Whitepaper

CNE™ Cannabinoid Nano-emulsification

2022 White Paper on CBD Conversion

MARCH 29

CBD CONVERSION

Authored by: CNE Labs, LLC

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Nondisclosure/Nondisclosure

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Should you accidentally obtain possession of any such Formula, You will promptly:

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b) discard, delete, destroy and
c) otherwise erase all such formulas in your possession.

Because of the sensitive nature of the CNE Labs Formula, any ultimate agreement between the Parties will contemplate liquidated damages for intentional disclosure, research, reverse-engineering, circumvention of CNE Labs process.

You may not attempt to replicate CNE™ Formula.

Aaron Miles

CNE™ Founder / Organic Chemist

At 12 years old Aaron Miles left home and decided he was going to make a better path for himself. A variety of jobs occupied his time until he stepped foot into Williams Bakery 1995. In 2002 Williams sent him to Kansas to attend 5 years of school at the American Institute in Baking. This is where he learned real time baking and management earning over 20 diploma plagues graduating top three as a master baker. In 2002 he had to developed 13 products of different bread lines and took a promotion in Seattle, Washington and became a master formulator.

In 2004 Aaron took a job with Grain Millers as a technical service manager developing products as an enzyme specialist. After a year he wanted to start his own business, so he rode the Atkins wave and started "Low Carb Wellness" formulating low carb bread goods. This led to forming another business producing live organic enzymes, NIS. Nestle was his first client and is still doing business with NIS today.

In 2006, as a consultant with Ammax Nutra Source, Aaron was challenged to create three products. First was Ninzi an antioxidant drink for MLM, second was a body cleanse drink for a nutritional supplement company, and third was a low-calorie sweetener, Luo Han Guo for coca cola. He watched the company accept the first check of 1.2 million and decided he would only work for himself from that point on.

Aaron's love for racing motorcycles took him as far as racing Isle of man in Ireland. This hobby won him two championships. He had to stay lean and hydrated to perform his best so he then was inspired to create another new company called Rawnrg where he could produce energy bars and electrolyte drinks which sales financially supported a trophy truck Baja racing team.

When Aaron's father became ill with a lung disease and bone degeneration and was in extreme, he invented a way to ingest cannabinoids safely and effective. Aaron saw an opportunity in the CBD and THC arena and decided to put Rawnrg on hold and pursue his two patents for cannabinoid conversion.

In 2019, Aaron obtained his first patent pending for nano emotion "water soluble CBD and THC. His second patent pending was obtained for CBD salt and Sugar. These patents and process secrets are the basis for forming CNE Labs, LLC and CNE™ Cannabinoid Nano Emulsification our trademarked process. Moose Tea Company, LLC was formed to introduce and make available exciting new CBD consumer products.

Aaron's role models are his grandfather, Wiley Coyote, and Speed Racer for, "Never Stop Trying" and "Where there is a will there is a way". "The rest is action of applications to the desired outcome of the evolution of the mind." After time, Wiley Coyote finally caught the Road Runner and look where we are now!!

CBD and Cannabinoid Nano-Emulsification = CNE™

Principle of CNE™ as it pertains to CBD oil conversion

CBD oil is the most commonly used form of cannabidiol. Although it has moderate bioavailability of 13-19%, that is not enough. Moreover, when people take CBD oil, lots of it go through first-pass metabolism (metabolism in the liver immediately after absorption), and a considerable amount of it is destroyed.

These issues related to CBD bioavailability forced researchers to look for alternative ways of delivering CBD to the body. Of course, CBD inhaling is one such excellent way. But then that is not for everyone. Thus, researchers came up with another technology that is creating water soluble CBD.

It is not as straight forward as it may sound. One cannot just directly dissolve CBD isolate or CBD distillate into the water. It is because the CBD molecule is lipophilic. That is, it loves oils but repels water. So, the only inconsiderable amount of CBD powder would dissolve in the water.

Nevertheless, researchers have long known various natural products in which oil molecules are suspended in water as if they are mixed with it. It is called emulsion. Some of the good examples could be milk and egg yolk. There are known ways of emulsification, and the same can be applied for producing water soluble CBD.

