Utjecaj spola i CYP polimorfizma na učinke nove formulacije kanabidiola u pacijenata s hipertenzijom

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2. POPIS OZNAKA I KRATICA

AUC – Površina ispod krivulje (engl. *area under the curve*)

ACC – Američki koledž kardiologije (engl. *American College of Cardiology*)

ACEi – Inhibitori angiotenzin konvertirajućeg enzima

AHA – Američko udruženje za srce (engl. *American Heart Association*)

BMI – indeks tjelesne mase-ITM (engl. body mass index)

CB1 – kanabinoidni receptor 1 (engl. *cannabinoid receptor 1*)

CB2 – kanabinoidni receptor 2 (engl. *cannabinoid receptor 2*)

CCB – blokatori kalcijskih kanala (engl. *calcium channel blockers*)

EMA – Europska agencija za lijekove (engl. *European Medicines Agency*)

ESC – Europsko kardiološko društvo (engl. *European Society of Cardiology*)

ESH – Europsko društvo za hipertenziju (engl. *European Society of Hypertension*)

FABPs – Proteini koji vežu masne kiseline (engl. *fatty acid-binding proteins)*

FDA – Agencija za hranu i lijekove Sjedinjenih Američkih Država (engl. *Food and Drug Administration*)

HALMED – Agencija za lijekove i medicinske proizvode

HPLC – tekuća kromatografija visoke učinkovitosti (engl. *High-performance liquid chromatography*)

IQR – interkvartilni raspon (engl. *interquartile range*)

ITM – indeks tjelesne mase (engl. *body mass index*-BMI)

MCT – CBD – formulacija srednjolančanih triglicerida-CBD (engl. *medium-chain triglycerides formulation)*

SD – standardna devijacija

SEDDS-CBD – samoemulgirajući sustav za isporuku lijeka (engl. *self-emulsifying drug delivery system)*

SNPs – polimorfizmi jednog nukleotida (engl. *single nucleotide polymorphisms,* SNPs)

VAS – vizualna analogna ljestvica

3. PREGLED OBJEDINJENIH RADOVA

Ova disertacija temelji se na objedinjenju sljedećih znanstvenih radova:

1. Batinić A, Sutlović D, Kuret S, Matana A, Kumrić M, Bozić J, Dujić Z. Trial of a Novel Oral Cannabinoid Formulation in Patients with Hypertension: A Double-Blind, Placebo-Controlled Pharmacogenetic Study. Pharmaceuticals (Basel). 2023 Apr 25;16(5):645.
2. Batinić A, Sutlović D, Kuret S, Burčul F, Kalajžić N, Matana A, Dujić G, Vrdoljak J, Kumrić M, Božić J, Dujić Z. Differences in Plasma Cannabidiol Concentrations in Women and Men: A Randomized, Placebo-Controlled, Crossover Study. Int J Mol Sci. 2023 Jun 17;24(12):10273.
3. Batinić A, Ćurković A, Bukić J, Žuntar I, Kuret S, Mimica B, et al. Knowledge and Attitudes of Cannabidiol in Croatia among Students, Physicians, and Pharmacists. Pharmacy (Basel). 2024;12(1).

3.1. UVOD

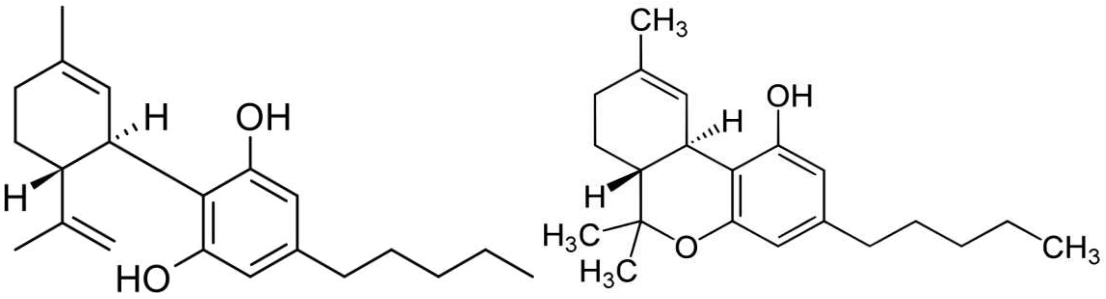
3.1.1. Kanabidiol (CBD)

Kanabidiol (CBD) je bioaktivni kanabinoid biljke konoplje (*Cannabis sativa* L.). U konoplji je izolirano preko 750 različitih spojeva, od kojih je preko 140 kanabinoida tj. fitokanabinoida, a biljka se koristi već tisućama godina u medicinske svrhe (1-3).

Izraz „medicinski kanabis“ odnosi se liječnički preporučenu upotrebu kanabisa i njegovih sastojaka, kanabinoida, za liječenje bolesti ili poboljšanje simptoma (4). THC (Δ^9-tetrahidrokanabinol) i CBD najznačajniji su i najistraživaniji fitokanabinoidi (5). Za razliku od THC-a, CBD uglavnom nema psihotropne učinke i danas ga veliki broj ljudi konzumira kao dodatak prehrani (6, 7).

Neki autori smatraju da je naziv „neintoksicirajući“ prikladniji od „nepsihoaktivnog“, s obzirom da CBD može modulirati simptome određenih neuropsihijatrijskih poremećaja (8, 9). Psihoaktivni učinci THC-a (osjećaj euforije, relaksacije, ali i psihomotorna i kognitivna disfunkcija) posljedica su njegova visokog afiniteta vezanja na endokanabinoidne receptore 1 i 2 (CB1 i CB2) (1).

A) CBD B) THC



Slika 1. Kemijske strukture kanabidiola i tetrahidrokanabinola.

Iako CB1 i CB2 receptore nalazimo u cijelom tijelu, CB1 su većinski lokalizirani u središnjem živčanom sustavu, dok su CB2 receptori uglavnom smješteni u imunološkom sustavu (10). Mnoštvo farmakoloških učinaka CBD-a posljedica je agonističkog i antagonističkog djelovanja na brojne druge receptore u organizmu (8, 11).

Istraživanja su pokazala općenito dobar sigurnosni profil CBD-a uz brojne zdravstvene koristi, zbog čega je upotreba CBD-a u stalnom porastu, posebno među mladima (7, 12-15). Dostupni dokazi upućuju na učinkovitost CBD-a kod raznih bolesti uključujući epileptičke napadaje (osobito u pedijatrijskih bolesnika), upalna stanja, neurodegenerativne i zloćudne bolesti, kroničnu bol, shizofreniju, psihoze te kardiovaskularne bolesti (16-22). Većina tih indikacija zahtijeva daljnja ispitivanja kako bi se potvrdila klinička učinkovitost. Uočena je dobra podnošljivost visokih doza (1500 mg do 6000 mg) pročišćenog oralnog pripravka CBD-a (100 mg/mL; Epidyolex®, GW Pharmaceuticals, Cambridge, UK) bez ozbiljnih nuspojava (samo s povremenim blagim do umjerenim nuspojavama kao što su mučnina, proljev, glavobolja i somnolencija), iako se radi o dozama koje su i do deset puta veće od standardno preporučenih doza (12).

Istraživanja pokazuju da zdrave odrasle osobe koje konzumiraju veće doze CBD-a (1500 mg na dan) mogu imati povišene jetrene enzime (ALT), što nije zanemarivo danas kada je sve više CBD proizvoda na tržištu dostupno u slobodnoj prodaji (23). Međutim, u konzumaciji manjih doza do 30 mg (uobičajena doza za CBD proizvode dostupne na tržištu bez recepta) nije uočen učinak na fiziološki značajne parametre jetre i bubrega (6). Prethodne studije pokazale su da je bioraspoloživost oralnih pripravaka CBD-a vrlo niska, pa su razvijeni pripravci koji imaju tehnološki napredne formulacije koje poboljšavaju bioraspoloživost i

omogućuju veće koncentracije CBD-a u plazmi (24-28). Ovisno o formulaciji, vrijeme maksimalne koncentracije i poluvrijeme eliminacije mogu se značajno razlikovati (6, 29).

U Hrvatskoj je Agencija za lijekove i medicinske proizvode (HALMED) odobrila lijek na bazi kanabidiola Epidyolex, koji su također odobrile *Food and Drug Administration* (FDA) i *European Medicines Agency* (EMA), uz dostupnost svih relevantnih podataka o lijeku, uključujući interakcije s drugim lijekovima (30-32). CBD je u Europskoj uniji registriran pod imenom Epidyolex, ali je u SAD-u službeno poznat kao Epidiolex. Marinol® (dronabinol), Syndros® (dronabinol) i Cesamet® (nabilon) tri su sintetska kanabinoida koje je odobrila FDA. Dronabinol i nabilon indicirani su kod mučnine i povraćanja, uglavnom kod malignih oboljenja. EMA je odobrila i Sativex® (oralni sprej, otopina) koji sadrži nabiksimol (THC:CBD=1:1), indiciran kod spastičnosti kod multiple skleroze.

Dosadašnje studije provedene u nekim zemljama pokazale su da je farmakološko znanje farmaceuta, liječnika, studenata, pacijenata i rekreativnih korisnika nedovoljno u pogledu kanabisa i lijekova koji potječu od kanabinoida (15, 33-36).

3.1.2. Utjecaj spola na metabolizam CBD-a

U rezultatima istraživanja utjecaja CBD terapije s obzirom na spol pronalaze se značajne razlike. Contin i sur. u svojem istraživanju provedenom na 43 ispitanika (24Ž i 19M) nisu pronašli razlike u CBD koncentracijama ispitanika s obzirom na spol (37). Aviram i sur. u svojoj studiji sugeriraju da je kod žena izražen veći rizik od nuspojava povezanih s upotrebom medicinskog kanabisa u liječenju kronične, nemaligne boli (38).

Novija studija na životinjskom modelu pokazala je da ženke štakora dosljedno pokazuju više razine CBD-a u mišićima, jetri i adipoznom tkivu nakon oralne konzumacije CBD-a. Kod ženki se Cmax povećala za 36%, a kod mužjaka se smanjila za 22%, 28 dana nakon posljednje doze (39). Maciel i sur. izvjestili su da su ženke miševa imale znatno veće koncentracije CBD-a u tkivu embrionalnog mozga nego mužjaci, dok su razine THC-a kod mužjaka bile više nego kod ženki. Ti podaci sugeriraju spolno ovisne transportne i/ili metaboličke razlike između THC-a i CBD-a u embrijima miševa (40). Varijacije u učincima kanabinoida mogu biti povezane s razlikom u distribuciji mišićne mase i masnog tkiva između muškaraca i žena. Rezultati studija na životinjama ukazuju na tkivno specifične farmakokinetičke interakcije i veće koncentracije CBD-a u plazmi kod ženki, međutim

nedostaje kliničkih ispitivanja na ljudima kako bi se utvrdilo postoje li razlike po spolu (40, 41).

Millar i sur. u svojem radu predlažu da se CBD treba uzimati oralno nakon obroka jer studije pokazuju da se C_{max} povećao nakon obroka bogatog mastima, tj. CBD se može otopiti u dijelu masti u hrani, koja povećava njegovu topljivost i apsorpciju, a posljedično i njegovu bioraspoloživost (42). Knaub i sur. izvijestili su da zdrave ispitanice mogu postići značajno veće koncentracije CBD-a od muških dobrovoljaca, ovisno o CBD formulaciji koja se konzumira (43). Osam zdravih i ženskih i muških sudionika dobilo je jednu dozu od 25 mg CBD-a oralno u novoj formulaciji: samoemulgirajućeg sustava za isporuku lijeka SEDDS-CBD (engl. *self-emulsifying drug delivery system*) ili formulaciju triglicerida srednjeg lanca MCT-CBD (engl. *medium-chain triglycerides formulation*). Žene su imale znatno veću površinu ispod krivulje (engl. *area under curve*-AUC) za MCT-CBD formulaciju od muškaraca. Međutim, muškarci su imali brži t_{max} nakon upotrebe SEDDS-CBD formulacije (43). Varijable koje kontroliraju metabolizam i akumulaciju uzete doze CBD-a su složene.

Proteini koji vežu masne kiseline (engl. *fatty acid-binding proteins*, FABPs) važni su u unutarstaničnom transportu CBD-a (44). Albumin je glavni prijenosnik CBD-a u izvanstaničnom prostoru jer je 90% CBD-a povezano s bjelančevinama. Žene, u odnosu na muškarce, imaju nižu srednju koncentraciju albumina u serumu u dobi od 20 do 60 godina. Takve informacije mogu biti korisne za optimizaciju doziranja oralnog CBD-a, posebno kod žena (45). Kako bi se najbolje razumjela potencijalna klinička korisnost CBD-a, potrebne su dodatne studije na ljudima koje bi istražile interakciju spola s učinkom liječenja CBD-om.

3.1.3. DehydraTECH™2.0 CBD

Lexaria Bioscience Corp., (Kelowna, BC, Kanada) je farmaceutska tvrtka koja je osmislila vlastitu tehnologiju isporuke lijekova tzv. DehydraTECH™, kojom se poboljšava način na koji aktivni farmaceutski sastojci (engl. *active pharmaceutical ingredients,* API) ulaze u krvotok promičući zdravije metode oralnog unosa i povećavajući učinkovitost aktivnih molekula topivih u mastima, čime se smanjuje ukupna doza. Patentirana formulacija razvijena je u skladu s načelima dobre proizvođačke prakse (engl. *Good Manufacturing Practices*, GMP) u Sjedinjenim Američkim Državama. Poboljšanog je sastava u odnosu na prethodnu TurboCBD™ formulacije (90 mg CBD; 1200 mg američkog ginsenga; 480 mg ginka bilobe;

300 mg organskog ulja konoplje) (25). Na temelju prethodnih istraživanja, za novu formulaciju, odabrana je doza od 300 mg CBD-a (6, 18, 25, 42, 46).

Dugolančane masne kiseline s visokim udjelom oleinske kiseline i pročišćeno ulje kanabidiola bez tetrahidrokanabinola patentiranim su postupkom dehidracije povezani s CBD-om. U formulaciju su dodani i praškasti organski sastojci u svrhu poboljšanja crijevne apsorpcije kao posljedica zaobilaska ili smanjenog prvog prolaska kroz jetru. Nova formulacija DehydraTECH^{TM}2.0 CBD punjena je u veganske gel kapsule veličine 00 s ciljnom jačinom od 25 mg CBD-a po kapsuli u studiji HYPER-H21-1, odnosno 75 mg CBD-a po kapsuli u studiji HYPER-H21-4. Nakon provedene kontrole kvalitete s pomoću tekuće kromatografije visoke učinkovitosti (engl. *High-performance liquid chromatography*, HPLC), analitičko izvješće potvrđuje deklarirani sastav. Formulacija generičkog CBD -a koja je korištena u prvoj studiji i placebo formulacije korištene u drugoj studiji pakirane su u potpuno jednake veganske gel kapsule veličine 00 u svrhu osljepljivanja. HPLC testiranjem potvrđeno je da se punjenje sastojalo samo od praškastog organskog supstrata bez prisustva aktivne tvari CBD-a.

3.1.4. Utjecaj CBD-a na kardiovaskularni sustav

Unatoč znatnom napretku u razumijevanju epidemiologije, patofiziologije i rizika povezanih s hipertenzijom, stope kontrole krvnog tlaka i dalje su niske u cijelom svijetu i daleko su od zadovoljavajućih diljem Europe. Postoji mnoštvo dokaza koji pokazuju da snižavanje krvnog tlaka (BP) može znatno smanjiti preuranjeni morbiditet i mortalitet (47-51). Prema važećim smjernicama Europskog kardiološkog društva (engl. *European Society of Cardiology,* ESC) i Europskog društva za hipertenziju (engl. *European Society of Hypertension*, ESH) hipertenzija se definira vrijednostima arterijskog tlaka većeg od 140/90 mmHg (51).

Tablica 1. Klasifikacija hipertenzije prema smjernicama Europskog kardiološkog društva (engl. *European Society of Cardiology,* ESC) i Europskog društva za hipertenziju (engl. *European Society of Hypertension*, ESH)

Klasifikacijska skupina	Sistolički tlak mmHg	Dijastolički tlak mmHg
Optimalni	< 120	< 80
Normalni	< 130	< 85
Visoki normalni	130–139	85–89
Stadij I hipertenzije (blaga)	140–159	90–99
Stadij II hipertenzije (umjerena)	160–179	100–109
Stadij III hipertenzije (teška)	≥ 180	≥ 110
Izolirana sistolička hipertenzija	≥ 140	< 90

Smjernice američkih društava, Američkog koledža kardiologije (engl. *American College of Cardiology*, ACC) i Američkog udruženja za srce (engl. *American Heart Association*, AHA), hipertenziju definiraju vrijednostima tlaka većih od 130/80 mmHg. Postavljanje različitih ciljnih vrijednosti tlaka očito je posljedica različite interpretacije rezultata SPRINT studije koja je ispitivala optimalne vrijednosti tlaka za smanjenje kardiovaskularnog mortaliteta i morbiditeta kod bolesnika s hipertenzijom, ali bez šećerne bolesti (52).

Utjecaj CBD-a na kardiovaskularni sustav kompleksan je i ovisi o mnogo čimbenika. Studije ukazuju da CBD ima terapijski potencijal u liječenju kardiovaskularnih bolesti kao što su moždani udar, infarkt miokarda, miokarditis, kardiomiopatije i kardiovaskularne komplikacije dijabetesa, što je povezano s vazodilatacijskim, kardioprotektivnim, antioksidativnim, protuupalnim i neuroprotektivnim svojstvima CBD-a (8, 22, 25, 53, 54).

Utjecaj CBD-a na kardiovaskularni sustav kod ljudi može ovisiti ne samo o dozi i trajanju primjene nego i o načinu uzimanja CBD-a (25, 46). Istraživanje na mladim dobrovoljcima nakon oralne upotrebe CBD-a u dozi od 90 mg nije utjecao na krvni tlak, otkucaje srca i cerebralnu perfuziju, no ista doza CBD-a inkapsuliranog kao TurboCBD™ (patentirana formulacija koja povećava bioraspoloživost CBD-a) rezultirala je smanjenjem dijastoličkog i srednjeg arterijskog krvnog tlaka (engl. *mean arterial pressure*, MAP) te

povećanjem cerebralne perfuzije (25). No, većina studija na ljudima i životinjama uglavnom ukazuju na minimalne učinke u fiziološkim uvjetima na kardiovaskularni sustav nakon akutnog i kroničnog doziranja CBD-a (20, 55-59).

Vazodilatacijski učinak CBD-a dokazan je u fiziološkim i patološkim uvjetima te je vjerojatno najdosljedniji učinak tog spoja na kardiovaskularni sustav (53, 60). Aktivacija endokanabinoidnog sustava opažena je u patološkim stanjima (61). Stresne situacije povezane su s povišenim krvnim tlakom i otkucajima srca. CBD može ublažiti oboje, ali također može poboljšati funkciju vaskularnog endotela i smanjiti arterijsku krutost (46). CBD bi mogao imati terapeutski potencijal u liječenju različitih kardiovaskularnih bolesti zbog protuupalnih i antioksidativnih svojstava, s obzirom na to da su oksidativni stres i upala bitni dijelovi njihove patogeneze (62-64).

3.1.5. Farmakogenetika CBD-a

Pojam farmakogenetika koristi se za proučavanje polimorfizama jednog nukleotida (engl. *Single nucleotide polymorphisms,* SNPs) ili drugih oblika genetskih varijanti koje su povezane s razlikama u odgovoru na lijekove, dok se farmakogenomika uglavnom koristi na razini cijelog genoma za proučavanje takvih varijanti.

Nije u potpunosti izvedivo definirati svih 38 milijuna jednonukleotidnih polimorfizama (SNPs) u ljudskom genomu. Prioritet su SNP-ovi za farmakogenomsku analizu te integracija varijabli kao što su informacije relevantne za lijek i put bolesti, potencijalna funkcionalnost SNP-ova duž puta i genetika diferencijacije populacije (65, 66). Pojedinci iste rase ili etničke pripadnosti imat će veću tendenciju nasljeđivanja više sličnih varijanti gena nego osobe različite etničke pripadnosti (67). Genetska varijabilnost enzima CYP rezultira enzimom s povećanom, normalnom, smanjenom ili nikakvom enzimskom aktivnošću (68).

Polimorfizmi u CYP genima kategoriziraju populaciju u fenotipove: slabe/spore metabolizatore (engl. *poor metabolizer*, PM), intermedijarne metabolizatore (engl. *intermediate metabolizer*, IM), normalne metabolizatore (engl. *normal metabolizer,* NM), brze metabolizatore (engl. *rapid metabolizer*, RM) i vrlo brze metabolizatore (engl. *ultrarapid metabolizer*, UM) (69, 70). Poznavanje učestalosti alela polimorfizama citokroma P450 u nekoj populaciji potrebno je za optimizaciju doziranja lijekova (69). CYP enzimi metaboliziraju većinu uobičajenih klinički propisanih lijekova (71).

Neki od najznačajnijih problema s oralnim CBD-om uključuju slabu bioraspoloživost, varijabilne farmakokinetičke profile i polimorfizme gena, koji mogu pridonijeti manjoj učinkovitosti, povećanju nuspojava i interakciji između lijekova, posebno pri višim dozama. Važno je odrediti što sve utječe na koncentraciju CBD-a u organizmu kako bi se moglo optimizirati doziranje. Genetski polimorfizmi utječu na populacijske razlike u odgovoru na lijekove, uključujući osjetljivost na nuspojave lijekova (65, 72-74). Bioraspoloživost CBD-a može ovisiti i o brzini metabolizma povezanog s genetskom varijabilnošću citokroma (CYP) P450: CYP2C19, CYP2C9 i CYP3A4 i čine između 20 i 70% ukupne aktivnosti citokroma P450 u jetri. Stoga, sposobnost kanabinoida da utječe na aktivnost ovih enzima može imati velike učinke na druge lijekove (75-78). U literaturi se spominje utjecaj polimorfizama gena na metabolizam CBD-a: CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3, CYP2C19*7 i CYP3A4. (65, 69, 79).

Proizvodi koji sadrže kanabinoide, zajedno s istodobno propisanim OTC, biljnim lijekovima ili lijekovima na recept, mogu se natjecati kao supstrat za isti metabolički enzim, što može rezultirati povećanjem koncentracije lijeka. Iako se dva ili više lijekova mogu natjecati kao supstrati za isti enzim, to se možda neće pokazati klinički značajnim ako postoji širok raspon sigurnih terapijskih koncentracija i povoljan toksikološki profil (76).

3.1.6. Cilj objedinjenih radova

Cilj je objedinjenih radova utvrditi kratkoročne i dugoročne učinke nove formulacije CBD-a (DehydraTECHTM2.0 CBD) u odnosu na generički CBD kod ispitanika s hipertenzijom. Klinička istraživanja upotpunjena su spoznajama o znanju i stavovima studenata, liječnika i ljekarnika o CBD-u.

Specifični ciljevi su:

1. Istražiti učinke i razlike nakon ingestije jedne doze DehydraTECH™2.0 CBD-a i generičkog CBD-a na krvni tlak pacijenata s neliječenom arterijskom hipertenzijom, usporediti učinak i povezanost generičkog CBD-a i DehydraTECH™2.0 CBD formulacije na polimorfizam u genima CYP P450.
2. Ispitati dugoročne učinke nove formulacije na koncentraciju CBD-a i njegovih metabolita [7-hidroksi-kanabidiol (7-OH-CBD) i 7-karboksi-kanabidiol (7-COOH-CBD)], koliko dugo ostaju u tijelu nakon ingestije zadnje doze te postoje li razlike po spolu u postignutim koncentracijama

3. Analizirati stavove i znanja liječnika, ljekarnika i studenata u Hrvatskoj o terapijskoj primjeni CBD-a i lijekova koji potječu od kanabinoida.

3.2. PREGLED METODOLOGIJE OBJEDINJENIH RADOVA

Istraživanja su provedena u Laboratoriju za integrativnu fiziologiju Medicinskog fakulteta Sveučilišta u Splitu, Laboratoriju kliničkih vještina Medicinsko laboratorijske dijagnostike Sveučilišnog odjela zdravstvenih studija Sveučilišta u Splitu te u Laboratoriju Kemijsko-tehnološkog fakulteta Sveučilišta u Splitu tijekom 2021. (1. studija) i 2022. godine (2. studija). Anketno istraživanje (3. studija) provodilo se od lipnja do srpnja 2023. godine.

3.2.1. Ispitanici

Prije uključivanja u istraživanje ispitanici su obaviješteni o postupcima i ciljevima istraživanja te su potpisali informirani pristanak.

Kriteriji uključenja za prvu studiju:

1. indeks tjelesne mase (BMI) 18,5–35 kg/m2
2. dob od 45 do 70 godina
3. manje od 150 minuta umjerene do snažne aktivnosti tjedno
4. normalni krvni tlak (120–129 mm Hg sistolički, 80–84 mm Hg dijastolički)
5. visoki normalni krvni tlak (130–139 mm Hg sistolički, 85–89 mm Hg dijastolički)
6. hipertenzija 1. ili 2. stupnja (prema važećim smjernicama Europskog kardiološkog društva za zbrinjavanje hipertenzije (engl. *European society of cardiology,* ESC)).

*Kriteriji uključenja za drugu studiju bili su isti, osim što su:

a. uključeni i bolesnici na antihipertenzivnoj terapiji (jedino ispitanici na ACEi i/ili CCB i/ili na diureticima)
b. dobna granica pomaknuta na raspon od 40 do 70 godina.

Iz studija su isključeni ispitanici koji su ispunili barem jedan od sljedećih kriterija:

1. pušenje (uključujući duhan i proizvode na bazi kanabisa)
2. sekundarni oblici hipertenzije
3. aktivna maligna bolest
4. bilo koji oblik dokumentirane srčane bolesti

5. šećerna bolest
6. kronična bolest bubrega
7. giht
8. kronična gastrointestinalna bolest
9. značajni psihijatrijski poremećaji
10. dijagnoza ili povijest epilepsije
11. trudnoća i/ili dojenje
12. primjena beta blokatora ili drugih antihipertenziva osim navedenih u uključnim kriterijima
13. bolest jetre, potvrđena na biokemiji krvi.

3.2.2. Ustroji studija

Prvi rad temelji se na randomiziranoj, placebo kontroliranoj, dvostruko zaslijepljenoj *crossover* studiji pod nazivom HYPER-H21-1 (80). Drugi rad temelji se na randomiziranoj, placebo kontroliranoj, trostruko zaslijepljenoj *crossover* studiji (81). Protokol drugog istraživanja prije početka rada s ispitanicima registriran je u Registru za klinička istraživanja ClinicalTrials.gov pod brojem NCT05346562 (od 6. travnja 2022.), a studija je registrirana pod nazivom HYPER-H21-4.

Prilikom prvog, inicijalnog posjeta (*screening*), potencijalni sudionici obaviješteni su o postupcima i ciljevima istraživanja. Tek nakon potpisivanja pismenog informiranog pristanka krenulo se u mjerenja te ispunjavanje upitnika. Primijenjen je upitnik za medicinski probir za potvrdu kriterija prihvatljivosti. Prikupljena su antropometrijska i fiziološka mjerenja (visina, tjelesna težina, opseg struka, krvni tlak).

