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RESEARCH ARTICLE

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Evaluation of pharmacokinetics and acute anti-inflammatory potential of two oral cannabidiol preparations in healthy adults

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Abstract

Cannabidiol (CBD) is a dietary supplement with numerous purported health benefits and an expanding commercial market. Commercially available CBD preparations range from tinctures, oils, and powders, to foods and beverages. Despite widespread use, information regarding bioavailability of these formulations is limited. The purpose of this study was to test the bioavailability of two oral formulations of CBD in humans and explore their potential acute anti-inflammatory activity. We conducted a pilot randomized, parallel arm, double-blind study in 10 healthy adults to determine differences in pharmacokinetics of commercially available water and lipid-soluble CBD powders. Participants consumed a single 30 mg dose, which is within the range of typical commercial supplement doses, and blood samples were collected over 6 hr and analyzed for CBD concentrations. Peripheral blood mononuclear cells (PBMCs) were collected at baseline and T = 90 min, cultured and stimulated with bacterial lipopolysaccharide (LPS) to induce an inflammatory response. Cell supernatants were assayed for IL-10 and TNF, markers of inflammation, using enzyme-linked immunosorbent assays. The water-soluble powder had C_{max} = 2.82 ng/ml, T_{max} = 90 min, and was approximately ×4.5 more bioavailable than the lipid-soluble form. TNF was decreased in LPS-stimulated PBMCs collected 90 min after CBD exposure relative to cells collected at baseline. This study provides pilot data for designing and powering future studies to establish the anti-inflammatory potential and bioavailability of a larger variety of commercial CBD products consumed by humans.

KEYWORDS
acute inflammation, cannabidiol, cannabinoids, pharmacokinetics

1 | INTRODUCTION

Cannabidiol (CBD), one of the major constituents of *Cannabis sativa* L., is a member of a group of C21 terpenophenolic compounds called phytocannabinoids (Brenneisen, 2007; Hanus, Meyer, Munoz, Taglialatela-Scafati, & Appendino, 2016; Taura, Sirikantaramas, Shoyama, Shoyama, & Morimoto, 2007). The structure of CBD is comprised of a resorcinol ring, a monoterpene moiety, and an alkyl side chain (Taura et al., 2007). In recent years, interest in this bioactive constituent of *C. sativa* has risen due to its diverse therapeutic potential

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and minimal reported adverse side effects (Iffland & Grotenhermen, 2017; Millar et al., 2019; Premoli et al., 2019). Proposed physiologic effects of CBD include anti-inflammatory, antioxidant, antipsychotic, anticonvulsant, anxiolytic, cytotoxic, and analgesic effects, which may occur through multiple signaling mechanisms, many of which are still poorly characterized (McPartland, Duncan, Di Marzo, & Pertwee, 2015; Zuardi et al., 2012). Some proposed receptor populations with which the compound interacts, both directly and indirectly, include canonical cannabinoid receptors CB1 and CB2, adenosine A2A receptors, numerous G protein-coupled receptors, opioid receptors, and the serotonin 1a receptor (Burstein, 2015; Laprairie, Bagher, Kelly, & Denovan-Wright, 2015; McPartland et al., 2015; Morales, Hurst, & Reggio, 2017). Moreover, there is evidence to suggest that CBD modulates certain enzymes, including those in the cytochrome P450 system, and interacts with many transient receptor potential channels (Iannotti et al., 2014; McPartland et al., 2015; Stout & Cimino, 2014; Yamaori, Kushihara, Yamamoto, & Watanabe, 2010).

With the legalization of cannabis products in much of the United States, the availability CBD-containing dietary supplements, foods, and beverages have expanded. To ensure consumer safety and awareness, it is important to gain a better understanding of basic information regarding bioavailability of oral CBD preparations, as well as their effects on the human body after acute and chronic exposure. Although there have been a few human studies on the pharmacokinetics of CBD (Millar, Stone, Yates, & O'Sullivan, 2018), much of the published information references inhaled rather than orally ingested forms and includes co-administration with tetrahydrocannabinol (THC), which may alter its pharmacokinetic and pharmacodynamic profiles (Bornheim, Kim, Li, Perotti, & Benet, 1995; Klein et al., 2011). Current studies reporting oral CBD administration suggest that its absorption time is similar to that of THC, with peak plasma levels detected from 60 to 120 min, but possibly as late as 6 hr postadministration (Ujváry & Hanuš, 2016). Bioavailability of oral CBD preparations is also variable (Harvey, 1991; Hawksworth & McArdle, 2004; Huestis, 2007; Ujváry & Hanuš, 2016). Cannabinoids can be altered by the stomach acid and metabolized by the gut microbiota, resulting in low circulating levels of the intact compound. Greater fecal excretion of CBD, compared to THC, has also been observed (Ujváry & Hanuš, 2016).

