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ORIGINAL ARTICLE

The effects of acute and sustained cannabidiol dosing for seven days on the haemodynamics in healthy men: A randomised controlled trial

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Background: In vivo studies show that cannabidiol (CBD) acutely reduces blood pressure (BP) in men. The aim of this study was to assess the effects of repeated CBD dosing on haemodynamics.

Methods: Twenty-six healthy males were given CBD (600 mg) or placebo orally for seven days in a randomised, placebo-controlled, double-blind, parallel study (n = 13/group). Cardiovascular parameters were assessed at rest and in response to isometric exercise after acute and repeated dosing using Finometer®, Vicorder® and Duplex ultrasound.

Results: Compared to placebo, CBD significantly reduced resting mean arterial pressure (P = .04, two-way ANOVA, mean difference (MD) −2 mmHg, 95% CI -3.6 to −0.3) after acute dosing, but not after repeated dosing. In response to stress, volunteers who had taken CBD had lower systolic BP after acute (P = .001, two-way ANOVA, MD −6 mmHg, 95% CI −10 to −1) and repeated (P = .02, two-way ANOVA, MD −5.7 mmHg, 95% CI −10 to −1) dosing. Seven days of CBD increased internal carotid artery diameter (MD +0.55 mm, P = .01). Within the CBD group, repeated dosing reduced arterial stiffness by day 7 (pulse wave velocity; MD −0.44 m/s, P = .05) and improved endothelial function (flow mediation dilatation, MD +3.5%, P = .02, n = 6 per group), compared to day 1.

Conclusion: CBD reduces BP at rest after a single dose but the effect is lost after seven days of treatment (tolerance); however, BP reduction during stress persists. The reduction in arterial stiffness and improvements in endothelial function after repeated CBD dosing are findings that warrant further investigation in populations with vascular diseases.

KEYWORDS

blood flow, blood pressure, cannabidiol, cardiovascular system, haemodynamics

Saoirse E. O'Sullivan and Timothy J. England joint last authors

The authors confirm that the Principal Investigator for this paper is Timothy J. England and that he had direct clinical responsibility for participants.

1 | INTRODUCTION

Cannabidiol (CBD), the second most abundant phytocannabinoid found in the *Cannabis sativa* plant, shows desirable effects in clinical conditions including anxiety and epilepsy.[1-3] Epidiolex® (CBD-based medicine) is already licenced and approved by the US Food and Drug Administration for children with Dravet and Lennox–Gastaut syndrome. CBD displays low affinity for **cannabinoid receptors** (cannabinoid receptor 1 and 2, CB_1 and CB_2) and activity at noncannabinoid receptor sites, including **transient receptor potential vanilloid receptors** (TRPs), **peroxisome proliferator-activated receptors** (PPARs), **G protein-coupled receptor 55** (GPR55) and **5-hydroxytryptamine** (5HT).[4,5]

Preclinical studies have shown beneficial effects of CBD on the vasculature.[5] CBD reduces cerebral vascular inflammation and associated dilatation induced by lipopolysaccharide in mice,[6] enhances blood–brain barrier permeability in in vitro models of stroke[7] and reduces infarct size in animal models of stroke.[8] These effects are mediated at least in part by peroxisome proliferator-activated receptor γ (PPARγ) and serotonin receptor, 5-hydroxytryptamine 1A ($5HT_{1A}$).[5] CBD also decreases myocardial infarct size in a rat model of ischaemia/reperfusion injury,[9] attenuates myocardial dysfunction and inflammation in animal models of diabetes through independent-cannabinoid receptor mechanisms,[10] and improves vasorelaxation in the femoral arteries of Zucker diabetic fatty rats via enhanced production of vasodilator COX-1/2-derived products acting at EP4 receptors.[11,12] Together, these studies suggest a potential benefit of CBD in cardiovascular disorders.

CBD also alters blood pressure (BP), heart rate (HR) and blood flow in animals; CBD increases heart rate and mean blood pressure in anaesthetised dogs,[13,14] causes bradycardia in conscious monkeys,[15] reduces blood pressure in rats with cardiac ischaemia[16] and increases cerebral blood flow in murine models of stroke.[17] Our recent systematic review and meta-analysis of the in vivo haemodynamic effects of CBD showed that acute and chronic dosing of CBD had no effect on haemodynamics under nonstress conditions, reduced the increase in BP and HR in response to stress, and increased cerebral blood flow in murine models of stroke.[18] This study also highlighted the limited number of studies investigating the haemodynamic and regional blood flow impact of CBD administration in humans. To help address this, we recently showed that a single dose of CBD (600 mg) causes a reduction in blood pressure at rest and in response to stress in healthy males.[19] The aim of this study was to establish if we could replicate the findings and examine whether the response was affected by repeated dosing with CBD.

What is already known about this subject

- Preclinical studies have shown that CBD causes vasorelaxation of isolated arteries and reduces vascular inflammation.
- In vivo studies show that CBD acutely reduces blood pressure in men.

What this study adds

- This study assessed the effects of repeated CBD dosing on haemodynamics, tolerance and other vascular endpoints in healthy males.
- Cannabidiol reduces blood pressure at rest after a single dose, in response to stress after a single and repeated doses, and potentially improves endothelial function and arterial stiffness.
- Further research is needed to investigate the cardiovascular effects of CBD in populations with cardiovascular disease.

2 | METHODS

2.1 | Study design

We performed a randomised, placebo controlled, double-blind, parallel group trial with each participant received an oral dose of either placebo (control) or CBD (600 mg per day) for seven consecutive days. The dose of CBD was chosen based on our recent study.[19] The trial was conducted in accordance with the Declaration of Helsinki and the International Conference of Harmonisation of Good Clinical Practice (ICH-GCP), was sponsored by the University of Nottingham, UK, and approved by the University of Nottingham's Faculty of Medicine & Healthy Sciences Research Ethics Committee (Reference No: E1411201). Written informed consent was taken from all participants, who were recruited between September 2017 and July 2018.

All measures, visits and analyses were performed blinded to treatment allocation. Randomisation was performed using computerised random number generation. TJE assigned participants and SRS carried out all study visits.

2.2 | Intervention

The placebo and CBD were provided by Phivida Neutrafuels (Phivida Organics, Wilmington DE 19808, United States) (Lot number 0717PV 0101).

2.3 | Primary outcome and sample size

In our previous study,[19] CBD reduced resting systolic blood pressure (SBP) by 6 mmHg in nine healthy male volunteers. The main aim of the study was to determine if the changes in BP, seen after a single dose, persisted after repeated dosing. We aimed to recruit 13 participants per group, which provided 80% power to detect a 5 mmHg

difference (a clinically meaningful reduction) in SBP (primary outcome) after 1 week between groups (assuming a standard deviation of 4.5 mmHg and alpha 0.05). All other vascular endpoints assessed in this study are considered as secondary outcomes.

