass ($r = -0.60$, $p = 0.017$), body mass ($r = -0.52$, $p = 0.048$); and % body fat ($r = 0.46$, $p = 0.082$). When these variables were considered together in forward stepwise regression, only fat free mass remained a significant predictor ($R^2 = 0.365$, $p = 0.017$) of T_{max}. For preparation 707, there were two more relations of significance: age with C_{max} ($r = 0.64$, $p = 0.011$) and with AUC_{0-t} ($r = 0.58$, $p = 0.024$). For preparation 178 most of the correlations between T_{max} and the above listed body characteristics were positive: body mass index ($r = 0.63$, $p = 0.016$), bone mineral content ($r = 0.61$, $p = 0.021$), fat free mass ($r = 0.46$, $p = 0.095$), and body mass ($r = 0.47$, $p = 0.091$). Forward stepwise regression revealed that the only significant predictor for T_{max} was body mass index ($R^2 = 0.397$, $p = 0.016$). Also, for 178, an additional relation of significance was height with AUC_{0-t} ($r = 0.58$, $p = 0.03$). For the rest of the CBD preparations, no significant associations between PK parameters and body composition characteristics were identified.

4.4. Heart Rate Variability

Heart rate variability was assessed immediately prior to and 60 min following CBD ingestion. Circulating CBD concentrations for preparations 178 and 203 were below the limit of quantitation at 60 min, thus heart rate variability data associated with these preparations were excluded from statistical analysis. The heart rate variability data are presented in Table 4.3. There were main effects of time (all $p < 0.05$) for heart rate (decreased), R-to-R interval (increased), peak frequency of the high frequency band (decreased), Poincaré plot standard

deviation perpendicular the line of identity (increased), and Poincaré plot standard deviation along the line of identity (decreased). In addition, there were several parameters with main effects of time that did not attain statistical significance ($0.05 < p < 0.08$) including, baseline width of the R-to-R interval histogram (increased), and the ratio of Poincaré plots standard deviation perpendicular to along the line of identity (decreased). There were no time x CBD preparation interactions (all $p > 0.10$). There were no appreciable changes in heart rate and blood pressure across the 4 h of data collection (Table 4.4).

Table 4.3. Heart rate variability prior to and 60 min following 30 mg cannabidiol ingestion.

Parameter	178		203		340		472		707	
	0	**60**	**0**	**60**	**0**	**60**	**0**	**60**	**0**	**60**
Time Domain										
HR (b/min) [a]	57 ± 9	56 ± 10	61 ± 10	59 ± 10	58 ± 11	55 ± 10	58 ± 10	56 ± 9	57 ± 9	55 ± 9
R-to-R_{Int} (ms) [a]	1076 ± 173	1110 ± 194	1011 ± 183	1049 ± 200	1061 ± 190	1113 ± 187	1069 ± 190	1106 ± 195	1075 ± 170	1119 ± 180
SDNN (ms)	112 ± 50	115 ± 57	126 ± 53	129 ± 53	126 ± 53	125 ± 46	133 ± 64	132 ± 53	123 ± 55	129 ± 47
RMSSD (ms)	100 ± 43	108 ± 56	118 ± 57	116 ± 50	113 ± 47	117 ± 41	121 ± 60	125 ± 55	113 ± 54	126 ± 49
R-to-R Triangular Index (ms)	19 ± 8	19 ± 6	17 ± 5	21 ± 6	21 ± 7	22 ± 8	22 ± 8	22 ± 10	21 ± 7	20 ± 8
TINN (ms) [b]	466 ± 174	525 ± 237	537 ± 167	605 ± 188	592 ± 252	624 ± 219	619 ± 215	708 ± 255	598 ± 242	697 ± 239
Frequency Domain										
VLF_{peak} (Hz)	0.037 ± 0.003	0.035 ± 0.005	0.037 ± 0.005	0.037 ± 0.004	0.035 ± 0.004	0.035 ± 0.004	0.036 ± 0.003	0.035 ± 0.005	0.037 ± 0.003	0.036 ± 0.005
LF_{peak} (Hz)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
HF_{peak} (Hz) [a]	0.21 ± 0.03	0.20 ± 0.04	0.21 ± 0.03	0.20 ± 0.01	0.22 ± 0.04	0.19 ± 0.02	0.21 ± 0.04	0.19 ± 0.01	0.21 ± 0.02	0.20 ± 0.02
Non-linear										
SI [b]	5.2 ± 2.4	4.6 ± 2.5	4.8 ± 1.9	3.9 ± 1.4	4.4 ± 2.2	3.9 ± 1.7	4.0 ± 1.6	3.5 ± 1.3	4.3 ± 2.0	3.5 ± 1.3
SD1 (%) [a]	34.3 ± 4.9	35.0 ± 3.8	34.5 ± 5.6	33.9 ± 4.0	33.7 ± 3.1	34.8 ± 3.8	34.2 ± 4.1	35.0 ± 3.6	34.5 ± 4.6	36.2 ± 5.4
SD2 (%) [a]	65.7 ± 4.9	65.2 ± 3.8	65.5 ± 5.6	66.1 ± 4.0	66.3 ± 3.1	65.2 ± 3.8	65.9 ± 4.1	65.0 ± 3.6	65.5 ± 4.6	63.9 ± 5.4
SD2/SD1 [b]	1.96 ± 0.37	1.89 ± 0.30	1.96 ± 0.37	1.98 ± 0.33	1.99 ± 0.26	1.91 ± 0.29	1.97 ± 0.35	1.89 ± 0.29	1.94 ± 0.36	1.82 ± 0.41