Svim uključenim ispitanicima uzeta je detaljna anamneza. Za mjerenje visine korišten je visinomjer (Seca, Birmingham, UK), dok je Tanita vaga (DC-360 S; Tanita, Tokyo, Japan) korištena za mjerenje težine, metaboličke dobi, visceralne masti i udjela masti, mišićnog tkiva i vode. ITM (engl. *body mass index*, BMI) izračunat je dijeljenjem vrijednosti tjelesne mase (kg) i kvadrata visine (m^2). Iskusni medicinski tehničar vadio je krv svakom od ispitanika iz kubitalne vene pomoću sterilne igle po pravilima standardne operativne procedure. Uzorci urina i plazme pohranjivali su se na -20°C te naknadno toksikološki analizirali.

Treći rad (82) temelji se na anketnom istraživanju koje je provedeno radi utvrđivanja znanja i stavova hrvatskih ljekarnika, liječnika i studenata zdravstvenih struka o CBD-u. Istraživanje je provedeno u lipnju i srpnju 2023. godine.

3.2.2.1. Postupci prve studije (HYPER-H21-1)

Sudionici koji ispunjavaju uvjete nasumično su razvrstani s pomoću web-usluge istraživačkog randomizatora (https://www.randomizer.org, pristupljeno posljednji put 16. travnja 2021.). Ispitanici su zatim dolazili u prostorije Zavoda za integrativnu fiziologiju Medicinskog fakulteta u Splitu u jutarnjim satima nakon cjelonoćnog posta od minimalno deset sati (bez hrane i pića, osim vode). Sudionicima studije je nakon petnaest-minutnog sjedenja i mirovanja izmjeren krvni tlak. Ukoliko je krvni tlak bio iznad 120/80 mm Hg, krenulo se s protokolom koji je trajao otprilike četiri sata.

Po dolasku svi su ispitanici dobili isti lagani obrok tj. pecivo, a vode su smjeli piti po volji. Masa peciva, koji je nabavljen i proizveden u trgovini Lidl Hrvatska, bila je 75g. Količina svih sastojaka po pecivu: ugljikohidrati 37,95 g (od čega saharoze 18,0 g); bjelančevine 3,9 g; sol 1,05 g; ukupne masti 15,3 g (od čega zasićene masti 2,625 g); energija (kcal) = 306,75 (1284,3 kJ).

Uzorak krvi uzimao se na početku, nakon 120 i 180 minuta od ingestije. Prilikom svakog vađenja uzeto je po 15 mL krvi. Uzorkovana krv odmah se centrifugirala (deset minuta na 3500 okretaja po minuti, na standardnoj temperaturi od 4°C). Ispitanici nisu smjeli konzumirati alkohol niti vježbati barem 24 sata prije istraživanja.

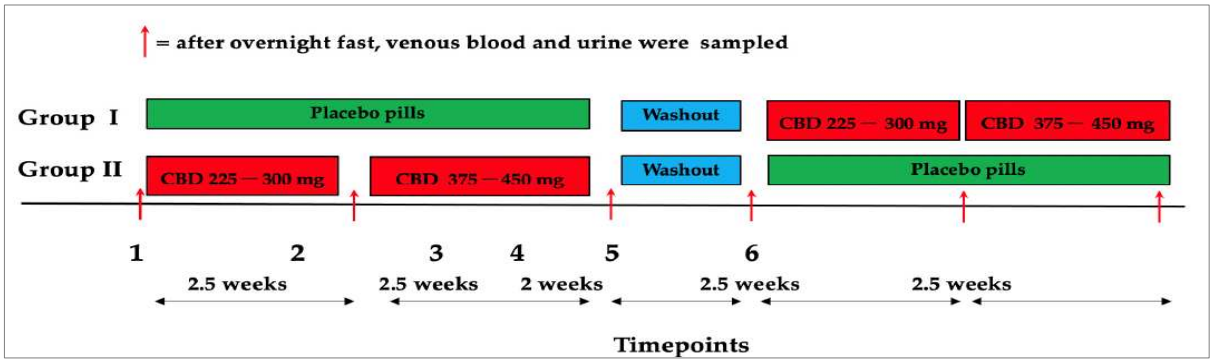
Za potrebe mjerenja krvnog tlaka u prvoj studiji korišten je tlakomjer WatchBP Home A (proizvođača Microlife AG Swiss Corporation, Widnau, Switzerland). Uzorkovana krv se nakon centrifugiranja pohranila na -20°C u svrhu naknadne toksikološke analize. Krvni tlak i broj otkucaja srca mjerili su se u triplikatu svakih deset minuta. Prije doziranja i svakih 90 minuta nakon toga korišteni su standardizirani upitnici za procjenu GI simptoma i anksioznosti (vizualna analogna ljestvica-VAS). Između prvoga i drugoga posjeta laboratoriju trebalo je proći najmanje četiri dana.

3.2.2.2. Postupci druge studije (HYPER-H21-4)

Odabrana su 62 sudionika s hipertenzijom (27 žena i 35 muškaraca) u dobi između 40 i 70 godina, od kojih je 30 ispitanika bilo na terapiji antihipertenzivima. Studija je trajala 12 tjedana.

Ispitanici su bili podijeljeni u dvije skupine, od kojih je prva skupina isprva uzimala placebo pet tjedana, pa onda CBD pet tjedana, dok je između bio period ispiranja (*washout*) u trajanju od dva tjedna. Druga skupina imala je obrnut protokol te je isprva uzimala kapsule CBD-a. Svi ispitanici posjetili su laboratorij šest puta kako bi im se uzeli uzorci krvi i urina.

U drugoj studiji analizirane su koncentracije CBD-a i njegovih metabolita 7-hydroxy-CBD (7-OH-CBD) i 7-carboxy-CBD (7-COOH-CBD) u uzorcima plazme i urina te određen polimorfizam određenih CYP 450 enzima. Cijeli protokol druge studije detaljno je opisan u radu Kumrić i sur. 2023 (83).



Slika 2. Dizajn studije HYPER-H21-4

3.2.2.3. Postupci treće studije

Treći rad temelji se na rezultatima anketnog istraživanja kojim se htjelo procijeniti znanja i stavove liječnika, ljekarnika i studenata o korištenju CBD-a u medicinske svrhe. U tu svrhu izrađena su dva upitnika: prvi za procjenu znanja i stavova liječnika i ljekarnika, a drugi za procjenu znanja i stavova studenata Medicinskog fakulteta, Farmaceutsko-biokemijskog i Zdravstvenih studija u Splitu, Zagrebu i Osijeku.

Ukupno je sudjelovalo 874 sudionika: 473 studenta, 100 liječnika (52 specijalista i 48 liječnika opće medicine) te 301 ljekarnik (16 sa završenom specijalizacijom i 285 ljekarnika bez specijalizacije). Ispitanici su ispunjavali anketne upitnike tijekom lipnja i srpnja 2023. godine. Upitnik za studente sadržavao je 20 pitanja, a za liječnike i ljekarnike 31 pitanje. Oba upitnika dizajnirana su na temelju prethodnih studija slične tematike (33, 35, 84-98).

3.2.3. Statističke metode

Prikupljeni podaci su kodirani, sortirani i pripremljeni za analizu pomoću softverskog paketa (SPSS Inc., Chicago, IL, SAD) verzije 28. U obradi podataka korištena je deskriptivna statistika za opis razdiobi kvantitativnih i kvalitativnih varijabli. Za provjeru normalnosti korišten je Kolmogorov–Smirnov test. Zbog nenormalne distribucija podataka kontinuirane varijable prikazane su kao srednja vrijednost ± standardna devijacija (SD) ili medijan (interkvartilni raspon, IQR), dok su kategoričke varijable prikazane kao apsolutni brojevi (N) i postotci (%). Razine CBD-a logaritamski su transformirane za postizanje normalne distribucije. CBD razine predstavljale su zavisne varijable, dok su CYP enzimi predstavljali nezavisne varijable.

Grupe su uspoređivane pomoću neparametrijskog Mann-Whitneyevog U testa, jednosmjernog ANOVA testa i t-testa. Multivarijatni model linearne regresije proveden je zasebno za CBD i DehydraTECH™2.0 CBD doze. Statistički značajnima smatrale su se *p*-vrijednosti < 0,05. Multivarijatna linearna regresija provedena je kako bi se procijenila povezanost CYP genotipova s razinama CBD-a u plazmi i urinu.

U trećem su radu razlike između skupina ispitivanih parametara mjerene s pomoću hi-kvadrata i Mann-Whitney U testa. Hi-kvadrat testovi korišteni su za usporedbu uobičajenih percepcija o CBD-u i obrazovanja među liječnicima, farmaceutima i studentima. Procjena minimalno potrebne veličine uzorka za navedene analize može se naći u izvornim radovima.

3.2.4. Etička načela

Kliničke studije iz ovog doktorskog rada provedene su u skladu s Helsinškom deklaracijom, a odobrilo ih je i Etičko povjerenstvo Medicinskog fakulteta Sveučilišta u Splitu (br. 2181-198-03-04-21-0001 za rad I, br. 2181-198-03-04-21-0091 za rad II, te br. 2181-228-103/1-47 za rad III). Svi ispitanici dali su pismeni informirani pristanak za sudjelovanje.

3.3. SAŽETI PREGLED REZULTATA OBJEDINJENIH RADOVA

Rezultati prve studije ukazuju da upotrebom Mann-Whitney testa nije primijećena statistički značajna razlika u koncentracijama CBD-a u plazmi nakon jednokratnog doziranja DehydraTECH™2.0 CBD formulacije i generičkog CBD-a. Međutim, koncentracija CBD-a u urinu 180 minuta nakon ingestije nove formulacije pokazala je statistički značajno veću koncentracijom CBD-a samo kod muškaraca ($p = 0,021$). Gotovo svaki ispitanik, dok je uzimao

DehydraTECH™2.0, imao je višu koncentraciju CBD-a u uzorcima plazme uzetim u 120. i 180. minuti, ali i u uzorcima urina u 180. minuti.

Od ukupno 24 ispitanika šest ih je bilo na terapiji lijekovima (levotiroksin, lorazepam, diazepam, celekoksib, acetil salicilna kiselina). Vrijednosti koncentracija CBD-a u njihovim uzorcima uglavnom odstupaju od srednjih vrijednosti. Prema rezultatima dosadašnjih istraživanja i dostupnim podacima metabolizam navedenih lijekova odvija se preko istih CYP P450 enzima (podaci dostupni na mrežnoj stranici: PharmGKB). Uočena je tendencija značajnijeg smanjenja srednjeg arterijskog tlaka (MAP) kod nove formulacije u usporedbi s generičkim CBD-om, posebno u prvih 20 minuta nakon doziranja ($p = 0.056$). S obzirom da se krv nije uzorkovala u toj vremenskoj točki, vrijednosti koncentracije CBD-a u plazmi ostaju nepoznate, dakle, nije poznato je li u 20. minuti od ingestije postignuta maksimalna koncentracija.

DehydraTECH™2.0 CBD imao je veći utjecaj na smanjenje dijastoličkog krvnog tlaka od CBD kontrole ($p = 0,025$ u 20. minuti). Smanjenje otkucaja srca bilo je statistički značajno za obje formulacije u 180. minuti mjerenja (CBD, $p = 0,024$; DehydraTECH™2.0 CBD, $p = 0,020$); Međutim, u 120. minuti samo DehydraTECH™2.0 CBD formulacija je polučila statistički značajno smanjenje otkucaja srca ($p = 0,048$). Mutirani aleli CYP2C19 smanjuju razinu CBD-a. Varijante CYP2C19*2 ($p = 0,037$) i CYP2C19*17 ($p = 0,022$) pokazale su statističku značajnost. Oba su bila negativno povezana s razinama CBD-a u mokraći (beta = -0,489 za CYP2C19*2 i beta = -0,494 za CYP2C19*17). Kod enzima CYP2C9*2*3 uočeno je da ispitanici sa sporim/slabim metabolizatorom (PM od eng. *poor metabolizer*), nakon konzumiranja DehydraTECH™2.0 CBD formulacije, imaju veću koncentraciju CBD-a u plazmi u 180. minuti.

U drugoj studiji prvi su put analizirane koncentracije CBD-a i metabolita tijekom 12 tjedana. Analizom koncentracije CBD-a u plazmi u prvoj vremenskoj točki mjerenja, nakon dva i pol tjedna konzumiranja CBD-a, uočeno je da su koncentracije CBD-a veće kod muškaraca nego kod žena. Kako je vrijeme odmicalo, a nakon ponovljenih većih doza u sljedećoj vremenskoj točki (nakon pet tjedana), koncentracija CBD-a bila je viša u žena (53,349 ng/mL) nego u muškaraca (41,171 ng/mL).

Linearnom regresijskom analizom uspoređivane su koncentracije CBD-a u plazmi s postotkom tjelesne masti kod oba spola. Kod muškaraca je pronađena značajna negativna korelacija nakon pet tjedana ($p = 0,03$). Nakon perioda ispiranja (dva tjedna) sudionici iz prve

skupine uzimali su placebo pet tjedana, tijekom kojih su testirani dva puta (nakon dva i pol te nakon pet tjedana). Svi ispitanici u obje ispitivane točke ostali su pozitivni na prisutnost metabolita 7-COOH-CBD. Dva i pol tjedna nakon početka uzimanja placeba rezultati su: 50,0% ih je bilo pozitivno na prisutnost CBD-a, a 18,35% na prisutnost 7-OH-CBD-a. Rezultati u zadnjoj točki mjerenja su sljedeći: 15,6% pozitivnih na CBD i 9,4% pozitivnih na 7-OH-CBD.

Rezultati naše studije pokazuju da su nakon prestanka uzimanja CBD-a i dvotjednog ispiranja koncentracije CBD-a u žena, u odnosu na muškarce, ostale znatno više. Više žena nego muškarca bilo je pozitivno na cirkulirajući CBD nakon ispiranja te dva i pol tjedna konzumiranja placebo kapsula. U zadnjoj točki mjerenja Grupe 1 koncentracija CBD-a u žena bila je 2,13 ng/mL, međutim, nijedan muškarac nije bio pozitivan na CBD. Koncentracija CBD-a u Grupi 1 smanjivala se u svakoj vremenskoj točki koja je slijedila nakon perioda ispiranja. Dakle, u četvrtoj, petoj i šestoj vremenskoj točki, 94% (16/17), 76% (13/17) i 29% (5/17) žena je imalo pozitivan nalaz na prisutnost CBD-a. U istim vremenskim točkama, 86% (12/14), 21% (3/14) i 0% (0/14) muškaraca je bilo pozitivno. Rezultati ukazuju na značajne razlike po spolu u koncentracijama CBD-a između vremenskih točaka 4 i 6 ($p < 0,05$).

Prisutnost CBD-a u plazmi kod žena uočena je i nakon 50 dana od zadnjeg uzimanja CBD preparata, za razliku od muškarca kod kojih nijedan uzorak plazme nije bio pozitivan na CBD ni na njegove metabolite. Prema prikupljenim podacima, postotak tjelesne masti kod žena bio je značajno viši u usporedbi s postotkom kod muškaraca (74%), dok su postoci mišićnog tkiva i vode kod žena u usporedbi s muškarcima bili znatno niži. Te vrijednosti vjerojatno objašnjavaju veće koncentracije CBD-a u plazmi žena u odnosu na muškarce u kasnijim vremenskim točkama mjerenja. Kod muškaraca je niži postotak masnog tkiva te smanjena akumulacija u adipoznom tkivu rezultirala višim koncentracijama CBD-a u krvotoku, koji je metaboliziran i izlučen urinom, tj. eliminiran iz tijela brže u usporedbi sa ženama.

Rezultati su pokazali da nije bilo povezanosti koncentracija CBD-a s bilo kojim genotipom. Ispitanici koji uzimaju terapiju za hipertenziju (inhibitore angiotenzin-konvertirajućeg enzima-ACE, blokatore kalcijskih kanala i tiazidne diuretike) nisu imali statistički značajnu razliku u koncentracijama CBD-a i njegovih metabolita u uzorcima u odnosu na ispitanike koji nisu na terapiji.

Statistički značajna razlika u koncentraciji CBD-a u urinu za vremensku točku 3 uočena je za enzim CYP2C9 kod muških sudionika. Muškarci s NM fenotipom imali su

statistički značajno više vrijednosti CBD-a od muškaraca s fenotipom IM (p = 0,025). Isti enzim u svim vremenskim točkama kod žena nije pokazao statistički značajne promjene.

Rezultati treće studije ukazuju na nedostatak znanja o CBD-u među objema skupinama s obzirom da 89,3% ljekarnika i liječnika, kao i 84,8% studenata, smatra da im je potrebna dodatna edukacija o CBD-u. Svaki peti ispitanik smatra da nema znanja o CBD-u. Preko 60% studenata i gotovo 60 % liječnika i ljekarnika smatra terapiju kanabidiolom učinkovitom.

Uočeno je da studenti značajno više koriste CBD-a (25,4%) u odnosu na liječnike i ljekarnike (16,2%), dok je značajno veći postotak liječnika i ljekarnika (43,0%, odnosno 47,8%) čitao znanstvene radove o CBD-u usporedbi sa značajno manjim postotkom studenata (17,5%).

Preko 70% ukupnog broja ispitanika izjavilo je da tijekom formalnog obrazovanja nisu imali saznanja o CBD-u. Na pitanje može li upotreba CBD-a smanjiti upotrebu opioida kod kronične boli, uočena je statistički značajna razlika u stavovima liječnika i ljekarnika. Samo 6% liječnika odgovorilo je pozitivno, dok čak 83,4% ljekarnika vjeruje da kanabidiol može smanjiti upotrebu opioida. Liječnici i ljekarnici uglavnom ne propisuju i/ili ne preporučuju CBD (92%, odnosno 83,1%) iako imaju dovoljno znanja o indikacijama, nuspojavama i interakcijama. Pretpostavlja se da je uzrok tomu, osim nesigurnosti u znanju, i visoka cijena proizvoda. Stoga je razumljivo da čak 83% liječnika i 85% ljekarnika smatra da bi zdravstveno osiguranje trebalo pokriti troškove lijeka.

3.4. RASPRAVA

Do sada nije provedena nijedna studija koja istražuje učinke samo jedne doze DehydraTECH™2.0 CBD-a na krvni tlak pacijenata s neliječenom arterijskom hipertenzijom uz usporedbu učinka generičkog CBD-a i DehydraTECH™2.0 CBD formulacije na polimorfizam u genima CYP P450. U drugoj studiji proširena su istraživanja ispitivanjem dugoročnih učinaka DehydraTECH™2.0 CBD formulacije na koncentracije CBD-a i njegovih metabolita [7-hidroksi-kanabidiol (7-OH-CBD) i 7-karboksi-kanabidol (7-COOH-CBD)] u plazmi i urinu. Proučavan je odnos postignute koncentracije CBD-a i njegovih metabolita u odnosu na spol, utjecaj genetskog polimorfizma na postignute koncentracije CBD-a i metabolita, ali i koliko dugo su CBD i njegovi metaboliti ostali u tijelu nakon zadnje doze.

DehydraTECH™2.0 CBD formulacija dobro se podnosi i ispitanici nisu prijavili nijednu ozbiljnu nuspojavu. Prijavljene su uglavnom blage nuspojave: gastrointestinalne

tegobe (proljev) i pospanost kod generičkog CBD-a te relaksacija bez pospanosti kod nove formulacije.

3.4.1. Koncentracije CBD u plazmi i urinu, utjecaj na krvni tlak

Rezultati prve studije pokazuju da nakon konzumiranja CBD-a nije bilo statistički značajne razlike u promjenama između sistoličkog i dijastoličkog krvnog tlaka u 120. i 180. minuti. Međutim, DehydraTECH™2.0 CBD je snižavao dijastolički tlak u odnosu na početnu vrijednost više nego generički CBD. To je bilo osobito vidljivo u prvih 10 do 20 minuta nakon doziranja, a statistička značajnost prikazana je u 20. minuti nakon ingestije.

Dodatno, DehydraTECH™2.0 CBD je imao tendenciju većeg smanjenja srednjeg arterijskog tlaka (MAP) od početne vrijednosti u odnosu na generičku kontrolu, osobito u prvih 20 minuta nakon doziranja. S obzirom na to da nismo uzimali uzorke krvi u 20. minuti nakon ingestije, ostaje nepoznato je li nova formulacija postigla maksimalne koncentracije u toj točki. Rezultati našeg istraživanja u skladu su s rezultatima prethodnih studija o utjecaju CBD-a na smanjenje krvnog tlaka (18, 25, 27). Za usporedbu, u prethodnoj kliničkoj studiji na ljudima bilo je potrebno 120 minuta za postizanje iste razine smanjenja MAP-a, uočavamo brži učinak nove, poboljšane formulacije (25).

Millar i sur. u svojem preglednom radu navode kako se CBD može otopiti u udjelu masti u hrani, povećavajući njegovu topljivost i apsorpciju, a posljedično i njegovu bioraspoloživost. Stoga, kako bi se pospješila optimalna apsorpcija, isti autori predlažu da se CBD treba uzimati oralno nakon obroka (42).

Uočeno je statistički značajno smanjenje brzine otkucaja srca (engl. *heart rate*, HR) za obje formulacije. Nakon 120 i 180 minuta DehydraTECH™2.0 CBD formulacija pokazala je statistički značajan pad HR-a, dok ga je generički CBD pokazao tek nakon 180 minuta.

Ovisno o formulaciji, vrijeme maksimalne koncentracije (t_{max}) i poluvrijeme eliminacije ($t_{1/2}$) mogu se značajno razlikovati. Abbotts i sur. ispitivali su farmakokinetiku pet različitih formulacija CBD-a u 14 ispitanika i otkrili da je $t_{1/2}$ između 106 i 246 min, a t_{max} između 30 i 90 minuta (6). Uspoređujući to s rezultatima naše studije pretpostavljamo da najveći učinak CBD-a na sniženje krvnog tlaka nije povezan s vremenom u kojem su postignute najveće koncentracije CBD-a u plazmi. Iako nije uočena statistički značajna razlika u koncentracijama obje testirane formulacije, vrijedno je napomenuti da se koncentracija CBD-a u plazmi obiju formulacija povećavala s vremenom. Nakon 120 minuta prosječne

koncentracije CBD-a bile su veće nakon ingestije nove formulacije, dok su nakon 180 minuta bile niže od generičkog CBD-a. Osim toga, nova je formulacija imala veću prosječnu koncentraciju u urinu na 180 minuta, što može biti posljedica njegove povećane apsorpcije i bržeg metabolizma u odnosu na generički CBD. Dakle, DehydraTECH™2.0 CBD formulacija ima bolji učinak na početno smanjenje dijastoličkog krvnog tlaka, MAP-a i otkucaja srca od iste doze generičkog CBD-a.

U drugoj smo studiji proširili ispitivanje koncentracija CBD-a i na njegove metabolite: 7-hidroksi-kanabidiol (7-OH-CBD) i 7-karboksi-kanabidol (7-COOH-CBD). CBD se uglavnom metabolizira u 7-COOH- CBD, kao što su već potvrdile prethodne studije (12, 99). U usporedbi s koncentracijom nakon dva i pol tjedna konzumacije CBD-a, koncentracija tog metabolita u plazmi nakon pet tjedana korištenja CBD-a bila je gotovo dva puta veća.

Perez-Acevedo i sur. u svojem su istraživanju izmjerili veće koncentracije 7-OH-CBD u urinu od 7-COOH metabolita (100). Naši rezultati ne podudaraju se s njihovima jer je koncentracija 7-OH-CBD u svim vremenskim točkama niža od izmjerene koncentracije 7-COOH- CBD, osim u drugoj točki mjerenja (nakon dva i pol tjedna od početka konzumacije). Međutim, valja napomenuti da je njihova studija vršila mjerenja unutar 24 sata. Taj se vremenski okvir razlikuje od našeg jer su mjerenja provedena nakon dva i pol te pet tjedana od početka konzumacije CBD-a. Omjer CBD/7-OH u plazmi se smanjio tijekom vremena u korist povećanja koncentracije metabolita.

Taylor i sur. u svojoj su studiji primijenili značajno više doze od naših (1500, 3000, 4500 i 6000 mg dnevno). Maksimalne koncentracije CBD-a i metabolita nisu porasle proporcionalno s povećanjem doza. Stoga, unatoč četverostrukom povećanju doze (s 1500 mg na 6000 mg), maksimalne koncentracije bile su samo 2,67 puta veće. Naši rezultati također ukazuju da povećanje metabolita nije proporcionalno povećanju uzete doze. Naime, pet tjedana od početka uzimanja CBD-a, u usporedbi s mjerenjem nakon dva i pol tjedna, u plazmi naših ispitanika primijećeno je povećanje od samo 50%.

3.4.2. Razlike po spolu

Rezultati studija HYPER-H21-1 i HYPER-H21-4 ukazuju na razlike po spolu u metabolizmu CBD-a. Sultan i sur. su, primjerice, izostavili žene iz svoje studije kako bi isključili mogućnost utjecaja razlika po spolu na rezultate, što je ujedno i glavno ograničenje njihova istraživanja (46). Sache-Seeboth i sur. su u svojoj studiji analizirali farmakokinetičke varijacije nakon jedne

oralne doze od 15 mg THC-a. Njihovi rezultati za maksimalne koncentracije i prosječnu površinu ispod krivulje ukazuju na razlike između žena i muškaraca. Kod žena su te vrijednosti bile oko 1,5 puta veće u usporedbi s vrijednostima kod muškaraca. Razlike nisu bile povezane s genotipom, nego su ovisile o volumenu distribucije tj. o postotku tjelesne masti (101).

Naše prvo istraživanje nije pokazalo statistički značajne razlike u koncentracijama CBD-a u plazmi i urinu kod generičkog CBD-a. Nakon konzumiranja nove formulacije, nije primijećena statistički značajna razlika u koncentracijama CBD-a u plazmi, dok su izmjerene koncentracija CBD-a u urinu muškaraca nakon 180 minuta bile značajno veće u odnosu na koncentracije kod žena. Posljednjih godina provedeno je mnogo istraživanja o farmakokinetici i farmakodinamici CBD-a i razlikama po spolu, ali su uglavnom bile kratke, nisu uključivale mnogo ispitanika oba spola ili nisu provedene na ljudima (39, 102). Knaub i sur. su uočili da zdrave ispitanice mogu postići veće koncentracije CBD-a u plazmi od zdravih muških dobrovoljaca (43).

Rezultati našeg istraživanja ukazuju na veći omjer CBD/7-OH-CBD kod muškaraca nego kod žena dva i pol tjedna od početka konzumacije CBD-a (1,97 prema 1,68), dok je gotovo jednak nakon pet tjedana (1,89 prema 1,87). U početnom mjerenju muškarci su imali više CBD-a u plazmi nego žene, vjerojatno zato što je dio CBD-a u žena već bio pohranjen u masnom tkivu.

Prema podacima našeg istraživanja postotak tjelesne masti kod žena bio je značajno viši u usporedbi s muškarcima, dok su postoci mišićnog tkiva i vode bili znatno niži. Žene su imale 74% veći udio masnog tkiva u odnosu na muškarce. Naši rezultati ukazuju na prisutnost CBD-a u plazmi kod žena i 50 dana nakon zadnjeg uzimanja CBD preparata, dok ni jedan uzorak kod muškaraca nije bio pozitivan na CBD. Razlika u masnom tkivu između muškaraca i žena vjerojatno je razlog za takve nalaze.

Rezultati naše studije podudaraju se s rezultatima studije koju su proveli Child i Tallon na životinjama (skupina od šest mužjaka i šest ženki štakora) tijekom 28 dana, dokazuju da se CBD nakuplja u ispitivanim tkivima, uključujući masno tkivo, mišiće i jetru. Najviše vrijednosti nađene su u masnom tkivu kod ženki (39). Čimbenici koji utječu na metabolizam i pohranjivanje CBD-a u tkivima su složeni. Proteini koji vežu masne kiseline (engl. *fatty acid-binding proteins,* FABPs) važni su u unutarstaničnom transportu CBD-a (44).

