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Antihypertensive effects of CBD are mediated by altered inflammatory response: A sub-study of the HYPER-H21-4 trial

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ABSTRACT

Despite the abundance of preclinical data, cardiovascular effects of cannabidiol (CBD) in humans remain controversial. HYPER-H21-4 trial was designed as a randomized, placebo-controlled, crossover trial aimed to explore the effects of chronic CBD dosing on ambulatory blood pressure (BP) and vascular stiffness in hypertensive individuals. In this pre-specified analysis of the trial we aimed to elucidate the anti-inflammatory effects of CBD by measuring the dynamic of serum cytokines. Specifically, interleukin 1β (IL-1β), IL-8, IL-6, IL-10, IL-18, Plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor alpha (TNF-α) and lectin-type oxidized LDL receptor 1 (LOX-1) were measured. A total of 65 patients with primary hypertension were included. After five weeks of oral CBD administration, but not of placebo, serum concentrations of IL-8, IL-10 and IL-18 were significantly lower in comparison to baseline concentrations ($p = 0.044$, $p < 0.001$ and $p < 0.001$, respectively). Serum levels of other cytokines showed no CBD-associated dynamic. Higher IL-8 and IL-10 baseline levels heralded higher diastolic BP reduction ($r = -0.478$, $p < 0.001$ and $r = -0.265$, $p = 0.034$, respectively), whereas the extent of reduction in IL-8 and IL-10 serum levels correlated with the extent of reduction in diastolic BP ($r = 0.434$, $p < 0.001$ and $r = 0.594$, $p < 0.001$, respectively). Overall, the results of the present analysis imply that the antihypertensive effects of CBD in patients with primary hypertension are accompanied by changes in serum concentrations of multiple cytokines.

1. Introduction

Cannabidiol (CBD) was first sequestered from the cannabis extract in 1940, and is one of the most abundant non-psychotropic phytocannabinoids (Pertwee, 2008 Jan; Adams et al., 1940). CBD has low infinity to the cannabinoid receptor 1 and receptor 2, but exerts a variety of direct and indirect effects (Pertwee, 2008 Jan; Thomas et al., 2007 Mar). Direct effects are mediated through receptors such as transient receptor potential vanilloid receptor 1 and peroxisome proliferator-activated receptors, whereas indirect effects are a result of modulation of endogenous metabolism and uptake of endocannabinoids, products of arachidonic acid and many others (Gonçalves et al., 2019 Feb 23; Premoli et al., 2019 May; Baron, 2018 Jul; Solowij et al., 2019; Campos et al., 2013 Jul). The eclectic nature of CBD explains its multidirectional properties, such as anti-inflammatory, antioxidant, immunomodulatory, anti-proliferative effects, which steered attention towards its therapeutic potential. Specifically, preclinical studies reported significant effects of CBD on vascular tone, vascular inflammation and endothelial function, which resulted in assessment of CBD hemodynamic effects in a clinical setting (Booz, 2011 Sep 1; Iuvone et al., 2009; Karimian Azari et al., 2020).

Arterial hypertension is a disorder with complex pathophysiological background that underlies multiple cardiovascular outcomes, the most important being ischemic heart disease and cerebrovascular disease (Forouzanfar et al., 2017 Jan 10). CBD is suggested to be a potential positive modulator of hypertension thanks to its vasodilatory properties

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(Wheal et al., 2017 May; Yan et al., 2010 Oct). Another property that may be of key importance in a potential antihypertensive activity of CBD is its impact on oxidative stress and inflammation, given the fact that these are central pathophysiological mechanisms of arterial hypertension. Despite abundant preclinical data, human studies concerning the vascular effects of CBD are scarce and mostly underpowered (Stanley et al., 2015 Sep 1; Dabiri and Kassab, 2021 Nov 12). Therefore, we conducted a randomized, placebo-controlled crossover trial which aimed to explore the effects of CBD on ambulatory blood pressure and vascular stiffness in hypertensive individuals (Dujic et al., 2023 Apr 21).

In the present study, we focused on the anti-inflammatory effects of CBD. Specifically, we explored whether the observed reduction in ambulatory blood pressure (BP) will be reflected by changes in serum concentrations of multiple cytokines, and whether baseline levels of cytokines will herald the reduction in BP.

2. Materials and methods

2.1. Study design and population

The HYPER-H21-4 trial was designed as a randomized, placebo-controlled, crossover study focused on evaluating the effects of CBD on ambulatory blood pressure and indices of arterial stiffness in patients with primary hypertension (Kumric et al., 2022 Jun 24). The complete course of study was conducted at the Department for Integrative Physiology and Department of Pathophysiology, University of Split School of Medicine, Split, Croatia from April 2022 to August 2022.