Data are mean ± SD. Data were collected over 10 min during paced breathing. R-to-Rint: R-to-R interval. SDNN: Standard deviation of N-to-N intervals. RMSSD: Root mean square of successive R-to-R interval differences. R-to-R Triangular Index: Integral of the density of the R-to-R interval histogram divided by its height. TINN: Baseline width of the R-to-R interval histogram. VLFpeak: Peak frequency of the very low frequency band (0.0033–0.04 Hz). LFpeak: Peak frequency of the low frequency band (0.04–0.15 Hz). HFpeak: Peak frequency of the high frequency band (0.15–0.4 Hz). SI: Stress Index. SD1: Poincaré plot standard deviation perpendicular the line of identity. SD2: Poincaré plot standard deviation along the line of identity. SD2/SD1: Ratio of SD2-to-SD1. [a] Denotes main effect of time ($p < 0.05$). [b] Denotes non-significant effect of time ($0.05 < p < 0.08$). There were no time x CBD preparation interactions (all $p > 0.10$). Circulating CBD concentrations for preparations 178 and 203 were below the limit of quantitation at 60 min, thus heart rate variability data associated with these preparations were excluded from statistical analysis

Table 4.4. Heart rate and blood pressure response to five different cannabidiol preparations.

Time	Var.	Cannabidiol Preparation (30 mg)				
		178	**203**	**340**	**472**	**707**
Base	HR	53 ± 8	58 ± 9	56 ± 16	54 ± 10	54 ± 10
	BP	115/70 ± 9/6	116/67 ± 12/8	115/71 ± 8/6	115/70 ± 9/6	115/72 ± 11/8
30	HR	57 ± 9	58 ± 9	54 ± 10	56 ± 12	55 ± 11
	BP	121/70 ± 11/8	120/71 ± 12/5	117/72 ± 11/7	117/70 ± 12/9	121/69 ± 12/9
60	HR	58 ± 9	56 ± 10	54 ± 9	55 ± 10	55 ± 12
	BP	119/72 ± 10/6	119/68 ± 5/7	118/71 ± 10/7	119/69 ± 11/5	120/71 ± 11/8
120	HR	64 ± 10	65 ± 11	62 ± 10	64 ± 10	64 ± 12
	BP	125/68 ± 9/5	122/70 ± 11/6	124/68	118/66 ± 10/7	125/69 ± 9/7
180	HR	65 ± 10	66 ± 11	65 ± 12	64 ± 11	63 ± 10
	BP	123/66 ± 12/9	121/68 ± 10/8	122/66 ± 12/7	123/68 ± 11/9	120/67 ± 12/7
240	HR	65 ± 12	67 ± 10	64 ± 13	66 ± 11	63 ± 9
	BP	124/66 ± 9/5	125/67 ± 9/4	122/67 ± 7/8	124/66 ± 11/7	119/68 ± 12/8

HR: Heart Rate (beats/minute). BP: Blood pressure (mmHg). Data are mean and SD.