Albumin je glavni prijenosnik CBD-a u izvanstaničnom odjeljku; 90% CBD-a je vezano za bjelančevine. Žene, u usporedbi s muškarcima, imaju nižu srednju koncentraciju

albumina u serumu u dobi od 20 do 60 godina (45). Ti se podaci mogu koristiti za personalizaciju doziranja oralnog CBD-a. Rezultati naše prve i druge studije koreliraju s obzirom na uočene razlike po spolu u izmjerenim koncentracijama cirkulirajućeg CBD-a.

3.4.3. SNP genotipizacija

Kako bi se uočila povezanost između koncentracija i metabolizma CBD-a s genetskom varijabilnosti citokroma P450, istraživanje je uključivalo analizu polimorfizma gena: CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3, CYP2C19*17 i CYP3A4 (103). Prethodne studije sugeriraju povezanost genetske varijabilnosti s metabolizmom CBD-a, a naši rezultati to djelomično potvrđuju (23, 103).

Rezultati naše prve studije pokazali su da nije bilo povezanosti koncentracija generičkog CBD-a ni s jednim fenotipom, dok je za DehydraTECH™2.0 CBD formulaciju uočena statistička povezanost između koncentracija u plazmi (u 180-oj min.) i fenotipa CYP2C9*2*3. Fenotip IM imao je najniže koncentracije CBD-a, zatim slijedi NM, dok je PM fenotip imao najviše vrijednosti koncentracija. Naši rezultati nisu pokazali značajne razlike u koncentracijama CBD-a s genotipovima svih pojedinačno testiranih SNP-ova, kao i njihovih kombinacija.

U svojoj *in vitro* studiji Beers i sur. su utvrdili važan utjecaj CYP2C9 i CYP2C19 u metabolizmu CBD-a, posebno za nastanak aktivnog metabolita 7-OH-CBD, dok CYP3A4 nije bio uključen u metabolizam 7-OH-CBD (103). U našoj je studiji najveća povezanost uočena za CYP2C9*2 i CYP2C19*17. Kod CYP2C9*2*3 enzima subjekti s fenotipom slabog metabolizatora (PM), u usporedbi s drugim fenotipovima, nakon konzumacije DehydraTECH™2.0 CBD formulacije imali su više koncentracija CBD-a u uzorku plazme uzetom u 180-oj min. Kao rezultat toga, preporuka je razmatranje fenotipa subjekta tijekom određivanja doze DehydraTECH™2.0 CBD formulacije.

Pokazalo se da se razina 7-COOH-CBD-a u urinu u vremenskoj točki 2 statistički značajno razlikuje kod žena. Naime, žene s NM fenotipom imale su najveće vrijednosti ispitivanog metabolita, dok su žene s RM fenotipom imale najniže vrijednosti. Pretpostavljamo da je to rezultat korištene formulacije, koja zaobilazi prvi prolaz kroz jetru, gdje se i nalaze ispitivani enzimi.

Nasrin i sur. u svojoj studiji sugeriraju da kanabinoidi i njihovi metaboliti mogu inhibirati neke CYP P450 enzime (79). Podaci o zdravim pojedincima koji su uzimali veće

doze CBD-a ukazuju na povećanje vrijednosti serumske alanin aminotransferaze (ALT), što korelira s oštećenjem jetre izazvane lijekovima (23). U trenutnoj studiji koriste se nekoliko puta niže doze, pa pretpostavljamo da jetreni enzimi nisu bili pogođeni. Potrebna su daljnja istraživanja kako bi se definirao utjecaj polimorfizma CYP P450 enzima na metabolizam CBD-a te iskristalizirale smjernice za personaliziranu terapiju.

3.4.4. Interakcije s lijekovima

U prvoj studiji, od ukupno 24 ispitanika 18 ih nije primalo nikakvu terapiju. Preostalih šest ispitanika bilo je na terapiji: dva ispitanika su uzimali po 25 μg levotiroksina ujutro; po jedan ispitanik je uzimao: 100 μg levotiroksina ujutro i 1 mg lorazepama navečer, 5 mg diazepama, 100 mg celekoksiba i 100 mg acetilsalicilne kiseline. Prema rezultatima dosadašnjih istraživanja i dostupnim podacima, oksidativni metabolizam diazepama i lorazepama posredovan je izoenzimima CYP3A4 i CYP2C19. Celekoksib primarno metabolizira CYP2C9, dok levotiroksin smanjuje aktivnost i ekspresiju CYP3A4 (podaci dostupni na web stranici: PharmGKB). Usporedbom koncentracija testiranih formulacija CBD-a u uzorcima plazme i urina navedenih ispitanika sa srednjim vrijednostima CBD koncentracija ostalih ispitanika, možemo uočiti da vrijednosti uglavnom odstupaju od srednjih vrijednosti. Zaključujemo da korištenje tih lijekova utječe na metabolizam CBD-a, što treba uzeti u obzir pri doziranju CBD dodataka prehrani u različitim formulacijama.

U drugoj studiji, od ukupno 62 sudionika s hipertenzijom (27 žena i 35 muškaraca) 30 ih je bilo na antihipertenzivnoj terapiji. Nije primijećena statistički značajna razlika u koncentracijama CBD-a i njegovih metabolita između ispitanika koji su bili na antihipertenzivnoj terapiji (inhibitori angiotenzin-konvertirajućeg enzima-ACEi, blokatori kalcijskih kanala i tiazidni diuretici) i onih koji nisu bili na terapiji.

3.4.5. Znanja i stavovi studenata, liječnika i ljekarnika o kanabidiolu

Prema našim saznanjima to je prva studija koja je istraživala percepcije i znanja o terapijskoj primjeni CBD-a među studentima u Splitu, Zagrebu i Osijeku te među ljekarnicima i liječnicima u Hrvatskoj. U prethodnim je studijama utvrđeno nedovoljno znanje o CBD-u među studentima medicine i zdravstvenim djelatnicima (33, 35, 85, 104, 105).

Naši rezultati ukazuju na manjkavost u znanju o CBD-u među objema skupinama s obzirom na to da 89,3% ljekarnika i liječnika te 84,8% studenata smatra da im trebaju dodatne

edukacije. Većina naših ispitanika također smatra da bi nastavni programi trebali sadržavati predavanja o korištenju CBD-a u medicinske svrhe (14, 33, 35, 104). Ispitanici se uglavnom slažu da je terapijsko uzimanje CBD-a korisno (63,6% studenata te 54,6% ljekarnika i liječnika), što je u skladu s rezultatima prethodnih studija o terapijskoj primjeni medicinskog kanabisa (34, 106). Goodman i sur. su u svojem anketnom istraživanju primijetili da se malo zna o potencijalnonim učincima CBD-a (107). Prema našem istraživanju samo je 31,5% studenata te 26,2% liječnika i ljekarnika svjesno rizika povezanih s uporabom CBD-a.

Prema rezultatima nacionalne ankete o upotrebi i stavovima o CBD-u u Francuskoj 30% sudionika nikada nije čulo za CBD (84). Svaki peti ispitanik našeg anketnog istraživanja nema saznanja o CBD-u. Statistički značajna razlika uočena je među skupinama u povezanosti s pitanjem o čitanju znanstvenih radova o CBD-u. Naime, samo 17,5% studenata je čitalo znanstvene radove o CBD-u, za razliku od značajno većeg postotka liječnika i ljekarnika (43% odnosno 47,8%).

Rezultati potvrđuju naša očekivanja da je konzumacija CBD-a povezana uglavnom sa studentima, koji su mlađa dobna skupina od profesionalaca, kao što su pokazala prijašnja istraživanja (84, 108). Najviše znanja o CBD-u među studentima su pokazali budući ljekarnici (84,8%) , a slijede ih studenti zdravstvenih studija (73,6%) te studenti medicine (70,7%). Slično istraživanje provedeno u Austriji ukazuje da su studenti medicine imali najviše znanja o kanabidiolu (35).

Liječnici i ljekarnici često povezuju odobrene FDA indikacije za CBD s onima za dronabinol i nabilon. Međutim, ljekarnici češće prepoznaju indikaciju za epileptičke napadaje u LGS-u (Lennox-Gastaut sindrom) i Dravet sindromu (36%) nego liječnici (23%), pri čemu je sudionicima uglavnom nepoznato da je tuberozna skleroza također među odobrenim indikacijama (31). Najčešće zabilježene nuspojave kanabidiola su somnolencija, smanjeni apetit, proljev i povraćanje. Znanje sudionika o nuspojavama općenito je dobro, s izuzetkom tahikardije koju je nuspojavom smatralo više od jedne trećine liječnika i ljekarnika. Prethodne studije pokazale su da CBD snižava otkucaje srca, dijastolički tlak i MAP (srednji arterijski tlak) bez izazivanja tahikardije (25, 46).

Za razliku od liječnika, ljekarnici znatno češće vjeruju da bi preporučivanje/propisivanje CBD-a moglo smanjiti upotrebu opioida za kroničnu bol, što neka istraživanja sugeriraju (109). McNabb i sur. objavili su u rezultatima svojeg istraživanja da veterani mogu smanjiti potrošnju farmaceutskih lijekova i drugih supstanci uzimanjem

ljekovitog kanabisa (109). Liječnici i ljekarnici slažu se da nemaju dovoljno znanja o korištenju CBD-a u medicinske svrhe (94% odnosno 88,7%). Zbog toga ni ne žele svojim pacijentima preporučiti njegovu upotrebu. Ispitanici se slažu da bi zdravstveno osiguranje trebalo pokriti troškove CBD-a kada ga liječnik propisuje kao terapiju. Epidyolex je trenutno dostupan samo uz ograničeni recept i u Hrvatskoj ga u cijelosti plaća pacijent (više od 1200 eura po bočici od 100 mL) (31).

3.5. ZAKLJUČCI

Svaki ispitanik uglavnom je imao višu koncentraciju CBD-a u svim analiziranim uzorcima nakon jednokratne ingestije DehydraTECH™2.0 CBD, dakle, utvrđena je bolja i brža apsorpcija CBD-a kod patentirane formulacije u odnosu na generički CBD.

Patentirana formulacija DehydraTECH™2.0 CBD pokazuje bolji učinak na početno smanjenje dijastoličkog krvnog tlaka i MAP-a u odnosu na generički CBD kod ispitanika nakon samo jedne doze od 300 mg.

Utvrđeno je da je smanjenje otkucaja srca (engl. *heart rate*, HR) bio primarni pokazatelj povišene koncentracije CBD-a, s tim da je sniženje HR bilo značajnije kod ispitanika koji su dobili DehydraTECH™2.0 CBD u odnosu na generički CBD.

Uočene su razlike po spolu u metabolizmu CBD-a: muškarci su imali statistički značajno više koncentracije CBD-a u uzorcima urina od žena nakon ingestije DehydraTECH™2.0 CBD-a. Kod dugoročne konzumacije CBD-a i zbog većeg postotka masnog tkiva žene su za razliku od muškaraca imale pozitivne nalaze CBD-a u plazmi 50 dana nakon posljednje ingestije CBD-a. Koncentracije CBD-a u plazmi kod muškaraca bile su u negativnoj korelaciji s količinom njihova masnog tkiva.

Preporučamo proizvodačima da na deklaracije proizvoda koji sadrže kanabinoide umetnu upozorenje o mogućnosti dugotrajnog zadržavanja u organizmu koja uzrokuje pozitivne rezultate testova za detekciju kanabinoida i do gotovo dva mjeseca iza posljednje ingestije.

Istovremeno konzumiranje lijekova čiji se metabolizam također odvija preko CYP2C9, CYP2C19 i CYP3A4 utječe na metabolizam CBD-a jer su vrijednosti njihovih koncentracija značajno odstupale od ostalih uzoraka. U optimizaciji doziranja CBD-a potrebno je uzeti u obzir cijelu terapiju koju pacijent koristi.

Polimorfizam citokroma P450 utječe na metabolizam kanabidiola, pa su potrebna daljnja istraživanja kako bi se doziranje moglo personalizirati i optimizirati.

Konzumacija CBD-a među ispitanicima povezana je uglavnom s mlađom dobnom skupinom tj. studentima, a manje sa starijima, tj. u našem slučaju sa zdravstvenim djelatnicima.

Nužno je unaprijediti obrazovne programe kako bi medicinski stručnjaci mogli raspolagati većim znanjima te preporučiti korištenje CBD-a pacijentima kada je potrebno.

Utvrđena je potreba za dodatnim edukacijama o pravilnoj i sigurnoj upotrebi CBD-a među zdravstvenim djelatnicima.

Ljekarnici i liječnici smatraju da bi zdravstveno osiguranje trebalo pokriti troškove liječenja CBD-om.

3.6. SAŽETAK

Cilj doktorskog rada bio je istražiti kratkoročne i dugoročne učinke nove formulacije DehydraTECH™2.0 CBD u odnosu na generički CBD i placebo kod ispitanika s hipertenzijom. Klinička istraživanja upotpunili smo spoznajama o znanju i stavovima studenata, liječnika i ljekarnika o CBD-u. DehydraTECH™2.0 CBD je patentirana formulacija kapsule koja povećava i ubrzava bioapsorpciju aktivnog sadržaja zbog poboljšanog lipofilnog sastava, što podrazumijeva patentirani proces kojim se dugolančane masne kiseline s visokim udjelom oleinske kiseline postupkom dehidracije povezuju s CBD-om, a za rezultat ima smanjen metabolizam prvog prolaza kroz jetru.

U prvoj studiji (HYPER-H21-1) prvi je put analiziran učinak patentirane formulacije DehydraTECH™2.0 CBD-a te uspoređen s učinkom generičkog CBD-a . Utvrđeno je da je smanjenje otkucaja srca bio primarni pokazatelj povišene koncentracije CBD-a, s tim da je nova formulacija pokazala značajniji učinak u odnosu na generički CBD. DehydraTECH™2.0 CBD formulacija pokazala je i bolji učinak na početno smanjenje dijastoličkog krvnog tlaka i MAP-a u odnosu na generički CBD. Za vrijeme uzimanja DehydraTECH™2.0 gotovo svaki ispitanik imao je višu koncentraciju CBD-a u uzorcima plazme i u uzorcima urina. Takav učinak DehydraTECH™2.0 CBD formulacije posljedica je bolje apsorpcije u odnosu na generički CBD-a.

Rezultati provedene studije ukazali su na razlike u metabolizmu CBD-a obzirom na spol. Muškarci su u odnosu na žene imali statistički značajno više koncentraciji CBD-a u uzorcima urina 180 minuta nakon ingestije DehydraTECH™2.0 CBD-a.

Od ukupno 24 ispitanika šest ih je bilo na terapiji lijekovima (levotiroksin, lorazepam, diazepam, celekoksib, acetil salicilna kiselina). Vrijednosti koncentracija CBD-a u njihovim uzorcima uglavnom odstupaju od srednjih vrijednosti. Zaključujemo da korištenje tih lijekova (ali i svih drugih lijekova koji se metaboliziraju preko istih CYP P450 enzima) utječe na metabolizam CBD-a, što treba uzeti u obzir pri optimizaciji doziranja CBD-a.

Tijekom i nakon studije nisu uočene ni prijavljene ozbiljne nuspojave. Prijavljene su samo one blage: proljev i relaksacija uz pospanost nakon generičkog CBD-a te relaksacija bez pospanosti nakon DehydraTECH™2.0 CBD.

Kako bi se uočila veza između postignute koncentracije CBD-a u uzorcima i metabolizma s genetskom varijabilnosti citokroma P450, istraživanje je uključivalo analizu polimorfizma gena CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3, CYP2C19*17 i CYP3A4. Kod enzima CYP2C9*2*3 uočeno je da ispitanici sa sporim/slabim metabolizatorom (PM od eng. *poor metabolizer*) nakon konzumiranja DehydraTECH™2.0 CBD formulacije imaju veću koncentraciju CBD-a u plazmi u 180. minuti.

U našoj drugoj studiji (HYPER-H21-4) prvi su put su analizirane koncentracije CBD-a i metabolita tijekom 12 tjedana. Analizom koncentracije CBD-a u plazmi u prvoj vremenskoj točki mjerenja, nakon dva i pol tjedna konzumiranja CBD-a, uočene su veće koncentracije CBD-a kod muškaraca nego kod žena. U sljedećoj vremenskoj točki (nakon pet tjedana) koncentracija CBD-a bila je viša u žena nego u muškaraca. Rezultati naše studije pokazuju da su nakon prestanka uzimanja CBD-a i dvotjednog ispiranja,koncentracije CBD-a u žena, u odnosu na muškarce, ostale znatno više. Zbog svoje visoke lipofilnosti CBD se lakše akumulira u lipofilnom okruženju, što utječe na kumulativnu koncentraciju i usporava eliminaciju. Postotak tjelesne masti kod žena bio je značajno viši u usporedbi s muškarcima, dok su postoci mišićnog tkiva i vode bili znatno niži, što vjerojatno objašnjava veće koncentracije CBD-a u plazmi žena u kasnijim vremenskim točkama mjerenja. Prisutnost CBD-a u plazmi kod žena uočena je čak i nakon 50 dana od zadnjeg uzimanja CBD preparata, za razliku od muškarca kod kojih ni jedan uzorak nije bio pozitivan na CBD ni na njegove metabolite. Kod muškaraca je niži postotak masnog tkiva te smanjena akumulacija u adipoznom tkivu rezultirala višim koncentracijama CBD-a u krvotoku te je metaboliziran i izlučen urinom, tj. eliminiran iz tijela brže u usporedbi sa ženama. U svim ispitivanim vremenskim točkama prisutnost metabolita 7-COOH-CBD bila je značajno veća kod žena u odnosu na muškarce.

Ispitanici koji uzimaju terapiju za hipertenziju (inhibitore angiotenzin-konvertirajućeg enzima-ACE, blokatore kalcijskih kanala i tiazidne diuretike) nisu imali statistički značajnu razliku u koncentracijama CBD-a i njegovih metabolita u uzorcima u odnosu na ispitanike koji nisu bili na terapiji.

U trećoj studiji prvi su put ispitane percepcije i znanja o terapijskoj primjeni CBD-a među studentima u Splitu, Zagrebu i Osijeku, kao i među ljekarnicima i liječnicima u Hrvatskoj. Rezultati ukazuju na nedostatak znanja o CBD-u među objema skupinama, s obzirom da većina ispitanika smatra da im je potrebna dodatna edukacija o CBD-u. Uočena je značajno veća upotreba CBD-a kod studenata u odnosu na liječnike i ljekarnike. Za razliku pak od studenata, značajno veći postotak liječnika i ljekarnika čita znanstvene radove o CBD-u.

Liječnici i ljekarnici uglavnom ne propisuju i/ili preporučuju CBD iako imaju dovoljno znanja o indikacijama, nuspojavama i interakcijama. Pretpostavljamo da je razlog, osim nesigurnosti u znanju, i visoka cijena proizvoda. Stoga je razumljivo da liječnici i ljekarnici općenito smatraju da zdravstveno osiguranje treba pokriti troškove lijeka.

Rezultati ovog doktorskog rada uključuju niz preporuka za ispravno doziranje i pravilnu primjenu nove formulacije kanabidiola.

Ključne riječi: kanabidiol, CBD, kanabidiol metaboliti, citokrom P450, CYP P450, farmakogenetika, SNP genotipizacija

3.7. SUMMARY

Dissertation title: The impact of gender and CYP polymorphism on the effects of the new cannabidiol formulation in patients with hypertension

This doctoral dissertation's objective was to investigate the short-term and long-term effects of the new DehydraTECH™2.0 CBD formulation compared to generic CBD and placebo in subjects with hypertension. We completed the clinical research with the knowledge and attitudes of students, physicians, and pharmacists about CBD.

The patented process that dehydrates long-chain fatty acids high in oleic acid with CBD, reduces the metabolism of the first pass through the liver. Therefore, the DehydraTECH™2.0

CBD formulation has increased and accelerated bioabsorption of the active content. The effects of the unique DehydraTECH™2.0 CBD formulation were evaluated and compared with the effects of generic CBD in our initial study (HYPER-H21-1).

The primary indication of increased CBD content was found to be a decrease in heart rate, with the novel formulation demonstrating a greater impact than generic CBD. Additionally, the DehydraTECH™2.0 CBD formulation demonstrated a superior impact on the initial lowering of MAP and diastolic blood pressure. Due to CBD's improved absorption, nearly every participant ingesting DehydraTECH™2.0 CBD displayed higher concentrations of CBD in their plasma and urine samples.

Based on gender, our study's findings revealed variations in CBD metabolism. In urine samples taken 180 minutes after ingesting DehydraTECH™2.0 CBD, men's CBD concentrations were significantly greater than women's.

Six of the 24 participants in the initial study were receiving medication therapy (levothyroxine, lorazepam, diazepam, celecoxib, and acetylsalicylic acid).

Their samples' CBD concentration values significantly differ from the mean values. We conclude that the metabolism of CBD can be affected by the consumption of these prescription drugs (as well as all other drugs metabolized by the same CYP P450 enzymes), and that this should be considered when calculating the optimal dosage of CBD.

During or following the research, no significant adverse effects have been noticed or reported. The only minor adverse effects were noted: relaxation with drowsiness and diarrhea after taking generic CBD, and relaxation without drowsiness after taking DehydraTECH™2.0 CBD.

To observe the relationship between the achieved concentration of CBD in the samples and the metabolism with the genetic variability of cytochrome P450, the study included the analysis of polymorphisms of CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3, CYP2C19*17 and CYP3A4 genes. After ingesting the DehydraTECH™2.0 CBD formulation, participants with a slow/weak metabolizer (engl. *poor metabolizer*-PM) at CYP2C9*2*3 enzyme showed increased CBD concentration in the plasma in 180 minutes.

For the first time, during a 12-week period, the concentrations of CBD and metabolites were analyzed in our second study (HYPER-H21-1).

Men had higher concentrations of CBD than women did, according to an analysis of the CBD concentrations in plasma at the first time point of testing, which was measured 2.5

weeks following CBD ingestion. At the next time point (after 5 weeks), the concentration of CBD was higher in women than in men. Even when they stopped taking their CBD medication and entered a two-week washout period, the study revealed noticeably greater CBD concentrations in women than men.

CBD accumulates more easily in a lipophilic environment because of its high lipophilicity. This slows down the elimination process and affects the cumulative concentration.

Women had much larger percentages of body fat than men did, but their percentages of muscle tissue and water were significantly lower. The increased levels of CBD in women's plasma at later test periods are presumably explained by this.

Unlike men, who did not have a single sample that was positive for CBD or its metabolites, the woman's plasma revealed that it contained CBD even 50 days after the last consumption of the CBD preparation. Men had a lower percentage of fat tissue than women, and since there was less CBD accumulation in fat tissue, there was more CBD in the bloodstream. In addition, CBD is eliminated in the urine, excreting from the body faster in men than in women. Compared to men, women had significantly higher levels of 7-COOH-CBD metabolites at all time points.

The concentrations of CBD and its metabolites in the samples did not differ significantly from the results for subjects who were not receiving therapy for hypertension, including angiotensin-converting enzyme-ACE inhibitors, calcium channel blockers, and thiazide diuretics.

Our third study examined the opinions and understandings of Croatian physicians and pharmacists, as well as students in Split, Zagreb, and Osijek, regarding the medicinal use of CBD. The majority of participants claimed that they needed more information regarding CBD, indicating that both groups lacked knowledge about the substance.

Students were found to consume CBD at a considerably higher rate than physicians and pharmacists. Compared to university students, a much higher proportion of doctors and pharmacists read scientific articles on CBD.

Even though they have considerable knowledge about the benefits, side effects, and interactions of CBD, medical professionals commonly do not prescribe or promote it.

We believe that, apart from a lack of knowledge, the explanation is the high price of the product. This is why medical professionals generally think that health insurance should cover the cost of this prescription drug.

The results of this doctoral thesis provide several recommendations for the correct dosage and proper application of the new cannabidiol formulation.

Keywords: cannabidiol, CBD, cannabidiol metabolites, cytochrome P450, CYP P450, pharmacogenetics, SNP genotyping

3.8. ŽIVOTOPIS

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STRANI JEZICI

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4. PRESLIKE RADOVA

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Trial of a Novel Oral Cannabinoid Formulation in Patients with Hypertension: A Double-Blind, Placebo-Controlled Pharmacogenetic Study

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Abstract: Cannabidiol (CBD) is a non-psychoactive cannabinoid, and available evidence suggests potential efficacy in the treatment of many disorders. DehydraTECH™2.0 CBD is a patented capsule formulation that improves the bioabsorption of CBD. We sought to compare the effects of CBD and DehydraTECH™2.0 CBD based on polymorphisms in CYP P450 genes and investigate the effects of a single CBD dose on blood pressure. In a randomized and double-blinded order, 12 emales and 12 males with reported hypertension were given either placebo capsules or DehydraTECH™2.0 CBD (300 mg of CBD, each). Blood pressure and heart rate were measured during 3 h, and blood and urine samples were collected. In the first 20 min following the dose, there was a greater reduction in diastolic blood pressure ($p = 0.025$) and mean arterial pressure MAP ($p = 0.056$) with DehydraTECH™2.0 CBD, which was probably due to its greater CBD bioavailability. In the CYP2C9*2*3 enzyme, subjects with the poor metabolizer (PM) phenotype had higher plasma CBD concentrations. Both CYP2C19*2 ($p = 0.037$) and CYP2C19*17 ($p = 0.022$) were negatively associated with urinary CBD levels (beta = −0.489 for CYP2C19*2 and beta = −0.494 for CYP2C19*17). Further research is required to establish the impact of CYP P450 enzymes and the identification of metabolizer phenotype for the optimization of CBD formulations.

Keywords: cannabidiol; blood pressure; CYP P450 genes; GC-MS analysis; SNP genotyping

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1. Introduction

Cannabidiol (CBD) is a bioactive cannabinoid of the plant *Cannabis sativa* L. Unlike tetrahydrocannabinol (THC; Δ^9-tetrahidrocannabinol), CBD has non-psychotropic effects and is consumed as a food supplement by millions of people today [1,2]. There are over 900 studies, reported on ClinicalTrials.gov, exploring the potential indications of CBD on Parkinson's disease, stroke, inflammations, epilepsy, chronic pain and many psychiatric conditions. CBD has a favorable low-affinity safety profile for CB1 and CB2 receptors that represent the primary binding site of THC (the primary psychoactive ingredient of cannabis) [3,4]. Available evidence suggests the effectiveness of CBD in various diseases including epileptic seizures (especially pediatric patients), inflammatory conditions, malignancies, chronic pain, schizophrenia, psychosis, and cardiovascular disease [5–14]. Stressful situations are associated with increased blood pressure and heart rate. CBD can alleviate both, but it can also improve vascular endothelial function and decrease arterial stiffness [15]. Good tolerability of high doses (1500 mg to 6000 mg) of purified oral CBD preparations (100 mg/mL; Epidiolex®, GW Pharmaceuticals, Cambridge, UK: pure CBD

oral solution) without serious side effects (only with occasional mild to moderate side effects such as nausea, diarrhea, headache and somnolence) has been demonstrated, although these are doses that are more than ten times higher than the standard recommended doses [16]. Safety has also been demonstrated at 20 mg/kg daily for 2–3 months [6]. The doses used in more recent studies are drastically lower and amount to about 5% of the maximum applied doses in tolerability trials. Healthy adults consuming CBD may experience elevated liver enzymes (ALT), which is not negligible today when more and more CBD products are available over-the-counter on the market [17]. Novel formulations for improving oral CBD delivery and efficacy are under development in the pharmaceutical industry [18]. Some preparations have technologically advanced formulations that improve bioavailability and allow higher concentrations of CBD in plasma. For instance, faster and higher absorption of CBD was shown in young volunteers after oral TurboCBD™ formulation, which led to a decrease in diastolic and mean arterial pressure and increase in cerebral perfusion [19]. In this study, we used DehydraTECH™2.0 CBD, a patented capsule that advances the earlier TurboCBD™ formulation (developed by Lexaria Bioscience Corp., Kelowna, BC, Canada) and increases the bioabsorption of the active content due to its enhanced lipophilic composition by bypassing (or reducing) first-pass liver metabolism [19–21]. The bioavailability of CBD in subjects depends also on the rate of metabolism associated with the genetic variability of cytochrome P450: CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3, CYP2C19*7 and CYP3A4 genes [22–28]. The main objective of this randomized, placebo-controlled crossover study was to investigate the effects of a single CBD dose on blood pressure in patients with untreated arterial hypertension and to compare the effect of generic CBD and DehydraTECH™2.0 CBD formulation on polymorphisms in CYP P450 genes. The hypothesis is that the hypotensive effects of CBD will be more apparent in advanced formulation DehydraTECH™2.0 CBD comparing to the generic CBD control tablets and that 300 mg of CBD is a safe dietary supplement for stage 1 or stage 2 hypertensive population.