Here, we present a pilot, randomized, parallel arm, double-blind study in healthy adults to establish preliminary data regarding bioavailability and persistence of two orally consumed CBD preparations. Participants were given a 30 mg powder packet of either a water-soluble or lipid-soluble CBD preparation dissolved in 8 oz water. CBD-infused beverages are a rising trend in the United States, and this dose falls within the 20–30 mg range unofficially adopted industry as a standard serving size. Blood was collected from an intravenous catheter at baseline and several time intervals postconsumption (T = 0, 15, 30, 45, 60, 90, 120, 240, and 360 min). As CBD is reported to reduce inflammation, we also examined the effects of this single oral dose of CBD on production of IL-10, an anti-inflammatory cytokine, and tumor-necrosis factor (TNF), which is a pro-inflammatory molecule, in LPS-stimulated peripheral blood mononuclear cells (PBMCs) collected at baseline and 90 min post-treatment with CBD. These data will contribute to the existing body of knowledge regarding CBD bioavailability and bioactivity as well as providing a platform for designing adequately powered future pharmacokinetic and anti-inflammatory studies.

2 | MATERIALS AND METHODS

2.1 | Study population

Ten healthy male and female adults (>21 years) were recruited into the study. Participants were recruited by word of mouth, through email, and social media platforms. The study was conducted according to the guidelines of the Declaration of Helsinki and Tokyo and the protocol was approved by the Colorado State University Institutional Research Board (Protocol #19-8667H). All participants provided written informed consent prior to study participation.

Eligible participants included individuals >21 years of age, who weighed more than 110 pounds and self-reported as healthy. Individuals taking certain medications known to have potential interactions with CBD (i.e., steroids, HMG-CoA reductase inhibitors, calcium channel blockers, antihistamines, antivirals, immune modulators, benzodiazepines, anti-arrhythmic, antibiotics, anesthetics, antipsychotics, antidepressants, anti-epileptics, beta blockers, proton pump inhibitors, NSAIDs, angiotensin II blockers, oral hypoglycemic agents, and sulfonylureas) were excluded from the study, as were those unable to tolerate prolonged periods of fasting (i.e., diabetics). Individuals that were pregnant or breastfeeding, reported food allergies, or that had been diagnosed with intestinal, liver, or renal diseases that would affect absorption or clearance of CBD were also excluded from the study.

2.2 | Study protocol

Participants were prescreened for eligibility by phone and those meeting inclusion criteria were scheduled for a clinic visit. Prior to arrival, participants were asked to (a) refrain from consuming any CBD-containing products for at least 3 days prior to their visit, and (b) fast for 6 hr prior to arrival in the clinic. Potential participants then underwent the informed consent process, and if they agreed to continue with the study, had an IV catheter placed. After collection of a baseline 10 ml blood sample (T = 0), participants were randomized to a treatment group using a random number generator in GraphPad. They were then asked to consume a 30 mg dose of CBD (Caliper CBD) in one of two forms, water-soluble, or lipid-soluble. Water-soluble CBD was prepared in the form of an emulsified, homogenized 2.5% CBD powder containing medium chain triglyceride (MCT) oil, modified food starch, and sorbitol and was consumed in an 8 oz glass of water. The lipid-soluble CBD was prepared in the form of a 2.5% CBD powder containing isolate, plus nonemulsified, nonhomogenized MCT oil, modified food starch, and sorbitol mixed into 8 oz water. The CBD extracts came from hemp plants certified under the Industrial Hemp Research Pilot Program by the Kentucky Department of Agriculture

(License number 16-10-01P). Additionally, third-party verification through ProVerde Laboratories (Milford, MA) showed that the extract lot used for testing contained 99.1% CBD and was free of THC, coliforms, yeasts, and molds. At T = 15, 30, 45, 60, 90, 120, 240, and 360 min post CBD consumption, a 3 ml aliquot of blood was collected from the IV catheter port and an additional 10 ml was drawn at baseline (T = 0) and T = 90 min for collection of PBMCs. In addition, supine blood pressure was measured on the nondominant arm prior to each blood collection using an automatic device (Professional Intellisense Blood Pressure Monitor, Omron Healthcare, Inc.). All participants remained in a hospital bed and were given access to television or reading material during the 6-hr course of the study. In addition, they were offered a standardized meal of sausage, orange juice, and a vegetarian breakfast burrito after the T = 90 min blood collection.