For the purpose of this pre-specified analysis we enrolled 65 patients with Grade 1 and Grade 2 primary hypertension (as defined by the contemporary guidelines of European Society of Cardiology for the treatment of hypertension), aged 40–70 years with body mass index (BMI) in the range from 18.5 to 35 kg/m^2 (Williams et al., 2018). Included patients were either newly discovered patients with hypertension receiving no treatment, or were previously treated with ACE inhibitors (either alone or in combination with calcium channel blockers and/or thiazide diuretics). Smoking of both tobacco and cannabis-based products, history of opioid use, secondary forms of hypertension, antihypertensive therapy other than stated above, active malignant disease, documented heart disease, chronic gastrointestinal and liver disease, chronic kidney disease, diabetes mellitus, gout, history of significant psychiatric disorders, history of any seizure disorder and unwillingness to sign the informed consent were considered exclusion criteria. Inclusion and exclusion criteria are further discussed in detail in the study protocol (Kumric et al., 2022 Jun 24).

The design, realization, and reporting followed the CONSORT guidelines for crossover studies (Dwan et al., 2019 Jul). The study was conducted according to the guidelines in the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of University of Split School of Medicine on 15th December 2021 (Class: 003–08/21–03/0003; Reg. No.: 2181–198–03–04–21–0091). Furthermore, the trial was registered at the ClinicalTrials.gov (NCT05346562) prior to the enrollment of the first participant. Prior to the enrolment in the trial, all included participants were informed about the procedures and the purpose of the research after which they provided individually written informed consent.

2.2. Interventions and trial visits

During the screening visit, basic anthropometric measurements were evaluated using an altitude meter (Seca, Birmingham, UK), a measuring tape (GIMA SpA, Bologna, Italy), and a bioimpedance scale Tanita DC-360 S (Tanita, Tokyo, Japan). Body mass index and waist-to-hip ratio were calculated according to standard formulas.

The details of randomization process, dosing and manufacturing of CBD formulation, and trial visits are discussed in the study protocol (Kumric et al., 2022 Jun 24). In brief, subjects were allocated in a 1:1 fashion to receive either CBD or Placebo for five weeks. After five weeks of dosing and subsequent two-week washout period, the participants who previously received CBD in the first five weeks were given placebo for five weeks and *vice versa*. CBD formulation that we used was DehydraTECH^{TM}2.0 CBD, a patented formulation by Lexaria Bioscience Corp., designed to increase the bioavailability of CBD (Dragun et al., 2023 Jun 8; Batinic et al., 2023 Apr 25; Batinic et al., 2023). The dose of CBD ranged from 225 to 300 mg in the first 2.5 weeks, with subsequent increase to 375–450 mg in the next 2.5 weeks, depending on the sex and weight of the participants.

The primary outcome of the present sub-analysis of the HYPER-H21-4 trial was change in serum concentrations of the inflammatory biomarkers during follow-up period. Specifically, interleukin 1β (IL-1β), IL-8, IL-6, IL-10, IL-18, Plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor alpha (TNF-α) and lectin-type oxidized LDL receptor 1 (LOX-1) were measured at four time points: at the start and at the end of CBD dosing period, and at the start and at the end of Placebo dosing period. The blood samples for biomarker analysis were obtained from the cubital vein following an overnight fast. Samples for inflammatory biomarker analysis were aliquoted and stored on −80 °C, whereas samples for baseline analysis (complete blood count, blood glucose, cholesterol…) were immediately analyzed. Inflammatory biomarker analysis was conducted using ProcartaPlex multiplex immunoassays (Thermo Fisher Scientific Inc., Waltham, Massachusetts, SAD). ProcartaPlex multiplex immunoassays make use of Luminex xMAP (multi-analyte profiling) technology to enable the simultaneous detection and quantitation of up to 80 protein targets in a single 25–50 μL sample of body fluids. Luminex technology uses differentially dyed capture beads for each target in a multiplex ELISA like assay. The beads are individually read using an xMAP instrument. The blood samples were analyzed by an experienced biochemist unaware of the participant allocation following standard protocols in the same institutional biochemical laboratory.

During each of the four visits, after obtaining blood samples, patients were instructed with Schiller BR102 plus PWA (Schiller AG, Baar, Switzerland), a continuous blood pressure monitor. The analysis was performed by two independent investigators blinded to the intervention arm, as per contemporary guidelines (a minimum of 70 % usable BP recordings), following the Casadei method (Williams et al., 2018; Winnicki et al., 1997 Apr).