2. Results

2.1. Visual Analog Scale (VAS)

A total of 24 subjects, 12 female and 12 male, participated in this study, and they were included for statistical analysis of the VAS data. The results are presented in Table 1.

Table 1. Visual analog scale (VAS) summary.

	Dose	BL	90 min	180 min	Dose	Time	Inter
GI	A	0 ± 0	0.8 ± 4.1	1.3 ± 6.1	0.154	0.464	0.579
	B	0 ± 0	0 ± 0	0.1 ± 0.6			
Anxiety	A	8.1 ± 14	3.3 ± 8.6	3.3 ± 8.6	0.523	0.083	0.663
	B	5.5 ± 10.1	3.2 ± 7.8	3.6 ± 7.9			
Sleepiness	A	7.1 ± 14.2	32.9 ± 27.6	29.1 ± 21.9	0.380	<0.001	0.763
	B	3.6 ± 8.5	26.8 ± 30.9	28.6 ± 28.2			

Data presented as mean ± SD. Abbreviation: SD, standard deviation; formulation A, CBD; formulation B, DehydraTECH™2.0 CBD; BL, baseline.

VAS was performed on a scale from 0 to 100—i.e., 0 being no distress and 100 being unbearable distress. While there does not appear to be any differences between the doses on VASs, there was a tendency for anxiety to decrease (main effect $p = 0.083$) and sleepiness to increase (main effect $p < 0.001$) with both doses across the 3 h. Statistical significance was assessed using a linear mixed model, with Bonferroni adjusted post hoc tests.

2.2. CBD Concentrations

The results of concentration CBD and DehydraTECH™2.0 CBD were statistically analyzed by measurement range (minimum and maximum values, median and interquartile range (IQR)) for every group of samples. The results are presented in Table 2.

Table 2. Descriptive statistics concentration of CBD (A) and DehydraTECH™2.0 CBD (B).

Samples n = 24		Minimum ng/mL	Maximum ng/mL	Median	IQR	Mann–Whitney U Test *p* Value
Plasma at 120 min.	A	5.91	225.22	73.73	99.80	0.853
	B	0.05	715.91	65.79	83.73	
Plasma at 180 min.	A	44.03	807.24	177.48	132.45	0.322
	B	51.22	612.98	134.78	105.76	
Urine at 180 min.	A	8.49	3678.50	545.76	1000.02	0.680
	B	12.16	3750.64	327.35	971.45	

Abbreviation: formulation A, CBD; formulation B, DehydraTECH™2.0 CBD, n, number of subjects exposed; IQR, interquartile range.

No statistical difference was found between the test participant' CBD concentrations for formulations A and B for any of the tested samples using the Mann–Whitney test. The investigated samples had p values greater than 0.05: for plasma samples at 120 min. ($p = 0.853$), for plasma samples at 180 min. ($p = 0.322$), and for urine samples at 180 min. ($p = 0.680$). In terms of sex, there was no statistically significant difference in the CBD concentrations in plasma and urine (formulation A). After the consumption of formulation B, no statistically significant difference was observed in plasma CBD concentrations, while the concentration of CBD in urine at 180 min, with respect to gender, showed a statistically significant difference with higher CBD concentration observed in men ($p = 0.021$). Figure 1 shows a comparison of plasma cannabidiol: CBD (dose A) and DehydraTECH™2.0 CBD (dose B) concentration in venous blood samples (**I** and **II**) and urine samples (**III**).

2.3. The Effect of CBD on Blood Pressure (BP)

The blood pressure and heart rate of all subjects after consuming different CBD formulations were measured every 10 min. Figure 2 shows a comparison of average changes in MAP (mean arterial pressure) in the measured time.

There was a tendency for relative mean arterial pressure (MAP) to be reduced to a greater extent from baseline with the DehydraTECH™2.0 CBD than the concentration matched, generic CBD control, most notably in the initial 20 min post-dosing.

Regardless of the fact that the plasma concentration was not measured 20 min after consumption, and it is unknown whether the maximum concentration was reached at that time, a DehydraTECH™2.0 CBD had greater influence on relative diastolic blood pressure drop than the generic CBD control (Figure 3).

This was most notable in the initial 10–20 min period post-dosing evidencing statistical significance at the 20 min timepoint ($p = 0.025$). Both formulations reduced systolic pressure throughout the whole measuring period (Figure 4), and there was no statistically significant difference in their effects on systolic pressure ($p > 0.05$).

Heart rate reduction was statistically different between the two studied at 180 min (CBD, $p = 0.024$; DehydraTECH™2.0 CBD, $p = 0.020$); however, at 120 min, only the DehydraTECH™2.0 CBD formulation was statistically different ($p = 0.048$) (Table 3). Compared to the baseline, no statistically significant difference was observed in changes in systolic and diastolic blood pressure at 120 and 180 min from the start for any of the tested formulations.

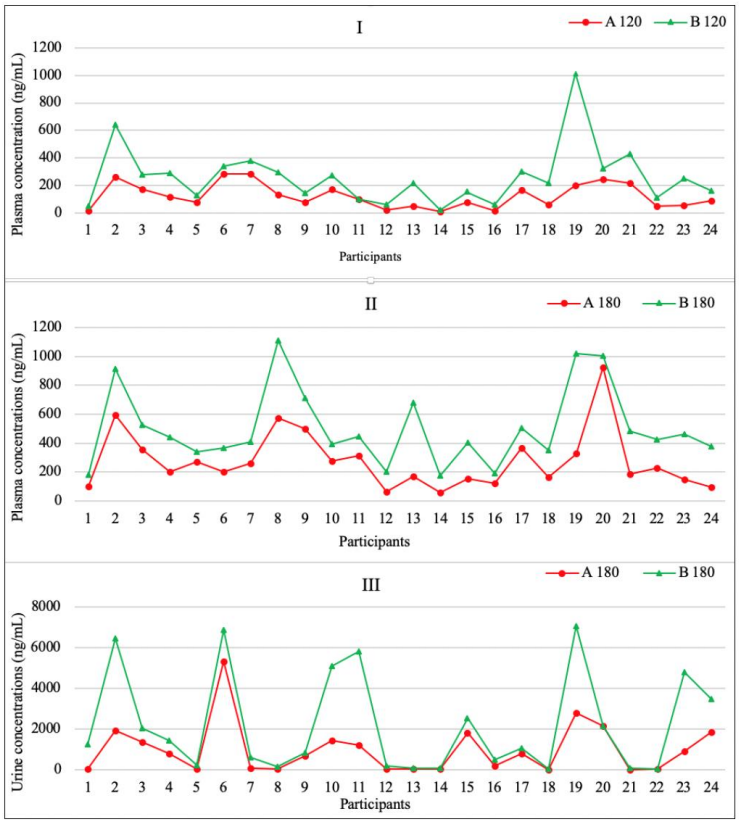

Figure 1. Plasma cannabidiol (CBD (dose A) and DehydraTECH™2.0 CBD (dose B)) concentration in venous blood (**I**,**II**) and urine (**III**). Individual data for 24 participants. Concentrations in plasma samples taken in 120th minute (**I**) and in 180th minute (**II**) after ingestion and in urine samples taken in 180th minute (**III**) after ingestion.

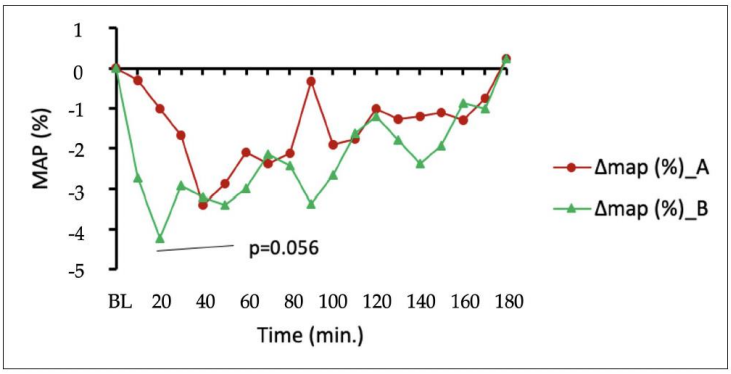

Figure 2. Changes in mean arterial blood pressure (MAP) between generic CBD control (dose A) and DehydraTECH™2.0 CBD (dose B). Data are grouped means (n = 24) with linear regression.

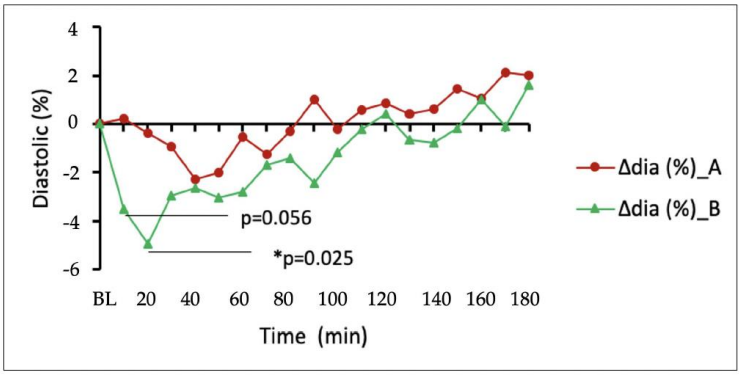

Figure 3. Changes in diastolic blood pressure between generic CBD control (dose A) and DehydraTECH™2.0 CBD (dose B). Data are grouped means (n = 24) with linear regression.

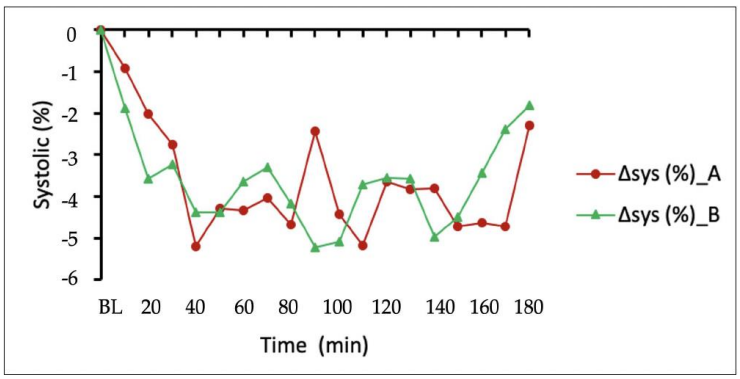

Figure 4. Systolic BP remained depressed throughout the entire 3 h duration of the study for both formulations.

Table 3. Blood pressure at baseline and 180 min from the start with CBD and DehydraTECH™2.0 CBD formulation.

CBD Formulation	Parameters	Baseline (Mean ± SD)	At 120 min (Mean ± SD)	At 180 min (Mean ± SD)	*p* * (Baseline—120 min)	*p* * (Baseline—180 min)
CBD (n = 24)	Diastolic BP (mmHg)	84.08 ± 8.53	84.58 ± 8.69	85.54 ± 8.78	0.842	0.562
	Systolic BP (mmHg)	136.21 ± 10.54	131.67 ± 10.36	133.5 ± 10.8	0.156	0.403
	Heart rate (bpm)	73.33 ± 11.02	66.54 ± 9.38	66.79 ± 8.41	0.238	0.024
DehydraTECH™2.0 CBD (n = 24)	Diastolic BP (mmHg)	84.38 ± 7.76	84.42 ± 6.58	85.58 ± 8.68	0.984	0.614
	Systolic BP (mmHg)	134.67 ± 9.25	130.17 ± 7.52	132.58 ± 9.82	0.071	0.453
	Heart rate (bpm)	74.33 ± 11.90	68.08 ± 9.34	66.96 ± 9.18	0.048	0.020

* one way ANOVA. Data presented as mean ± SD. Abbreviation: SD, standard deviation; CBD, cannabidiol.

2.4. SNP Genotyping

The results of genotyping of six investigated SNP loci in DNA samples of participants are given in Table 4. According to the Hardy–Weinberg equilibrium, there was no statistically significant difference for any of the studied SNPs (*p* value was in range from 0.243

to 0.959). The results for the minor allele frequency (MAF) are consistent with the data presented in the PharmGKB resource [29].

Table 4. Genotype and allele frequencies (%) by loci for single-nucleotide polymorphisms (SNPs) of analyzed genes. Data for 24 participants.

Gene	SNP	Genotype	N	Minor Allele	Minor Allele Frequency (MAF) %	Hardy–Weinberg Equilibrium	
						p Allele Frequency	χ^2 *p*-Value
CYP3A4	rs2740574	TT CT CC	23 1 0	C	2.08	0.977	0.011 0.917
*CYP2C9*2*	rs1799853	CC CT TT	17 6 1	T	16.67	0.833	0.24 0.624
*CYP2C9*3*	rs1057910	AA AC CC	19 4 1	C	12.50	0.875	1.361 0.243
*CYP2C19*2*	rs4244285	GG GA AA	19 5 0	A	10.42	0.896	0.325 0.569
*CYP2C19*3*	rs4986893	GG GA AA	24 0 0	A	0	1.00	0.000 0.569
*CYP2C19*17*	rs12248560	CC CT TT	15 8 1	T	20.83	0.792	0.003 0.959

2.5. Association of CBD Concentrations with CYP Genotype and Phenotype of Subjects

Since none of the concentration values from formulation A and B plasma samples taken at 120 and 180 min from the start and formulation A and B urine samples taken at 180 min were normally transformed, a logarithmic transformation of the data was performed in order to produce a normal distribution. A linear regression model was performed for each of the samples log (formulation A and B plasma samples taken at 120 and 180 min) and log (formulation A and B urine samples taken at 180 min) as dependent variables, and the independent variables were CYP2C9*2, CYP2C9*3, CYP2C19*2 and CYP2C19*17.

For formulation A, no model (for log values of plasma samples at 120 and 180 min and urine samples at 180 min) was statistically significant: *p* values were, respectively, $p = 0.821$, $p = 0.738$ and $p = 0.196$.

For formulation B, the linear regression model (for log values of formulation B urine samples at 180 min) was also adjusted for gender.

Without distribution by phenotype, the linear regression model was not significant for log values of formulation B plasma samples at 120 min ($p = 0.863$) and the log values of formulation B plasma samples at 180 min ($p = 0.979$). For the formulation B log values of urine samples at 180 min, the model was statistically significant ($p = 0.028$, R2 = 0.3332). The variants CYP2C19*2 ($p = 0.037$) and CYP2C19*17 ($p = 0.022$) showed statistical significance. Both were negatively associated with urinary CBD levels (beta = −0.489 for CYP2C19*2 and beta = −0.494 for CYP2C19*17). Each mutated allele reduces the level of CBD.

By combining the CYP2C9*2 and CYP2C9*3 as well as CYP2C19*2 CYP2C19*17 genotypes, phenotypes were created for each subject, and thus, phenotypes are also related to drug metabolism (Figure 5) [25,30].

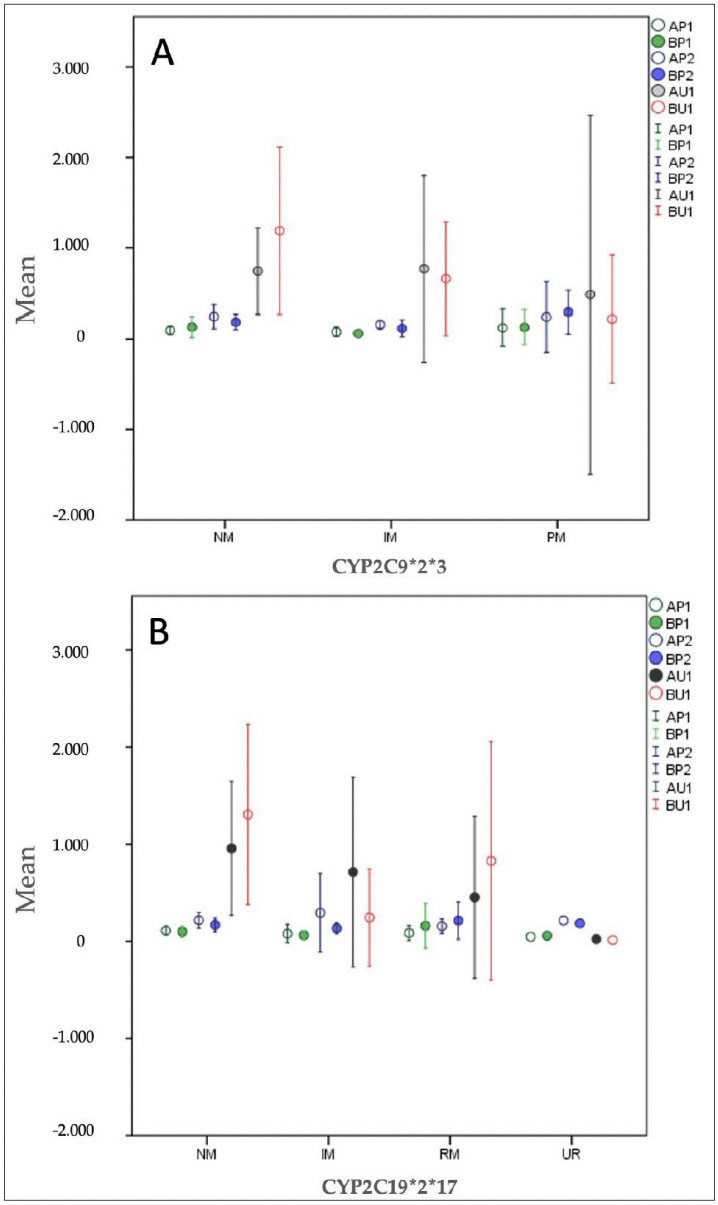

Figure 5. Mean values of CBD plasma (AP1 and BP1 at 120 min; AP2 and BP2 at 180 min) and urine (AU1 and BU1 at 180 min) concentrations (ng/mL) after consumption of formulation A and B classified by CYP2C9*2*3 phenotype: normal metabolism (NM), intermediate metabolism (IM) and poor metabolism (PM)) (**A**) and CYP2C19*2*17 phenotype: normal metabolism (NM), intermediate metabolism (IM), rapid metabolism (RM) and ultra-rapid metabolism (UR), (**B**) (n = 24).

According to the CYP2C9*2*3 enzyme phenotype, there were 13 (54%) subjects with normal metabolism (NM) (genotype *1/*1), eight (33%) with intermediate metabolism (IM) (genotype *1/*2 or *1*3) and 3 subjects (13%) with poor metabolism (PM) (genotype *2/*2, *2/*3 or *3/*3). According to the CYP2C19*2*17 enzyme phenotype, there were 11

(46%) subjects with normal metabolism (NM) (genotype *1/*1), 5 (21%) with intermediate metabolism (IM) (genotype *1/*2 or *2/*17), 7 subjects (29%) with rapid metabolism (RM) (genotype *1/*17) and 1 subject (4%) with ultra-rapid metabolism (UR) (*17/*17).

The variants CYP2C19*2 and CYP2C19*17 showed statistical significance, considering the concentrations of CBD. Therefore, the relationship between changes in blood pressure and their variants has been explored. It has been noted that there is a statistically significant decrease ($p = 0.033$), comparing with baseline, for diastolic pressure for CYP2C19*2. Regarding the genotype of the individuals, there was no statistically significant correlation between CBD concentrations and changes in blood pressure and heart rate for CYP2C19*17.

3. Discussion

3.1. CBD Effect on Cardiovascular System

This double-blinded, placebo-controlled study examined the impact of acute dosing of a concentration-matched CBD and DehydraTECH™2.0 CBD formulation in male and female subjects with stage 1 or 2 arterial hypertension. Our results showed that after consuming the tested formulations, there was no statistically significant difference in the changes between systolic and diastolic blood pressure at 120 and 180 min. Additionally, DehydraTECH™2.0 CBD tended to lower relative diastolic pressure from baseline more than the concentration-matched, generic CBD control. This was particularly noticeable in the first 10 to 20 min after dosage, with statistical significance being shown at the 20 min timepoint. Additionally, the DehydraTECH™2.0 CBD had a propensity to also reduce relative mean arterial pressure (MAP) from baseline more than the concentration-matched, generic CBD control, particularly in the first 20 min after dosage ($p = 0.056$). It should be noted that the concentration was not measured at the 20th minute, and it is unknown whether the DehydraTECH™2.0 CBD formulation had maximum plasma concentrations at that time. However, the effect on diastolic blood pressure and MAP lowering was most evident at that time. Because of the improved properties of DehydraTECH™2.0 CBD, bioabsorption and bioavailability are increased [19–21]. Perhaps it is reasonable to infer that the maximum concentration, and hence the effect, is reached sooner.

A statistically significant decrease was observed for heart rate (HR) for both formulations. At 120 and 180 min, the DehydraTECH™2.0 CBD formulation showed a statistically significant drop in HR, whereas concentration-matched CBD only showed it at 180 min. By comparison, in the previous human clinical study, 120 min were required to achieve the same level of MAP reduction, demonstrating the superior rapidity of onset of the DehydraTECH™2.0 CBD formulation used in the present study, relatively speaking [19].

As the DehydraTECH™2.0 CBD formulation is improved and bypasses the metabolism of the first pass through the liver, it very likely affected the speed of effect of such a formulation. Depending on the formulation, the time of maximum concentration and half-life might differ significantly [1]. For example, Abbotts et al. [1] examined the pharmacokinetics of six different formulations of CBD in 14 subjects and found that the median Tmax was between 30 and 90 min, and that of t1/2 was between 106 and 246 min. In contrast to the timepoint of the greatest BP-lowering effect of CBD, plasma concentrations were higher at later timepoints, e.g., in 180 min samples. Therefore, we assume that the maximum effect of CBD on lowering blood pressure is not related to the moment of maximum plasma concentrations, which we suspect we failed to detect and measure for the DehydraTECH™2.0 CBD formulation in this study if it likely occurred earlier than the 120 and 180 min timepoints of our plasma analyses. Bonomo et al. in their study of nine subjects determined that the concentration of all tested analytes increased up to 2 h after administration [31]. They observed significant inter- and intra-individual variability. The results of our study showed that maximum concentrations were reached after 2 h (120 min). In addition, there was a distinction between the respondents in our findings. While the interindividual differences in the 180th minute were less significant, subjects 2 and 19 had considerably higher levels of CBD in their plasma at the 120th minute than the other participants. These results will

undoubtedly need to be further investigated in order to improve CBD supplement dosage and produce a longer-lasting effect without requiring the enhancement of single dosages.

Sultan et al. (2020.) examined the effects of CBD on 26 men and found that the BP-lowering effect of CBD was lost after repeated dosing because tolerance probably developed while endothelial function improved [15]. We assume that this is the reason why in our study, the diastolic pressure values at 180 min were higher than the values at the baseline point, although the CBD concentrations of both formulations were higher at the 180th minute than at the baseline and at the 120th minute [15].

The dose of 300 mg of CBD was chosen based on the results of previous studies [1,5,15,19,32]. The achieved concentrations are shown in Table 2. Although no statistically significant difference was observed in the concentrations of both tested formulations, it is worth noting that the concentration of CBD in plasma of both formulations increased with time. At 120 min, the average concentrations of DehydraTECH™2.0 CBD formulations were higher, while at 180 min, they were lower than those of the concentration-matched CBD. In addition, the new formulation had a larger average urine concentration at 180 min, which may be due to its increased bioresorption [33]. However, with the DehydraTECH™2.0 CBD formulation, almost every subject had a higher plasma CBD concentration (at 120 min and plasma and urine samples at 180 min). This effect seems to be due to the DehydraTECH™2.0 CBD formulation having a higher bioavailability than concentration-matched CBD. Only the urine samples showed a statistically significant difference between the CBD concentrations of the DehydraTECH™2.0 CBD formulation when the gender was taken into consideration. Men specifically had larger CBD concentrations than the female participants (in plasma samples at 120 and 180 min as well as in urine samples at 180 min). It is interesting that Sultan et al. excluded female participants from their study in order to rule out the possibility that gender differences could affect CBD's effect [15]. However, at the same time, this was their study limitation, and our study provides experimental evidence in sex differences in CBD metabolism.

The heart rate was the primary indicator of elevated CBD concentrations, which steadily dropped, with a remark that the results following intake of the DehydraTECH™2.0 CBD formulation were marginally greater than the CBD concentration-matched formulation. Furthermore, it was observed that the DehydraTECH™2.0 CBD formulation had a better effect on the initial reduction in diastolic blood pressure, MAP and heart rate than the CBD concentration-matched formulation.

3.2. SNP Genotyping

Multiallelic genetic polymorphisms depend on ethnicity and are often associated with variations in drug response among populations [23,24,26,34], which in their combinations lead to different pharmacogenetic phenotypes. The results of the polymorphisms of the subjects from this study are consistent with the results found in the PHARMGKB resource [29]. The phenotype of the study subjects, as measured by their MAF frequencies, is consistent with the Caucasian–European population. This is because the CYP2C9*2*3 and CYP2C19*2*17 gene polymorphisms are combinations which result in different phenotypes.

Our results showed that there was no association of CBD concentrations of concentration-matched CBD formulation with any phenotype, while for the DehydraTECH™2.0 CBD formulation, a statistical association was observed between the concentrations of plasma samples extracted at 180 min and the CYP2C9*2*3 phenotype. The IM phenotype had the lowest level, followed by NM, and the PM phenotype had the highest level.

In order to observe the connection between concentrations and metabolism with the genetic variability of cytochrome P450, the research included the analysis of polymorphism CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3, CYP2C19*17 and CYP3A4 genes [22,35].

Although numerous studies have suggested an association of genetic variability with CBD metabolism, our results did not fully confirm these findings [17,35]. Our results showed that there was no significant difference in CBD concentrations with genotypes of all SNPs tested individually, unlike combinations.

The results obtained for CYP3A4 and CYP2C19*3 genes were not statistically significant. Beers et al. conducted an in vitro study in which they examined the influence of CYP-450 enzymes on CBD metabolism [35]. Their results showed that both CYP2C9 and CYP2C19 are important participants in the metabolism of CBD to the active metabolite 7-OH-CBD, while CYP3A4 was not involved in the formation of 7-OH-CBD. In our study, the highest association was observed for CYP2C9*2 and CYP2C19*17. In the CYP2C9*2*3 enzyme, subjects with the poor metabolizer (PM) phenotype, compared to other phenotypes, after consuming the DehydraTECH™2.0 CBD formulation had a higher plasma concentration of CBD in 180 min. As a result, consideration for the subject's phenotype should be given while determining the dosage of DehydraTECH™2.0 CBD formulation.