2.3 | Plasma extraction

CBD was extracted from 50 μl of thawed plasma by adding 200 μl of cold (−20°C) 100% acetonitrile (spiked with 60 ng/ml of d3-CBD) and vortexing at RT for 5 min. A 200 μl aliquot of water was added and vortexed for an additional 5 min. One milliliter of 100% hexane was added to each sample and vortexed for a final 5 min. Phases were separated by centrifugation at 1000*g* for 15 min at 4°C. The organic phase was removed (~900 μl per sample), and placed in new glass vials. Samples were concentrated to dryness under nitrogen gas (N_2) and resuspended in 60 μl of 100% acetonitrile.

2.4 | CBD detection and quantification by UPLC–MS/MS

Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) was performed on a Waters Acquity M-Class Ultra High Pressure Liquid Chromatography (UPLC) coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer. Chromatographic separations were carried out on a Waters HSS T3 C18 UPLC column (2.1 mm x 50 mm, 1.7 μM). Mobile phases were 99.9% acetonitrile, 0.1% formic acid (B), and water with 0.1% formic acid (A). The analytical gradient was as follows: time = 0 min, 30% B; time = 1.0 min, 30% B; time = 2.5 min, 100% B; time 3.5 min, 100% B; time 4.0 min, 30% B. Total run time was 6 min. Flow rate was 350 μl/min and injection volume was 2.0 μl. Samples were held at 6°C in the autosampler, and the column was operated at 45°C. The MS was operated in selected reaction monitoring mode, where a parent ion is selected by the first quadrupole, fragmented in the collision cell, then a fragment ion selected for by the third quadrupole. Product ions, collision energies, and cone voltages were optimized by direct injection of an individual synthetic standard. Interchannel delay was set to 3 ms. The MS was operated in negative ionization mode with the capillary voltage set to 2.4 kV. Source temperature was 150°C and desolvation temperature 550°C. Desolvation gas flow was 800 L/hr, cone gas flow was 150 L/hr, and collision gas flow was 0.2 ml/min. Nebulizer pressure (nitrogen) was set to 7 Bar and argon was used as the collision gas. All raw data files were imported into Skyline (MacCoss Lab, Department of Genome Sciences, University of Washington, Seattle, WA) and peak areas extracted for CBD and d3-CBD. Quantitation of analyte in plasma samples was based on linear regression of calibration curves and extrapolation using the analyte peak area to internal standard peak area ratios. Authentic standard CBD was spiked into 0.05 ml aliquots of pooled plasma (study participants at T = 0) from 0 ng/ml to 1,000 ng/ml. Each spiked standard curve sample was extracted in the presence of d3-CBD as described for the unknown and QC pooled samples. Calibration curve was analyzed in triplicate (r^2 > 0.998). The limit of detection of the assay was 0.188 ng/ml and was calculated as the standard error divided by the slope of the calibration curves multiplied by 3.3. The limit of quantitation was 0.627 ng/ml and was determined as the lowest concentration within the linear portion of the calibration curves with an accuracy within 15% of the nominal concentration. Accuracy and precision of the calibration curves were within 15%; the inter- and intra-day coefficient of variation was less than 5% (File S1).

2.5 | Isolation of PBMCs

Ten ml of whole blood was collected from antecubital veins into ethylenediaminetetraacetic acid treated vacutainer tubes at baseline (T=0) and 90 min postexposure to CBD. PBMCs were isolated from the whole blood within 6 hr of collection via density centrifugation and a series of washes. Initial whole blood was diluted with ×1 PBS + 2% fetal bovine serum (FBS, Atlas Biologics) at a 1:1 ratio and transferred into 50 ml SepMate Tubes (STEMCELL Technologies) with 17 ml density gradient medium, Lymphoprep (STEMCELL Technologies). Tubes were centrifuged (10 min, 1,200*g*, RT) and PBMCs were poured off and diluted with equivalent volume of ×1 PBS + 2% FBS and centrifuged at 300*g* for 8 min. Tubes were decanted and pelleted cells were resuspended in equivalent volume of ×1 PBS + 2% FBS for final wash and centrifugation. Cells were counted using cell counting chambers on a Cellometer Auto T4 (Nexcelom), resuspended in CryoStor and placed in a Mr. Frosty container at −80°C for 12–24 hr before final storage in liquid nitrogen.