Chapter 5. DISCUSSION

The goals of the current study were to compare the pharmacokinetics of five oral CBD preparations over 4 h, to examine the relationship between body composition and CBD pharmacokinetics, and to explore the influence of acute CBD ingestion on heart rate variability. Our primary findings were: (1) compared with most of the other preparations, the preparation comprising 5% CBD concentration liquid (preparation 707; Table 4.2) evoked the fastest T_{max}, and the greatest C_{max}; (2) within each CBD preparation, there was considerable variability in the calculated pharmacokinetic parameters; some of the variability for some of the preparations could be explained by body size and composition; and, (3) CBD had only a modest effect on some of the parameters used to describe heart rate variability.

5.1 Pharmacokinetics of CBD Preparations

The high degree of variability in CBD absorption and the resulting pharmacokinetic parameters following CBD ingestion have been previously described (Hobbs et al., 2020; Izgelov et al., 2020; Knaub et al., 2019; Millar et al., 2020; Millar et al., 2018). Potential explanations for variable, and often poor, CBD absorption have pertained to properties of the CBD itself and also variability brought about by the significant first pass metabolism. To counter these potential absorption hurdles, CBD preparations have been modified, with mixed success, in a variety of ways, including generation of synthetic CBD (Izgelov et al., 2020), creation of water-soluble CBD powders (Hobbs et al., 2020), development of self-emulsifying delivery systems (Izgelov et al., 2020; Knaub et al., 2019), encapsulation of CBD within gelatin matrix pellets (Atsmon, Heffetz, et al., 2018), and liposomes (Verrico et al., 2020). One of the common ways of improving the bioavailability of dietary supplements is the use of additional ingredients that can either enhance gut absorption or favorably alter the biotransformation of the active

substance in the liver and other tissues. In our study, four out of five CBD preparations contained additional ingredients. The two preparations with the lower bioavailability contained either pure CBD (preparation 203) or only one additional ingredient (MCT oil, in preparation 178) (Table 3.2). Medium-chain triglycerides (MCT) have been shown to facilitate absorption of lipophilic substances in the GI tract (Feng et al., 2020; Yao et al., 2019), and are used in nutraceutical and pharmaceutical preparations both for bioavailability enhancement of other active ingredients and for their own multiple physiological properties in managing certain health conditions (Kharat, Du, Zhang, & McClements, 2017; Y. Y. Lee et al., 2021). Based on the results of our study, MCT oil by itself only moderately increased CBD bioavailability, since preparation 178 evoked slightly higher C_{max} and AUC than preparation 203, but still lower than the other preparations. Preparation 707 appeared to be the superior preparation as, compared with most of the others, it generally evoked the fastest T_{max}, greatest C_{max}, and largest AUC. Besides MCT oil, it included gum arabic and citric acid as additional ingredients. Gum arabic is widely used in food and pharmaceutical industries as stabilizer and emulsifier (Ward, 2000). It has excellent functional properties such as high solubility and low viscosity, and has shown to increase the bioavailability of lipophilic substances (Lamsen et al., 2020).

Perhaps more important than comparisons within the current study are comparisons of the preparations to others described within the literature. From all the published up to date information on CBD pharmacokinetics we extracted data from 14 studies and presented 27 sets of pharmacokinetic data that are comparable to our study (Table 1.1). There, only 9 sets of data pertain to low oral CBD doses of 30 mg or less. Our study added five more sets of PK data to this review (Table 5.1). Preparation 707 from the current study appears to have the fastest T_{max}, but a smaller C_{max} when compared with the self-emulsifying drug delivery system (SEDDS). The