Water soluble CBD isolate or distillate still require the use of oil. It is because CBD simply does not mix well with water. So, how to make water soluble CBD? The starting point in creating water soluble CBD is mixing it with oil. Then comes the next step, which is breaking these CBD oil particles into tiny (nano) droplets so that they can remain suspended in the water. The resulting solution is a nano-emulsion.

There are many ways of breaking CBD oil into smaller particles. But the most common strategy used is deploying either high shear mixers or high energy ultrasonic sound waves. There are special machines in which this whole process is carried out. This equipment can break CBD oil into nanoparticles, small enough to get homogeneously suspended in the water, and remain stable for quite a long time.

Usually using ultrasonic waves are regarded better than high shear mixers (moving oil and water mixture at high velocity). However, most producers use a combination of these methods. One can start with high shear mixers and then use ultrasound homogenizers to produce even better and more stable nano-emulsion. These nanoparticles are so small that they can readily penetrate through various membranes, including intestinal membranes. These particles can also escape the first-pass metabolism in the liver in large amounts. All this means far greater bioavailability of CBD.

There are other well-known ways of boosting the bioavailability of various natural extracts. One such method is using liposomes as carriers. Liposomes form a bubble-like structure over natural extracts like CBD resulting in better absorption.

Although there is no doubt that liposomal CBD has better bioavailability than CBD oil, it is still inferior to water soluble nano-emulsion. It is because the size of CBD droplets in nano-emulsion is extremely small. In contrast, liposomes are 10 to a hundred times larger than nano-emulsion droplets. Thus, despite the benefits of improved bioavailability, liposomal technology is much inferior to water soluble nano-emulsion. Both offer bigger efficacy, but nano, being smaller, is also absorbed better.

When choosing water soluble CBD, one does not have to compromise on the range of options. Like non-water-soluble options, water soluble CBD is available as isolate, distillate, and even full-spectrum.

Water soluble CBD isolate – quite like CBD isolate powder or CBD crystals, it contains CBD without other cannabinoids and is THC-free. It is loved by many due to its perceived better safety profile and a wide number of applications. It may be a better fit for certain kinds of products like edibles, CBD for older adults and athletes, and so on. Nonetheless, it is less potent due to the lack of entourage effect.

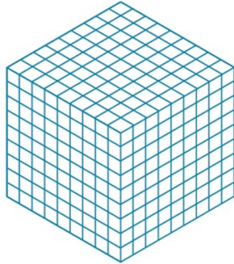
Water soluble CBD distillate – is perhaps an optimal solution for most needs. It contains CBD, other cannabinoids, and terpenes but is free from THC. Thus, it is generally regarded as quite potent and safe for special population groups who do not want any THC like athletes.

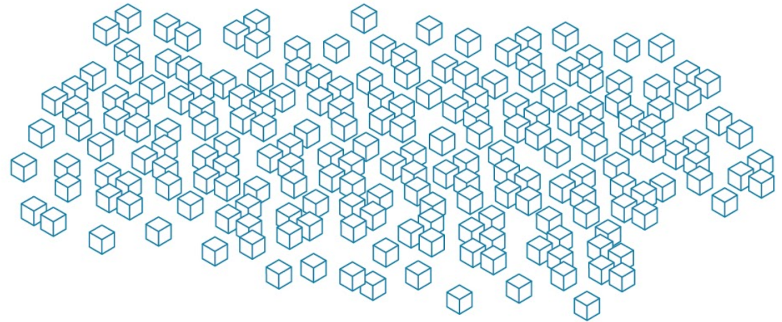
Full-spectrum water soluble CBD – as the name suggests is CBD, plus other cannabinoids, terpenes, along with traces of THC. THC is only in the permissible levels, which is usually below 0.2%. Therefore, it is a very small amount of THC to cause any mind-altering effect. However, full-spectrum water soluble CBD wholesale might be better for producing products for severe conditions like anxiety, sleep disorders, and so on.