2.4 | Participants

For this study, healthy men with no underlying medical conditions, not on regular medications and not exposed to cannabis within the last month were included. Women were excluded due to the potential gender differences in response to CBD.[20,21]

2.5 | Cardiovascular parameters

Haemodynamic parameters were assessed noninvasively after days 1 and 7 of receiving the drug. BP and other parameters, including HR, stroke volume (SV), cardiac output (CO), ejection time (ET) and total peripheral resistance (TPR), were measured at rest and during isometric exercise using a Finometer® (SMART Medical, Gloucestershire, UK). Aortic blood pressure was measured from the brachial site by pulse wave analysis (PWA).

2.6 | Arterial stiffness

Arterial stiffness was measured through pulse wave velocity (PWV) between the carotid and femoral anatomical sites using a Vicorder® (SMART Medical, Gloucestershire, UK).

2.7 | Blood flow and endothelial function

Duplex ultrasound and Cardiovascular suite Quipu (SMART Medical,Gloucestershire, UK) software were used to measure blood vessel diameter (D) and blood flow (BF) velocities of the common carotid artery (CCA), internal carotid artery (ICA) and brachial artery (BA); and endothelial function through flow mediation dilatation (FMD) of the brachial artery was assessed using a linear probe (L15–4 MHz) of high resolution ultrasound (Terason 3200 T, SMART Medical, Gloucestershire, UK).[22-24] For the CCA, the Doppler sample volume was placed 1–2 cm proximal to the carotid bifurcation. The ICA Doppler sample was placed 1–2 cm distal to the bifurcation or at the most distal segment where the artery is uniform.[25] Doppler information for the brachial artery was measured 1–2 cm proximal to the angle in which the brachial artery dives in to cubital fossa. The diameters of the CCA, ICA and BA were measured at the same location in which the Doppler information was measured using automated edge-detection software (Cardiovascular suite Quipu). The BF volume within the ICA, CCA, and BA was calculated using following equation: BF volume (L/min) = ((peak systolic velocity/2) × area × 60)/1000 to compare relative flow volumes among the participants in the present study. The area (A) was determined from the artery diameter (D) using the equation $A = D^2 \times 0.78$.[26,27] The left middle cerebral artery (MCA) was assessed by measuring the blood flow velocities using a low frequency transcranial Doppler (TCD) probe (2 MHz) of the Sonora Transcranial Doppler System (Viasys Healthcare, Pennsylvania, United States).

2.8 | 24-hour ambulatory blood pressure monitoring

24-hour ambulatory blood pressure monitoring (ABPM; TM-2430) was used to provide continuous blood pressure measurements away from the clinical assessment room. The device was set to take measurements every 30 minutes through the day (9 am–11 pm) and every hour through the night (11 pm–8 am).[28] Participants were given the option to wear the ABPM device on day 6 and were asked to keep their arm still at the level of their heart when the device started to take a reading.[29]

2.9 | Study assessment

Each participant attended a clinical assessment room at the Division of Medical Sciences and Graduate Entry Medicine & Health, University of Nottingham in the Royal Derby Hospital three times plus an additional optional visit for the ABPM.

Vascular function can be affected by medications, food supplements, caffeine, smoking and temperature.[30,31] Participants were asked to attend having fasted overnight, with no vitamin supplementations for 72 hours and avoiding exercise, caffeine, alcohol and cigarette smoking for the previous 24 hours.[32]

During the initial visit, subjects were screened for eligibility and provided written informed consent. On day 1 of receiving the drug or placebo, height and weight were measured and participants were asked to lie in supine position with their head slightly elevated on a bed for 15 minutes. The Finometer® was then attached to the participant's left index finger and cardiovascular parameters were recorded continuously for 3 hours at rest. Baseline readings were recorded over 15 minutes before the participants took either placebo or CBD (600 mg). Two hours later, the effects of the drug/placebo on blood flow, endothelial function, aortic blood pressure and arterial stiffness were measured. During these 2 hours, participants were allowed to watch television, use a computer or read. Two hours after drug administration, participants were asked to perform isometric handgrip (IHG) stress exercise for 3 minutes[33] by using a dynamometer with their right hand while the Finometer® was continuously recording. The assessment on both study visits (days 1 and 7) took place in the same assessment room at a temperature ~22 °C. On day 7 (the last day of receiving the drug), the same protocol was followed.