2.3. Statistical analysis

SPSS statistics (version 29.0, IBM, Chicago, IL, USA) and Prism 6 for Windows® (version 9.4.1, GraphPad, La Jolla, CA, USA) were used for data analysis and graphical representation. Kolmogorov-Smirnov test was used to evaluate the normality of distribution. Mean and standard deviation, mean and standard error of mean (with reported 95 % CI), and median with interquartile range were used for qualitative data, as deemed appropriate. Qualitative data were expressed as a whole number and percentage and analyzed using chi-squared test, whilst quantitative variables were compared with Student's *t*-test or Mann-Whitney *U* test, depending on data distribution. Friedman's test with *post hoc* Conover test was used to assess the dynamic of inflammatory biomarkers during each respective period. A variable named Δ_{CBD}DBP was introduced, and it represented the difference between diastolic blood pressure at the end of CBD dosing period and diastolic blood pressure at the start of the CBD dosing period. Accordingly, variables Δ_{CBD}IL-8, Δ_{CBD}IL-10 and Δ_{CBD}IL-18 were defined in a same fashion. Correlations between the aforementioned variables were explored using Spearman's rank-order correlation analysis. The r correlation coefficient (rho) and two-tailed significance (p) values were reported in this analysis. The statistical significance was set at $p < 0.05$ for all comparisons.

3. Results

For the present analysis, 65 patients with Grade 1 or Grade 2 hypertension were included. Population was mostly consisted of male participants 65 (%), with an average age of 54.9 years, and mean arterial pressure of 104.3 ± 11.0 mmHg. Baseline characteristics of patients were outlined in Table 1.

After five weeks of CBD administration, serum concentrations of IL-8 were significantly lower in comparison to baseline concentrations (0.29 [10.85–19.05] vs. 0.26 [0.21–0.38] pg/mL, $p = 0.044$). After five weeks of Placebo dosing, such dynamic was not observed (0.29 [0.19–0.41] vs. 0.29 [0.19–0.44] pg/mL, $p = 0.824$) (Fig. 1a).

After five weeks of CBD administration, serum concentrations of IL-10 were significantly lower in comparison to baseline concentrations (0.29 [0.23–0.37] vs. 0.23 [0.19–0.26] ng/mL, $p < 0.001$). After five weeks of Placebo dosing, such dynamic was not observed (0.28 [0.20–0.33] vs. 0.26 [0.23–0.35] pg/mL, $p = 0.909$) (Fig. 1b).

After five weeks of CBD administration, serum concentrations of IL-18 were significantly lower in comparison to baseline concentrations (8.00 [5.47–11.24] vs. 5.09 [3.40–7.23] pg/mL, $p < 0.001$). After five weeks of Placebo dosing, such dynamic was not observed (7.56 [10.82–19.78] vs. 5.95 [3.80–10.61] pg/mL, $p = 0.186$) (Fig. 1c).

On the other hand, serum concentrations of PAI-1, LOX-1 and TNF-α showed no significant dynamic during either CBD or Placebo dosing period (p = 0.229, p = 0.943 and p = 0.061, respectively) (Fig. 2a, 2b and 2c). Serum concentrations of IL-1β and IL-6 significantly reduced after both CBD and Placebo dosing, but no difference was found between change observed after CBD and after Placebo period (Fig. 2d and 2e).

Serum concentrations of IL-8 at the start of CBD period moderately correlated with Δ_{CBD}DBP (r = -0.478, $p < 0.001$) (Fig. 3a), whereas serum IL-10 concentrations exhibited weak correlation with Δ_{CBD}DBP (r = -0.265, $p = 0.034$) (Fig. 3b).

Δ_{CBD}IL-8 and Δ_{CBD}IL-10 positively correlated with Δ_{CBD}DBP (r = 0.434, $p < 0.001$ and r = 0.594, $p < 0.001$, respectively) (Fig. 4). Meanwhile, no such correlation was observed for Δ_{CBD}IL-18 (r = -0.138, $p = 0.293$).

Table 1
Baseline characteristics of the study population.

Parameters	Total (n = 65)
Age, years	54.9 ± 7.3
Female sex, n (%)	28 (43.1)
Time since AH diagnosis, years	4 (2–6)
Therapy, n (%)	
ACEi	15 (48.4)
ACEi + CCB	12 (38.7)
ACEi + thiazide diuretic	4 (12.9)
No therapy	34 (52.3)
Baseline ambulatory BP	
Systolic BP, mmHg	134.4 ± 13.3
Diastolic BP, mmHg	84.1 ± 10.4
Mean arterial pressure, mmHg	104.3 ± 11.0
Body mass index, kg/m^2	28.3 ± 3.3
Total cholesterol, mmol/L	5.6 ± 1.0
LDL-C, mmol/L	3.4 ± 0.9
HDL-C, mmol/L	1.4 (1.2–1.7)
Triglycerides, mmol/L	1.3 (0.9–1.8)
Aspartate transaminase, U/L	23 (19–28)
Alanine transaminase, U/L	23 (18–31)
Gamma-glutamyl transferase, U/L	18 (13– 25)
Creatinine, μmol/L	74.5 ± 15.7
Blood glucose, mmol/L	5.1 (4.8–5.5)

Data presented as mean ± SD, n (%) or median (IQR). Abbreviations: AH: arterial hypertension; ACEi: angiotensin-converting enzyme inhibitors; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; CCB, calcium channel blockers; CV: cardiovascular; BP: blood pressure.