favorable CBD bioavailability of the SEDDS formulation was attributed to augmented solubility within the GI tract. Additional contribution of our study to the current knowledge of CBD pharmacokinetics are calculations of several PK parameters that were not commonly recorded at lower doses of administration: $t_{½}$, K_a, K_e, and V_d. Half-life ($t_{½}$), the amount of time it takes to decrease the circulating concentration to half of its initial value, is a very important consideration for CBD use in the context of pharmacokinetic research, where undetectable baseline circulating CBD concentrations can be critical for the valid derivation of pharmacokinetic parameters. Most of the $t_{½}$ values in our study fall within the previously established range of 1.09 to 2.54 hours (Table 5.1) with the exception of preparation 472, which showed somewhat larger value of 5.18 h. This is further illustrated by its low K_e value, denoting a slower rate of elimination. Due to the lack of comparative data, we can only speculate that additional ingredients in this preparation (sorbitol and modified food starch) could potentially influence the rate of CBD elimination.

The important considerations when evaluating CBD formulations are the therapeutic goal and intended use of the drug. If the indication for the CBD is to treat acute pain, then a faster T_{max} and higher C_{max} may be desirable and may also help to decrease the risk of overdose due to premature repeat self-administration. Alternatively, as a chronic treatment for anxiety, a larger AUC and slower rate of elimination may be preferable if a user follows a regular dosing schedule. Also, the other, less obvious, pharmacokinetic effects of specific CBD formulations should be considered.

Table 5.1. Comparison of pharmacokinetic parameters of current preparations with previously reported studies of ingestible cannabidiol doses 5-30 mg.

Study	Formulation, Administration, CBD Single Dose (mg)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-t} (hr x ng/mL)	AUC_{0-inf} (hr x ng/mL)	$t_{½}$ (hr)	K_a (1/hr)	K_e (1/hr)	V_d (L)
(Nadulski, Pragst, et al., 2005)	Oral capsule (CBD+THC) 5.4 mg	0.99	0.93	4.35					
(Nadulski, Sporkert, et al., 2005)	Oral capsule (CBD+THC) 5.4 mg	1.0	0.95						
(Guy & Robson, 2004)	GW oral capsule (CBD+THC) 10 mg	1.27	2.47	5.76	6.03	1.09			
(Cherniakov et al., 2017)	Oral capsule (CBD+THC) 10 mg	1	2.1	6.9					
(Atsmon, Heffetz, et al., 2018)	PTL101* CBD oral capsule 10 mg	3	3.22	9.64	10.31	2.95		0.1	
(Atsmon, Cherniakov, et al., 2018)	PTL401*(CBD+THC) oral capsule 10 mg	1.25	2.94	9.85	10.52	3.21		0.29	
(Knaub et al., 2019)	Oral capsule MCT-CBD** 25 mg	3.0	3.05	9.51	19.23				

(Knaub et al., 2019)	Oral capsule SEDDS-CBD*** 25 mg		13.53	27.15	32.63				
(Hobbs et al., 2020)	Caliper CBD water soluble 30 mg	0.9	2.82	6.80	7.94	2.54	1.68	0.66	
(Hobbs et al., 2020)	Caliper CBD lipid soluble 30 mg	1.5	0.65	1.51	1.64	2.30	1.14	0.72	
	Preparation 178 CBD 30 mg	3.29	2.20	4.58	-	-	0.32	-	-
	Preparation 203 CBD 30 mg	3.39	1.29	2.30	-	-	0.24	-	-
	Preparation 340 CBD 30 mg	1.28	3.54	7.81	13.81	2.20	1.19	0.40	7428
	Preparation 472 CBD 30 mg	1.53	2.88	6.32	9.96	5.18	1.87	0.27	20178
	Preparation 707 CBD 30 mg	0.70	5.57	9.12	10.77	1.42	1.43	0.56	8024

CBD: Cannabidiol. T_{max}: the time to maximum concentration. C_{max}: the maximum concentration. $AUC_{0\text{-}t}$: the area under the curve representing total cannabidiol exposure between 0 and end of data collection. $AUC_{0\text{-}inf}$: an estimate of the total exposure to cannabidiol over time. $t_{½}$: the amount of time it takes to decrease the circulating concentration to half of its initial value. K_a: the rate at which the cannabidiol is absorbed into the body. K_e: the rate at which the cannabidiol is removed from the body. V_d: the volume of distribution, an estimate of the degree to which cannabidiol is distributed in the body tissue *vs*. the plasma. * PTL- gelatin matrix pellets technology-based formulation; ** MCT-CBD – medium-chain triglycerides; *** SEDDS-CBD – self-emulsifying drug delivery system.