There are numerous benefits of water-soluble CBD:

- It is a cost-effective way of enhancing the bioavailability of CBD, which helps keep the cost of CBD products low and yet improve its effectiveness.
- Water soluble CBD is proven in human studies to have reliably better pharmacokinetics. In human studies, water soluble CBD was more bioavailable than CBD oil. Thus, it is considerably better than other technologies like liposomal or the addition of other herbal extracts.
- Convenience for both users and producers. For users, water-soluble products are easier to use and carry. For producers also mean ease of adding CBD to various formulations.
- It starts acting faster, and thus, those who use water soluble CBD always prefer it.

Water soluble CBD achieves a much higher concentration in various organs, especially when used for prolonged intervals. It means that people may achieve health effects that they could not get from other types of CBD products.





CBD Oil Before Nanotechnology

CBD Oil After Nanotechnology

Smaller particle size for quicker absorption

- CNE™ uses registered DEA Labs to insure the highest level of accuracy and efficiency.
- Each batch is analytically tested and CNE Labs certified (add the certified water-soluble logo)
- CNE™ uses its own patent pending process not a third-party process.

Human Clinical Trials on CBD in 2020

Potential Superfood

2020 has been a big year for cannabidiol (CBD), a non-intoxicating extract of the hemp plant. Sales of the supplement may reach up to $2.1 billion this year, according to a study by industry analysts Hemp Business Journal, and anecdotal reports claim that CBD is helpful for all kinds of conditions.

Also see: The State of Cleaning Business in Toronto Amidst COVID-19

For decades, properly researching the therapeutic potential of the hemp plant was considered taboo and seen as a sort of "hippie pseudoscience." Now the stigma has lifted and respectable mainstream scientists are rushing to make up for lost time and research exactly what CBD is most effective for. Here are just a couple of the most recently clinical trial outcomes that have been published in the National Library of Medicine at the time of this writing in 2020 (and late 2019):

1. Peripheral Neuropathy

Scientists at the Scripps Mercy Hospital and University of Missouri School of Medicine conducted a clinical trial of 29 patients with symptoms of peripheral neuropathy. 15 patients were randomized to the CBD group and given a topical CBD oil product. The scientists found that there was "a statistically significant reduction in intense pain, sharp pain, cold and itchy sensations" and did not get any reported adverse effects.

2. Infant Epilepsy

A very small study published in the *European Journal of Pediatric Neurology* studied three infants with Epilepsy of Infancy with Migrating Focal Seizures (EIMFS) who were given pharmaceutical grade CBD. Two of the infants showed no benefit and quit the study. One infant got no benefit in reduction of seizure frequency experienced less seizure intensity.

Also read: How to Use CBD Oil to Relieve Pain

3. Absorption & Anti-inflammatory Effects of "Water Soluble" CBD

A 2020 study by scientists from Colorado State University focused on 10 healthy adults and measured their response to water-soluble vs. fat soluble CBD powders. They found that water-soluble CBD was approximately 4.5x more bioavailable … and that exposure into CBD in general may decrease markers of inflammation.

4. CBD May Help Alleviate Drug Cravings

A November 2019 study published in the *Journal of American Psychiatry* measured the effect that CBD had on heron addicts' cravings for the drug when shown a supply of narcotics (visual 'drug cues'). Opiate addicted patients who took CBD has "*significantly reduced … craving and anxiety*" when exposed to drug cues compared to those who took placebo. According to Dr. Bomi Joseph, a proponent of using hops CBD rather than sources derived from hemp, this study suggests that CBD should be further studied as a potential treatment option for opiate addiction. Also see: How CBD Calms Anxiety

5. CBD's Synergistic Effects with Anti-Epileptic Drugs

Prescription CBD, called Epidiolex, is currently of great interest to mainstream medicine because it is the first ever natural, botanical drug approved by the FDA for the treatment of epilepsy. It has shown to be highly effective for treating Lennox-Gastaut and Dravet syndrome epilepsy. Researches needed to test how CBD and its major metabolites interreacted with 3 major synthetic epilepsy drugs *clobazam*, *stiripentol*, and *valproate.* In 2020, Scientists from Greenwich Biosciences, the maker of Epidiolex, ran some clinical trials on humans and found that CBD taken along with these epileptic drugs caused slight changes in how bioactive the drugs were and in exposure to their metabolites. It concluded that CBD was 'moderately well tolerated,' at least as well as the other drugs, and there were no deaths or other serious adverse events.