4. Discussion

In this pre-specified analysis of the HYPER-H21-4 trial we demonstrated that five weeks of oral CBD supplementation led to reduction in serum levels of IL-8, IL-10 and IL-18. Moreover, the extent of 24 h DBP reduction moderately correlated with the extent of serum IL-8 and IL-10 reduction, whilst higher IL-8 and IL-10 serum levels at baseline heralded larger decrease in 24 h DBP. On the other hand, no significant dynamic in serum levels of PAI-1, LOX-1 and TNF-α was observed, whereas IL-1β and IL-6 reduced similarly after CBD and Placebo dosing.

CBD binds many molecular targets in the cardiovascular system (Gonca and Darıcı, 2015 Jan; Mishima et al., 2005 May; Kossakowski et al., 2019 May). Notwithstanding, cardiovascular effects of this non-intoxicating constituent of *Cannabis sativa* L. remain elusive. Preclinical data on rats and *in vitro* models demonstrated vasodilatory effects and improvement of endothelial function, but BP in resting conditions was not affected by CBD supplementation in most rodent models (Stanley et al., 2013 Feb). For instance, Remiszevski et al. recently demonstrated that chronic administration of CBD in rats did not lead to observable reduction of BP in either primary or secondary hypertension, despite the fact that CBD exhibited effect on lipid metabolism, oxidative stress and endocannabinoid system (Remiszewski et al., 2020 Feb 14). In stressful condition however, CBD was shown to reduce both heart rate and BP, as demonstrated in a meta-analysis by Sultan et al. (Sultan et al., 2017 Feb). Human studies that investigated cardiovascular effects of CBD so far are mostly underpowered and focus on acute, rather than chronic CBD administration. Results of these studies are unequivocal, but suggest that CBD blunts BP response during cold pressor test, improves flow-mediated dilation, and reduces arterial stiffness upon repeated dosing, whereas the BP-reducing effect seems to weaken upon repeated dosing (Arout et al., 2022 Jan; Jadoon et al., 2017; Sultan et al., 2020 Jun). Our study group explored the effects of two similar patented formulations of CBD on cardiovascular system, both of which were designed to increase its bioavailability. In a small placebo-controlled crossover study conducted on healthy males (n = 12), TurboCBD^{TM} formulation diminished mean arterial pressure and increased cerebral perfusion in comparison to baseline and standard CBD formulation in the same dose (90 mg) (Patrician et al., 2019 Nov). Furthermore, prior to HYPER-H21-4 trial, we conducted two smaller studies using the DehydraTECH^{TM}2.0 CBD formulation. In a pharmacogenetic study conducted on hypertensive population (n = 24) we demonstrated that DehydraTECH^{TM}2.0 CBD reduced DBP more than standard CBD formulation after acute dosing (Batinic et al., 2023 Apr 25). In the other study, focused on the effects of DehydraTECH^{TM}2.0 CBD on ambulatory BP, vascular stiffness and heart rate variability (HRV), we showed that CBD (150mgevery8h) reduces arterial stiffness (≈ 0.7 m/s), systolic BP (≈ 5 mmHg), and mean arterial pressure (≈ 3 mmHg) when compared to the placebo formulation in patients with newly discovered hypertension (Dragun et al., 2023 Jun 8). On the other hand, no observable effects of CBD on physical activity, sleep patterns and HRV were noted. Finally, HYPER-H21-4 trial was a randomized, placebo-controlled, crossover study (n = 70) in which it was demonstrated that chronic administration of CBD reduces ambulatory BP without affecting indices of arterial stiffness (Dujic et al., 2023 Apr 21). In the subsequent analyses of the HYPER-H21-4 trial we aimed to get an insight into underlying mechanisms that might explain the CBD-mediated ambulatory BP reduction. For instance, we demonstrated that serum levels of catestatin, an inhibitor of catecholamine secretion, and urotensin-II, a potent vasoactive peptide, both decreased following five weeks of CBD oral supplementation (Kumric et al., 2023 Aug; Kumric et al., 2023 Apr).

Anti-inflammatory and antioxidant properties of CBD have been widely explored. Mechanisms that explain such properties are heterogeneous owing to many direct and indirect effects of CBD (Peyravian et al., 2020 Aug). Most direct effects of CBD are not a consequence of cannabinoid receptor (CB) binding, for which CBD can also act as a negative allosteric modulator (CB_1) and inverse agonist (CB_2). Apart