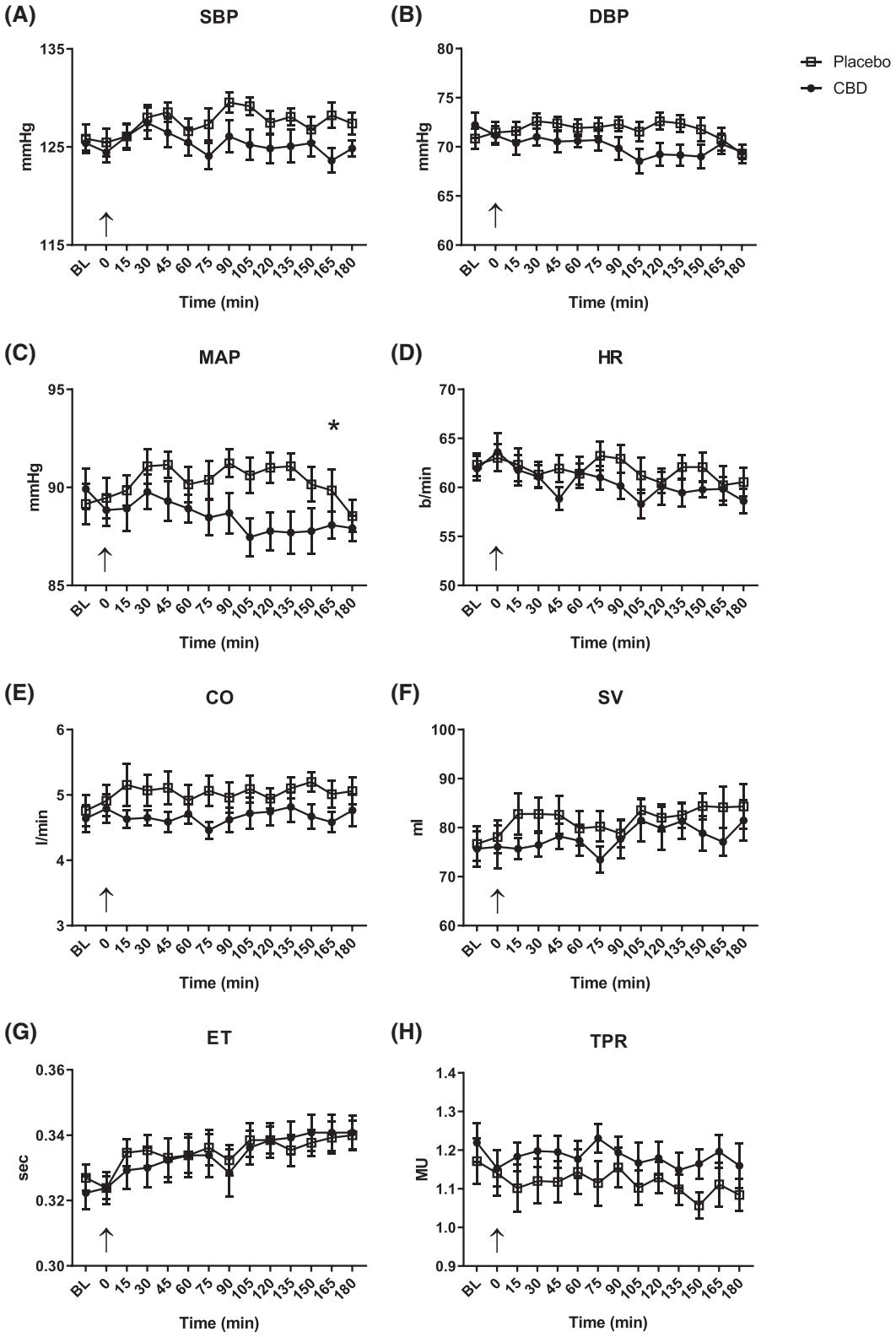

FIGURE 1 Cardiovascular parameters at rest after acute dosing (day 1). Systolic blood pressure (SBP) (A), diastolic blood pressure (DBP) (B), mean arterial pressure (MAP) (C), heart rate (HR) (D), cardiac output (CO) (E), stroke volume (SV) (F), ejection time (ET) (G) and total peripheral resistance (TPR) (H) were measured continuously for 3 hours after drug ingestion (↑) using a Finometer (n = 13). Closed circles indicate the CBD group and opened squares the placebo group. Analysis by repeated measures two-way ANOVA (*$P \leq .05$); data are presented as mean ± SEM

CBD/placebo tolerance was assisted with a subject diary including any side effects they may have experienced.

2.10 | Statistical analysis

All statistical analysis was performed using IBM SPSS Statistics (Version 21.0, IBM Corp., Armonk, NY, USA) and PRISM 7 (GraphPad, Software, La Jolla, CA, USA). The level of significance was set at 0.05. Variables were tested for normality by assessing the data distribution on a histogram. Repeated measures of two-way ANOVA determined the effect of treatment on BP and other cardiovascular parameters measured with the Finometer®. Sidak's post hoc tests were used to determine changes at specific time points. For all variables, paired and unpaired *t* tests were used for comparison between related and independent samples, respectively.

2.11 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the archived in the Concise Guide t IUPHAR/BPS Guide to PHARMACOLOGY,[34] and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[35]

3 | RESULTS

Twenty-six healthy men were recruited (13 subjects in each group). Eleven of the 26 subjects were previous cannabis users (CBD, n = 8; placebo, n = 3), but were abstinent from cannabis for a period ranging from 2 months to 1 year prestudy. The age, weight, height and body mass index (BMI) of participants who received CBD were 26.3 ± 5.6 years, 80.1 ± 9.9 kg, 1.8 ± 0.1 m and 25 ± 2, respectively; and of participants who received placebo were 27 ± 6 years, 82.1 ± 9.8 kg, 1.8 ± 0.1 m and 25.7 ± 2.9 (mean ± SD).

3.1 | BP and other cardiovascular parameters

Acute dosing of CBD treatment at rest did not significantly reduce SBP (P = .08) or diastolic BP (DBP, P = .09) (Figure 1A,B), but significantly reduced mean arterial pressure (MAP) (P = .04, two-way ANOVA, Figure 1C; mean difference (MD) −2 mmHg, 95% CI −3.6 to −0.3), without affecting other cardiovascular parameters compared to placebo (Figure 1D–H). There was no effect on SBP, DBP or MAP at rest following chronic CBD dosing (Figure 2), suggesting the development of tolerance. Participants' SBP readings obtained pre-CBD/placebo on days 1 and 7 are presented in Supporting Information Table SS1 and Supporting Information Figure S1.

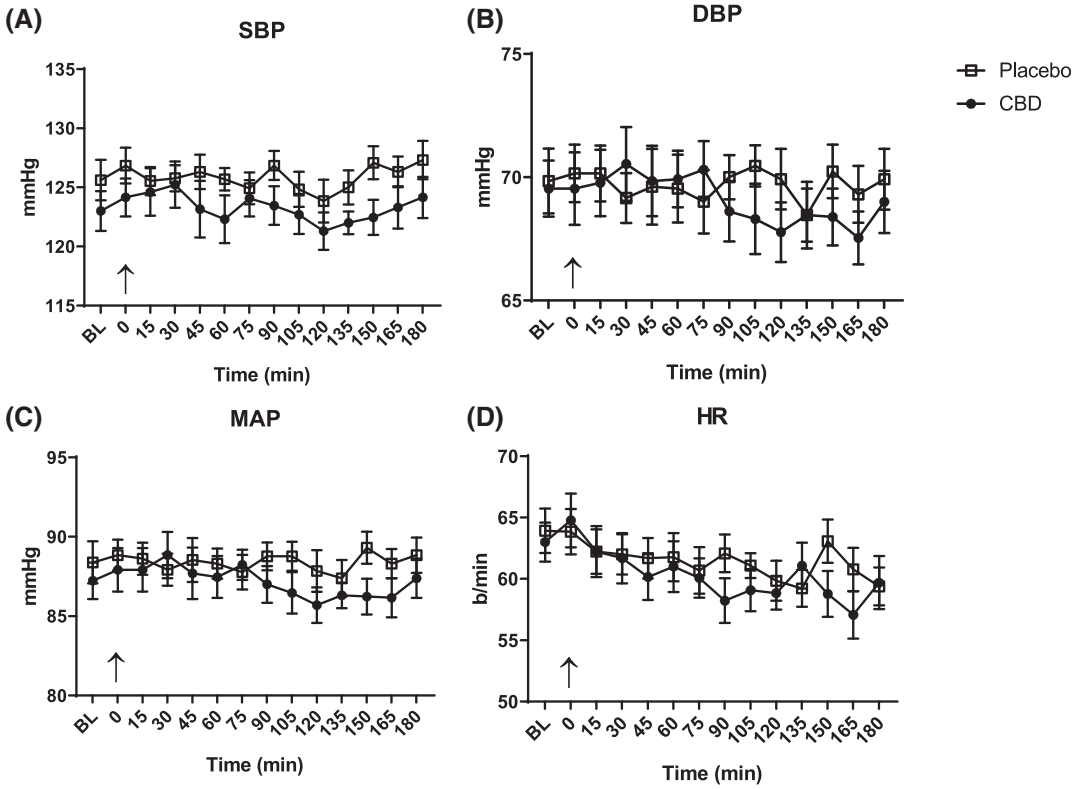

FIGURE 2 Cardiovascular parameters at rest after 7 days of CBD treatment. Systolic blood pressure (SBP) (A), diastolic blood pressure (DBP) (B), mean arterial pressure (MAP) (C) and heart rate (HR) (D) measured continuously after drug ingestion (↑) using a Finometer on day 7 (n = 13). Closed circles indicate the CBD group and opened squares the placebo group; mean ± SEM. No significant difference between the treatment groups