6. CBD's Potency When Combined with Food, Milk & Alcohol

CBD is a fat-soluble & alcohol cannabinoid: it mixes well into oil or alcohol but doesn't dissolve into water. In 2020 scientists at Greenwich Biosciences, the maker of Epidiolex prescription CBD, examined the effect of large (750mg) doses of CBD to 4 different groups of healthy adults who took CBD alongside different foods and drinks. Those who took CBD on an empty stomach were the baseline of this experiment. CBD taken with alcohol had higher-than-baseline absorption. CBD taken with whole milk or low-calorie meals had moderate absorption, and CBD taken with a high-fat/calorie meal had up top 5 times higher absorption than the baseline. This study suggests that patients who want higher absorption of CBD may wish to take it with high calorie meals and those who are sensitive to its effect may want to try it on an empty stomach.

While 2020 has come up with a few smaller clinical trials that established what CBD could be further investigated for and a couple of larger trials on CBD absorption and coadministration with epilepsy drugs – the best is yet to come with CBD clinical trials. There are currently trials taking place on *autism, COVID-19, prostate cancer, bipolar depression* and *PTSD* that are currently underway. Stay tuned for exciting new findings of what CBD may be effective for… the scientific journey to discover the full potential of plant cannabinoids is just getting started!

References

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Cannabidiol Preparations

Jack M. Hobbs[1‡], Allegra R. Vazquez[1‡], Nicholas D. Remijan[2], Roxanne E. Trotter[1], Thomas V. McMillan[1], Kimberly Freedman[1],Yuren Wei[1], Keith A. Woelfel[3], Olivia R. Arnold[4], Lisa M. Wolfe[5] , Sarah A. Johnson[1*], and Tiffany L. Weir[1,*]

[1] Department of Food Science and Human Nutrition, Food and Nutrition Clinical Research Laboratory, Colorado State University, Fort Collins, CO 80523

[2]Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO 80523

[3]Caliper Foods, Commerce City, CO 80022

[4]Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO 80523

[5]Proteomics and Metabolomics Facility, Office of the Vice President, Colorado State University, Fort Collins, CO 80523

[‡] These authors contributed equally to the study.

*Corresponding Authors:

Sarah A Johnson; email: sarah.johnson@colostate.edu; phone: (970) 491-3807

Tiffany L. Weir; email: tiffany.weir@colostate.edu; phone: (970) 491-4631

ABSTRACT

Cannabidiol (CBD) is a dietary supplement with numerous purported health benefits and an expanding commercial market. CBD preparations range from tinctures, oils, and powders to foods and beverages. Despite its use, there is a paucity of information regarding bioavailability of these formulations. We conducted a pilot randomized, double blind study in 10 healthy adults to determine differences in absorption times and amounts between a water and lipid-soluble powder. Blood samples were collected over six hours in fasted participants and analyzed for CBD concentrations. Peripheral blood mononuclear cells (PBMCs) were collected at baseline and T=90 and assayed for markers of inflammation. In participants consuming the water soluble formulation, CBD began to appear as quickly as 15 minutes post-consumption peaking at around one hour. This formulation was ~4.5x more bioavailable than the lipid soluble treatment. Participants did not experience any changes in heart rate, blood pressure, or pulse pressure throughout the study. TNF was decreased in LPS-stimulated PBMCs after CBD exposure (combined data from both preparations) relative to cells collected at baseline. Future studies will establish the bioavailability of a larger variety of CBD delivery formats, and target additional inflammatory markers to clarify CBD's biologic effects.

Cannabidiol (CBD), one of the major constituents of *Cannabis sativa L.* (*C. sativa),* is a member of a group of C21 terpenophenolic compounds called phytocannabinoids [1-3]. The structure of CBD is comprised of a resorcinol ring, a monoterpene moiety, and an alkyl side chain [3]. In recent years, interest in this constituent of *C. sativa* has risen due to its seemingly diverse therapeutic potential and minimal

adverse side effects [4-6]. Proposed physiologic effects of CBD include anti-inflammatory, antioxidant, antipsychotic, anticonvulsant, anxiolytic, cytotoxic, and analgesic effects, which occur through multiple signaling mechanisms, many of which are still poorly characterized [7-8]. 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 [8-11]. 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 [8, 12-14].