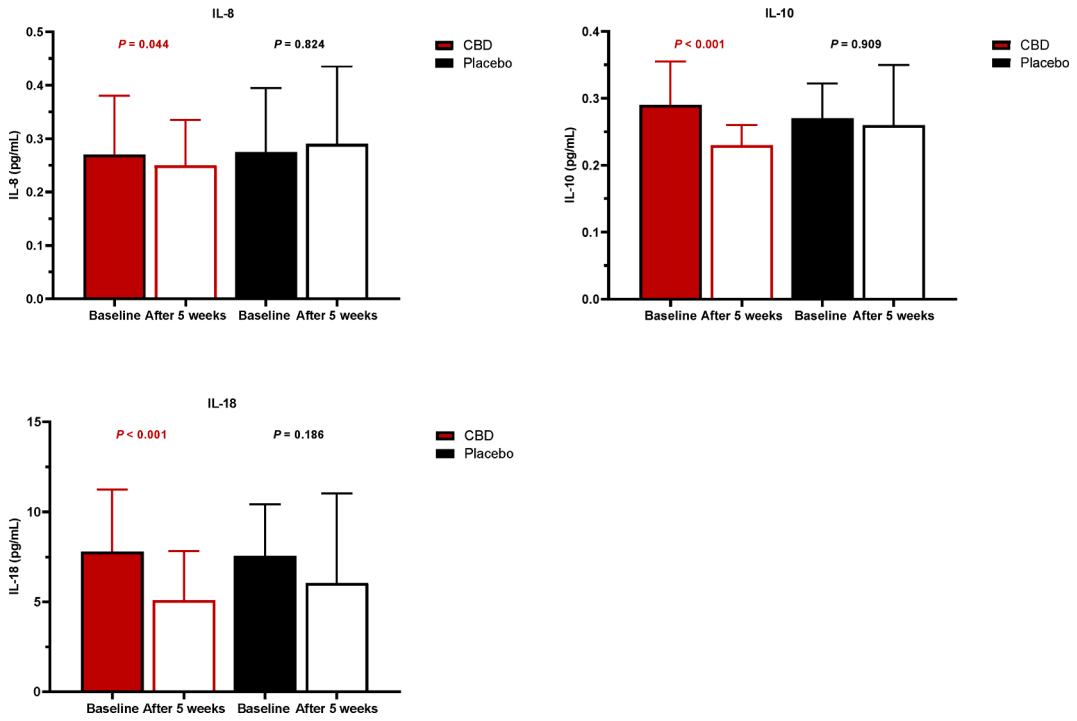

Fig. 1. Comparison between changes in serum concentrations of cytokines during CBD period and during Placebo period: a) IL-8b) IL-10c) IL-18. Abbreviations: CBD: cannabidiol, IL: interleukin. Data is presented as median and IQR. *Friedman test with *post hoc* signed-rank Wilcoxon test.

from CB_1/CB_2, direct effects of CBD are mediated through peroxisome proliferator-activated receptor γ (PPARγ), transient receptor potential ankyrin subfamily member 1 (TRPA1), vanilloid subfamily members 1–4 (TRPV1–4), 5-HT_{1A} and 5-HT_{2A}, but many others as well (Gonca and Darıcı, 2015 Jan; Mishima et al., 2005 May; Kossakowski et al., 2019 May). Indirect effects, particularly the effect on modulation of endogenous metabolism and uptake of endocannabinoids, seem to be important in regards to anti-inflammatory effects of CBD (Peng et al., 2022 Apr). For instance, Petrosino et al. showed on a model of allergic contact dermatitis that CBD dose-dependently inhibits poly-(I:C)–induced release of IL-6, IL-8, and TNF-α in a manner reversed by CB_2 and TRPV1 antagonists (Petrosino et al., 2018 Jun). Although the effect mediated by TRPV1 is unsurprising considering the aforementioned capability of CBD to stimulate it (Iannotti et al., 2014 Nov 19), as CBD exhibits only low affinity for CB_2 and as CBD was known to increase andanamide (AEA), the authors concluded that AEA could mediate the anti-inflammatory effect of CBD at this receptor. In addition, TRPV1-mediated effect may in part be attributed to endocannabinoid as well, since AEA is a full TRPV1 agonist (Di Marzo and De Petrocellis, 2012 Dec 5). PPARγ participates in the modulation of inflammation, thus inhibiting pro-inflammatory gene expression and pro-inflammatory mediators secretion such as IL-1β, IL-6 and TNF-α (Hou et al., 2012). Therefore, PPARγ agonists such as CBD (directly and by increasing AEA) might also play an anti-inflammatory role by inhibiting the NFκB-mediated transcription of downstream genes (Vallée et al., 2017 Oct 1). Importantly, pro-inflammatory cytokines IL-1β, IL-6 and TNF-α were shown to upregulate the expression of CB_1 and CB_2, which suggests that manipulation of CB receptors might have therapeutic value in inflammatory states (Jean-Gilles et al., 2015 May). A recent systemic review of *in vivo* studies showed that CBD alone or in combination with THC exerts an anti-inflammatory effect *in vivo*, whereas THC alone does not lead to reduction in pro-inflammatory or increase in anti-inflammatory cytokines (Henshaw et al., 2021 Jun).