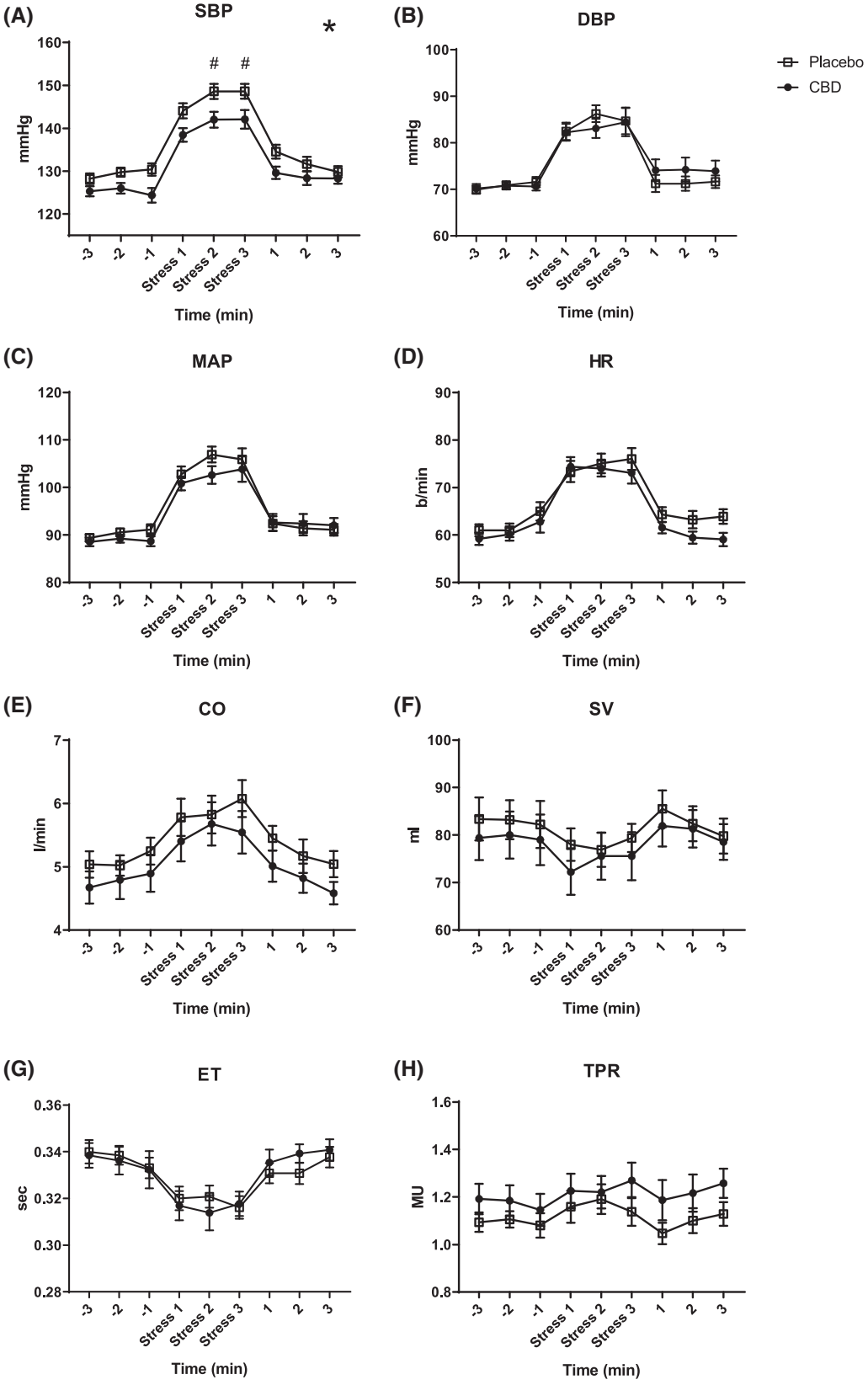

FIGURE 3 Cardiovascular parameters in response to exercise stress after acute dosing (day 1). Systolic blood pressure (SBP) (A), diastolic blood pressure (DBP) (B), mean arterial pressure (MAP) (C), heart rate (HR) (D), cardiac output (CO) (E), stroke volume (SV) (F), ejection time (ET) (G) and total peripheral resistance (TPR) (H) measured continuously using a Finometer on day 1 pre, during and post isometric exercise stress (n = 13). Closed circles indicate the CBD group and opened squares the placebo group. Analysis by repeated measures two-way ANOVA (*$P \leq .05$); mean ± SEM (#$P \leq .05$ using Sidak post hoc analysis between CBD and placebo groups)