With the legalization of cannabis products in much of the United States, the availability CBD-containing dietary supplements, foods and beverages has 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. To date, there have been very few human studies on the pharmacokinetics of CBD [15], and 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 [16-17]. Current studies reporting oral CBD administration suggest that its absorption time is similar to that of THC, with peak plasma levels detected from 60-120 minutes, but possibly as late as 6 hours post-administration[18]. Bioavailability of oral CBD preparations is also variable[18-21]. 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[18].

Here we present a pilot, two-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 and blood was collected from an intravenous catheter at several time intervals post-consumption. 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 TNF, which is a pro-inflammatory molecule, in LPS-stimulated peripheral blood mononuclear cells (PBMCs). 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 pharmacokinetic studies.

RESULTS

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 ten individuals completed the study (Table 1). Participants ranged in age from 22-51 years old and the majority (8/10) were in the normal weight BMI range (20-24.9). A total of 6 females and 4 males completed the study and each treatment group had an equal number of male and female participants. There were no significant differences in the average characteristics (height, weight, BMI, height) between treatment groups.

Plasma CBD Levels After Oral Ingestion. CBD from oral preparations was rapidly detected in the blood, with initial increases occurring as quickly as 15 minutes after ingestion. Plasma concentrations were approaching baseline levels by 6 hours post-ingestion. There was a significant difference in

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)*

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 formulation treatment group had a larger C_{max} (2.82 ng/mL) than the treatment group given the lipid soluble treatment group (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} value for the lipid soluble formulation was estimated at 90 min post ingestion (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 about 4.5-fold greater than that of the lipid-soluble formula. Despite the increased bioavailability of the water-soluble formula, there was still a high level of inter-individual variability and it appeared that absorption occurred completely in the upper gastrointestinal (GI) tract in some individuals as evidenced by a single absorption peak, while in others there were two peaks, suggestive of both upper GI and colonic absorption (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).

Figure 1. Plasma levels of 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.

Treatment	Tmax (min)	Cmax (ng/mL)	AUC $_{T=0-360}$	AUC$_{inf}$
Water-soluble CBD	54	2.82	408.11	476.1
Lipid-soluble CBD	90	0.65	90.52	98.5

Power calculations. One purpose of this study was to establish baseline data for calculating the sample size needed to detect differences in rate and amount of absorption of the two CBD preparations for future studies. Using the group means (mean A=2.44 and mean B=0.38) and variance (SD=2.17) in plasma CBD concentrations at T=30 minutes, we determined that a sample size of 17 participants per group would be needed to detect a statistically significant difference in this parameter with 80% power and a Type I error rate of 0.05.

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 (Supplemental Figure 1A-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).

Markers of inflammation in LPS-stimulated and non-stimulated 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 non-stimulated PBMCs at T=0 and T=90. 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 non-stimulated (Supplemental Figure 2A and B) or stimulated cells (Supplemental Figure 2C and 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 non-stimulated cells (Figure 2B) and the suppression of TNF was not significantly correlated with plasma CBD levels at T=90.

Figure 2. Levels of TNF and IL-10 at baseline and 90 min post-CBD consumption in LSP stimulated (A) and non-stimulated (B) PBMCs.

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 inter-individual 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 30mg 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 800mg dose provided in oral capsules to 8 volunteers that were habitual cannabis smokers had an average T*max* of 3 hours, suggesting it was primarily absorbed in the lower GI tract[22]. Likewise, an oral preparation in a gelatin format also had an average T*max* of 3 hours for a 10 mg dose and 3.5 hours for a 100 mg dose [23].

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 hours to approximately 4 hours when participants were in a fed versus fasted state [24]. Research designed to evaluate the reasons for differential absorption between the upper and lower GI tract is needed.