2.6 | PBMC culturing and stimulation

Frozen PBMCs were thawed for 2 min in a 37°C water bath and added to a 15 ml conical tube with warm culture media (1x RPMI-1640 [Corning], 10% [Atlas Biologics], 1% penicillin/streptomycin [100 U/ml penicillin and 100 μg/ml streptomycin; HyClone]). Cells were centrifuged (25°C, 300*g*, 8 min) and supernatant was decanted, followed by two additional wash cycles. Cells were then plated in duplicate at 1–2 x 10^6 cells/ml. After a 24-hr rest period, cells were counted and adjusted to reach a desired concentration of 5 x 10^5 cells/250 μl. One of the duplicate wells was spiked with bacterial LPS at a total concentration of 1 μg/ml. Experimental control wells included a media control and a media + LPS control. Cells were incubated at 37°C and 5% CO_2 for 48 hr. Postincubation, the plate

was centrifuged (400*g*, 5 min, 4°C), and supernatant was collected and frozen at −80°C until further analysis.

2.7 | Cytokine quantification

Interleukin-10 (IL-10) and human TNF enzyme-linked immunosorbent assays were performed in duplicate using supernatants from LPS stimulated and nonstimulated PBMCs using commercially available kits according to the manufacturer's instructions (Boster Biological Technology).

2.8 | Pharmacokinetic calculations

Pharmacokinetics values were established using Phoenix WinNonlin 2018 software (Certara, NJ). Area under the concentration curve (AUC) was calculated using the trapezoidal method and relative bioavailability (F*rel*) of the two administered preparations was calculated as the ratio: AUC Treatment A/AUC Treatment B.

2.9 | Statistical analysis

Comparisons between treatment groups were calculated using two-tailed *t* tests and longitudinal comparisons within treatment groups were analyzed by repeated measures ANOVA. A *p*-value <0.05 was considered to be statistically significant.

3 | RESULTS

3.1 | Participant demographics

Eleven individuals underwent a screening process to confirm eligibility and were enrolled in the study. One participant dropped from the study prior to their clinic visit and thus 10 individuals completed the study (Table 1). Participants ranged in age from 22 to 51 years old and the majority (8/10) was in the normal weight BMI range (20–24.9). A total of six females and four males completed the study with three females and two males in each study arm. There were no significant differences in the average characteristics (height, weight, BMI, height) between treatment groups.

3.2 | Plasma CBD levels after oral ingestion

CBD from oral preparations was rapidly detected in the blood, with initial increases occurring as quickly as 15 min after ingestion. Plasma concentrations were approaching baseline levels by 6 hr postingestion. There was a significant difference in absorption between the two treatment preparations. The water-soluble preparation resulted in significantly higher levels of plasma CBD detected at the 45–120 min time point range compared to the lipid-soluble preparation (T = 45, p = 0.044; T = 60, p = 0.035; T = 90, p = 0.026; T = 120, p = 0.015; Figure 1a). The water soluble CBD treatment group had a larger C_{max} (2.82 ng/ml) than the treatment group given the lipid soluble CBD (C_{max} = 0.645 ng/ml). The predicted time to peak concentration (T_{max}) for the water soluble formulation was 54 min while the T_{max} for the lipid soluble formulation was estimated as 90 min postingestion (Table 2). The AUC of these preparations was also significantly different between groups (AUC p = 0.013; Figure 1b) and using a ratio of the AUCs, the relative bioavailability of the water-soluble formula was determined to be ~4.5-fold greater than that of the lipid-soluble CBD. As expected, the water soluble CBD also had a longer half-life ($t_{1/2}$) in plasma and significantly higher absorption rate (K_a), although the elimination rate of the two formulations was similar (K_e). The volume of distribution (V_d) was higher for the lipid soluble CBD, indicating higher volumes in tissue relative to plasma. Despite the increased bioavailability of the water-soluble formula, there was still a high level of interindividual variability (Figure 1c). This variability was much less apparent in the lipid-soluble format; however, concentrations in plasma after consumption of that preparation were near or below the theoretical limit of detection (LOD; 0.188 ng/ml) throughout the course of the study (Figure 1d).