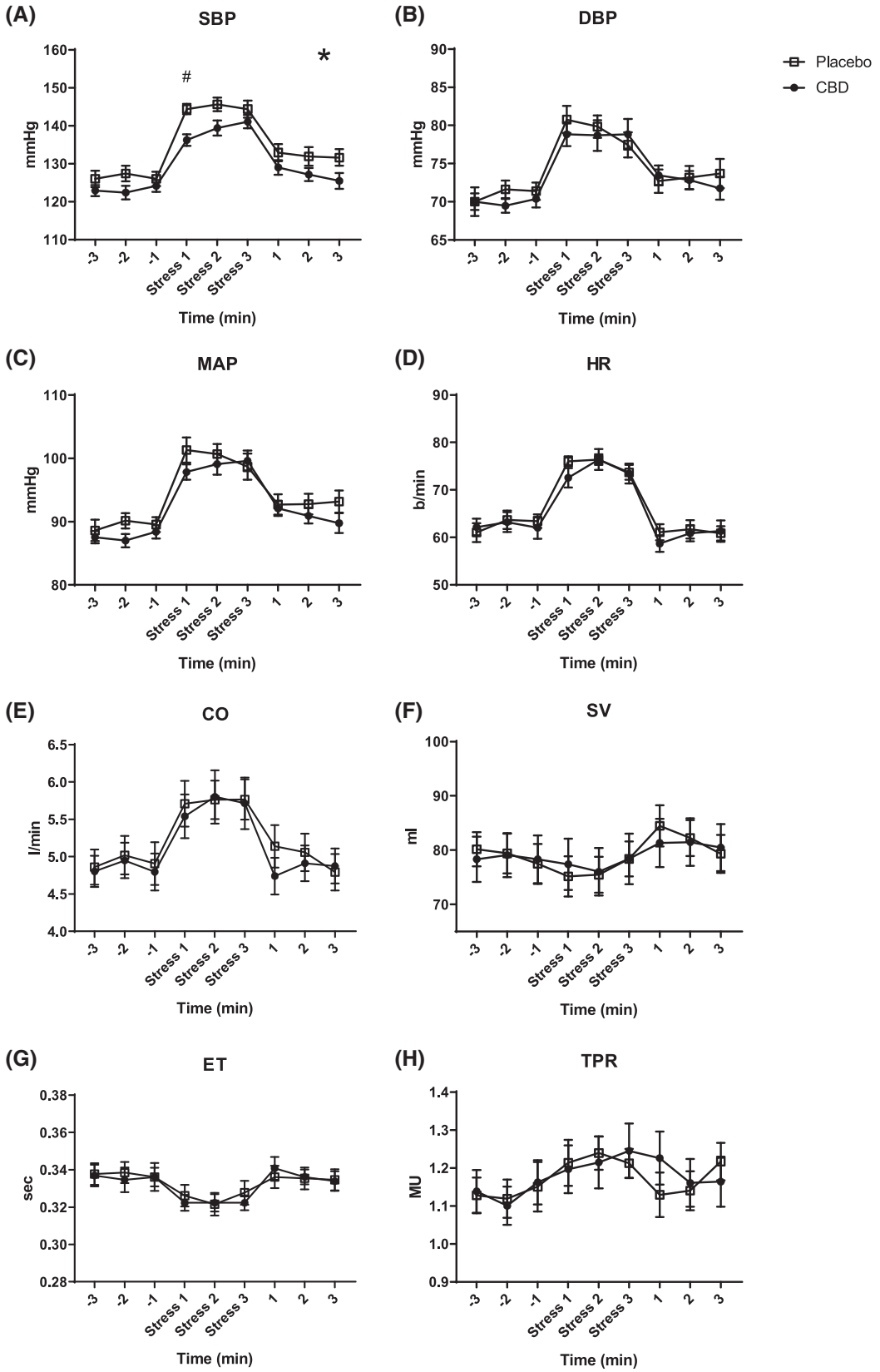

FIGURE 4 Cardiovascular parameters in response to exercise stress after 7 days of CBD treatment. Systolic blood pressure (SBP) (A), diastolic blood pressure (DBP) (B), mean arterial pressure (MAP) (C), heart rate (HR) (D), cardiac output (CO) (E), stroke volume (SV) (F), ejection time (ET) (G) and total peripheral resistance (TPR) (H) measured continuously using a Finometer on day 7 pre, during and post isometric exercise stress (n = 13). Closed circles indicate the CBD group and opened squares the placebo group. Analysis by repeated measures two-way ANOVA (*$P \leq .05$); mean ± SEM (#$P \leq .05$ using Sidak post hoc analysis between CBD and placebo groups)

Isometric exercise caused a significant increase in BP in the two treatment groups (Figures 3 and 4). SBP was significantly lower in the CBD-treated group compared to the placebo group after single (P = .001, two-way ANOVA, Figure 3A; MD −6 mmHg, 95% CI −10 to −1) or repeated CBD dosing (P = .02, two-way ANOVA, Figure 4A; MD −5.7 mmHg, 95% CI −10 to −1). Sidak post hoc analysis showed that acute CBD dosing significantly lowered SBP at minutes 2 (P = .02, MD −6.6 mmHg, 95% CI −12.7 to −0.4, Figure 3A) and 3 (P = .03,MD −6.5 mmHg, 95% CI −12.7 to −0.3, Figure 3A) during stress. After 7 days of CBD dosing, the mean difference in SBP compared to placebo was −8 mmHg (95% CI −15.7 to −0.57, P = .02, Figure 4A) at the first minute of stress.

3.2 | 24-hour ambulatory blood pressure monitoring

After 6 days of treatment with CBD, there was no difference in the 24-ABPM between the two treatment groups (Figure 5 A–C).