To date, the absolute bioavailability of CBD preparations relative to intravenous delivery has not yet been reported in humans. 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 were consumed by 12 healthy individuals and resulted in peak plasma CBD concentrations of ~5 ng/mL[25]. Guy and Flint [26] reported that sublingual drops containing 20 mg of CBD had a C*max* of 2.17 ng/ml and Nadulski et al [27] 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 hours prior to consuming the CBD-containing beverage and remained fasted for 90 min post consumption, which may have decreased the overall bioavailability of the CBD, despite decreasing the time to maximum absorption. Absorption and elimination curves, as well as the total maximum concentration, showed a great deal of inter-individual variability, which could potentially be due to interpersonal differences in interactions between the CBD and the meal provided at the 90 minute 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 [28]. Thus, the difference that we observed in bioavailability of the two tested preparations is likely due to differences in solubility in the water 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 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 [29]. 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 [30]. 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[11, 31]. 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 a pro-inflammatory elicitor. To our knowledge, this is the first study to report 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[32-33]. 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 [33]. 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 cytokine production under certain conditions[34-35]. 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, IL-5, IL-23, and GCSF. Chen et al. exposed mice to the HIV envelope glycoprotein 120 (HIV_{gp120}) to explore the effects of CBD on T-cell responses. They found that the introduction of CBD following sub-optimal cellular stimulation with low concentrations of P/I Phorbol ester/Ionomycin (P/I) 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 necessitate further research. In addition to conducting adequately powered human pharmacokinetic studies on various doses and preparations of CBD, further exploration into the potential benefits of CBD's anti-inflammatory properties should include more broadly profiling cytokine responses in humans and testing specific human populations, such as those suffering from irritable bowel syndrome or other conditions that are characterized by high levels of inflammation. Additionally, systematic studies are needed to better understand the mechanisms though which CBD exerts these effects.

EXPERIMENTAL SECTION

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 (ie. 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.

Study protocol. Participants were screened for eligibility by phone and those meeting inclusion criteria were scheduled for a clinic visit. Prior to arrival, participants were asked to 1) refrain from consuming any CBD-containing products for at least 3 days prior to their visit, and 2) fast for 6 hours 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 cannabidiol (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 non-emulsified, non-homogenized 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 minutes, 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 minutes for collection of PBMCs. In addition, supine blood pressure was measured on the non-dominant 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-hour 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 blood collection.

Plasma extraction. Cannabidiol 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 room temperature 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 re-suspended in 60 µL of 100% acetonitrile.

CBD detection and quantification by UPLC-MS/MS. LC-MS/MS was performed on a Waters Acquity M-Class 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 minutes. 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 (SRM) 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. Inter-channel 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, Washington) 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. The calibration curve was an 8 point standard curve of CBD

generated in matrix background (human plasma at T=0) with concentrations ranging from 0 ng/mL - 1000 ng/mL (3.2X dilution series). All calibration curves were linear over the range of concentrations tested ($r^2 > 0.998$). The limit of detection of the assay was 0.3 ng/mL and was calculated as the standard error divided by the slope of the linear regression of the calibration curves multiplied by 3.3. The limit of quantitation was 1 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%.

Isolation of PBMCs. Ten mL of whole blood was collected from antecubital veins into ethylenediaminetetraacetic acid (EDTA) treated vacutainer tubes. PBMCs were isolated from the whole blood within six hours of collection via density centrifugation and a series of washes. Initial whole blood was diluted with 1x Phosphate Buffer Solution (PBS) + 2% Fetal Bovine Serum (FBS, Atlas Biologics) at a 1:1 ratio and transferred into 50 mL SepMate Tubes (STEMCELL Tehcnologies) with 17 mL density gradient medium, Lymphoprep (STEMCELL Technologies). Tubes were centrifuged for 10 min at 1200 x g at room temperature. The separated plasma and PBMCs were poured off and diluted with equivalent volume of 1x PBS + 2% FBS and centrifuged at 300 x g for 8 min. Tubes were decanted and pelleted cells were resuspended in equivalent volume of 1x PBS + 2% FBS for final wash and centrifugation. Cells were counted using cell counting chambers (Nexcelom) and a Cellometer Auto T4 (Nexcelom) to calculate appropriate volume of cell freezing media, CryoStor (STEMCELL Technologies). Pelleted cells were re-suspended in CryoStor and placed in a Mr. Frosty container at -80C for 12-24 hours before final storage in liquid nitrogen.