TABLE 1 Participant demographics

	Sex	Height (cm)	Weight (kg)	BMI	Age
Water-soluble CBD	F	172.72	68.6	23	45
	M	182.88	61.4	18.3	22
	F	167.64	90.9	32.3	27
	F	160.02	63.6	24.8	24
	M	180.34	85.9	26.4	51
Average (±*SD*)		172.72 (±9.3)	74.09 (±13.4)	24.96 (±5.1)	33.8 (±13.3)
Lipid-soluble CBD	M	185.42	76.4	22.2	33
	M	180.34	75.0	23	26
	F	170.18	58.2	20	32
	F	171.45	55.9	19	23
	F	170.18	59.1	20.4	22
Average (±*SD*)		175.51 (±7.0)	64.90 (±9.9)	20.92 (±1.6)	27.2 (±5.1)

Abbreviation: BMI, body mass index; CBD, cannabidiol.

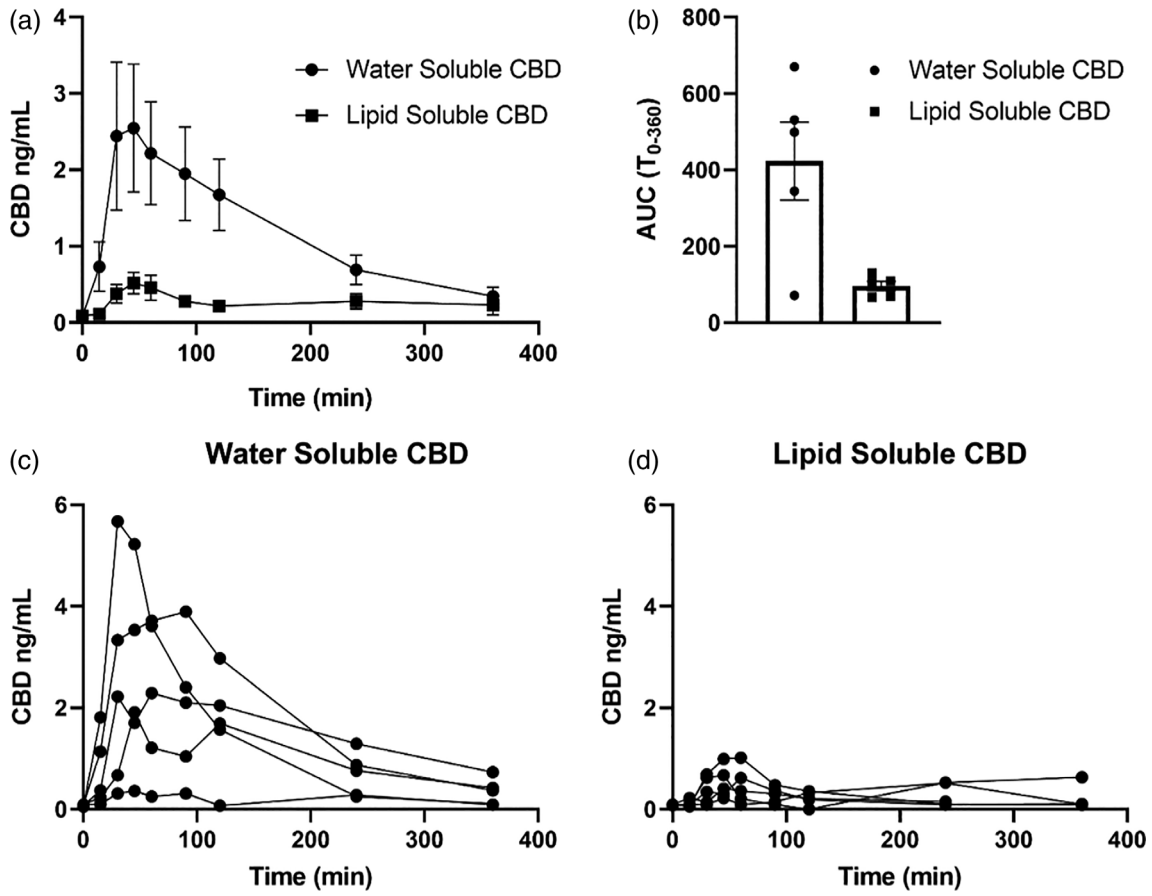

FIGURE 1 Plasma levels of cannabidiol (CBD) measured over six hours (a). Values are averages for each treatment group (n = 5) ± *SEM*. Average area under the curve (AUC) for each group measured by the trapezoidal method (b). Error bars represent *SEM*. Detection of plasma CBD by individual for water soluble (c) and lipid soluble (d) oral treatments