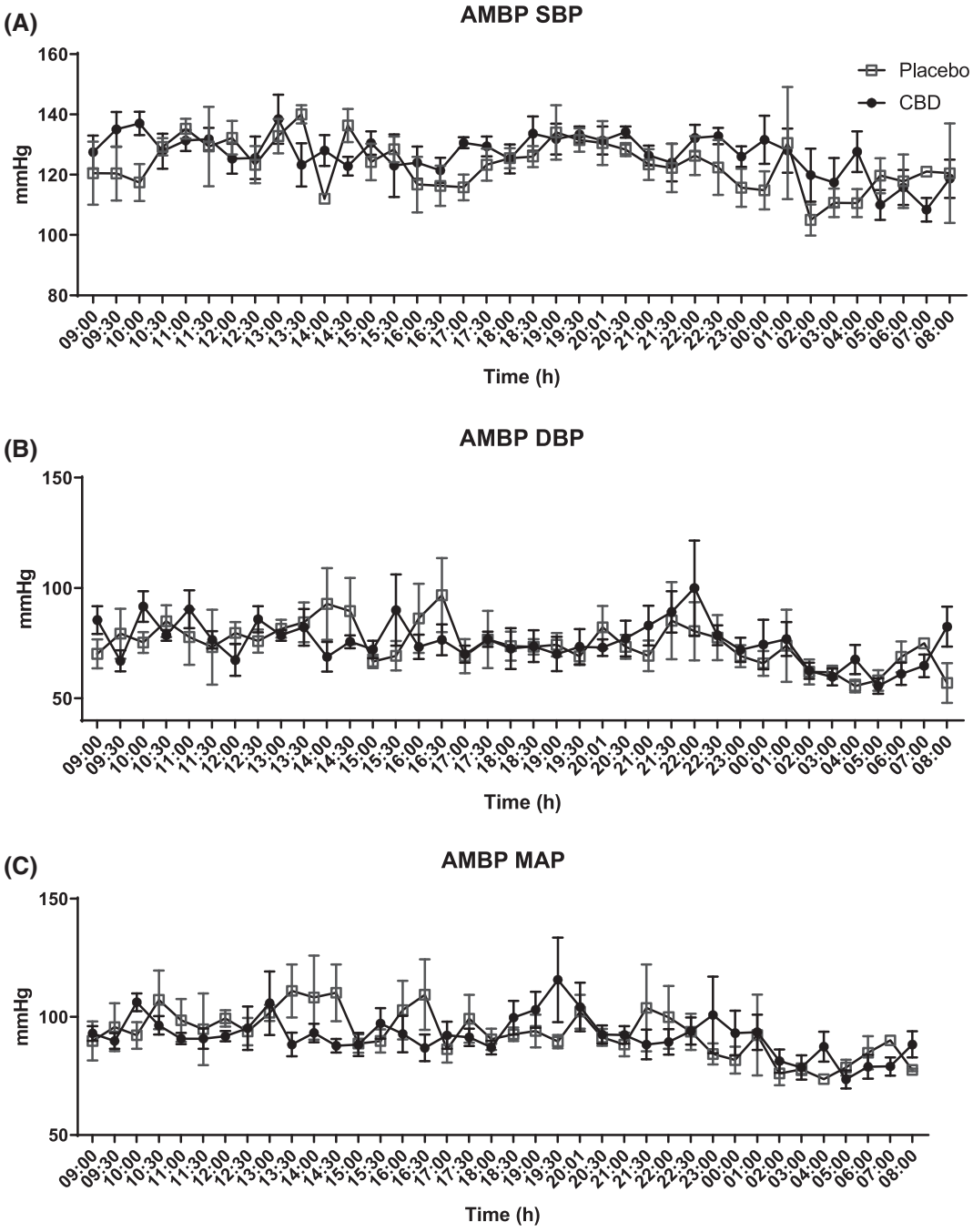

FIGURE 5 24-hour ambulatory blood pressure monitoring (ABPM) at rest after 6 days of CBD treatment. Systolic blood pressure (SBP) (A), diastolic blood pressure (DBP) (B) and mean arterial pressure (MAP) (C) were measured using an ambulatory blood pressure device for 24 hours on day 6 of receiving the treatment (CBD group n = 10 and placebo group n = 7). Closed circles indicate the CBD group and opened squares the placebo group; mean ± SEM. No significant difference between the treatment groups

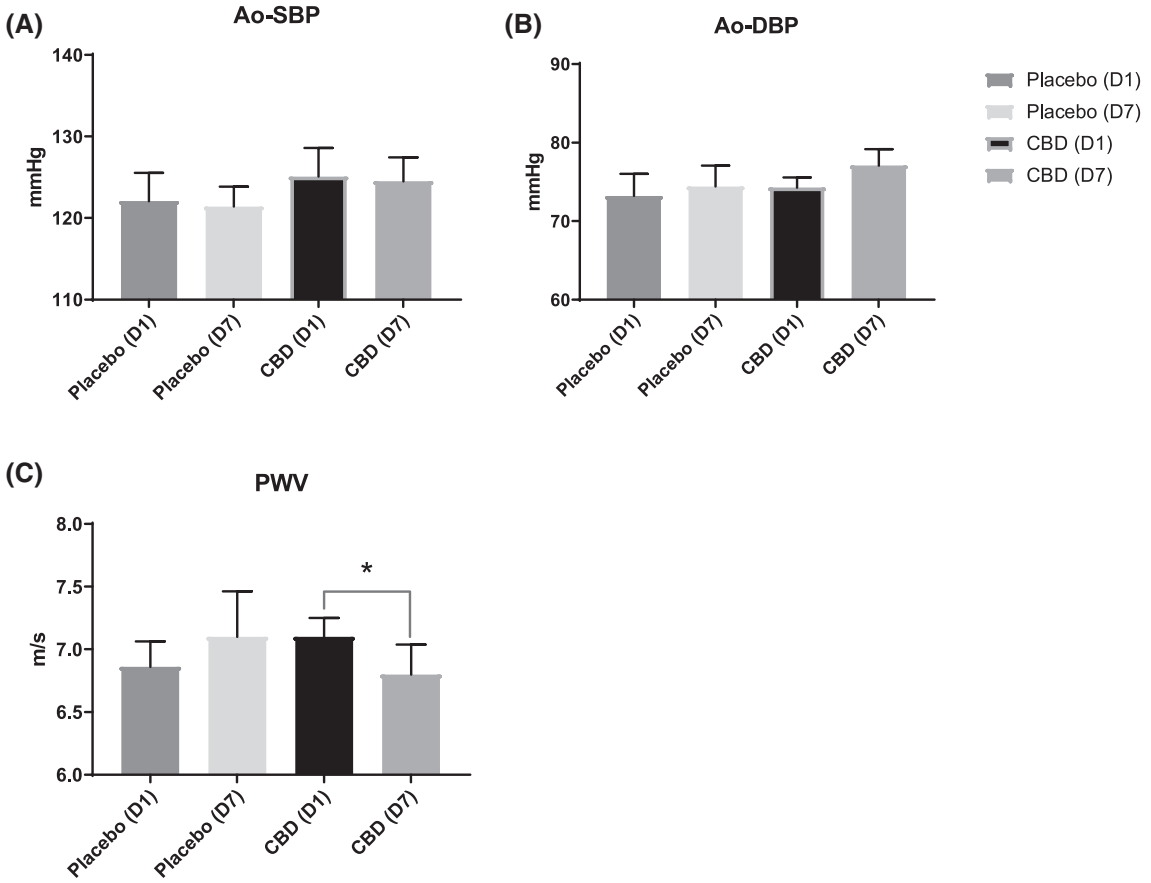

FIGURE 6 Aortic blood pressure and arterial stiffness after the first (day 1) and seventh dose of CBD. Aortic systolic blood pressure (Ao-SBP) (A), aortic diastolic blood pressure (Ao-DBP) (B) (CBD group n = 9 and placebo group n = 8 and n = 9 on days 1 and 7, respectively) and pulse wave velocity (PWV) (D) (n = 10) measured using a Vicorder 2 hours after acute dosing on day 1 (D1) and chronic dosing on day 7 (D7); mean ± SEM (*$P \leq .05$ using a paired *t* test between related groups post drug ingestion)

3.3 | Aortic blood pressure and arterial stiffness

There was no difference in aortic BP (CBD n = 9; placebo n = 8 on day 1 and n = 9/group on day 7, Figure 6A,B) or arterial stiffness (n = 10 in each group, Figure 6C) between the two treatment groups at rest after acute or repeated dosing. The paired *t* test showed that CBD dosing for 7 days significantly reduced arterial stiffness (PWV: MD −0.44 m/s, 95% −0.01 to 0.9, $P = .05$, Figure 6C) compared to a single CBD dosing received on day 1.

3.4 | Blood flow and endothelial function

CBD dosing for 7 days increased ICA diameter (MD +0.55 mm, 95% CI 0.1 to 0.9, $P = .01$, Figure 7B) without causing a significant increase in ICA blood flow volume (BFV) ($P = .07$) compared to placebo at rest. Repeated CBD dosing also significantly increased ICA diameter (MD +0.43 mm, 95% CI −0.8 to 0, $P = .05$, Figure 7B) and significantly increased FMD (MD +3.5%, 95% CI −6.2 to −0.6, $P = .02$, n = 6 in each group, Figure 8C) compared to the same group treated with CBD after a single dose on day 1. No differences were seen in MCA velocity or CCA and BA parameters between the two groups post treatment (see Supporting Information Figure S2A–G).