3.3. Medication Influence

Out of all 24 participants, 18 of them were not receiving any therapy. The following medications were being taken by the six subjects: two subjects were taking 25 µg of levothyroxine daily, one subject regularly was taking 100 µg of levothyroxine along with 1 mg of lorazepam, one subject was using 5 mg of diazepam, one subject was using 100 mg of celecoxib, and one subject regularly was taking 100 mg of acetylsalicylic acid per day. According to the results of previous research and available data, the oxidative metabolism of diazepam and lorazepam is mediated by CYP3A4 and CYP2C19 isoenzymes [36]. Celecoxib is primarily metabolized by CYP2C9 [37]. Levothyroxine reduces the activity and expression of CYP3A4 and may influence the pharmacokinetics of concomitant CYP3A substrate drugs [38].

By comparing the concentrations of CBD of the tested formulations in the plasma and urine samples of the mentioned subjects with the mean values from Table 5, it was observed that the values mostly deviate from the mean values. We conclude that the use of these drugs affects the metabolism of CBD, which needs to be taken into consideration of optimizing the dosage of CBD as a dietary supplement in different formulations.

Table 5. Anthropometric summary of participants.

	n	Age (Years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
Females	12	55 ± 8	167 ± 4	81 ± 9	29.2 ± 3.8
Males	12	52 ± 6	182 ± 6	101 ± 15	30.3 ± 4.2
Total	24	54 ± 7	175 ± 9	91 ± 16	29.7 ± 4

Data presented as mean ± SD. Abbreviation: SD, standard deviation; n, number of subjects exposed (n = 24); BMI, body mass index.

3.4. Side Effects

DehydraTECH™2.0 CBD and the concentration-matched, generic CBD control were well tolerated by all subjects, with no serious side effects observed or reported. Ingestion of the concentration-matched, generic CBD control, on the other hand, resulted in mild side effects in some of the volunteers, namely gastrointestinal distress including diarrhea (n = 2). Some volunteers reported relaxation and sleepiness (n = 2) after generic CBD control, while relaxation without sleepiness was reported after DehydraTECH™2.0 CBD (n = 2).

3.5. Study Limitations

Limitations of the study include the relatively small number of subjects, acute intervention, no placebo control and no determination of CBD metabolite concentrations. Additionally, the limiting factor for the interpretation of the results is that the concentration in the plasma was not determined more frequently after consumption. Therefore, our follow-up study has now included a larger sample size, placebo control, longer intervention (5 weeks) and the determination of the concentration of the main metabolites of CBD [21].

4. Materials and Methods

4.1. Participants

Twenty-seven untreated participants with reported or measured stage 1 or stage 2 hypertension (13 female and 14 male) were recruited, and 24 completed all experimental parts.

4.2. Anthropometrics and Background

One participant (1 female) dropped out because of syncope, and two participants (2 males) dropped out because their BP was too low (under normal values of 120/80 mm Hg) at the start of the study. Exclusion criteria included: body mass index under 35 kg/m^2 (according to the National Institute of Health: BMI of 18.5–24.9 kg/m^2 is considered normal weight; BMI of 25–29.9 kg/m^2 is considered overweight; BMI of >30 kg/m^2 is considered obesity), secondary forms of hypertension, if they had any previous history of kidney, gastrointestinal (GI), liver, cardiopulmonary or cerebrovascular disease, epilepsy, diabetes, pregnant or breastfeeding, clinically diagnosed anxiety or depression or if they were taking estrogen supplementation or other prescription drugs or over-the-counter supplements. Participants were also excluded if they were current smokers or had any history of smoking, opioid use or using vapor-based products and medical or recreational use of cannabis. Trained athletes were also excluded. Inclusion criteria included: normal or overweight (body mass index between 18 and 35 kg/m^2), between the ages of 45 and 70; under 150 min of moderate-to-vigorous activity per week, reported or measured elevated blood pressure (120 to 129 mm Hg systolic and less than 80 mm Hg diastolic), stage 1 hypertension (130/80 to 139/89 mmHg) or stage 2 hypertension (140/90 to 159/99 mmHg) [39]. Participant background information are presented in Table 6.

Table 6. Participant background summary.

Current smokers (n)	0
Used to smoke (n (%))	6
Years since smoked	20 ± 10; range: 6–33
Pack years when smoking	13 ± 5; range: 5–20
Uses CBD (n (%))	1 †
On medications (n (%))	6
Diazepam (n; dose)	1 (5 mg)
Levothyroxin (n; dose)	3 (25 µg and 100 µg)
Lorazepam (n; dose)	1 (1 mg)
Celecoxib (n; dose)	1 (200 mg)
Acidum salicylicum (n; dose)	1 (100 mg)

Abbreviation: n, number of subjects exposed; CBD, cannabidiol. † Participant had not used CBD products for the 43 days preceding the study. Out of the medications, only Levothyroxin was taken on day of test.

The study design, reporting and implementation followed the CONSORT guidelines and a flow diagram (Figure 6).

Both researchers and subjects were blinded to the performed conditions on testing days by having a research associate who dispatched the capsules to participants according to the randomization schedule and was not involved in any other aspect of the study. When reporting to the laboratory in the morning, participants were in a fasted state (no food or drinks for at least 10 h).

4.3. Research Design

This was a double-blinded (Participant, Investigator), cross-over study in which 24 eligible volunteers (12 males and 12 females) visited the laboratory on three occasions.

On the first initial visit (screening), potential participants read through the information and consent form, and they provided written informed consent before any measurements. Following informed consent, the Medical Screening Questionnaire was administered to confirm eligibility criteria. Anthropometric and physiologic measurements were collected (height, body weight, waist circumferences, blood pressure) for baseline participant

characterization. All subjects were instructed to keep a food and physical activity diary in the 24 h preceding their second laboratory visit and to replicate food consumption and physical activity in the 24 h preceding the last visit. Subjects were instructed to refrain from caffeine and alcohol-containing drinks for 24 h before each laboratory visit, and dietary logs were collected and corroborated. Eligible participants were then randomized. The sequence of conditions the participant received was generated by a research randomizer web-service (https://www.randomizer.org, accessed on 16 April 2021).

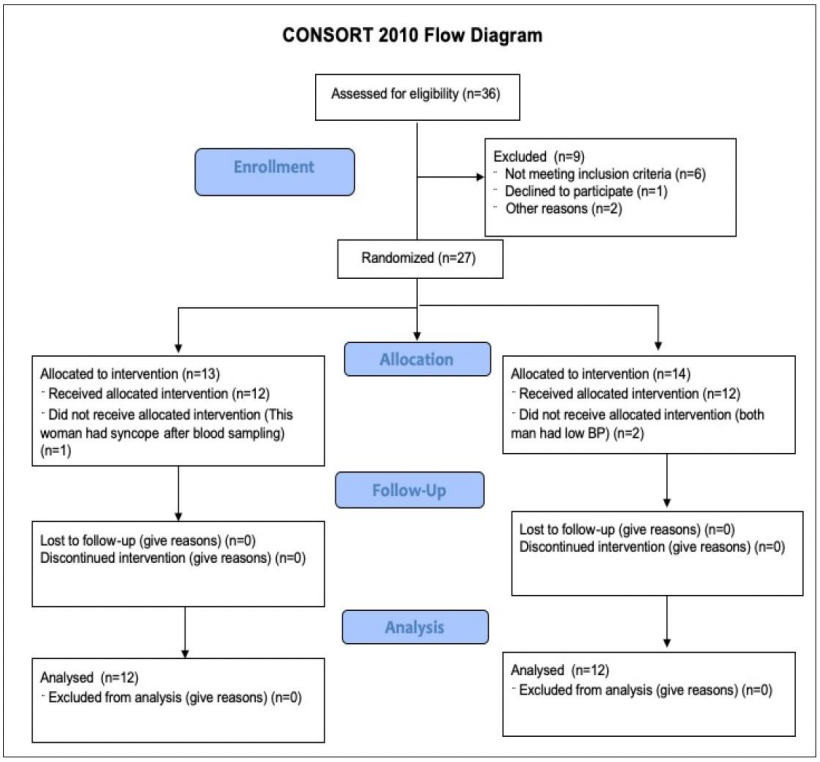

Figure 6. CONSORT flow diagram.

Second and third visits were experimental trials, each separated by at least 4 days. There were three venous blood samples (15 mL each) collected; one sample was obtained at baseline and the others were obtained at 120 and 180 min following ingestion of the capsules (Figure 7). Blood was drawn into lithium–heparin and EDTA tubes for the measurement of plasma samples before being frozen in a −20 °C research refrigerator. Blood pressure and heart rate were measured in triplicate every 10 min throughout the study. Before dosing and every 90 min thereafter, standardized questionnaires were used to assess GI (gastrointestinal) symptoms and anxiety (visual analog scale—VAS).

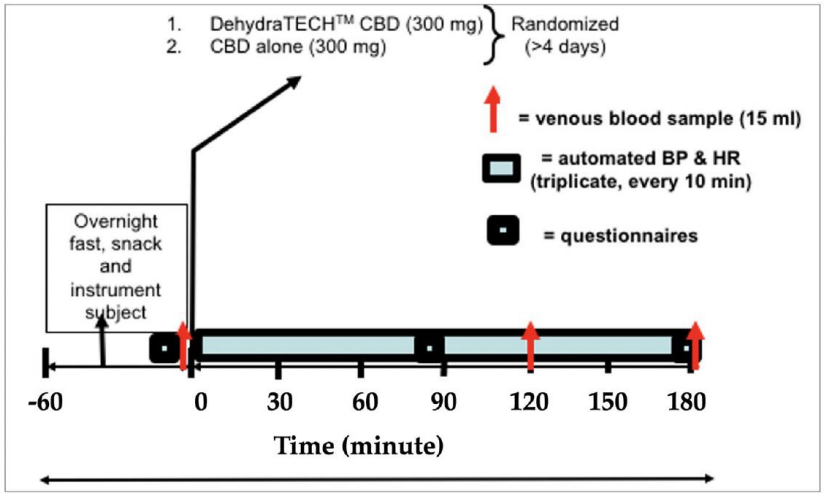

Figure 7. Schematic for Study. The full planned protocol lasted <4 h. Participants arrived in the laboratory after an overnight fast. A snack high in fat (muffin) was provided upon initial arrival. A 15 mL blood sample (red arrows) was collected at baseline and following 120 and 180 min. Automated blood pressure and heart rate were obtained, in triplicate, every 10 min. Before dosing and every 90 min thereafter, standardized questionnaires were used to assess GI symptoms and anxiety. See text below for dosing details.

The second visit was scheduled at least 24 h after the first visit to the laboratory, and participants were required to be in the laboratory for at least 3.5 h. Participants had to report to the laboratory after an overnight fast (>10 h). A snack high in fat (muffin) was provided upon initial arrival. Water intake was allowed ad libitum. Upon arrival at the laboratory, participants had to sit down for at least 15 min before an intravenous cannula was inserted into the area of the cubital fossa. Blood sampling was repeated; one sample was obtained at baseline and the others were obtained at 120 and 180 min following ingestion of the capsules. Blood was drawn into lithium–heparin and KEDTA vacutainers for analysis of plasma samples. Plasma was separated (centrifuged at 4 °C on 3500 rpm for 10 min) before being frozen in a −20 °C research refrigerator. Blood pressure and heart rate were measured in triplicate every 10 min throughout the study. Office blood pressures were measured during each visit according to guidelines for blood pressure measurement, using WatchBP Home A (Microlife AG Swiss Corporation, Widnau, Switzerland). Blood pressure was measured in the seated position 3 times during each visit, and the mean value was reported. Urine samples were collected upon arrival each morning and at the end of the protocol. Two urine samples (10 mL) and plasma samples (1.5–2 mL) were used for further analysis. The 24 h food log was reviewed, and a copy was provided to participants who were asked to repeat the exact diet 24 h before the following visit. Subjects were reminded not to perform exercise or to consume alcohol 24 h before the next visit. Subjects returned fasted to the laboratory at least 4 days after the second visit. Adherence to the 24 h diet was confirmed upon arrival in the laboratory. Participants were instructed to identify any deviations in diet from the food log before beginning the previous trial. The protocol was repeated on the third visit, with the participant receiving one of the other two conditions.

4.4. Supplementation and Dosing

Each participant received in a randomized and double-blinded order: cannabidiol-matched placebo tablets (labeled as substance ***A***) (12 capsules with 25 mg of active substance each, with total amount of 300 mg of CBD) and DehydraTECH™2.0 CBD (labeled as substance ***B***) (12 capsules with 25 mg of active substance each, with total amount of 300 mg

of CBD). Each visit was separated by 14 ± 3 days (range: 11–22). For dose A, participants consumed the capsules 35 ± 16 min after eating the snack (before which they had fasted for 10.3 ± 4.8 h). For dose B, participants consumed the capsules 32 ± 10 min after eating the snack (before which they had fasted for 9.8 ± 4.6 h).

There was no significant difference between these timings between doses ($p = 0.642$ for capsule consumption time; $p = 0.538$ for fasted time; paired *t*-test). It should be noted that: 19 participants had the snack on both visits; two participants did not have a snack on either visit; three subjects only had a snack on one visit (two were dose A, and one was dose B). For both experimental visits, the ingestion of capsules was coordinated to occur at approximately the same time of day. Only three subjects had a between-visit ingestion time >45 min. However, the timing of testing for these three participants still coincided with both either being in the morning or in the afternoon.

DehydraTECH™2.0 CBD delivery technology is a patented capsule formulation that increases the bioabsorption of the active content due to its enhanced lipophilic composition, including a patented process by which long-chain fatty acids, high in oleic acid, are associated through a dehydration process procedure with the CBD. It is believed that this proprietary process assists the human GI system in the uptake of the CBD via bypassing (or reducing) first-pass liver metabolism; therefore, in the short term, it was speculated that this approach allows for higher volumes of CBD to enter the system more rapidly, circumventing first-pass liver metabolism than what is otherwise achieved with generic forms of CBD [19,21,33].

4.5. Sample Collection and Storage

Blood samples were collected in lithium heparin Vacutainers. Within 10–15 min of collection, plasma was separated (centrifuged at 4 °C on 3500 rpm for 10 min) and stored at −20 °C. All samples were labeled with the study number for each patient, test session number, date and time. Three plasma samples (1.5–2 mL) and two urine samples (5–10 mL), per patient, were sent directly to the laboratory for analysis.

4.6. Standard Solution

Cannabidiol standard solutions (CBD, 1 mg/mL certified reference material; Lipomed AG, Arlesheim, Switzerland) were prepared by dilution with acetonitrile to a final concentration of 10 µg/mL and were used to prepare calibration samples. To establish linearity, a calibration curve was calculated by analyzing drug free plasma and urine samples spiked with CBD at the concentrations of 10, 25, 50, 100, 250 and 500 ng/mL.

4.7. CBD Extraction

Proteins in plasma samples (1 mL aliquots) were precipitated with 1.25 mL of ice-cold acetonitrile. After mixing, samples were centrifuged (2600 rpm for 2 min) and supernatant was centrifuged with 1 mL dd H_2O, as well as the urine sample, which was added to preconditioned solid phase extraction (SPE) columns with CBD specific cartridges (United Chemical Technologies, Styre Screen SSTHC06Z, Bristol, PA, USA; for both, plasma and urine extraction protocol were carried out according to the manufacturer's instructions). The column was rinsed with 1 mL of dd H_2O and dried under high vacuum (~20 inches). CBD was eluted with a 3 mL mix of hexane: ethyl acetate: acetic acid (49:49:2, *v*/*v*) and dried under nitrogen. Samples were reconstituted with 150 µL ethyl acetate [19].

4.8. GC-MS Analysis

CBD concentration was determined by a gas chromatograph model 8890 GC (Agilent Inc., Santa Clara, CA, USA) coupled to the tandem mass spectrometer model 7000D GC/TQ GC-MS/MS (Agilent Inc., Santa Clara, CA, USA) equipped with an automatic liquid injector model 7693A (Agilent Inc., Santa Clara, CA, USA). A non-polar HP-5MS UI column (dimensions: 30 m long, 0.25 mm inner diameter and 0.25 mm stationary phase layer thickness, Agilent Inc., Santa Clara, CA, USA) was used.

The initial column temperature of 200 °C was held for 1 min, then ramped to 290 °C at 12 °C min^{-1}, and then ramped again to 310 °C at 30 °C min^{-1} and held to the total run time of 14.1 min. For the carrier gas, ultrapure-grade helium was used at the flow rate of 1.5 mL min^{-1}. The volume of the analyzed sample was 1 µL, and samples were injected using the splitless mode with an injection temperature of 250 °C. GC/MS analysis was performed using single ion monitoring mode (SIM mode) with CBD characteristic ions of 231, 246, 314, 232 and 121 m/z [40,41].

4.9. SNP Genotyping

Blood samples for DNA analysis were collected at regular check-ups and stored in EDTA tubes. DNA was isolated with a commercial genomic DNA isolation kit (High Pure PCR Template Preparation Kit, Version 27, Cat. No. 11796828001, Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. Extracted DNA was quantified using a Qubit 4 fluorimeter (Thermo Fischer Scientific, Waltham, MA, USA).

Using the TaqMan® SNP genotyping assay (Thermo Fischer Scientific, Waltham, MA, USA) and an Applied Biosystems 7500 Realtime polymerase chain reaction (RT-PCR) system (Applied Biosystems, Foster City, CA, USA), we genotyped for the following single-nucleotide polymorphisms (SNPs): rs1799853; C_25625805_10; *CYP2C9*2*, rs1057910; C_27104892_10; *CYP2C9*3*, rs 4244285; C_25986767_70 *CYP2C19*2*, rs 4986893; *CYP2C19*3*, rs 12248560; C_469857_10 *CYP2C19*17*, and rs2740574; C_1837671_50 *CYP3A4* [21,30,31]. RT-PCR and allelic discrimination analyses were performed according to the manufacturer's instructions in a 25 mL reaction volume. The temperature program for RT-PCR was 60 °C for 1 min and 95 °C for 10 min, which was followed by 50 cycles of 92 °C for 15 s and 60 °C for 90 s. SNP genotypes were determined using instrument software with the manual allele call option.

4.10. Statistical Analysis

The Kolmogorov–Smirnov test was used for normality checking. Due to the non-normal distribution of the data, continuous variables are presented with the median (interquartile range, IQR) and categorical variables are presented with frequencies (percentages). Groups were compared using the non-parametric Mann–Whitney U test, one-way ANOVA test and paired samples *t*-test [42]. Furthermore, multivariate linear regression analysis was performed to assess the association of CYP genotypes with CBD levels in the plasma and urine. The multivariate linear regression model was carried out separately for CBD and DehydraTECH™2.0 CBD doses. CBD levels were logarithmically transformed to achieve normal distribution. In the model, logarithmically transformed CBD levels were dependent variables, while *CYP2C9*2*, *CYP2C9*3*, *CYP2C19*2*, and *CYP2C19*17* were independent variables. The model was adjusted for sex. Correlations between changes in the systolic and diastolic blood pressure as well as heart rate with the CBD levels were determined using the Spearman rho correlation coefficient. *p*-values of less than 0.05 were considered statistically significant. Statistical analysis was performed using Statistical Package Software for Social Science, version 28 (SPSS Inc., Chicago, IL, USA).

Using an online calculator, the Hardy–Weinberg equilibrium, χ^2 and p values were calculated [43].

5. Conclusions

In conclusion, this research has detected a significant reduction in diastolic blood pressure and MAP in the first 20 min after ingestion of DehydraTECH™2.0 CBD formulation exclusively, while both tested formulations led to a decrease in heart rate. After 180 min of DehydraTECH™2.0 CBD formulation intake, male subjects had higher levels of CBD in their urine compared to female subjects. In the CYP2C9*2*3 enzyme, subjects with the poor metabolizer (PM) phenotype, after consuming the DehydraTECH™2.0 CBD formulation, had a higher plasma concentration of CBD in 180 min.

In future scientific endeavors, it would be optimal to conduct additional trials on a higher number of subjects to measure CBD concentrations and its metabolites after ingesting the novel DehydraTECHTM2.0 CBD formulation in order to better understand the subtle metabolic differences observed thus far.

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Differences in Plasma Cannabidiol Concentrations in Women and Men: A Randomized, Placebo-Controlled, Crossover Study

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Abstract: The potential therapeutic benefits of cannabidiol (CBD) require further study. Here, we report a triple-blind (participant, investigator, and outcome assessor) placebo-controlled crossover study in which 62 hypertensive volunteers were randomly assigned to receive the recently developed DehydraTECH2.0 CBD formulation or a placebo. This is the first study to have been conducted using the DehydraTECH2.0 CBD formulation over a 12-week study duration. The new formulation's long-term effects on CBD concentrations in plasma and urine, as well as its metabolites 7-hydroxy-CBD and 7-carboxy-CBD, were analyzed. The results of the plasma concentration ratio for CBD/7-OH-CBD in the third timepoint (after 5 weeks of use) were significantly higher than in the second timepoint (after 2.5 weeks of use; $p = 0.043$). In the same timepoints in the urine, a significantly higher concentration of 7-COOH-CBD was observed $p < 0.001$. Differences in CBD concentration were found between men and women. Plasma levels of CBD were still detectable 50 days after the last consumption of the CBD preparations. Significantly higher plasma CBD concentrations occurred in females compared to males, which was potentially related to greater adipose tissue. More research is needed to optimize CBD doses to consider the differential therapeutic benefits in men and women.

Keywords: DehydraTECH2.0 CBD; CBD metabolites; LC-MS analysis; SNP genotyping

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1. Introduction

A non-intoxicating and safely tolerated component of cannabis, cannabidiol (CBD), has been shown by numerous authors to have the ability to treat a variety of clinical conditions; therefore, therapeutic benefits of CBD in specialized populations continue to develop [1–4]. Previous studies have demonstrated that the oral bioavailability of CBD is very low [5,6]. Methods for accelerating the transport of ingested CBD to the bloodstream and preventing first-pass (hepatic) metabolism have been created through the use of inventive dietary supplements with various lipid formulations [7–11]. As an example, TurboCBD^{TM} (90 mg CBD; 1200 mg American ginseng; 480 mg ginkgo biloba; 300 mg organic hemp oil), which was consistent with higher peak CBD bioavailability, was associated with an increase in cerebral perfusion and a slight drop in blood pressure compared to baseline and the 90 mg control [8]. In their study, Kumric et al. found that serum catestatin and mean arterial

pressure (MAP) levels were reduced after a 5-week treatment with DehydraTECH^{TM}2.0 CBD formulation [10]. Dujic et al. found that in participants with untreated and treated hypertension, long-term CBD administration lowers ambulatory blood pressure [12]. In our prior research, only the DehydraTECH^{TM}2.0 CBD formulation significantly decreased diastolic blood pressure and mean arterial pressure (MAP) in the first 20 min after ingestion of single dose, while both tested formulations (CBD and DehydraTECH^{TM}2.0) led to a decrease in heart rate [13]. Since there were no serious adverse effects in either study, it was clear that the formulation was safe and acceptable [12,13]. We intended to expand on our previous study in which male subjects had more CBD in their urine than female subjects did [13]. There is still a huge lack of knowledge and understanding of sex-related differences in CBD therapy. Many researchers have explored how cannabis consumption differs depending on biological sex [14,15]. The research results are contradictory because some findings suggest that there is no significant sex difference in CBD concentrations in patient populations [16]. The findings of the study by Aviram et al. suggest that women are more at risk for medical-cannabis-related adverse events (AEs), probably due to the inherent sex effect [17]. Child and Tallon used an animal model of oral CBD consumption in their research and found that female rats consistently displayed higher levels of CBD in muscles and the liver. They have also found that this relationship was present in adipose tissue with low and medium CBD doses [18]. Maciel et al. reported that female mice had considerably higher CBD concentrations in their embryonic brains than males did [19]. These findings indicate tissue-specific pharmacokinetic interactions and higher plasma CBD concentrations in females than in males. Since there are a lack of human data, studies examining sex differences on the effects of CBD in clinical trials are required [20]. Genetic variants are linked to variations in population responses to CBD [21]. Cytochrome P450 (CYP) 2C19, CYP2C9, and CYP3A4 are mainly responsible for the metabolism of CBD [22–26]. In the current study, we analyzed the availability (i.e., circulating concentration) of the improved DehydraTECHTM2.0 CBD formulation in a larger number of participants with treated and untreated arterial hypertension (AH). This research was conducted based on our prior studies. The primary aim of this research was to examine the long-term effects of the new formulation on CBD concentrations as well as its metabolites [7-hydroxy-cannabidiol (7-OH-CBD) and 7-carboxy-cannabidol (7-COOH-CBD)]. Cannabinoid metabolism may be important for research because variations in the effects of cannabinoids may be connected to changes in the distribution of muscle mass and adipose tissue between men and women. Therefore, this research aimed to examine the relationship between the dose consumed and the concentrations achieved in relation to sex. The secondary goal of the study was to determine if there was a connection between CYP P450 enzyme polymorphism and the metabolism of DehydraTECH^{TM}2.0 CBD formulation, as well as how long CBD and its metabolites remained in the body after the last dose.

2. Results

2.1. CBD and Metabolites Concentrations

Descriptive statistics (including minimum and maximum values, median, and interquartile range (IQR) for the CBD concentrations and metabolites, 7-OH-CBD and 7-COOH-CBD) are presented in Table 1. The baseline results for CBD concentrations and its metabolites showed a value of 0.0 for all individuals. Therefore, baseline data are not shown in further results.

Two timepoints (timepoints 2 and 3 for Group II and timepoints 5 and 6 for Group I) were utilized to compare the variations in CBD and CBD metabolite ratios and concentrations in plasma and urine using the Wilcoxon Signed Ranks Test. The results of the plasma concentration ratio for CBD/7-OH-CBD in the third timepoint (after 5 weeks of use) were significantly higher than in the second timepoint (after 2.5 weeks of use) $p = 0.043$. Likewise, the values of urine concentrations for 7-COOH-CBD in timepoint 3 were considerably higher than in timepoint 2, $p < 0.001$. Other variables did not show statistical significance.

Table 1. Descriptive statistics for the CBD concentrations and metabolites 7-OH-CBD and 7-COOH-CBD in plasma and urine samples and plasma ratio. The findings only apply to samples taken after ingesting CBD at two different timepoints after 2.5 and 5 weeks. Timepoints 5 and 6 for Group I and timepoints 2 and 3 for Group II. In the following text, we will refer to them as timepoint 2 (2 or 5—after 2.5 weeks) or timepoint 3 (3 or 6—after 5 weeks). N = 62.