TABLE 2 Pharmacokinetic parameters of CBD in plasma

	T_{max} (min)	C_{max} (ng/ml)	AUC T = 0–360	AUCinf	$t_{1/2}$ (min)	K_a (1/min)	K_e (1/min)	V_d (L)
Water soluble CBD	54	2.82	408.11	476.1	152.35	0.028	0.011	32,445
Lipid soluble CBD	90	0.65	90.52	98.5	137.95	0.019	0.012	63,334

Note: T_{max} is the time to maximum concentration. C_{max} is the maximum amount detected in plasma. $AUC_{T=0-360}$ is the area under the curve representing total drug exposure during the duration of the study and the AUC_{inf} estimates the total drug exposure over time. $t½$ is the amount of time it takes to reduce a compounds to half its initial value. K_a is the rate at which the drug is absorbed into the body. K_e is the rate at which the drug is removed from the body. V_d is the volume of distribution. This represents the degree to which a drug is distributed in the body tissue vs plasma.
Abbreviation: CBD, cannabidiol.

3.3 | Blood pressure measures

Because a previous study reported acute hypotensive effects on blood pressure after CBD administration in healthy adult males, we measured this parameter prior to each blood collection. We saw no significant changes in either systolic or diastolic blood pressure over time for either the entire cohort or the individual treatment groups (Figure S1a–c). Furthermore, we did not detect any significant changes in heart rate or pulse pressure after CBD administration for either the entire dataset or within treatment groups (data not shown).

3.4 | Markers of inflammation in LPS-stimulated and nonstimulated PBMCs

To assess the effects of an acute oral dose of CBD on inflammation, we measured TNF and IL-10 levels in supernatants from LPS stimulated and nonstimulated PBMCs collected at T = 0 and T = 90 min. When comparing these parameters between treatments (water or lipid soluble preparations; n = 4 and n = 5, respectively), we saw no statistically significant differences in TNF or IL-10 production in nonstimulated (Figure S2A,B) or stimulated cells (Figure S2C,D) between

T = 0 and T = 90. However, when combining the data from both treatments (n = 9) to examine the effects of CBD more broadly, we did observe a significant suppression of the pro-inflammatory marker TNF in LPS-stimulated cells at T = 90 compared to baseline (p = 0.021; Figure 2A). No differences were noted in nonstimulated cells (Figure 2B) and the suppression of TNF was not significantly correlated with plasma CBD levels at T = 90.

4 | DISCUSSION

Here, we show data from a pilot study that suggests that different oral formulations of CBD vary in their bioavailability and that a high degree of interindividual variability in absorption exists. In this study, a water soluble CBD preparation had 4.5x greater bioavailability than a lipid soluble preparation when consumed as a 30 mg dose dissolved in an 8 oz glass of water. In this treatment group, the average T_{max} was around 54 min, which is considerably faster than other published reports. An 800 mg dose provided in oral capsules to eight volunteers that were habitual cannabis smokers had an average T_{max} of 3 hr, suggesting it was primarily absorbed in the lower GI tract (Haney et al., 2016). Likewise, an oral preparation in a gelatin format also had an average T_{max} of 3 hr for a 10 mg dose and 3.5 hr for a 100 mg dose (Atsmon et al., 2018). These discrepancies with the current study may be a result of increased bioavailability of the tested preparation, but may also be due to the fact that participants were fasted prior to administration. Stott et al. reported that the T_{max} was delayed from 1.4 hr to approximately 4 hr when participants were in a fed versus fasted state (Stott, White, Wright, Wilbraham, & Guy, 2013).