3.5 | Side effects

Side effects reported post CBD administration included lack of appetite on day 4 (n = 1), headache on day 3 (n = 1), insomnia on days 2 and 3 (n = 1), hyperactivity on days 2 and 3 (n = 1), and dysuria on days 5 and 6 (n = 1). Following placebo administration, subjects reported migraine on day 4 (n = 1) and lightheadedness on day 6 (n = 1).

4 | DISCUSSION

The aim of this study was to investigate the effects of acute and repeated dosing of CBD on the cardiovascular system in healthy males. Compared to a placebo, CBD significantly reduced MAP at rest but not after repeated dosing. In response to isometric exercise, there

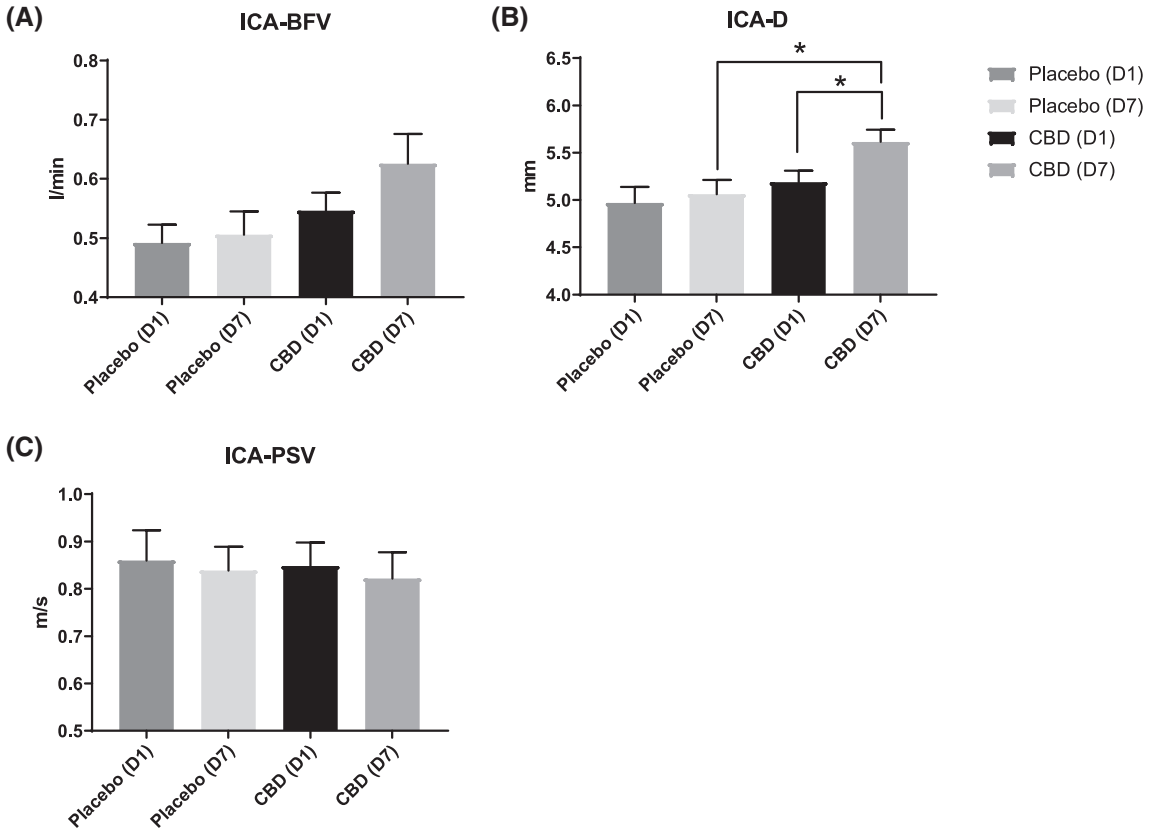

FIGURE 7 Internal carotid artery response after the first (day 1) and seventh dose of CBD. Internal carotid artery blood flow volume (ICA-BFV) (A), diameter (ICA-D) (B) and peak systolic velocity (ICA-PSV) (C) (n = 13) measured using ultrasound 2 hours after acute dosing on day 1 (D1) and chronic dosing on day 7 (D7); mean ± SEM (*$P \leq .05$ using a paired *t* test between related groups and an unpaired *t* test between independent groups post drug ingestion)

was a lower SBP response after both acute and repeated dosing. We also found that repeated CBD dosing increased ICA diameter and may improve endothelial function and arterial stiffness. These findings suggest that CBD might benefit vascular function, particularly under stress, and further work is required to investigate the effects of CBD in humans under pathological conditions.

We previously showed that a single dose of CBD decreases resting BP and increases HR in cannabis naïve men.[19] The effect on BP was reproduced in the present study, but this effect was lost after repeated dosing, suggesting that tolerance develops. However, the increase in HR post-CBD was not reproduced here. THC, the major component of cannabis, can induce tachycardia in humans through CB_1 activation.[36] It is possible that CBD could induce tachycardia through a similar mechanism, although CBD stimulates several receptors and is a weak CB_1 agonist. Explaining the difference in findings on the effect of CBD on HR between our two studies is challenging as the protocols were so similar in terms of dose, population (young males) and methods of measurement. However, variability in 'activity' during the resting phase could be contributory (eg reading versus watching the TV). Furthermore, unmeasured variables such as ethnicity and genetic factors (polymorphisms) may account for the difference and deserves attention in future studies. Of note, post hoc analyses did not show any differences in HR between previous cannabis users and those who were cannabis naïve.

The reduction in BP may be secondary to CBD anti-anxiolytic properties[2,37] preventing the increase in BP which was seen in the placebo group on the first day of receiving the treatment (see Supporting Information Figure S3). A number of previous human studies, in which their primary aim was not to investigate the effect of CBD on BP, reported that there were no changes on BP or HR post CBD administration. It is important, however, to note that these studies assessed BP and HR by taking a single measurement manually at different time points post treatment, whereas cardiovascular parameters were measured continuously for 3 hours post treatment in the present study.