5.2 Effect of Body Composition

An influence of body size and composition on CBD pharmacokinetics has been previously speculated but to our knowledge is currently undocumented. In the current study we used DEXA to quantify body composition. Some of the inter-personal variability, for some of the preparations, could be explained by body size and composition as reflected by correlations between several of the DEXA-derived variables and CBD pharmacokinetic parameters. Preparation 707 showed a negative correlation between T_{max} and several parameters of body composition: bone mineral content, lean mass, fat free mass, and body mass. Since these are interdependent variables, they were considered together in forward stepwise regression, and fat free mass remained a significant predictor of T_{max}, which means that individuals with higher fat free mass achieve C_{max} faster. The shorter time to peak concentration in adults with greater fat free mass may reflect a greater rate of clearance by metabolically active and relatively well-perfused tissues (such as skeletal muscle). It is noteworthy that, fat free mass did not predict C_{max} or AUC for preparation 707. Interestingly, for this preparation, age positively correlated with C_{max} and AUC, which implies that older individuals have greater drug exposure at the same dose. This tendency is harder to explain since absorption in the GI tract doesn't seem to improve with age but rather the opposite (VanPutte et al., 2020). We can speculate that younger individuals may endure greater losses of active substance at first-pass metabolism due to higher activity of xenobiotic enzymes. Alternatively, older adults may have slower rate of clearance due to reduction in lean skeletal muscle mass, well perfused and metabolically active tissue that facilitates clearance. Since older adults might be a group in which CBD would be particularly helpful if they suffer from pain or other conditions that are applicable to CBD treatments, studies that evaluate CBD pharmacokinetics specifically in this population group may help to establish normative values for CBD formulations for older adults. Our study was neither designed nor

statistically powered to compare CBD pharmacokinetics in younger individuals *vs*. older adults. More studies are warranted to explore age-related differences. Preparation 178, with one of the slower T_{max}, showed positive correlation between T_{max} and body mass index. It is possible that for preparations absorbed more slowly (i.e., slower rate of entry into the blood), body size (and presumably blood volume) may contribute to time to peak circulating concentration. Interestingly, preparation 203, also with slow T_{max}, didn't exhibit a similar tendency. Since 203 also achieved relatively low C_{max} and AUC, we can speculate that very limited gut CBD absorption for this preparation may have rendered its distribution in the body (and thus the dependence on body size and blood volume) less important. From a broader perspective, CBD bioavailability may be determined not only by preparation/formulation, but perhaps also by body size and composition; however, interaction between multiple variables both in preparation and body composition make it impossible to give any clear recommendations to the user based on currently available data. More research is warranted for establishing future dosing guidelines for therapeutic purposes, with additional considerations to be given to age, body composition, physiological characteristics, and the overall state of health of the consumer.

5.3 CBD Influence on Heart Rate Variability

An additional goal of the current study was to explore the influence of acute CBD ingestion on heart rate variability, the inconsistencies in the periods of time separating consecutive cardiac cycles. It is an important predictor of future cardiac events (Tsuji et al., 1996; Zbilut & Lawson, 1988) and, in some instances, longevity (Hernandez-Vicente et al., 2020). While there are many methods and different parameters used to quantify heart rate variability, generally speaking, greater variability is considered desirable. To our knowledge, this is the first exploration of a potential influence of acute CBD on heart rate variability in adult

humans. One previous study has examined the short-term influence of CBD (administered as a hemp oil extract) on heart rate variability (Lopez et al., 2020) but the experimental design did not include an acute measurement. Other studies have compared heart rate variability in users and non-users of *Cannabis sativa* L. (i.e., CBD plus THC and other potentially active ingredients), thus making assertions as to the independent influence of CBD difficult (Schmid, Schonlebe, Drexler, & Mueck-Weymann, 2010). A recent study evaluated cannabidiol expectancy effects on acute stress and anxiety in healthy adults (Spinella, Stewart, Naugler, Yakovenko, & Barrett, 2021). The authors measured HRV in subjects following administration of CBD-free oil on two occasions: during one session, the subjects were falsely informed that the oil contained CBD and in the other session, that the oil was CBD-free. Since no actual CBD was administered in that study, the effect on HRV reflects only psychological anticipatory reactions that were dependent, as to the authors' conclusions, on *a priori* beliefs regarding the anxiety-dampening effects of CBD.