The dynamic of cytokines in the present study is somewhat challenging to interpret. The reduction of IL-8 and IL-18 are mostly in line with the available data. IL-8 and IL-18 are both pro-inflammatory cytokines implicated in the pathogenesis of arterial hypertension and hypertension-induced vascular disease (Apostolakis et al., 2009 Dec 1; Rabkin, 2009 Mar). Studies suggest that IL-8 is implicated in the establishment and preservation of the inflammatory micro-environment of the insulted vascular wall, and that IL-18 can alter endothelial function and induce vascular smooth muscle cell migration and/or proliferation, thus causing the vascular changes that occur in arterial hypertension (Henshaw et al., 2021 Jun). Early reports indicated that TNF-α-induced IL-8 release is inhibited by cannabinoids through activation of cannabinoid CB_2 receptor, and subsequent studies demonstrated that CBD also leads to reduction of IL-8, most probably as a combination of direct and AEA-mediated effects on CB_2 (Petrosino et al., 2018 Jun; Yndart Arias et al., 2023 May 5; Ihenetu et al., 2003 Jan 1). Concomitant reduction of IL-8 with DBP, and the fact that higher IL-8 baseline levels heralded higher BP drop neither confirm nor denies that suppression of inflammation underlies BP reduction. It is likely that patients who had significant hypotensive response have more pronounced IL-8 expression, and thus, bigger reduction in IL-8 serum levels upon CBD supplementation. On the other hand, similar effects of CBD on IL-10 do not correlate well with the available data, as most preclinical studies showed that CBD stimulates rather than inhibits IL-10 production (Al-Ghezi et al., 2019 Nov; Borrelli et al., 2009 Nov; Borrelli et al., 2013 May 1; Sonego et al., 2018 Nov). It is worth noting that two *in vivo*, and one *in vitro* study reported IL-10 reduction upon CBD dosing (Britch et al., 2020 Jun; Vuolo et al., 2015; Turner et al., 2021 Aug). Although the relationship between CBD supplementation and IL-18 was not previously explored, the reduction may be interpreted in light of the established CBD-induced anti-inflammatory effects. Finally, what further impedes the interpretation of our results is lack of data concerning the effect of CBD on cytokine profile in humans.

Implication of CBD in function of virtually every organ system has also raised concern about its toxic effects. Fortunately, an abundance of accumulated data suggests that unlike THC, CBD does not lead to significant adverse cardiovascular effects, such as tachycardia or even acute coronary events (Pacher et al., 2018 Mar; Subramaniam et al., 2019). Furthermore, a meta-analysis exploring the toxicity of CBD