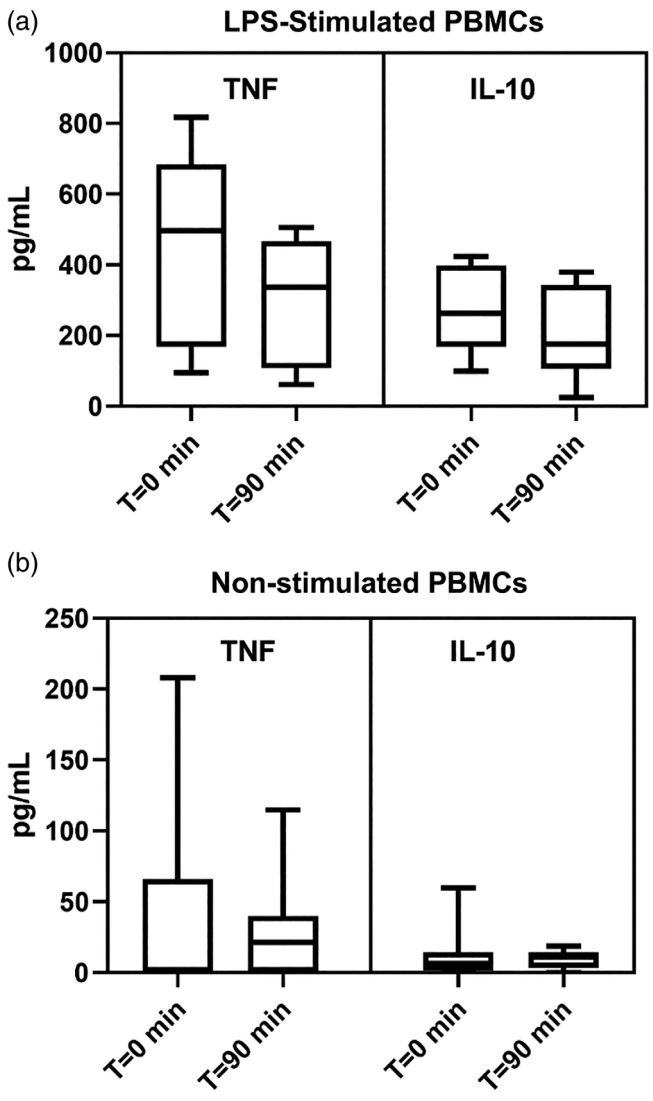

FIGURE 2 Levels of TNF and IL-10 at baseline and 90 min post-CBD consumption in LSP stimulated (a) and nonstimulated (b) PBMCs. TNF levels were significantly different at T=90 compared to T=0 in stimulated PBMCs (p>0.05). CBD, cannabidiol; PBMCs, Peripheral blood mononuclear cells; TNF, tumor-necrosis factor

In the current study, we also observed a great deal of interindividual variability in the CBD pharmacokinetics, particularly in the water soluble CBD treatment group. It is interesting to note that some individuals appeared to have a single absorption peak, while in others there were two peaks, suggesting that some exposure may occur after entero-hepatic recirculation. In addition, the V_d of CBD in our study was high for both treatment groups, suggesting a high level of the compound partitioning to tissue relative to the plasma. Other studies have reported that slow release of THC from lipid storage compartments and extensive entero-hepatic circulation are responsible for the long half-life of this compound in habitual cannabis users (Johansson, Agurell, Hollister, & Halldin, 1988). Therefore, it is likely that these factors would have an impact on the pharmacokinetics of CBD as well, and further studies are needed to assess how factors such as an individual's physiology, previous exposure, and so on can impact the absorption and elimination of CBD.

We observed a plasma C_{max} of 2.82 ng/ml for the water soluble powder, which is similar to average maximum concentrations reported in other human studies using a similar dose and/or oral delivery method. Chocolate cookies spiked with 40 mg CBD and 20 mg of THC consumed by 12 healthy individuals resulted in peak plasma CBD concentrations of ~5 ng/ml (Agurell et al., 1981). Guy and Flint (2004) reported that sublingual drops containing 20 mg of CBD had a C_{max} of 2.17 ng/ml and Nadulski et al. (2005) reported a C_{max} of 0.93 ng/ml for a 5.4 mg oral capsule, although they also reported a slight increase in C_{max} to 1.13 ng/ml in participants that were fed. In the current study, the participants were fasted for 6 hr prior to consuming the CBD-containing beverage and remained fasted for 90 min postconsumption, which may have decreased the overall bioavailability of the CBD, despite also decreasing the time to maximum absorption. Absorption and elimination curves, as well as the total maximum concentration, showed a great deal of interindividual variability, which could potentially be due to interpersonal differences in interactions between the CBD and the meal provided at the 90 min time point. Finally, it is important to note that the C_{max} of the lipid-soluble form that we tested was 0.645 ng/ml, which is much lower than most previous reports. For example, a study in rats showed that oral delivery of CBD with lipids resulted in about 3x greater bioavailability than a lipid-free form (Zgair et al., 2016). Thus, the difference that we

observed in bioavailability of the two tested preparations is likely due to differences in solubility in the delivery matrix, reducing the actual delivered dose. As the popularity of CBD-infused beverages and drink powders increases, it is important to take these differences into consideration in product design.