Variable		Timepoint 2 (after 2.5 weeks)			Timepoint 3 (after 5 weeks)		
		Min	Max	Median (IQR)	Min	Max	Median (IQR)
Plasma concentration (ng/mL)	CBD	10.53	329.88	23.81 (20.36)	18.36	149.97	47.1 (35.53)
	7-OH-CBD	2.72	127.23	13.15 (13.90)	6.40	109.93	30.01 (28.19)
	7-COOH-CBD	195.79	5415.25	837.15 (883.48)	575.72	9300.95	1589.77 (2786.08)
Plasma ratio	CBD/−7OH-CBD	0.62	5.67	1.96 (1.30)	0.62	6.19	1.84 (1.87)
	CBD/7-COOH-CBD	0.005	0.20	0.03 (0.02)	0.007	0.12	0.03 (0.03)
	7OH-CBD /7COOH-CBD	0.006	0.08	0.02 (0.007)	0.007	0.03	0.01 (0.008)
Urine concentration (ng/mL)	CBD	0	9.93	0 (0)	0	6,59	0 (0)
	7-OH-CBD	0	81.86	0 (11.074)	0	66,35	0 (7.43)
	7-COOH-CBD	0	43.87	2.92 (5.68)	0	265,15	9.06 (9.813)

Abbreviations: CBD, cannabidiol; 7-OH-CBD, 7-hydroxy-CBD; 7-COOH-CBD, 7-carboxy-CBD; IQR, interquartile range.

In terms of sex, the concentrations of the metabolite 7-COOH-CBD in the urine sample at timepoint 3 were considerably higher in both women and men than they were at timepoint 2 ($p = 0.027$). The median at timepoint 2 was 2.92 ng/mL, while at timepoint 3 it was 9.06 ng/mL.

Figure 1 shows sex differences in mean CBD plasma concentrations at timepoints 2 (after 2.5 weeks of consumption) and 3 (after 5 weeks of consumption). The results demonstrated a significant difference in median CBD concentration for timepoint 3. The median for women was 53,349 ng/mL, whereas the median for men was 41,171 ng/mL. At timepoint 2, there was no significant difference in the median CBD concentrations.

CBD plasma concentrations were compared to body fat percentage in both sexes using linear regression analysis (Figure 2). For the male participants, a slightly negative trendline was found, but it was statistically significant only after 5 weeks ($p = 0.0346$).

The results for the concentrations of CBD and metabolites were zero at the baseline (timepoint 1 or timepoint 4, depending on whether CBD or placebo was taken first), with the exception of the group of participants who took CBD first and subsequently placebo (N = 31). Therefore, we aimed to establish whether CBD and/or its metabolites were present only in patients from Group II (who received CBD before placebo). The results are shown in Table 2. After the washout, the participants in Group II were examined, and the results revealed the presence of CBD and 7-OH-CBD metabolites in some of them (90.6% and 37.5%, respectively), while the presence of the 7-COOH-CBD metabolite was confirmed in all of the subjects. Following that, the participants took a placebo for 5 weeks, during which they went through testing twice (after 2.5 and 5 weeks). A smaller percentage of the participants were positive for the presence of CBD and 7-OH-CBD during both tests; however, all subjects remained positive for the presence of 7-COOH-CBD metabolites. In

total, 50.0% of them were positive for the presence of CBD and 18.35% were positive for the presence of 7-OH-CBD after 2.5 weeks, while 15.6% and 9.4% were positive after 5 weeks.

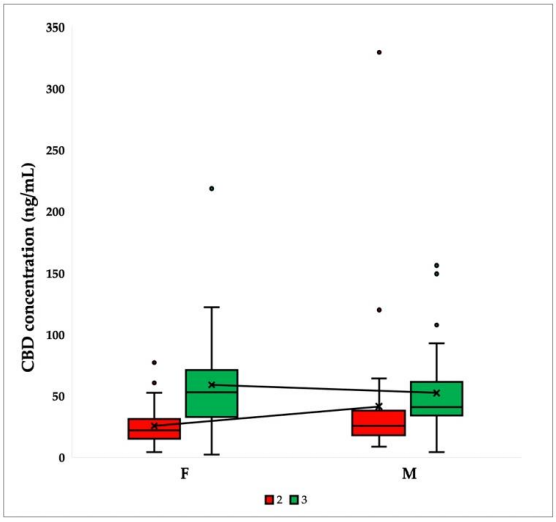

Figure 1. Comparison of CBD plasma concentrations at timepoints 2 (2.5 weeks after CBD ingestion) and 3 (5 weeks after CBD ingestion) stratified by sex (female—F and male—M). Box plots show the median, interquartile range (box), and total range (whiskers). Average values are connected by lines (N = 62).

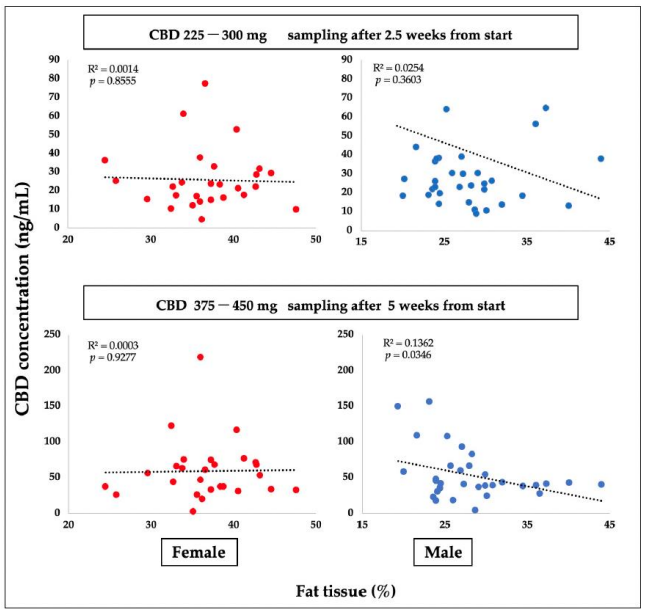

Figure 2. A graphical representation of the results of a linear regression analysis of pairs of dependent and independent variables: The concentration of CBD is represented by the dependent variable on the y axis, while the proportion of adipose tissue in female (F) and male (M) participants is represented by the independent variable on the x axis (N = 62).

Table 2. Descriptive statistics for the CBD concentration and metabolites 7-OH-CBD and 7-COOH-CBD in plasma and urine samples at timepoints 4–6 (2.5 and 5 weeks after placebo capsules ingestion). The results refer only to Group II participants (N = 31) for which samples were collected while taking placebo capsules.

Variable		Timepoint 4 (after Washout)			After Placebo Capsule Ingestion					
					Timepoint 5 (after 2.5 Weeks)			Timepoint 6 (after 5 Weeks)		
		Min	Max	Median (IQR)	Min	Max	Median (IQR)	Min	Max	Median (IQR)
Plasma concentration (ng/mL)	CBD	1.58	10.63	4.91 (3.15)	1.97	5.2	3.51 (1.34)	0	3.71	0 (2.17)
	7-OH-CBD	0	1.85	0.91 (0.78)	0	1.34	0.77 (1.07)	0	1.109	0 (0)
	7-COOH-CBD	3.87	66.85	18.77 (24.28)	2.81	59.11	11.19 (16.05)	1.42	28.94	7.36 (8.93)
Urine concentration (ng/mL)	CBD	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0 (0.0)
	7-OH-CBD	0.0	1.20	0.0 (0.0)	0.0	5.11	0.0 (0.0)	0.0	0.93	0.0 (0.0)
	7-COOH-CBD	0.0	73.81	0.0 (0.69)	0.0	316.24	0.0 (0.41)	0.0	8.17	0.0 (0.0)

Abbreviations: CBD, cannabidiol; 7-OH-CBD, 7-hydroxy-CBD; 7-COOH-CBD, 7-carboxy-CBD; IQR, interquartile range.

The results revealed a significant difference in the median concentration of CBD in plasma at timepoints 4 (after washout) and 6 (5 weeks after placebo capsule ingestion) (Figure 3). The median for women at timepoint 4 was 5.994 ng/mL, while the median for men was 3.375 ng/mL. At timepoint 6, the CBD concentration in women was 2.13 ng/mL, but no men were CBD positive.

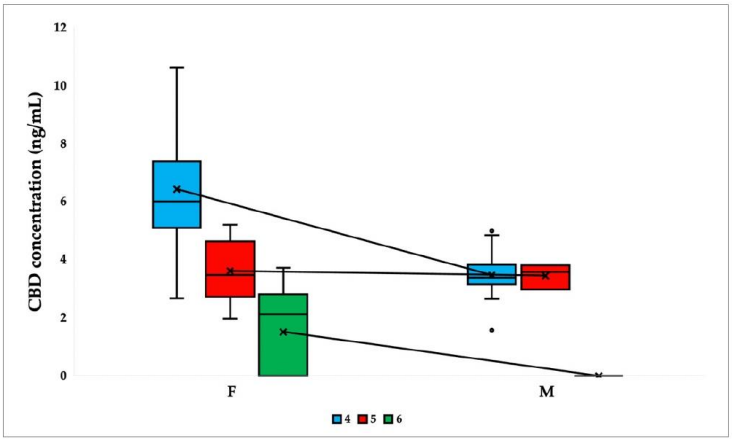

Figure 3. Comparison of CBD plasma concentrations at timepoints 4 (after washout), 5 (2.5 weeks after placebo capsule ingestion), and 6 (5 weeks after placebo capsule ingestion), stratified by sex (female—F and male—M). Box plots show the median, interquartile range (box), and total range (whiskers). Average values are connected by lines (N = 31).

The concentration decreased in each timepoint that followed. Men and women had statistically different concentrations at practically all timepoints. In every instance where there was a statistically significant difference, the CBD or its metabolites' plasma concentrations were higher in females compared to males.

Thus, at timepoints 4, 5, and 6, 16/17 (94%), 13/17 (76%), and 5/17 (29%) of the women tested positive for the presence of CBD. At the same timepoints, 12/14 (86%), 3/14 (21%), and 0/14 (0%) of the men were positive (Figure 4). The findings revealed a significant sex difference in CBD concentrations between timepoints 4 and 6 ($p < 0.05$). For any timepoint, there was no statistically significant difference in 7-OH-CBD concentrations, although there was a difference in 7-COOH-CBD concentrations across all timepoints.

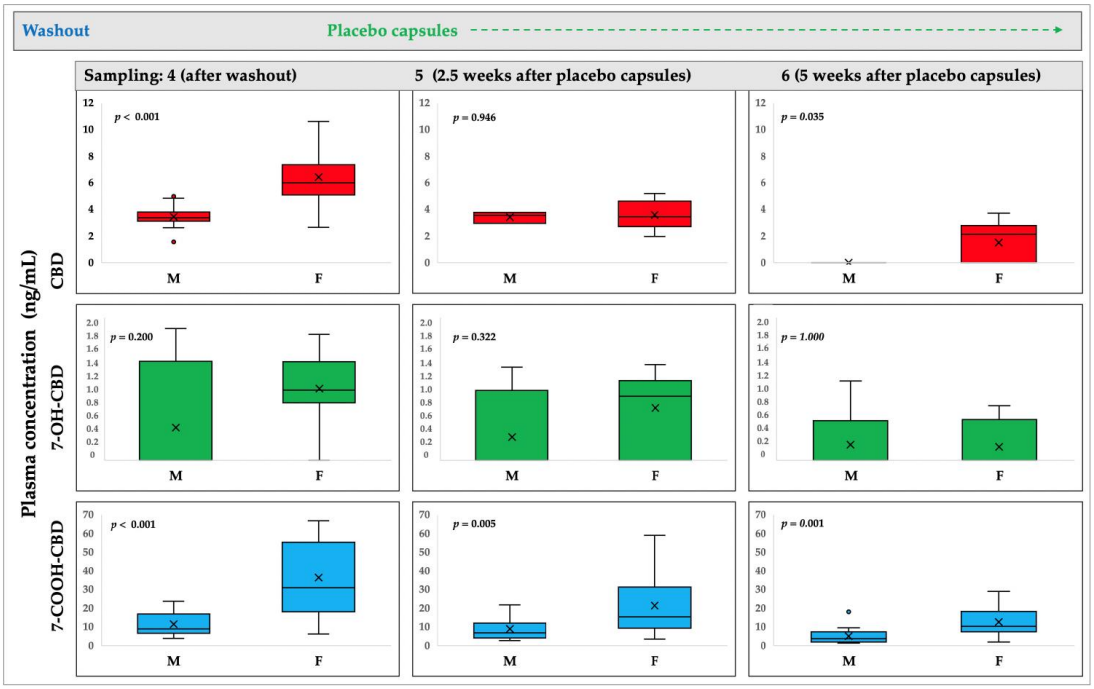

Figure 4. The Mann–Whitney test was used to compare the concentrations of CBD, 7-OH-CBD, and 7-COOH-CBD (ng/mL) in females (F) and males (M) at three different timepoints (4—after washout, 5—2.5 weeks after placebo capsules ingestion, and 6—5 weeks after placebo capsule ingestion). Box plots show the median, interquartile range (box), and total range (whiskers). Statistical significance is defined as $p < 0.05$.

Using linear regression analysis, plasma CBD concentrations were compared to body fat % in both sexes (Figure 5). The analysis only included patients who first consumed CBD and then placebo, and it refers to CBD concentrations in plasma after washout following placebo capsule intake. Female participants had a modestly positive trendline that was statistically significant after washout ($p = 0.021$). A slight positive correlation factor was found following the last testing, 5 weeks after the placebo capsules. No statistical significance was observed in men, but it should be noted that there were significantly fewer CBD-positive results in men at all timepoints indicated.

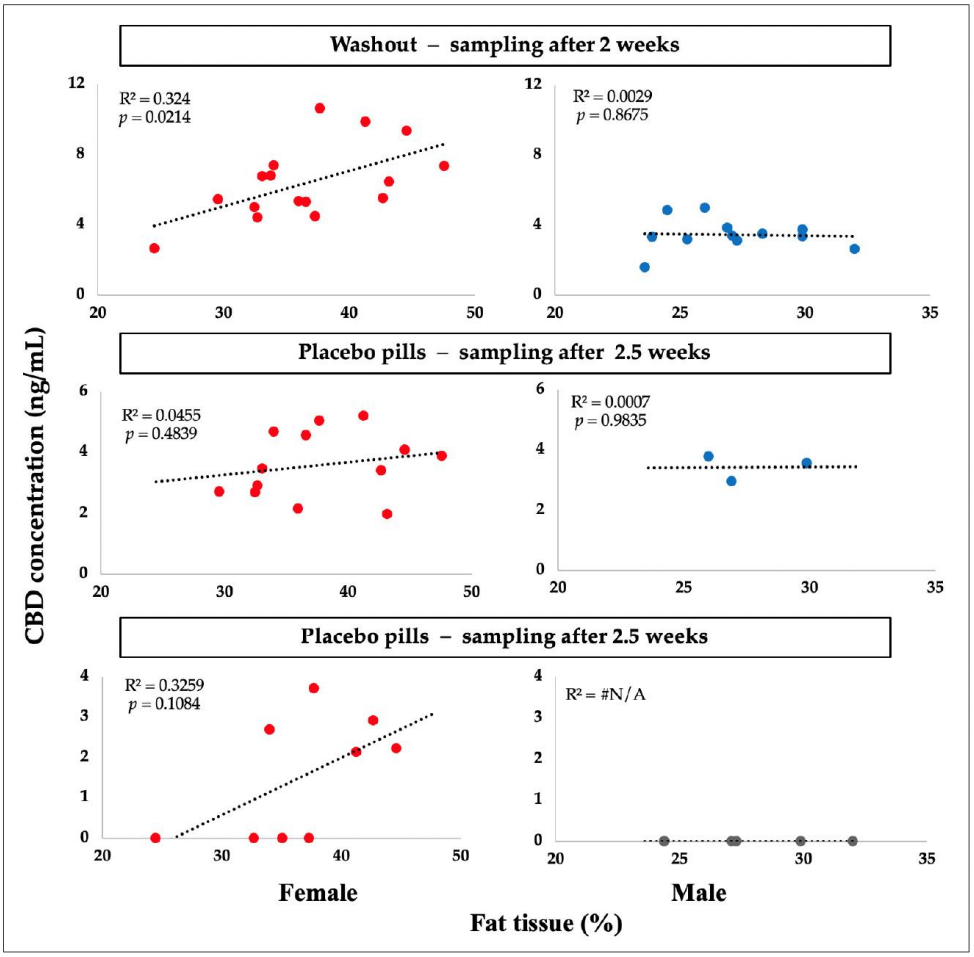

Figure 5. A graphical representation of the results of a linear regression analysis of pairs of dependent and independent variables: The concentration of CBD is represented by the dependent variable on the y axis, while the proportion of adipose tissue in female (F) and male (M) participants is represented by the independent variable on the x axis (N = 31).

2.2. Association of Concentrations with CYP Genotype and Phenotype of Subjects

In total, 39% of the participants had a normal metabolism (NM) (genotype *1/*1), 56% had an intermediate metabolism (IM) (genotype *1/*2 or *1*3), and 5% had a poor metabolism (PM) (genotype *2/*2, *2/*3 or *3/*3), according to the CYP2C9*2*3 enzyme phenotype.

The CYP2C19*2*17 enzyme phenotype revealed that 39% of subjects had a normal metabolism (NM) (genotype *1/*1), 24% had an intermediate metabolism (IM) (genotype *1/*2 or *2/*17), 32% had a rapid metabolism (RM) (genotype *1/*17), 3% had an ultra-rapid metabolism (UR) (*17/*17), and 2% had a poor metabolism (PM) (genotype *2/*2).

Subjects were divided into categories for the CYP3A4 enzyme based on genotype. Consequently, 92% of them were wild type and 8% were heterozygous. No statistical significance was seen in the concentration differences at any timepoint when the CYP2C9 and CYP2C19 phenotypes were considered.

A statistically significant difference in the concentration of CBD in urine for timepoint 3 was seen for the CYP2C9 enzyme in male participants. Men with the NM phenotype had higher CBD values than males with the IM phenotype ($p = 0.025$). For any of the tested time intervals, the same enzyme in women showed no statistically significant changes. For any of the examined timepoints, no statistical difference was seen for the CYP2C19 enzyme in men. The level of 7-COOH-CBD in urine at timepoint 2 was shown to differ among women, though. The NM phenotype had the highest values, whereas the RM phenotype had the lowest values ($p = 0.031$).

At timepoint 2, the CBD/7-COOH-CBD concentration ratio had a statistically significant result for CYP3A4 ($p = 0.037$). In comparison to the wild type, the heterozygous genotype had a greater ratio of CBD/7-COOH-CBD.

3. Discussion

In this research, for the first time, steady-state CBD concentrations in plasma and urine samples were monitored over a 12-week period (84 days) with volunteers with hypertension. No statistical difference was observed in the CBD and CBD metabolites concentrations of participants who consumed and participants who did not consume angiotensin-converting enzyme (ACE) inhibitors, calcium blockers, and thiazide diuretics.

We found that the amount of CBD in the second 2.5 weeks of intake (5 weeks after the beginning of CBD consumption) increased by 50% compared to the first dose. CBD is mainly metabolized to 7-carboxy metabolites, as already confirmed by previous studies [2,27]. This final metabolite was found in plasma. When compared to the concentration after 2.5 weeks of CBD consumption, the metabolite's concentration in plasma was nearly two times greater after 5 weeks of CBD use.

In contrast to our study, Perez-Acevedo et al.'s findings demonstrated that the concentration of 7-OH-metabolites in urine was higher than 7-COOH metabolites [28]. However, it should be noted that their study carried out measurements within 24 h, so the metabolite was not excreted to a great extent. This timeframe is in contrast to our study, in which measurements were carried out after 2.5 and 5 weeks from the start of CBD consumption, and with daily consumption. The plasma CBD/7-OH ratio decreased over time, which is in favor of an increase in metabolite concentration.

In their study, which lasted 7 days, Taylor et al. administered doses of 1500, 3000, 4500, and 6000 mg per day [2], which was significantly higher than in our study. The maximum concentrations of CBD and metabolites did not increase proportionally with increasing dose. Therefore, despite a four-fold increase in the dose (from 1500 mg to 6000 mg), the maximum concentrations were only 2.67 times higher. According to the findings of our study, only a 50% increase in CBD concentration was observed in our subjects' plasma after 5 weeks of taking the supplement compared to the measurement taken after 2.5 weeks.

3.1. Sex Difference

By analyzing CBD plasma concentrations at the first measurement timepoint, after 2.5 weeks of CBD consumption, it was observed that CBD concentrations were higher in men than in women. As time progressed, and after repeated higher doses at the next timepoint (after 5 weeks), the concentration of CBD was higher in women than in men. Broadly consistent with these observations, in their rat study, Child and Tallon found that after 28 days of taking CBD, C_{max} increased by 36% in females, while it decreased by 22% in males [18].

The results of our study show that after stopping CBD intake and a two-week washout, CBD concentrations in female subjects remained significantly higher. More females than males tested positive for circulating CBD after the washout and 2.5 weeks of consuming the placebo capsules. Five weeks after taking the placebo, no men tested positively. In all subjects, at all examined timepoints, the presence of 7-COOH-CBD metabolites was significantly higher in women compared to men. Differences in concentration in favor of women may be due to their higher fat content compared to men. Due to its high

lipophilicity, CBD disperses more readily in a lipophilic environment and is subsequently partly deposited, which affects cumulative concentration and slows elimination. According to Review by Millar et al. [29], C_{max} increased during the fed state and in lipid formulations, i.e., CBD can dissolve in the fat content of food, increasing its solubility and absorption, and consequently its bioavailability. Therefore, in order to promote optimal absorption, the same authors suggested that CBD should be administered orally after a meal.

Knaub et al. reported that healthy female volunteers may achieve higher CBD concentrations than healthy male volunteers, depending on the CBD formulation ingested [11]. Eight healthy female and eight healthy male participants were selected to receive a single dose of 25 mg of CBD orally in either a novel self-emulsifying drug delivery system formulation (SEDDS-CBD) or a medium-chain triglycerides formulation (MCT-CBD). Females had considerably greater area under curve (AUC) for the MCT-CBD formulation than males (for comparison, DehydraTECH™2.0 CBD has long-chain fatty acids associated with the CBD); however, for the SEDDS-CBD formulation, males had a more rapid t_{max} [11]. In our study, the CBD/7-OH-CBD ratio was higher in males than in women at 2.5 weeks following CBD consumption (1.97 vs. 1.68), but it was nearly equal after 5 weeks (1.89 vs. 1.87). It could be concluded that in the initial measurement, males had more CBD in their plasma than women, possibly because some of the CBD in women had already been stored in adipose tissue. Seeboth et al. [30], in their study involving 16 women and 27 men, analyzed the pharmacokinetic variations in a single oral dose of 15 mg of THC. Their results for maximum concentrations and average area under the curve showed differences between women and men. Consistent with the current findings, in women, these values were about 1.5 times higher than in men. These differences were not related to genotype or phenotype differences, but to differences in the volume of distribution, which is influenced by differences in the percentage of body fat.

A statistical correlation was observed with the percentage of body fat in the subjects. After 5 weeks of CBD ingestion, male individuals had a negative correlation, while females after washout had a positive correlation. According to the results of our study, the values of body fat percentage of female subjects were significantly higher compared to men, while at the same time, the percentages of muscle tissue and water were significantly lower. This likely explains the difference in the higher plasma CBD concentrations achieved at the later measurement timepoints. The findings of the study by Child and Tallon, which was carried out on rats (groups of six males and six females) over the course of 28 days, demonstrated that CBD accumulated in the tissues that were examined, including fat, muscle, and the liver, with a suggestion that higher values were found in adipose tissue and in female rats [18].

There has been a lot of research in recent years on the pharmacokinetics and pharmacodynamics of CBD and the differences between the sexes, but the studies were either acute, did not involve many subjects of either sex, or were not conducted on humans [14,18].

Our findings revealed that the presence of CBD in the plasma was evident in female participants 50 days after the last intake of CBD preparations. The difference in fat tissue between men and women is likely the reason for such findings. The fat tissue content of our study participants differed significantly in favor of women. It was 74% higher. Child and Tallon's research on rats of both sexes, showed significant changes in concentrations related to the time of the measurements [18]. CBD was administered to rats every day for 28 days. C_{max} was similar in both sexes, and on the first day, it was 3.7% higher in female rats. On the 28th day, female rats compared to male rats had a 66% higher concentration. The variables controlling the metabolism and tissue accumulation of ingested CBD are complex. Fatty acid binding proteins are important in the intracellular transport of CBD [31]. Albumin is the major transporter of CBD in the extracellular compartment; 90% of CBD is linked to proteins. Women, compared to men, have a lower mean concentration of albumin in their serum at the age of 20–60 years [32]. Such information can be used to personalize oral CBD dosing and maximize therapeutic doses in women.

In our previous study [13], in the 180th minute after ingestion, significantly higher concentrations of CBD in urine were found in male subjects than in female subjects. The results obtained are in accordance with the results of this study. In male subjects, due to a lower percentage of fat tissue, a lower accumulation of CBD was achieved, and consequently, a higher concentration of CBD remained in the bloodstream. Most of it from the bloodstream was metabolized and excreted in the urine, i.e., it was eliminated from the body more quickly. Also, it is stated [32] that the level of albumin may be responsible for the achieved concentrations of CBD. Perhaps the larger albumin content accelerated metabolism and, as a result, excretion, resulting in a lower plasma CBD concentration.

3.2. Implications

A positive screening test for the presence of cannabinoids long after the cessation of consumption should also be noted as information on CBD products and should serve as a caution to all future users. In particular, it is well known that, in the majority of nations, if a person's test is positive for the presence of cannabinoids, they are not permitted to operate machines or a motor vehicles [33,34]. We also observe that in certain situations, testing is only conducted using quick and insufficiently precise screening tests. Since this relates to CBD or its inactive metabolites, 7-OH-CBD and 7-COOH-CBD, it is essential to mention this in the consumption instructions so that if users are tested with rapid screening tests and are still positive, they know to insist on confirmatory tests before possible sanctions.

3.3. CYP Genotype and Phenotype

Our results showed that there was no association of CBD concentrations of concentration-matched CBD formulation with any phenotype or genotype. An exception was observed only for the concentration of CBD in men after 5 weeks of consumption, and related to the CYP2C9 enzyme phenotype. Men with the NM phenotype had significantly higher values of CBD in urine compared to subjects with IM phenotype. Also, a difference in the CYP2C19 enzyme phenotype was observed among female subjects. Although this was compared to subjects with the RM phenotype, subjects with the NM phenotype had greater values here as well. Perhaps this is the result of the formulation used, which bypasses the first pass through the liver, where the tested enzymes are located. There are also studies that have shown that cannabinoids and their metabolites can inhibit some P450 enzymes [35], which could be a basis for further discussion and additional research. It should be mentioned that some studies have demonstrated that healthy individuals who ingested doses of CBD of 1500 mg/day revealed an increase in serum alanine aminotransferase (ALT) values, which correlate with drug-induced liver damage. In the current study using several-fold-lower CBD dosage serum, liver enzymes were not affected [36].

3.4. Study Limitations

A limitation of the study is that serum albumin levels were not measured for all participants. Hormonal status was not included to better understand the difference between the sexes.

4. Materials and Methods

4.1. Participants and Ethical Approval

For this study, we selected 62 hypertensive participants (27 women and 35 men) aged between 40 and 70 years. The research was carried out in accordance with the Helsinki Declaration. Before being included in the trial, all participants provided written informed permission, and the trial was approved by the Ethics Committee of the Faculty of Medicine at the University of Split on 15 December 2021 (Class: 003-08/21-03/0003; Registration number: 2181-198-03-04-21-0091). The HYPER-H21-4 trial is registered on ClinicalTrials.gov as NCT05346562. The study's inclusion and exclusion criteria were described in our prior article [9].

4.2. Anthropometrics and Background

The Tanita scale (DC-360 S; Tanita, Tokyo, Japan) was used to measure weight, metabolic age, visceral fat, and the proportions of fat, muscle tissue, and water. Body mass index was calculated as weight divided by squared height. Results are shown in Table 3.