PBMC Culturing and Stimulation. All cell processing was performed aseptically. A complete culture medium containing 1x RPMI-1640 (Corning), 10% fetal bovine serum (FBS; Atlas Biologics), 1% penicillin/streptomycin [100 U/mL penicillin and 100 µg/mL streptomycin](HyClone) was warmed to 37°C in a water bath. Frozen PBMCs were rapidly thawed for 2 min in a 37°C water bath, followed by an inversion of the cryovial to resuspend the PBMC sample in the media. Samples were then added to a 15 mL conical tube and warm media was added at a rate of 1mL/second to the conical tube containing the sample. Cells were centrifuged (25°C, 300g, 8 min) then the supernatant was decanted, followed by a repeated wash cycle for a total of two wash periods. Cells were then plated for a recovery period at 1-2 x 10^6 cells/mL.

After a 24-hour rest period, cells were counted and adjusted to reach a desired concentration of $5x10^5$ cells/250µL. A 247.5 µL aliquot of the cell-containing solution was added to each well of a U-bottom, 96-well, untreated plate to prevent cell adherence. An LPS working solution was created to give a final concentration of 100 µg/mL by diluting the original 1 mg/mL stock solution of LPS into sterile PBS. Appropriate wells were spiked with LPS by adding 2.5µL of the LPS working solution to achieve a final LPS concentration of 1 µg/mL/well. Experimental control wells included a media control and a media + LPS control. Cells were incubated at 37°C and 5% CO_2 for 48 hours. Post incubation, the 96 well plate was centrifuged (400 g, 5 min, 4°C), and supernatant was collected and frozen at -80°C until further experimentation.

Cytokine Quantification. Interleukin-10 (IL-10) and human tumor-necrosis factor alpha (TNF) enzyme-linked immunosorbent assays (ELISA) were performed using supernatants from both LPS stimulated and non-stimulated PBMCs using commercially available kits (Boster Biological Technology). Each ELISA

reaction was performed in duplicate. Absorbance values for ELISA plates were collected and averaged and target analytes were quantified by fitting to standard curves as described in the manufacturer's protocols.

Pharmacokinetic calculations. Maximum concentration (C*max*) and the time to maximum concentration (T*max*) values were established using Phoenix WinNonlin 2018 software (Certara, New Jersey). 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*.

Statistical analysis. Comparisons between treatment groups were calculated using one-way ANOVA and longitudinal comparisons within treatment groups were analyzed by repeated measures ANOVA. A p-value <0.05 was considered to be statistically significant. The required sample size to detect a statistically significant difference with 80% power and $\alpha=0.05$ in a parallel 2-arm study was calculated using the group means and variance in CBD absorption at T=30.

Associated Content

The following supplemental files are included:

-Supplemental figures 1A-C and 2A-D

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Author Information

Study design (KAW, SAJ, LMW, TLW), conduct of the clinical trial (ARV, JMH, RET, TVH), sample processing and data analysis (JMH, KF, YW, ORA, LMW, SAJ, TLW), drafting the manuscript (JNH, ARV, NDR, SAJ, LMW, TLW), and reviewing the manuscript (all authors).

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CBD oil is the most commonly used form of cannabidiol. Although it has moderate bioavailability of 13-19%, that is not enough. Moreover, when people take CBD oil, lots of it go through first-pass metabolism (metabolism in the liver immediately after absorption), and a considerable amount of it is destroyed.

These issues related to CBD bioavailability forced researchers to look for alternative ways of delivering CBD to the body. Of course, CBD inhaling is one such excellent way. But then that is not for everyone. Thus, researchers came up with another technology that is creating water soluble CBD.

Overview:

CNE Labs uses a patented and proprietary process to create water-soluble concentrate ("Concentrate") from cannabinoid distillates. If you have expressed a desire to purchase Concentrate from CNE Labs, and CNE Labs has expressed a desire in producing and selling Concentrate to you (collectively "the Parties" or "Party"). Given the complexities of interstate cannabis law, all production must be completed on-site in the state in which the end-product shall be marketed and sold.