In contrast to a recent placebo-controlled crossover study in nine healthy males that reported significant reduction in systolic blood pressure after a single oral dose of CBD, we saw no significant changes in either systolic or diastolic blood pressure (Jadoon, Tan, & O'Sullivan, 2017). However, there were a few key differences in that study compared to our study, including their use of a 600 mg (pharmaceutical) dose of CBD and assessment by more sensitive and accurate Doppler measures for blood pressure. Additionally, that study was performed in healthy males whereas we included both men and women. Their study also reported that blood pressure decreases were accompanied by an increase in heart rate and maintained cardiac output. We saw no significant changes in heart rate and pulse pressure after CBD administration, which is consistent with another report examining the physiologic effects of CBD compared to THC in humans (Martin-Santos et al., 2012). In that study, a 10 mg dose of THC raised heart rate while a 600 mg dose of CBD in the same subjects did not elicit this effect. Thus, while the impact of CBD on cardiovascular parameters is still uncertain, it is unlikely that the amount typically found in food and beverage products would have any hypotensive effects in healthy individuals with normal blood pressure. Nonetheless, more research is needed to further elucidate the impact of CBD intake on blood pressure and cardiovascular health.

Regarding its anti-inflammatory potential, CBD reportedly has multiple mechanisms of action, which result in the reduction in levels of pro-inflammatory compounds (Burstein, 2015). Because our test population consisted of primarily normal weight, healthy individuals who likely had low baseline levels of inflammation, we collected PBMCs and challenged them with LPS, a pro-inflammatory elicitor. To our knowledge, this is the first study to report CBD-mediated suppression of the pro-inflammatory cytokine, TNF, in LPS-stimulated human PBMCs; although CBD has previously been shown to reduce TNF in LPS-exposed animal models (Carrier, Auchampach, & Hillard, 2006; Weiss et al., 2006). Mechanistically, this may occur through enhanced adenosine signaling via the A2A receptor, as the effect was abolished in mice treated with an A2A receptor antagonist (Carrier et al., 2006). However, other mechanisms may be involved and further investigation is warranted.

Contrary to the claims above, other reports have indicated that CBD can elevate TNF or other pro-inflammatory cytokines under certain conditions (Chen et al., 2012; Karmaus, Wagner, Harkema, Kaminski, & Kaplan, 2013). Karmaus et al. observed that orally delivered CBD led to the enhancement of LPS-induced pulmonary inflammation in mice. They concluded that CBD increased pro-inflammatory cytokine mRNA production, including TNF, among others. Chen et al. exposed mice to the HIV envelope glycoprotein 120 to explore the effects of CBD on T-cell responses. They found that the introduction of CBD following suboptimal cellular stimulation with low concentrations of Phorbol ester/Ionomycin or soluble anti-CD3 plus soluble anti-CD28 antibodies (sCD3/CD28) caused T-cell production of IL-2 and IFN-γ to increase. Together, these reports add weight to the idea that CBD's effect on cytokine production is dependent on multiple variables, including the type and magnitude of cellular stimulation. In conclusion, both the increasing popularity of CBD-containing products and the mounting evidence for beneficial physiologic effects of CBD suggest further research is necessary to ensure consumer safety and maximize health promoting benefits.

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CONFLICT OF INTEREST

K.A.W. is the head of R&D at Caliper Foods. The principle investigators S.A.J. and T.L.W. and other authors are not affiliated with Caliper Foods in any manner and do not declare any conflict of interest.

AUTHOR CONTRIBUTIONS

K.A.W., S.A.J., L.M.W., and T.L.W. designed the study. A.R.V., J.M.H., R.E.T., and T.V.H. conducted the clinical trial. J.M.H., K.F., Y.W., O.R.A., L.M.W., S.A.J., and T.L.W. sample processing and data analysis. J.M.H., A.R.V., N.D.R., S.A.J., L.M.W., and T.L.W. drafted the manuscript. All authors reviewed the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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