Table 3. Antropometric summary and descriptive statistics of participants.

Variable		Min	Max	Median (IQR)	Sex Difference Mann–Whitney Test
Age	F	43	70	55 (12)	$p = 0.230$
	M	42	69	54 (12)	
Weight	F	62.00	101.70	78.30 (15.10)	$p < 0.001$
	M	79.30	126.80	95.90 (14.30)	
Body Mass index (BMI)	F	20.88	33.87	28.43 (4.96)	$p = 0.659$
	M	24.55	34.97	28.17 (5.08)	
Fat tissue (%)	F	24.50	47.60	36.60 (6.80)	$p < 0.001$
	M	19.30	44.00	27.10 (6.20)	
Muscle tissue (%)	F	49.70	71.60	60.10 (6.40)	$p < 0.001$
	M	53.30	76.60	69.30 (5.60)	
Body water (%)	F	37.00	52.50	42.20 (4.60)	$p < 0.001$
	M	41.00	54.90	49.40 (3.50)	
Metabolic age (years)	F	35.00	77.00	58.00 (14.00)	$p = 0.795$
	M	40.00	82.00	60.00 (13.00)	
Fat free mass (kg)	F	42.30	59.40	51.10 (8.10)	$p < 0.001$
	M	60.00	89.00	70.50 (8.80)	
Visceral fat (kg)	F	4.00	14.00	9.00 (4.00)	$p < 0.001$
	M	8.00	31.00	12.00 (4.00)	

Data presented as minimum, maximum, median, and interquartile range (IQR).

Of the total number of participants, 14 were taking angiotensin-converting enzyme (ACE) inhibitors, 13 were taking ACE inhibitors and calcium blockers, and three were taking ACE inhibitors and thiazide diuretics.

4.3. Research Design

The participants were randomly divided into two groups (Figure 6). For five weeks, participants randomized to the first group (placebo, then cannabidiol) received placebo capsule matching a cannabidiol one. After a two-week washout period, participants received cannabidiol in the following doses: 225 to 300 mg (depending on the sex and weight of participants) divided three times per day for the first 2.5 weeks, then 375 to 450 mg (depending on the sex and weight of participants) divided three times for the next 2.5 weeks. Participants in the second group (cannabidiol, then placebo) received cannabidiol in the amounts of 225 to 300 mg divided three times daily for the first 2.5 weeks and 375 to 450 mg divided three times daily for the next 2.5 weeks. Participants received cannabidiol-matched placebo capsules for the next five weeks after a two-week washout period. For blinding reasons, the DehydraTECH™2.0 CBD formulation and the placebo substrate powder were packed into capsules of comparable dimensions and shape. A detailed description of the formulation, intake, and overall regimen of the study was described in a previous paper [9].

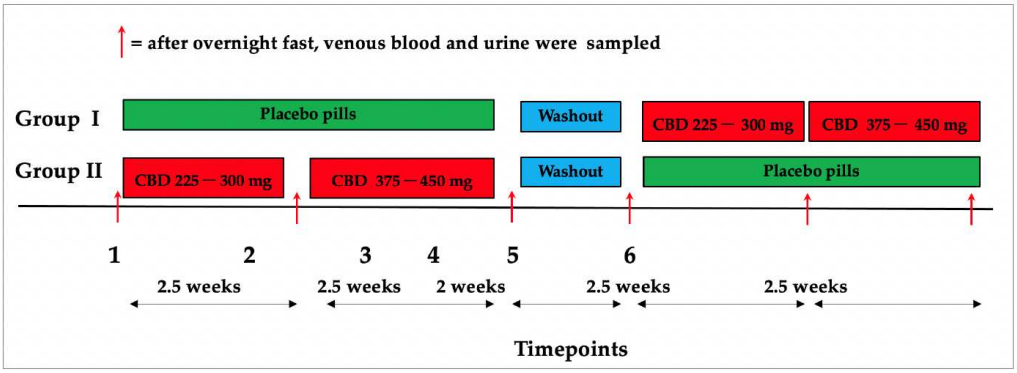

Figure 6. Research design. DehydraTECH™2.0 CBD (225 to 300 mg split over three times daily for the initial 2.5 weeks and 375 to 450 mg split over three times for the following 2.5 weeks). Participants repeated tests for a further five weeks under varied circumstances after a two-week washout. Three times in each study arm, participants visited the laboratory on a total of six occasions, each after an overnight fast.

4.4. Sample Collection and Storage

After a 12 h fast, venous blood samples were collected from the antecubital vein in each individual. Plasma was separated (centrifuged at 4 °C on 3500 rpm for 10 min) and kept at −20 °C within 1–2 h of collection. Each sample was marked with the study number, the test session number, the date, and the time for each patient. Per patient, two urine samples (5–10 mL) and three plasma samples (1.5–2 mL) were sent directly to the lab for evaluation. All plasma and urine samples were tested in the same laboratory in accordance with proper laboratory practices, and analysts were unaware of the distribution of patients in the studied groups.

4.5. Plasma and Urine Samples Extraction

Proteins in plasma samples (1 mL aliquots) were precipitated with 1.25 mL of ice-cold acetonitrile. After mixing, samples were centrifuged (2600 rpm for 2 min) and 1.5 mL of supernatant with 1 mL d.d. H_2O, as well as urine sample, was added to preconditioned solid phase extraction (SPE) columns with CBD specific cartridges (United Chemical Technologies, Styre Screen SSTHC063, Bristol, PA, USA; for both, plasma and urine extraction protocol were carried out according to the manufacturer's instructions, Bristol, PA, USA). Subsequently, the column was rinsed with 1 mL dd H_2O and dried under high vacuum (~20 inch). CBD was eluted with a 3 mL mixture of hexane/ethyl acetate/acetic acid (49:49:2, *v*/*v*) and dried under nitrogen. Samples were reconstituted with 150 µL mix of acetonitrile/d.d. H_2O (1:1, *v*/*v*).

4.6. Standard Solutions

Standards used for qualitative and quantitative determination of cannabidiol (CBD) and its metabolites 7-hydroxy-cannabidiol (7-OH-CBD) and 7-carboxy-cannabidol (7-COOH-CBD) were purchased from Cerilliant, Sigma Aldrich (Round Rock, TX, USA), as certified reference solutions in methanol 1 mg/mL each (Product codes: C-045-1ML, C-180-1ML and C-181-1ML, respectively).

4.7. LC-MS Analysis

Measurements were performed on the UHPLC-MS/MS (Ultimate 3000RS equipped with TSQ Quantis MS/MS detector, Thermo Fischer Scientific, Waltham, MA, USA). Heated electrospray ion source (H-ESI) was set to a positive ion mode with spray voltage of 4000 V, while Sheat, Aux, and Sweep gasses were set to 50, 25, and 2 arbitrary units

(Arb), respectively. Ion transfer tube was set to 325 °C and Vaporizer temperature was set to 280 °C. Collision-induced dissociation (CID) gas pressure was constant at 1.5 mTorr. Sample injection volume was 1 µL. Separation was performed on Accucore C18 column (150 mm × 2.1 mm, 2.6 µm, Thermo Fischer Scientific, Waltham, MA, USA) equipped with Accucore C18 guard column (10 mm × 2.1 mm, 2.6 µm) using gradient elution with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Solvent gradient was programmed as follows: 0 min 60% B, 8 min 68% B, 8.1 min 95% B, 9.1 min 95% B, 9.3 min 60% B, and 11 min 60% B. Total run time was 11 min. Column flow was 0.5 mL/min. Column temperature was held at 40 °C, while autosampler temperature was held at 15 °C. Needle was washed in solvent mixture comprised of acetonitrile/methanol/water/formic acid = 40:40:20:2 (*v*/*v*).

Quantitative analysis of CBD, 7-OH-CBD, and 7-COOH-CBD was performed using external calibration curves for each analyte (ranging from 0 ng/mL to 2000 ng/mL). Each compound was introduced to the MS/MS system separately to optimize fragment transitions, collision energies, and ion focusing lens voltages, which were all performed using Chromeleon MS tuning console v. 7.2.10 (23925) and are shown in the following Table 4.

Table 4. Quantitative and qualitative transitions used for analysis of CBD and its metabolites.

Compound	Q1 (m/z)	Q3 [a] (m/z)	Q3 [b] (m/z)	RF (V)	CE (eV)	CE (eV)	RT (min)
CBD	315.5	193.1	259.2	115	21	18	5.57
7-OH-CBD	313.5	201.1	193.2	134	22	22	1.48
7-COOH-CBD	345.5	327.2	299.3	109	14	18	1.33

Abbreviations: CBD—cannabidiol; 7-OH-CBD—7-hydroxy-CBD; 7-COOH-CBD—7-carboxy-CBD; Q1—precursor ion; Q3—product ion (a—quantifying, b—qualifying); RF—focusing lens voltage; CE—collision energy; RT—retention time.

Limit of detection (LOD) and limit of quantification (LOQ) were estimated using Blank Samples approach. Ten (10) blank samples were analyzed and LOD was calculated as $3.9*(s_{y,bl}/b)$, where $s_{y,bl}$ represents the standard deviation of the blank signal for each analyte and b represents the slope of the appropriate calibration curve. LOQ was estimated as 3.3*LOD. Both LOD and LOQ and the appropriate calibration curve equations are shown in the following Table 5.

Table 5. Quantitative and qualitative transitions used for analysis of CBD and its metabolites.

Compound	LOD (ng/mL)	LOQ (ng/mL)	Calibration Curve Equation	R^2
CBD	0.60	1.98	y = 3.1186x	0.995
7-OH-CBD	0.20	0.66	y = 2.7706x	0.998
7-COOH-CBD	0.20	0.66	y = 9.6546x	0.998

Abbreviations: CBD—cannabidiol; 7-OH-CBD—7-hydroxy-CBD; 7-COOH-CBD—7-carboxy-CBD; LOD—limit of detection; LOQ—limit of quantification; R^2—correlation coefficient.

4.8. DNA Analysis and SNP Genotyping

From the baseline and regular check-ups, blood samples for DNA research were taken and kept in EDTA tubes. DNA isolation, DNA quantification, and SNP determination were performed according to our previous study [13].

4.9. Statistical Analysis

The Kolmogorov–Smirnov test was used for normality checking. Due to the non-normal distribution of the data, continuous variables are presented with the minimum, maximum, and median (interquartile range, IQR), and categorical variables are presented

with frequencies (percentages). Groups were compared using the non-parametric Mann–Whitney U test, linear regression analysis, and Wilcoxon Signed Ranks test. *p*-values of less than 0.05 were considered statistically significant. Furthermore, multivariate linear regression analysis was performed to assess the association of CYP genotypes with CBD levels in the plasma and urine. Statistical analysis was performed using Statistical Package Software for Social Science, version 28 (SPSS Inc., Chicago, IL, USA).

5. Conclusions

CBD's effect is affected by its bioavailability, and larger concentrations of CBD in plasma or serum correlate well with it. In order for a person to achieve a higher concentration, it is not necessary to exclusively take more daily doses. We assume that a longer period of intake has a greater impact on more effective concentrations than a higher daily dose. Over a longer period of time, women achieve a higher plasma concentration of CBD.

CBD plasma concentrations in men were negatively correlated with their amount of adipose tissue. It can be assumed that the rate constant of absorption and elimination of CBD in men compared to women is higher, which may mean faster metabolism of CBD in men and consequently lower CBD concentrations.

To examine the concentrations and effects of CBD over a period of several months, we believe that further research is needed. We found no studies in the literature relating to differences in CBD concentrations in participants based on sex hormone status. The majority of the test subjects in our study were postmenopausal. We believe that this parameter should be researched further as well. Additionally, it would be beneficial to look into the effects of combining CBD with medications that are more frequently used, such as those that decrease pain, fever, or other symptoms.

Author Contributions: Conceptualization, Z.D., J.B. and D.S.; methodology, Z.D., D.S., G.D., J.V., A.B., F.B., N.K. and S.K.; validation, Z.D. and D.S.; formal analysis J.B., M.K., G.D., J.V., A.B., F.B., N.K., S.K. and A.M.; investigation, J.B., M.K., A.B., S.K. and A.M.; resources, Z.D.; writing—original draft preparation, A.B., D.S., S.K., A.M., F.B., N.K., G.D., J.V. and Z.D.; writing—review and editing, Z.D., A.B. and D.S.; visualization, A.B. and D.S.; supervision, Z.D.; project administration, J.B., M.K. and Z.D.; funding acquisition, Z.D. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was approved by the Ethics Committee of the University of Split School of Medicine on 15 December 2021. (Class: 003-08/21-03/0003; Reg. No.: 2181-198-03-04-21-0091). All procedures conformed to the Declaration of Helsinki.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Article

Knowledge and Attitudes of Cannabidiol in Croatia among Students, Physicians, and Pharmacists

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Abstract: Due to cannabidiol's health benefits and absence of serious side effects, its use is constantly growing. This is a survey-based cross-sectional study that was conducted to determine Croatian pharmacists', physicians', and students' knowledge and attitudes about cannabidiol (CBD). Two questionnaires were created, one for students and the other for physicians and pharmacists. Our participants (in total 874: 473 students and 401 physicians and pharmacists) generally had positive attitudes towards CBD therapy as approximately 60% of them believe that CBD treatment is generally efficacious. Participants had positive attitudes toward the therapeutic value of CBD, especially pharmacists and pharmacy students (63.8% and 72.2%, respectively). Pharmacists were significantly more convinced that CBD could reduce the use of opioids prescribed for chronic pain ($p < 0.05$). Only 17.5% of students had read scientific papers about CBD, compared to a significantly higher percentage of physicians and pharmacists (43.0% and 47.8%, respectively) ($p < 0.05$). This study revealed a gap in knowledge regarding CBD, since 89.3% of pharmacists and physicians, as well as 84.8% of students, believe they need more education about CBD. We conclude that it is important to improve the educational curricula so that medical professionals can recommend CBD use to their patients when needed.

Keywords: cannabidiol; knowledge; attitudes; pharmacists; physicians; students; Croatia

1. Introduction

The hemp plant (lat. *Cannabis sativa* L.) is a widely used plant, and its subspecies Indian hemp (lat. *Cannabis sativa* L. *subsp. indica*), i.e., marijuana, is considered one of the most commonly consumed recreational drugs worldwide, especially among young adults [1,2]. There are more than 750 identified cannabis chemicals, including more than

100 cannabinoids. Cannabidiol (CBD) and delta-tetrahydrocannabidiol (THC) are the two most important and widely studied components [3,4]. Cannabis is associated with recreational drugs due to THC, which is known as the primary psychoactive component of the plant [5]. However, CBD has no psychotropic effects and has a confirmed safety profile [6,7]. Due to its numerous health benefits and lack of significant negative side effects, CBD use and product marketing are constantly increasing [8,9]. National regulations for the use of CBD vary around the world. The use of CBD as a dietary supplement is allowed in many countries as long as the THC content is below 0.3% in the United States and 0.2% in Europe [10].

Currently, the Food and Drug Administration (FDA) has approved only one purified, prescription CBD medicine (Epidiolex®, 100 mg/mL, oral solution). This drug has been designated as an "orphan drug" (a medication used to treat rare disorders). Epidiolex is indicated as adjunctive therapy for seizures associated with Lennox-Gastaut syndrome (LGS) or Dravet syndrome (DS) in combination with clobazam in patients $\geq$ 2 years old and as adjunctive therapy for seizures associated with tuberous sclerosis complex (TSC) also in patients $\geq$ 2 years old [11–13]. Marinol® (dronabinol), Syndros® (dronabinol), and Cesamet® (nabilone) are three synthetic cannabis-related pharmacological products, also approved by the FDA. Dronabinol is a synthetic delta-9- tetrahydrocannabinol (THC), which is considered the psychoactive intoxicating component of cannabis (i.e., the component responsible for the "high" people can experience when using cannabis). The use of dronabinol is indicated for nausea and vomiting associated with malignancies and for the treatment of anorexia associated with weight loss in patients with acquired immunodeficiency syndrome (AIDS) [11,14]. Nabilone (a synthetic with a THC-like chemical structure) is indicated for the treatment of nausea and vomiting associated with cancer chemotherapy in patients who have not responded adequately to conventional antiemetic treatments. These medications are available in the United States only with a prescription from a licensed healthcare provider [11]. The European Medicines Agency (EMA), has approved the use of Epidyolex® (cannabidiol) for the same indications accepted by the FDA [15]. CBD is marketed as Epidyolex in the European Union, but it is officially known as Epidiolex in the USA. In addition, the EMA has also approved Sativex®, an oromucosal spray (solution), containing two extracts of *Cannabis sativa* L., folium cum flore (cannabis leaf and flower), which contain almost the same amount of THC and CBD [16]. Sativex is indicated as a treatment to improve symptoms in adult patients with moderate to severe spasticity due to multiple sclerosis (MS) who have not responded adequately to other anti-spasticity medications and who show clinically significant improvement in symptoms associated with spasticity during an initial trial of therapy. The FDA has not yet approved Sativex in the United States.

An increasing body of evidence-based information available, including multiple CBD human research, now supports the long-standing use of cannabis and CBD products to treat a variety of medical conditions: symptoms of chronic pain, inflammation, cardiovascular disease, mental health issues, spasticity associated with multiple sclerosis and malignancies without serious side effects [17,18]. With the development of novel CBD formulations, smaller doses may lead to increased absorption and, consequently, greater health benefits [19–23].

Nowadays, people are becoming more aware and interested in the natural medicinal aspects of CBD, as it is becoming more widely available in cosmetics and dietary supplements. In Croatia, the Agency for Medicinal Products and Medical Devices (HALMED) has approved the FDA and EMA-approved cannabinoid-based medication Epidyolex, and all relevant details about the medicine, including interactions with other medicines, are available [24]. The use of unapproved cannabis and cannabis-derived products may have unpredictable and unintended consequences, including serious safety risks, considering that there are CBD products of questionable quality and with inconsistent labelling on the market [11,25]. According to recent studies, young adults have a positive perception of CBD despite having limited knowledge of its evidence base or regulation [26]. Several

studies have shown that the pharmacological knowledge of pharmacists, physicians, students, patients, and recreational users in other countries is insufficient regarding cannabis and cannabinoid-derived drugs [12,26–32]. This study aimed to analyze the attitudes and knowledge of physicians, pharmacists, and students in Croatia about the therapeutic use of cannabis and cannabinoid-derived medicines.

2. Materials and Methods

A cross-sectional survey study was conducted from 30 June to 30 July 2023. Two questionnaires were developed for this study, one to assess the knowledge and attitudes of physicians and pharmacists about the use of CBD for medical purposes and another to assess the knowledge and attitudes of students. The sample of students included medical, pharmacy, and health science students. Both questionnaires were developed by the researcher and were based on a literature review of this particular topic [10,12,28,32–46].

2.1. Surveys Design

The questionnaire for the students consisted of 20 questions and the physicians' and pharmacists' questionnaire consisted of 31 questions. The questions were divided into 5 categories: general questions, self-assessment knowledge questions, researcher-identified knowledge questions, CBD experience questions, and attitude assessment questions about CBD use (Table 1). Attitudes and knowledge regarding CBD were assessed using a 5-point Likert scale (from strongly disagree to strongly agree), yes/no questions, and categorical questions (with one or more choices).

Table 1. Surveys design: categories and questions for respondents.

Question Category	Physicians' and Pharmacist' Questionnaire	Students' Questionnaire
General	Gender, profession, specialization, years of work in practice, county of residence	Gender, study program, year of study
Questions represented in both questionnaires		
Knowledge self-assessment	Do you have knowledge about CBD? Through my formal education, I had an education about CBD. I think that I need more education about CBD. I am aware of CBD use risks. I am aware of CBD use benefits.	
Researcher-assessed knowledge	CBD is bad for health. CBD treatment is efficacious. CBD has positive effects on physical health. CBD has positive effects on mental health. CBD helps patients with chronically debilitating conditions. CBD is physically addictive. CBD is psychologically addictive. Using CBD can lead to addiction to other opioids and drugs. CBD causes a feeling of euphoria. Have you ever read a scientific paper about CBD?	
CBD experience	Have you ever consumed CBD?	
Attitudes about CBD use	The educational curricula of Physicians, health professionals, and pharmacists should include subjects on the use of CBD for medical purposes.	
Questions presented in physicians' and pharmacists' questionnaire only		
Knowledge self-assessment	I believe that I have enough knowledge about the use of CBD for medical purposes and that I can recommend it to patients.	

Table 1. *Cont.*

Question Category	Physicians' and Pharmacist' Questionnaire Students' Questionnaire
Researcher-assessed knowledge	FDA has approved CBD drugs for nausea associated with chemotherapy, chronic neuropathic pain, EPI attacks in Lennox-Gastaut and Dravet syndrome, depressive disorders, Parkinson's disease, tuberous sclerosis, and pain in malignant diseases. ** Side effects of CBD are anemia, tachycardia, diarrhea and vomiting, glaucoma, decreased appetite, hyperglycemia, and somnolence. ** Medications that have moderate or severe interactions with CBD include: paracetamol, valproat, omeprazole, karbamazepin, ibuprofen, rifampicin, amoksicilin, everolimus, klobazam, fehidramin. ** Conditions that require caution when using CBD are cardiac arrhythmia, hepatocellular damage, glaucoma, somnolence, cancer, reduced body weight, pregnancy, suicidal behaviour, somnolence, and sedation. **
CBD experience	Have you ever recommended/prescribed CBD to your patients?
Attitudes about CBD use	I support the use of CBD in palliative patients, cancer pain relief, side effects of chemotherapy, multiple sclerosis, neuropathic pain, chronic pain, PTSD, insomnia, Crohn's disease, glaucoma, hepatitis C, muscle spasticity, HIV, traumatic brain injury, ALS, Alzheimer's disease, anorexia, Parkinson's disease, migraine. ** I believe that recommending/prescribing CBD could reduce the use of opioids in chronic pain. I believe that health insurance should cover the cost of CBD if a doctor prescribes it as therapy.

** Check all that apply.

Surveys were created and distributed using Google Forms online survey administration software offered by Google. The open survey link was sent to physicians and pharmacists across Croatia and students at the Universities of Split (medical, pharmacy, and health students), Zagreb (pharmacy students), and Osijek (medical students). The sample size was determined using the SurveyMonkey sample size calculator [47]. The confidence level was 95% with a margin of error of 5%.

With a target population of 2000 students and 20,000 physicians and pharmacists, the required sample was 323 for students and 377 for physicians and pharmacists. The final sample consisted of 874 participants, of whom 473 were students and 401 were physicians and pharmacists.

The survey was completely anonymous and voluntary, and it was approved by the Ethical Committee of the University Department of Health studies at the University of Split on 26 June 2023 (Class: 029-03/23-08/01; Registration number: 2181-228-103/1-47).

2.2. Statistical Analysis

Data analysis utilized descriptive statistics to describe responses to survey items. The differences between the groups of study parameters were measured using the Chi-square and Mann-Whitney U tests. Chi-square tests were utilized for comparisons of common perceptions about knowledge and education about CBD between physicians, pharmacists, and surveyed students. Differences and relationships were considered to be statistically significant at p-value < 0.05. Statistical analysis was performed using Statistical Package Software for Social Science, version 26 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Demographic Data

General statistics of study participants who completed the online survey were presented in Tables 2 and 3. Among demographic data, tables showed participants' knowledge self-assessment and researcher-assesses knowledge. Our study consisted of a total of 874 participants; students (N = 473), physicians' and pharmacists' [N = 401: 100 physicians (52 specialists and 48 general practitioners) and 301 pharmacists (16 specialized pharmacists and 285 pharmacists without specialization)]. The majority of all respondents were female, as in other similar research [12,32,36]. The percentage of female students

was 78.65% and the percentage of males was 21.35%. The Croatian Bureau of Statistics reports that 151,827 students were enrolled for the academic year 2022/2023 57.9% of them were female students, and 42.1% were males [48]. The study included responses from 100 physicians from both genders: females (70.0%) and males (30.0%). Among pharmacists' the proportion of female participants was 85.0% and for males was 15.0%. A representative sample of participants took part in the survey, the Croatian Medical Chamber has 16,089 members (63% female), and the Croatian Chamber of Pharmacists has 4325 members (88.6% female) [49,50].

Table 2. Study sample general characteristics and knowledge self-assessment of Croatian students (*N* = 473), physicians (*N* = 100), and pharmacists (*N* = 301).

Variable		Students *N* (%)	Physicians' and Pharmacists' *N* (%)	Difference between Groups χ^2 Test
Gender	F	372 (78.6)	326 (81.3)	$p = 0.330$
	M	101 (21.4)	75 (18.7)	
Study program	Medical	150 (31.7)	-	
	Pharmacy	198 (41.9)	-	
	Health	125 (26.4)	-	
Year of study program Medical 1–6; Pharmacy 1–5	1	111 (23.5)	-	
	2	92 (19.5)	-	
	3	92 (19.5)	-	
	4	71 (15.0)	-	
	5	50 (10.6)	-	
	6	57 (12.1)	-	
Years of work in practice physicians'/pharmacists' (*N* = 401)	1–5	-	135 (33.7)	
	6–10	-	50 (12.5)	
	11–20	-	101 (25.2)	
	21–30	-	65 (16.2)	
	31–40	-	34 (8.5)	
	>40	-	16 (4.0)	
Do you have knowledge about CBD?	Yes	361 (76.3)	316 (78.8)	$p = 0.382$
	No	112 (23.7)	85 (21.2)	
Through my formal education, I had an education about CBD.	Yes	127 (26.8)	90 (22.4)	$p = 0.136$
	No	346 (73.2)	311 (77.6)	
Have you ever read a scientific paper about CBD?	Yes	83 (17.5)	187 (46.6)	$p < 0.05$
	No	390 (82.5)	214 (53.4)	
Have you ever consumed CBD?	Yes	120 (25.4)	65 (16.2)	$p < 0.05$
	No	353 (74.6)	336 (83.8)	
The educational curricula of Physicians, health professionals, and pharmacists should include subjects on The use of CBD for medical purposes.	1	11 (2.3)	14 (3.5)	$p < 0.05$
	2	15 (3.2)	9 (2.2)	
	3	67 (14.2)	39 (9.7)	
	4	143 (30.2)	85 (21.2)	
	5	237 (50.1)	254 (63.3)	

Table 2. *Cont.*

Variable		Students *N* (%)	Physicians' and Pharmacists' *N* (%)	Difference between Groups χ^2 Test
I think that I need more education about CBD.	1	17 (3.6)	10 (2.5)	$p < 0.05$
	2	11 (2.3)	15 (3.7)	
	3	44 (9.3)	18 (4.5)	
	4	108 (22.8)	61 (15.2)	
	5	293 (61.9)	297 (74.1)	
I am aware of CBD use risks.	1	88 (18.6)	61 (15.2)	$p < 0.05$
	2	96 (20.3)	78 (19.5)	
	3	140 (29.6)	157 (39.2)	
	4	91 (19.2)	70 (17.5)	
	5	58 (12.3)	35 (8.7)	
I am aware of CBD use benefits.	1	50 (10.6)	33 (8.2)	$p < 0.05$
	2	75 (15.9)	36 (9.0)	
	3	144 (30.4)	162 (40.4)	
	4	155 (32.8)	136 (33.9)	
	5	49 (10.4)	34 (8.5)	

Participants agreement level: 1—strongly disagree; 2—disagree; 3—neutral; 4—agree; and 5—strongly agree.

Table 3. Researcher assessment of knowledge and differences between analyzed groups: Croatian students (*N* = 473), physicians (*N* = 100), and pharmacists (*N* = 301).