Requirements:

You must provide the following constituent ingredients and facilities for on-site production in that company's home state:

- ❑ High-quality cannabinoid distillate, approximately three liters for a 45-liter run
- ❑ Production facilities that include
  - Proper site licensure in compliance will all state and local laws regarding the production and storage of CBD, cannabis, marijuana, cannabinoid distillates and/or any of the by-products of same
  - Power supply for 110V and 220V
  - Three-part sink
  - Running potable water
  - Secured temporary storage of equipment/Palate sized footprint
  - Tamper proof environment and room for production, minimum 12x12 ft.
- ❑ All necessary licensure in compliance with all state and local laws regarding the purchase, sale, transport, production, manufacturing, packaging and storage of CBD, CBD, cannabis, marijuana, cannabinoid distillates and/or any of the by-products of same

***

Please Note: procurement of the foregoing requirements does not constitute **partial performance** for the purposes of causing this non-binding letter of intent to mature into contract. TO's duty to procure the foregoing requirements is merely illustrative of the simple yet necessary parts that are required by CNE Labs in order to complete this novel and proprietary process.

* * *

Production:

In order to create the intended product, CNE Labs shall provide all required equipment, technology and knowledge to create the intended product on-site.

Purchase Price:

Product Price:

You will pay CNE Labs according to the final concentration and volume of the Concentrate produced; the concentration shall be evaluated by an independent third-party laboratory at your expense, with the written analysis report to be delivered jointly to the Parties, on the following schedule:

Volume of Concentrate Produced	Price Rate (as a price per milligram of CBD per milliliter of Concentrate)
45 – 90 L	$0.05 per mg CBD per mL Concentrate produced
90 – 136 L	$0.04 per mg CBD per mL Concentrate produced
> 136 L	$0.03 per mg CBD per mL Concentrate produced

Illustration A. (For demonstration purposes only.)

NOTES:

- CNE Labs will not produce a batch of Concentrate smaller than 45 liter
- Volumes are calculated in metric for conversion purposes, but the Parties have discussed the volume of Concentrate in terms of gallons produced. One gallon is approximately 3.785 liters
- *By way of Illustration A* of the foregoing table, if 50 L of Concentrate is produced, and an independent third-party laboratory determines that batch of Concentrate contains 60 mg CBD per mL, the price of that Concentrate is $150,000 ($0.05 per mg CBD per mL. Fifty liters (50,000 mL) of 60 mg/mL Concentrate at $0.05)
- *By way of Illustration A* of the foregoing table, if 100 L of Concentrate is produced, and an independent third-party laboratory determines that batch of Concentrate contains 60

mg CBD per mL, the price of that Concentrate is $240,000 ($0.04 per mg CBD per mL. One hundred liters (100,000 mL) of 60 mg/mL Concentrate at $0.04)

- *By way of Illustration A* of the foregoing table, if 150 L of Concentrate is produced, and an independent third-party laboratory determines that batch of Concentrate contains 60 mg CBD per mL, the price of that Concentrate is $270,000 ($0.03 per mg CBD per mL. One hundred fifty liters (150,000 mL) of 60 mg/mL Concentrate at $0.03)

<u>Production Session Cost:</u>

For each production session after the first production session, you will reimburse CNE Labs for reasonable travel expenses incurred. Such travel expenses shall be calculated using the travel expenses incurred during the prior production session. "Reasonable travel expenses" will include roundtrip airfare for two to three persons; rental vehicle during the production session; cost of shipping necessary equipment and technology to the production facility; and reasonable lodging expenses. "Reasonable travel expenses" shall not include dining, entertainment or incidental expenses.

<u>Timing:</u>

Purchase Price shall be due in two installments, the first as a "Deposit" in the amount of $75,000 due upon arrival of the CNE Labs team at the production facilities that You have provided; the balance due within fifteen (15) days of receiving the written analysis report; or twenty (20) days of production of the Concentrate, whichever is later. The balance due will be the total Product Price of the Concentrate as described above, plus Production Session Costs described above, less the deposit already paid upon arrival.

<u>Disclosure:</u>

Shelf life 6 to 12 months Store in Dry/Cool conditions.

The Parties would have the right to renegotiate the Purchase Price in accordance with market forces on 180 days'-notice.