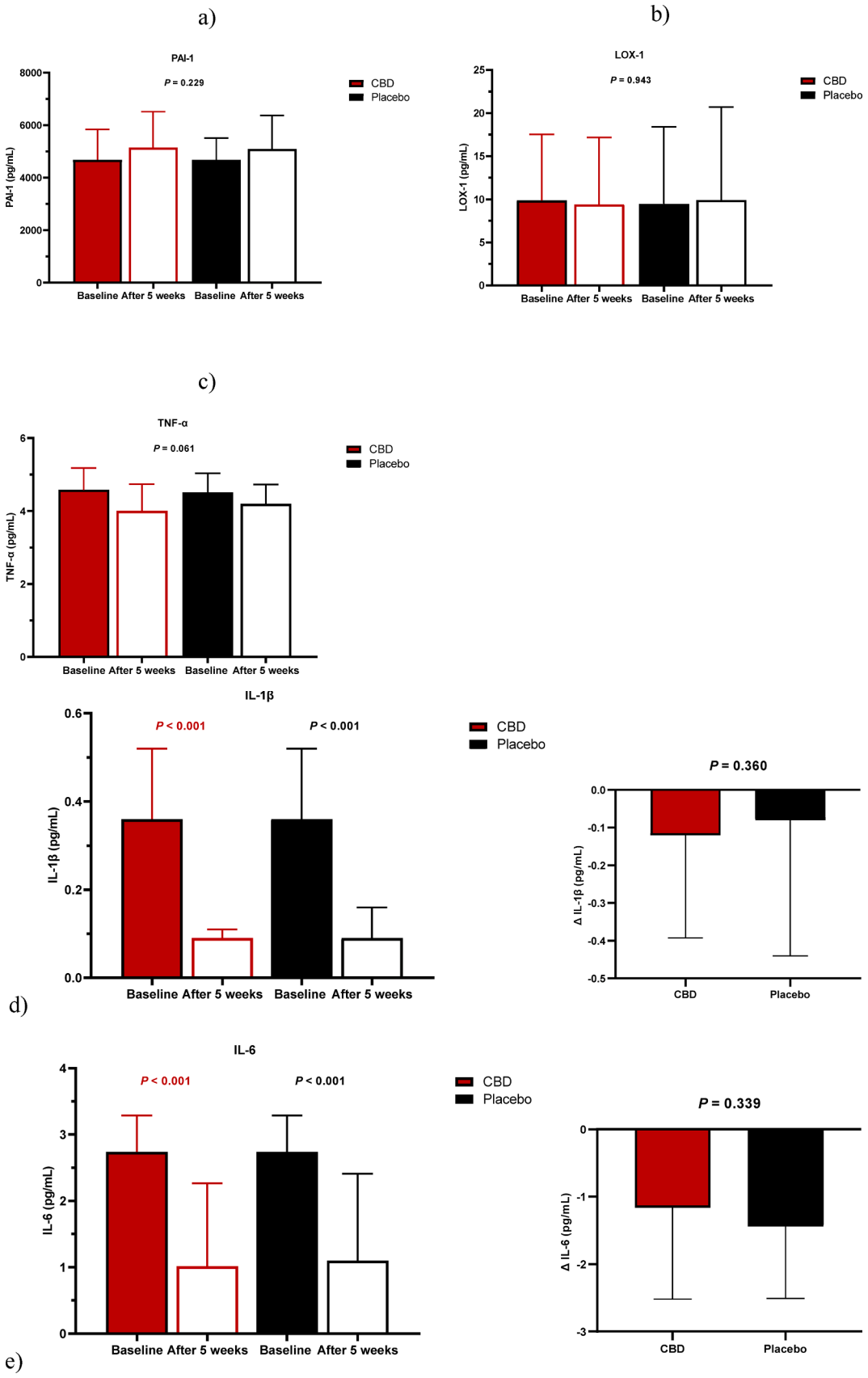

Fig. 2. Comparison between changes in serum concentrations of cytokines during CBD period and during Placebo period: a) PAI-1b) LOX-1c) TNF-α d) IL-1β e) IL-6. Abbreviations: CBD: cannabidiol, IL: interleukin, LOX-1: lectin-type oxidized LDL receptor 1, Plasminogen activator inhibitor-1, TNF-α: tumor necrosis factor alpha. Data is presented as median and IQR. *Friedman test with *post hoc* Wilcoxon signed-rank test.

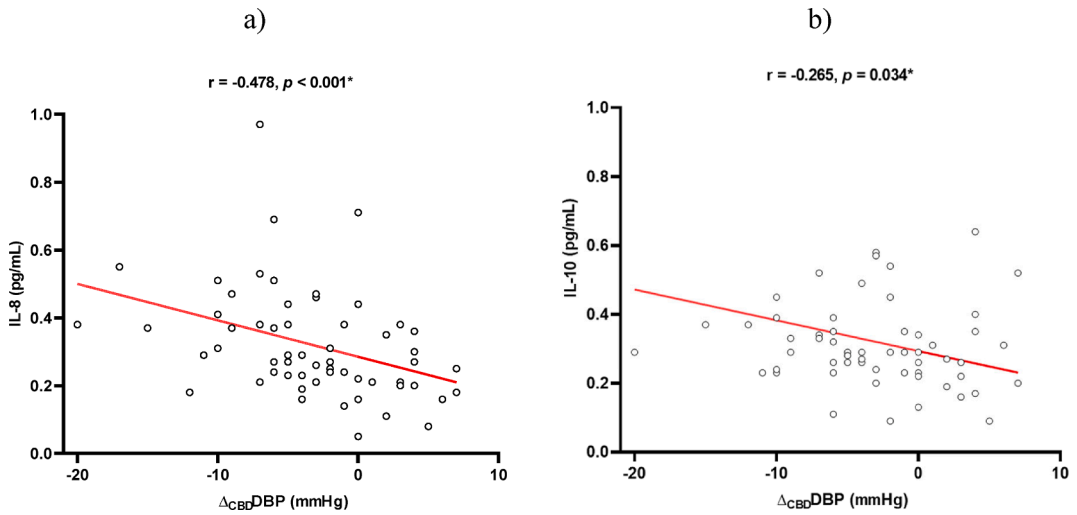

Fig. 3. Correlation between Δ_{CBD}DBP and cytokine serum concentrations (N = 65): a) IL-8b) IL-10. Abbreviations: r: correlation coefficient; CBD: cannabidiol; DBP: diastolic blood pressure; IL: interleukin. *Spearman's rank correlation coefficient.