In the current study, acute CBD ingestion decreased heart rate, peak frequency of the high frequency band, and Poincaré plot standard deviation along the line of identity. CBD ingestion increased R-to-R interval and Poincaré plot standard deviation perpendicular to the line of identity (Table 4.3). Poincaré plots are a useful tool in the heart rate variability analysis arsenal; they are often able to recognize patterns and rhythms in the R-to-R interval data that are sometimes overlooked using spectral analysis (Blake, Shaw, Culshaw, & Martinez-Pereira, 2018; Brennan, Palaniswami, & Kamen, 2002). From an interpretation perspective, the standard deviation perpendicular to the line of identity reflects short-term heart rate variability and is thought to be regulated by parasympathetic (vagal) input, while the standard deviation along the line of identity reflects total heart rate variability and is determined by a combination of

sympathetic and parasympathetic input (Brennan et al., 2002). When considered together, the ratio of these two variables (i.e., the SD2/SD1 ratio; Table 4.3) represents sympathovagal balance. In light of the opposing direction of change in these Poincaré plot data, in addition to the small magnitude of change in the other heart rate variability parameters, it appears likely that acute CBD evokes only a modest, and physiologically irrelevant effect on heart rate variability, at least when studied at rest.

Our study has several limitations. Since our primary focus was the pharmacokinetics of the different CBD preparations and the potential interaction with body size and composition, we did not include a placebo control within our experimental design. Thus, any conclusions with respect to our exploration of the acute influence of CBD on heart rate variability must be considered with caution. For example, it is plausible that CBD does not exert any acute influence on heart rate variability, and we are simply reporting on the influence of 60 min of semi-recumbent rest. The second consideration is related to our study inclusion/exclusion criteria. In our subject recruitment we did not differentiate between habitual cannabis or CBD users and non-users; the requirement was 72-hour abstention from using any products containing CBD before each visit. Our rationale was based on the published data that the terminal elimination half-life of CBD administered to fasted humans varies between 6 and 32 h (Devinsky et al., 2014; Hosseini et al., 2021) depending on mode of administration and CBD preparation. Therefore, our choice of a minimum 72-hour washout/abstention period between consecutive laboratory visits seemed reasonable. Also, all baseline (time 0) concentrations for all CBD preparations, for all participants, were below the limit of quantitation. Nevertheless, after a 12-hour fast even such minimal activity as being subjected to study procedures during the first fasting hour of blood sampling could possibly involve oxidation of some body fat. As a

lipophilic substance, CBD can potentially accumulate in adipose tissue in a manner similar to that previously reported for THC (Johansson et al., 1989; Wong et al., 2013). Therefore, CBD released from fat deposits during the first hour after baseline blood sampling could potentially contribute to plasma concentrations beyond the dose established by the study protocol. However, considering well known high inter-individual variability of CBD bioavailability, the confounding effect of previous cannabis use can be considered negligible.

CONCLUSION

The objectives of the current study were to assess the bioavailability of five different oral preparations of CBD, to examine the relationship between body composition and pharmacokinetics, and to explore the acute influence of CBD ingestion on heart rate variability. In relation to bioavailability, we identified a CBD preparation that compared well with respect to other preparations described in the literature, and with the other preparations incorporated within our design. Furthermore, we demonstrated that some of the pharmacokinetic parameters of this superior preparation were influenced by body size, body composition, and the age of the consumer. These findings highlight the need to optimize CBD preparations and personalize dosing strategies in order to confirm the physiological relevance of CBD as a potential therapeutic agent. Finally, we documented, for the first time, a modest effect of acute CBD ingestion on selected parameters of resting heart rate variability in healthy adults, alleviating any potential concerns that CBD may unfavorably impact autonomic regulation of heart rate.

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