Variable	* Participants Agreement Level	Students *N* (%)	Physicians' and Pharmacists' *N* (%)	Difference between Groups χ^2 Test
CBD is bad for health.	1	73 (15.4)	104 (25.9)	$p < 0.05$
	2	121 (25.6)	123 (30.7)	
	3	205 (43.3)	133 (33.2)	
	4	43 (9.1)	31 (7.7)	
	5	31 (6.6)	10 (2.5)	
CBD treatment is efficacious.	1	7 (1.5)	8 (2.0)	$p = 0.408$
	2	25 (5.3)	29 (7.2)	
	3	140 (29.6)	133 (33.2)	
	4	200 (42.3)	157 (39.2)	
	5	101 (21.4)	74 (18.5)	
CBD has positive effects on physical health.	1	21 (4.4)	11 (2.7)	$p = 0.158$
	2	37 (7.8)	29 (7.2)	
	3	225 (47.6)	168 (41.9)	
	4	140 (29.6)	145 (36.2)	
	5	50 (10.6)	48 (12.0)	

Table 3. *Cont.*

Variable	* Participants Agreement Level	Students *N* (%)	Physicians' and Pharmacists' *N* (%)	Difference between Groups χ^2 Test
CBD has positive effects on mental health.	1	41 (8.7)	21 (5.2)	$p = 0.092$
	2	75 (15.9)	49 (12.2)	
	3	181 (38.3)	164 (40.9)	
	4	126 (26.6)	127 (31.7)	
	5	50 (10.6)	40 (10.0)	
CBD helps patients with chronically debilitating conditions.	1	5 (1.1)	5 (1.2)	$p = 0.546$
	2	18 (3.8)	12 (3.0)	
	3	120 (25.4)	112 (27.9)	
	4	194 (41.0)	175 (43.6)	
	5	136 (28.8)	97 (24.2)	
CBD is physically addictive.	1	74 (15.6)	92 (22.9)	$p < 0.05$
	2	98 (20.7)	89 (22.2)	
	3	168 (35.5)	143 (35.7)	
	4	88 (18.6)	46 (11.5)	
	5	45 (9.5)	31 (7.7)	
CBD is psychologically addictive.	1	40 (8.5)	71 (17.7)	$p < 0.05$
	2	58 (12.3)	70 (17.5)	
	3	164 (34.7)	136 (33.9)	
	4	125 (26.4)	77 (19.2)	
	5	86 (18.2)	47 (11.7)	
Using CBD can lead to addiction to other opioids and drugs.	1	87 (18.4)	126 (31.4)	$p < 0.05$
	2	88 (18.6)	91 (22.7)	
	3	155 (32.8)	115 (28.7)	
	4	87 (18.4)	38 (9.5)	
	5	56 (11.8)	31 (7.7)	
CBD causes a feeling of euphoria.	1	81 (17.1)	118 (29.4)	$p < 0.05$
	2	86 (18.2)	100 (24.9)	
	3	177 (37.4)	126 (31.4)	
	4	77 (16.3)	38 (9.5)	
	5	52 (11.0)	19 (4.7)	

* Participants agreement level: 1—strongly disagree; 2—disagree; 3—neutral; 4—agree; and 5—strongly agree.

Participants were not paid for their participation as it was voluntary. Croatian medical studies last six years, while pharmacy and public health studies have five-year programs (with the exception of the 3-year basic public health studies). Croatian students from all years of study enrolled in the academic year 2022/2023 were included in this survey.

Respondents include physicians and pharmacists with a wide range of professional experiences, from one to more than forty years.

3.2. Results of Respondent's Knowledge about CBD

In our study, we did not find any differences between groups of participants where more than 70% (76.3% and 78.8%) of respondents believe they have general knowledge

about CBD (Figure 1 and Table 2). Interestingly, more than 70% (73.2% and 77.6%) of them answered that they did not have a formal education about it.

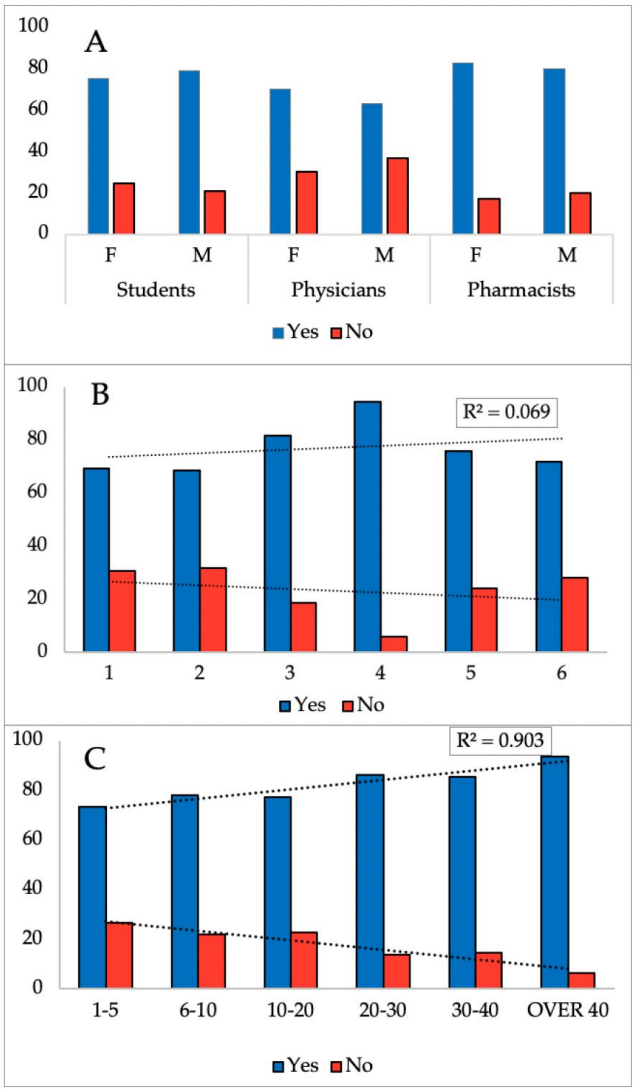

Figure 1. Percentage of participants' knowledge about CBD. On the y-axis, the percentage of respondents' responses is displayed. The x-axis is shown as follows: (**A**) shows the distribution of respondents by gender (F—female and M—male) for the student, doctor, and pharmacist groups; (**B**) shows the respondents' years of study for the student group; and (**C**) shows the respondents' number of years of working experience for the doctor and pharmacist group. The answer trend Yes is positively correlated: in the examined group of students from 1 to 4 years of study (**B**) and in the group of doctors and pharmacists depending on the years of work experience.

The majority of participants, with the exception of pharmacists, were generally neutral regarding whether CBD is harmful to health. A significant difference in perceptions of CBD's hazards was not observed between all student groups ($p = 0.059$) while a statistical difference was observed between students and a group of physicians and pharmacists (as

well as between a group of physicians and pharmacists) ($p < 0.05$). For participants agreeing with level 1 strongly disagree, there was a statistically significant difference between all respondents' responses ($p < 0.05$) (Figure 2A and Table 3).

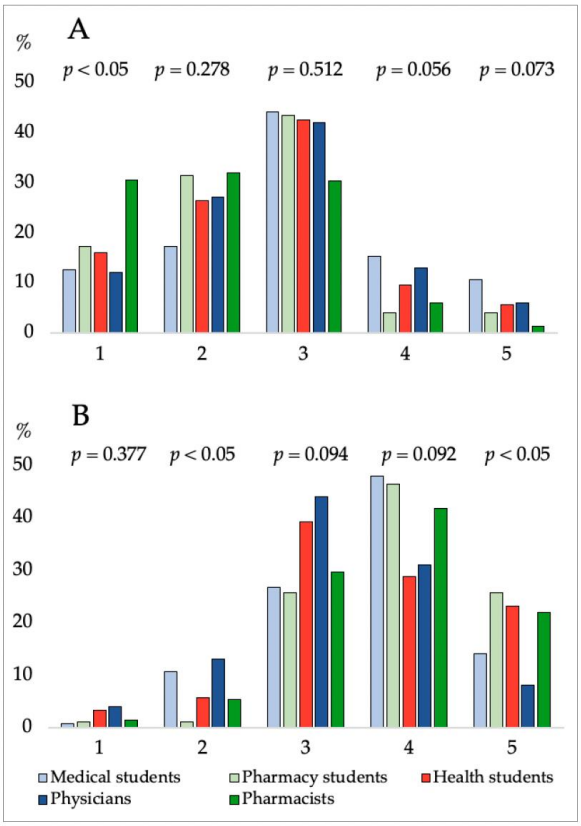

Figure 2. Percentage of participants' attitudes regarding questions: (**A**)—shows the answers to the questions "CBD is bad for health", (**B**)—shows the answers to the questions "CBD treatment is efficacious" Difference between groups: χ^2 Test. On the y-axis, the percentage of respondents' responses is displayed. On the y-axis is displayed participants agreement level: 1—strongly disagree; 2—disagree; 3—neutral; 4—agree; and 5—strongly agree.

A significant difference ($p < 0.05$) was found regarding attitudes toward the efficacy of CBD therapy between all groups of students (Figure 2B) and between pharmacists and physicians. A statistically significant difference was also observed between the responses of all respondents for participants' agreement level 2-disagree and 5-strongly agree ($p < 0.05$). (Figure 2B). In general, participants had high and very high attitudes toward the therapeutic value of CBD, especially pharmacists and pharmacy students (63.8% and 72.2%, respectively).

3.3. Results of the Questionnaire Presented Only for Physicians' and Pharmacists'

In Figures 3–7 and Table 4 results of the questionnaire presented only for physicians and pharmacists were shown. Figures 3 and 4 showed no significant difference between the group of physicians and pharmacists regarding the FDA-approved indications for CBD as well as their knowledge of CBD side effects.

Additionally, as shown in Figure 5. there was not a significant difference between the groups of physicians and pharmacists regarding knowledge about CBD and drug interactions. Also, physicians and pharmacists are aware of the need for caution while utilizing CBD for specific medical conditions except for reduced body weight (only 11% of physicians and 20% of pharmacists) as it was shown in Figure 6.

Finally, there wasn't a significant difference in the attitudes between the two groups of participants, physicians, and pharmacists, regarding support of the use of CBD for different medical conditions as it was presented in Figure 7. Most pharmacists and physicians support the use of CBD for malignant conditions and in palliative patients. Furthermore, pharmacists are significantly more convinced that CBD could reduce the use of opioids prescribed for chronic pain, as it was shown in Table 4. Both test groups generally agree that they do not have enough knowledge about the use of CBD for medical purposes and therefore can't recommend it to patients. Pharmacists and physicians support health insurance coverage for CBD use.

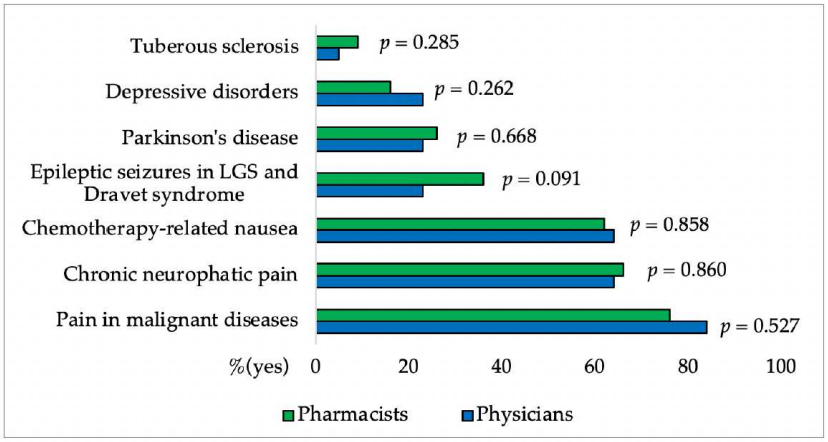

Figure 3. Percentages of pharmacists' and physicians' affirmative responses regarding the FDA-approved indications for CBD (LGS: Lennox-Gastaut syndrome). The difference between groups for each indication was made using the χ^2 Test.

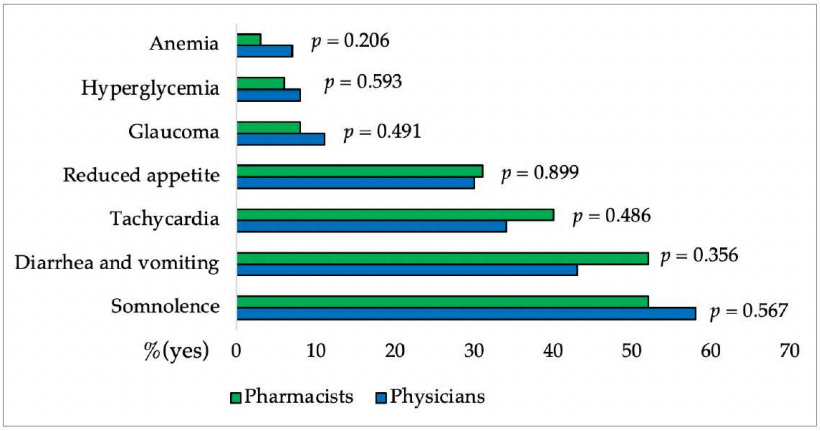

Figure 4. Percentages of pharmacists and physicians' affirmative responses regarding the CBD side effects. The difference between groups for each side effect was made using the χ^2 Test.

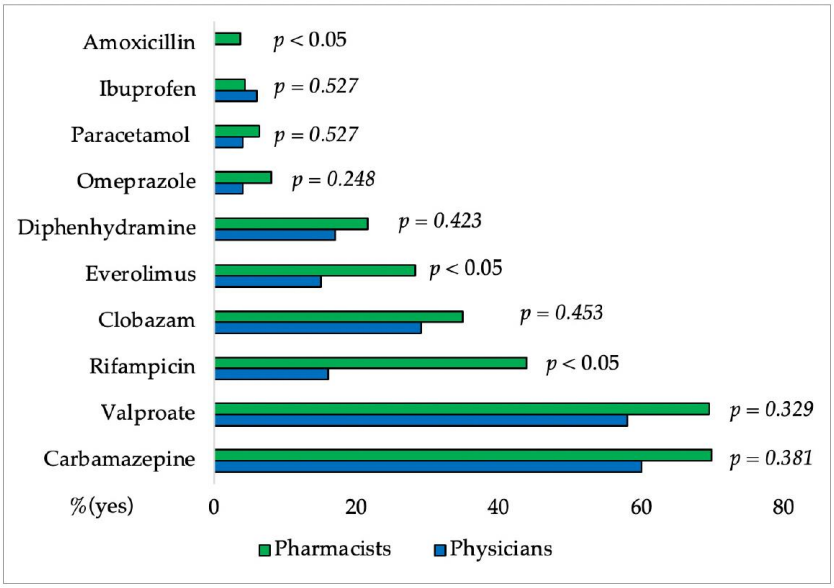

Figure 5. Percentages of pharmacists and physicians' affirmative responses regarding CBD and drug interactions. The difference between groups for each drug interaction was made using the χ^2 Test.

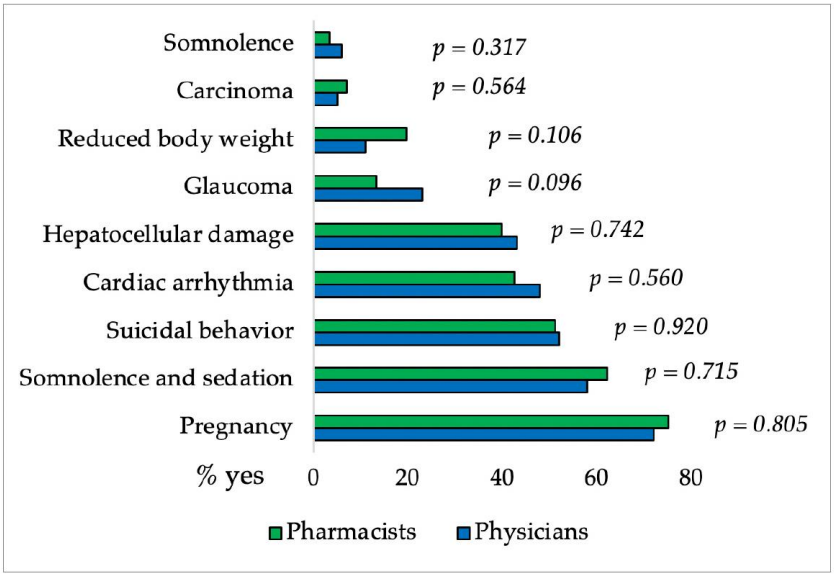

Figure 6. Percentages of pharmacists' and physicians' affirmative responses regarding different medical conditions when ingestion of CBD requires caution. The difference between groups for each medical condition was made using the χ^2 Test.

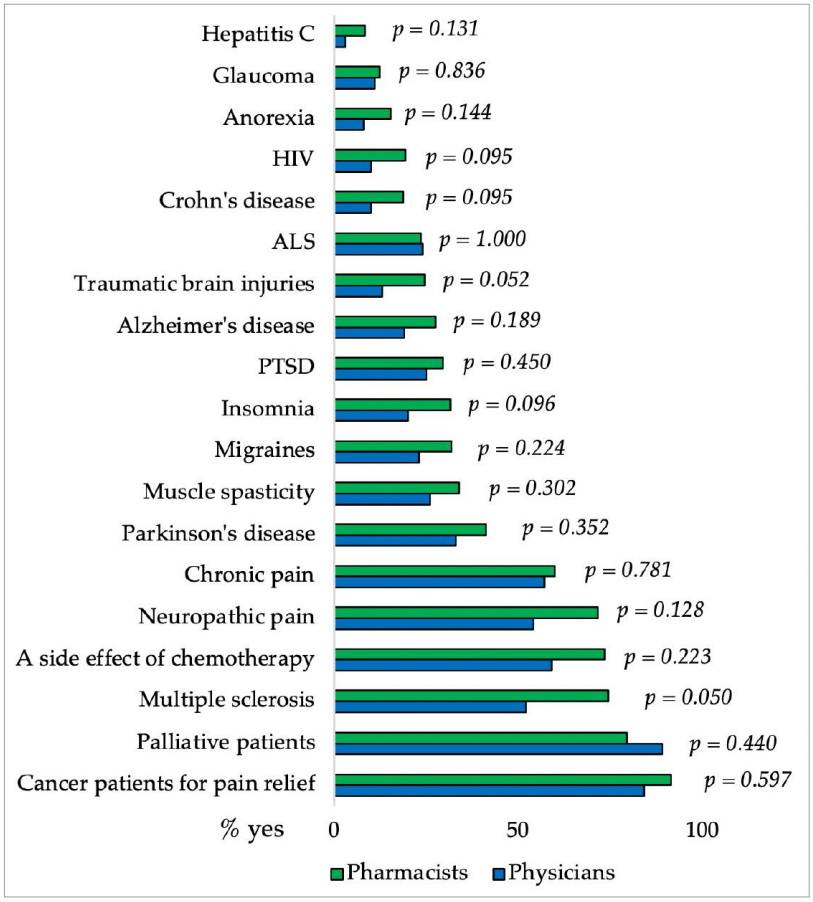

Figure 7. Percentages of pharmacists and physicians who support the use of CBD for different medical conditions. The difference between groups for each medical condition was made using the χ^2 Test.

Table 4. Differences between physicians' and pharmacists' attitudes and knowledge about prescribing/recommending the use of CBD. The difference between groups was made using the χ^2 Test.

Variable		Physicians *n* (%)	Pharmacists *n* (%)	Differences between Groups (χ^2 Test)
Question 25 I believe that recommending/prescribing CBD could reduce the use of opioids in chronic pain.	Yes	6 (6)	251 (83.4)	$p < 0.05$
	No	94 (94)	50 (16.6)	
Question 26 I believe that I have enough knowledge about the use of CBD for medical purposes and that I can recommend it to patients.	Yes	6 (6)	34 (11.3)	$p = 0.126$
	No	94 (94)	267 (88.7)	

Table 4. *Cont.*

Variable		Physicians *n* (%)	Pharmacists *n* (%)	Differences between Groups (χ^2 Test)
Question 27 Have you ever recommended/prescribed the use of CBD to patients in your practice so far?	Yes, just once	5 (5)	25 (8.3)	$p = 0.108$
	Yes, more than once	1 (1)	19 (6.3)	
	Yes, often to patients with specific diagnoses	2 (2)	7 (2.3)	
	No	92 (92)	250 (83.1)	
Question 28 I believe that healthcare insurance should cover the cost of CBD if a doctor prescribes it as therapy.	Yes	83 (83)	256 (85.0)	$p = 0.623$
	No	17 (17)	45 (15.0)	

4. Discussion

4.1. Questions Presented in Both Questionnaires

To the best of our knowledge, this is the first study that explored perceptions and knowledge regarding the therapeutic use of CBD among students in Split, Zagreb, and Osijek, as well as among pharmacists and physicians in Croatia.

This study revealed a gap in knowledge regarding CBD, among both groups since 89.3% of pharmacists and physicians, as well as 84.8% of students, believe they need more education about CBD. As in previous studies [2,12,28,31], most of our respondents also believe that curricula should include lectures on the use of CBD for medicinal purposes. In addition, we also find that 63.6% of students and 54.6% of pharmacists and physicians agreed that taking CBD as therapy is beneficial; this finding is consistent with published research in which participants revealed generally positive attitudes toward medical cannabis therapy [8,27]. Participants in the Schilling et al.'s study [27] revealed a positive attitude toward CBD products as a therapeutic alternative, as they reported positive outcomes and expressed an interest in learning more about CBD from their physicians. Approximately 40% of all our participants believe that CBD use has positive effects on physical and mental health, while about 60% of them believe that CBD treatment is generally efficacious.

However, Goodman et al. [8] observed that little is known about the potential negative effects of CBD. According to our study, only 31.5% of students and 26.2% of physicians and pharmacists considered they were aware of the risks associated with CBD use.

Almost a quarter of all respondents have no knowledge of CBD. This is unexpected among healthcare professionals, considering how widespread CBD products are on the market today. This information is also unexpected from an academic perspective, as nowadays over 500 research on potential indications of CBD have been reported on ClinicalTrials.gov, a well-known website and online database of clinical research studies and information about their results that provide information to the public, researchers and health care professionals (https://clinicaltrials.gov/, accessed on 2 December 2023). The results of our study are consistent with the results of a nationwide survey on CBD use and attitudes in France, where 30% of participants had never heard of CBD [10]. Regarding a question about reading scientific literature on CBD, there was a significant difference between groups as only 17.5% of students had read scientific papers about CBD, compared to a significantly higher percentage of physicians and pharmacists (43% and 47.8%, respectively).

Due to students' significantly greater CBD use than physicians and pharmacists, the results confirm our expectations that CBD consumption is associated with students, who are a younger age group than professionals, as was shown in previous studies [10,51]. Our results show that among all students, pharmacists (84.8%) have the most knowledge about CBD, followed by health (73.6%) and medical students (70.7%). This result is in contrast

to the same conducted in Austria where medical students had the most knowledge about CBD [28].

4.2. Specific Knowledge of CBD among Physicians and Pharmacists

Based on the study's results, physicians and pharmacists frequently link the FDA-approved indications for CBD with the ones for dronabinol and nabilone. However, the indication for epileptic seizures in LGS (Lennox-Gastaut syndrome) and Dravet syndrome was more often recognized by pharmacists (36%) than by physicians (23%), with participants mostly unaware that tuberous sclerosis is also among indications [24].

Physicians and pharmacists more often indicated pain associated with malignant diseases, chronic neuropathic pain, and chemotherapy-related nausea as a possible FDA-approved indication, although FDA-approved indications are only seizures associated with Lennox-Gastaut syndrome (LGS) or Dravet syndrome (DS) and for seizures associated with tuberous sclerosis complex.

Somnolence, reduced appetite, diarrhea, and vomiting are the most commonly reported side effects of CBD. Participants' knowledge was generally good, with the exception of tachycardia, which was selected by more than one-third of physicians and pharmacists. Previous studies have shown that CBD lowers heart rate, diastolic pressure, and MAP (mean arterial pressure) without causing tachycardia [19,52]. Participants mainly recognize carbamazepine, valproate, rifampicin, clobazam, and everolimus as drugs that have the greatest interactions with CBD. Although there was no significant difference in knowledge, pharmacists were more familiar with interactions. Medical conditions that require caution when taking CBD (somnolence and sedation, suicidal behaviour, hepatocellular damage) were generally well-known to respondents. They are mostly unaware that caution is required even with reduced body weight (only 20% of pharmacists and 11% of physicians are aware) and that heart arrhythmia does not require caution when dosing CBD (43% of pharmacists and 48% of physicians).

In contrast to physicians, pharmacists are significantly more likely to believe that recommending/prescribing CBD could reduce opioid use for chronic pain, as some research suggests [53]. In their study, McNabb et al. [53] proved that the consumption of pharmaceutical medications and other substances by veterans could potentially be reduced due to medicinal cannabis. Physicians and pharmacists agree that they do not have enough knowledge about the use of CBD for medical purposes and, therefore, cannot recommend it to their patients (94% and 88.7%, respectively).

Knowledge about CBD was found to be insufficient among medical students and healthcare professionals in the prior studies [12,28,31,32,54]. Participants agree that health insurance should cover the cost of CBD when a physician prescribes it as a therapy. Epidyolex is now available only with a restricted prescription and is entirely paid for by the patient in Croatia (more than 12 hundred euros per bottle of 100 mL) [24].

4.3. Study Limitations

This cross-sectional study has certain limitations. Despite the representative sample of participants, we were limited to a small percentage of physicians compared to the total number of physicians in Croatia, in contrast to the substantial number of participants-pharmacists. With the large final sample size, we believe that the effects were partially reduced. Another limitation was that students from other biomedical faculties in Croatia were not included in the survey.

5. Conclusions

The results of our survey indicate that current and future healthcare professionals involved in the process of patients' medication, medical, pharmacy, and health students, as well as physicians and pharmacists, believe they need additional education on the proper and safe use of CBD. Therefore, it is indispensable to improve the educational curricula so that medical professionals have more knowledge and can recommend CBD use to their

patients when needed. Nevertheless, physicians and pharmacists have shown that although they have close to enough knowledge about the indications, side effects, and interactions of CBD, they hardly prescribe and/or recommend it.

We assume that the reason for this, in addition to the uncertainty in knowledge, is the high price of the product. Therefore, it is understandable that physicians and pharmacists generally support that health insurance should cover the cost of the medicine. Further research is required to gain a more comprehensive understanding of the specific challenges and factors influencing knowledge gaps in these areas.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pharmacy12010002/s1, Attitudes and knowledge of doctors and pharmacists about the use of medical CBD.

Author Contributions: Conceptualization, D.S., A.B., A.C. and J.B., methodology, D.S., A.B., A.C., J.B., I.Ž., B.M., L.G.-O. and Z.D.; analysis A.B., A.C., N.K., S.K., B.M., A.S. and G.D.; writing—original draft preparation, A.B., D.S., J.B. and A.C.; writing—review and editing, D.S., A.B., I.Ž., L.G.-O., A.V. and Z.D.; visualization, A.B. and D.S.; supervision, D.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was approved by the Ethics Committee of the University Department of Health Studies, University of Split on 26 June 2023. (Class: 029-03/23-08/01; Reg. No.: 2181-228-103/1-47). All the procedures conformed to the Declaration of Helsinki.

Informed Consent Statement: Informed consent was obtained from all the subjects involved in the study. Participation in the anonymous questionnaire was considered consent based on notice at the beginning of the questionnaire.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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