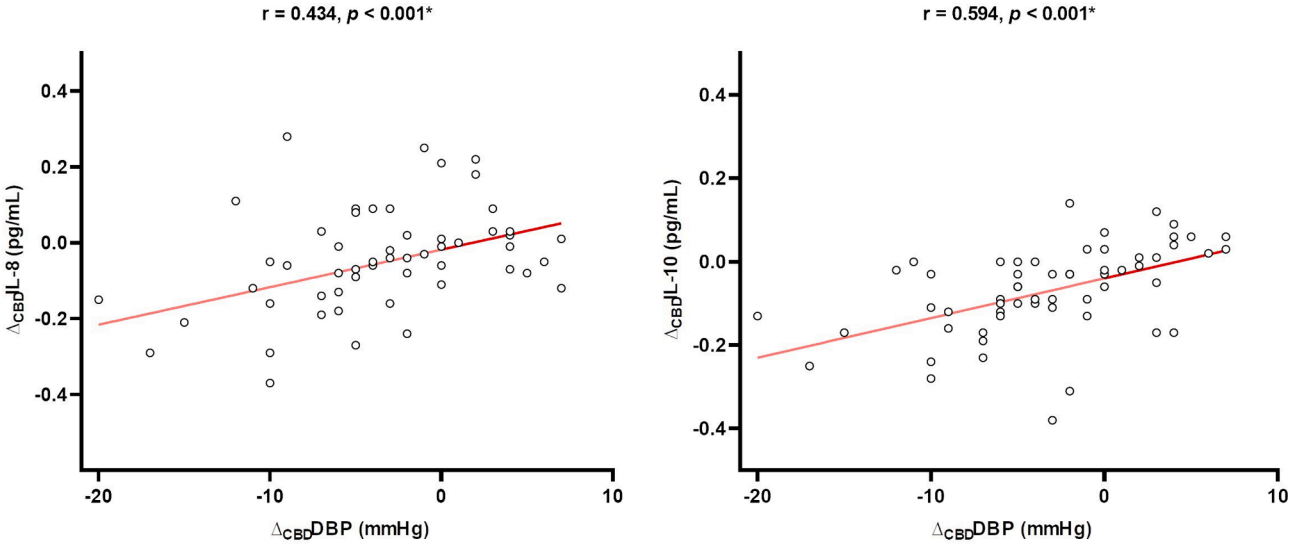

Fig. 4. Correlation between Δ_{CBD}DBP and Δ of cytokine serum concentrations (N = 65): a) Δ_{CBD}IL-8b) Δ_{CBD}IL-10. Abbreviations: r: correlation coefficient; CBD: cannabidiol; DBP: diastolic blood pressure; IL: interleukin. *Spearman's rank correlation coefficient.

demonstrated that acute CBD dosing does not lead to adverse events, whereas chronic dosing can result in mild to moderate adverse events (Larsen and Dosage, 2020 Mar). The insights from our trial support these notions as no participant reported serious or even moderate adverse event, whereas mild adverse effects, such as diarrhea, nausea, hypersomnia and headache were present in only 8 participants (Dujic et al., 2023 Apr 21). However, it has to be addressed that CBD is not completely devoid of adverse events, especially in very high doses (usually confined to preclinical studies) at which CBD can induce hormonal changes, exhibit cardio and hepatotoxic effects, and lead to decreased fertility (Huestis et al., 2019). In addition, studies concerning the adverse effects of chronic CBD administration are scarce and further data is needed to draw appropriate conclusions. Another important matter worth addressing is the drug-drug interaction, as CBD interacts with some common medications, such as bupropion, propofol and lorazepam (Zendulka et al., 2016).

Several limitations of the present study should be noted. First, the study was conducted in a single center, and did not include patients who are not of European descent. Second, whether the observed effects are applicable to other CBD formulations remains elusive, as only one CBD formulation was used in the present study. Finally, we did not include patients with severe hypertension which impedes us from translating the findings to patients with more severe forms of hypertension.

In conclusion, chronic CBD supplementation led to reduction in serum concentrations of IL-8, IL-10 and IL-18, and no CBD-associated dynamic was observed for of IL-1β, IL-6, PAI-1, LOX-1 and TNF-α. Moreover, the extent of 24 h DBP reduction moderately correlated with the extent of serum IL-8 and IL-10 reduction, whilst higher IL-8 and IL-10 serum levels at baseline heralded larger decrease in 24 h DBP. Whether these results imply that BP reduction is explained by changes in inflammatory response or merely indicate that patients with favorable BP-reducing response have more pronounced expression of the aforementioned cytokines, and thus, more significant reduction in serum levels following CBD-supplementation remain elusive.

Ethical statement

The study was conducted according to the guidelines in the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of University of Split School of Medicine on 15th December

2021 (Class: 003–08/21–03/0003; Reg. No.: 2181–198–03–04–21–0091). Furthermore, the trial was registered at the ClinicalTrials.gov (NCT05346562) prior to the enrollment of the first participant. Prior to the enrolment in the trial, all included participants were informed about the procedures and the purpose of the research after which they provided individually written informed consent.

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CRediT authorship contribution statement

Hrvoje Urlic: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Marko Kumric:** Visualization, Investigation, Formal analysis, Writing – original draft. **Goran Dujic:** Visualization, Methodology, Investigation, Formal analysis. **Josip Vrdoljak:** Visualization, Investigation, Formal analysis, Writing – original draft.. **Daniela Supe-Domic:** Visualization, Investigation, Formal analysis, Writing – original draft.. **Zeljko Dujic:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Josko Bozic:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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