A CBD dose of 600 mg was chosen based on our previous study.[19] A wide range of doses have been used in other conditions, highlighted in our recent systematic review,[38] including epilepsy, schizophrenia, dystonia, social anxiety and post-traumatic stress disorders. The average CBD dose that reported positive effects in the assessed conditions ranged between 14 and 23 mg/kg/d. A CBD dose of 14 mg/kg/d would be equivalent to a dose of 1120 mg in an 80 kg human (the mean weight of participants in the present study). Overall, there is limited information on the efficacy, pharmacokinetics and

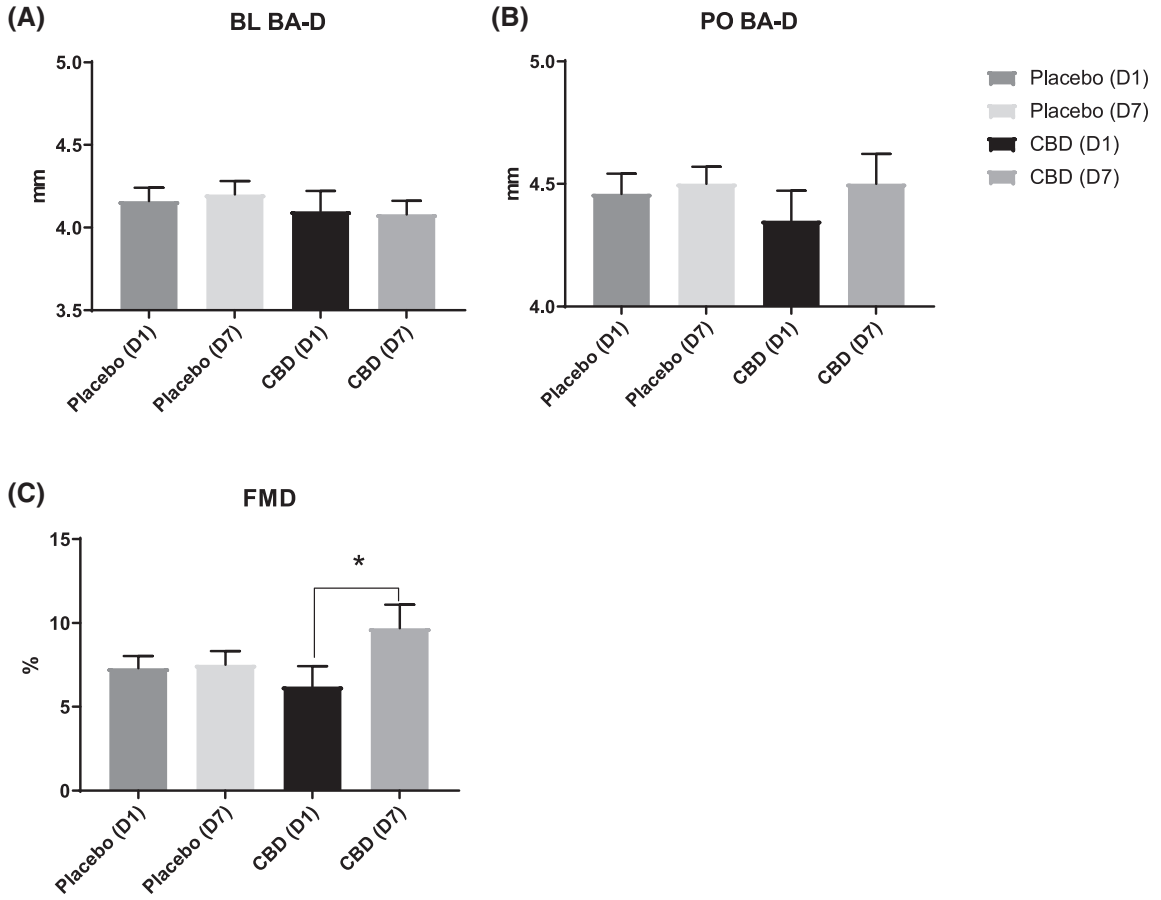

FIGURE 8 Endothelial function response after the first (day 1) and seventh dose of CBD. Brachial artery diameter (BA-D) at baseline (BL) (A) and post-occlusion (PO) (B), and flow mediation dilatation (FMD) (C) (n = 6) measured using ultrasound 2 hours after acute dosing on day 1 (D1) and chronic dosing on day 7 (D7); mean ± SEM (*$P \leq .05$ using a paired *t* test between related groups post drug ingestion)

pharmacodynamics of cannabis-derived medicines,[39] and dose-escalation trials in people with cardiovascular diseases are required.

In response to stress-induced hypertension, our findings showed that CBD reduced the increase in BP during isometric exercise after acute and repeated dosing, as we previously observed after a single dose.[19] We note that for the hand-grip experiment, the groups start to diverge prior to exercise initiation with no difference between groups at baseline −3 minutes prestress (Sidak's post hoc analyses, $P = .8$) compared to −1 minute ($P = .06$). It is feasible that CBD also suppresses the anticipation of the expected stress and therefore the groups diverge from each other prior to the exercise itself. Preclinical studies on animal models of stress showed that CBD reduces anxiety through the activation of $5HT_{1A}$ and CB_1 receptors.[40,41] CBD activation of $5HT_{1A}$ in rats also reduces the increase in BP induced by stress.[42,43] Our systematic review and meta-analysis also suggested that CBD alters BP under stressful conditions, but not under resting conditions.[18] In this study, CBD's hypotensive effect in response to stress persisted after repeated dosing, a finding that warrants further research into conditions affecting the cardiovascular system.

Our results indicate, for the first time in humans, that CBD increases ICA diameter with an associated tendency to increase ICA-BFV. This effect was only observed after repeated and not after single dosing. A related effect of CBD has been reported in preclinical studies,[44,45] in that neuroprotection induced by CBD in murine models of stroke was seen in association with an increase in cerebral blood flow, with no tolerance developing after chronic treatment for 14 days.[45,46] These effects were inhibited with the administration of $5HT_{1A}$ antagonist.[45,47] Another study in piglets with brain injury reported that $5HT_{1A}$ and CB_2 have a role in CBD induced neuroprotection.[44] Our study showed that CBD had no effect in the MCA blood flow velocities assessed using TCD; however, testing MCA velocity at a single time point in our small sample is unlikely to be sufficiently sensitive to detect subtle changes in cerebral autoregulation in a healthy brain. This was an exploratory measure since it is recognised that CBD can alter regional cerebral blood flow in other conditions, eg in social anxiety disorder.[48] Taken together, the effects we have seen that CBD has on BP acutely, under stress, on endothelial function and on arterial stiffness, in conjunction with pre-clinical stroke data showing improvements in CBF, suggests further investigation in a stroke population is warranted.

Repeated CBD administration for 7 days increased FMD and may improve endothelial function. This should be interpreted with caution, however, since (i) this difference was observed within the CBD group (day 1 *vs* day 7) rather than between CBD and placebo

groups; (ii) the change occurred in the absence of a BP change over 7 days; and (iii) we do not have any pretreatment PWV values in either group. CBD stimulates the production of nitric oxide (NO), causing endothelium-dependent vasorelaxation of isolated human arteries through CB_1 activation,[49] and a meta-analysis on the association of FMD and NO demonstrates that FMD is significantly mediated by NO.[50] Therefore, CBD may improve endothelial function through a NO-dependant mechanism via the activation of CB_1. The same mechanism might be responsible for the potential reduction in arterial stiffness seen after repeated doses of CBD; PWV is a recognised surrogate marker of cardiovascular disease with a 1 m/s in PWV decrease correlating with a reduction in the risk of cardiovascular events by 10%.[51] This signal of reduction in arterial stiffness and the improvement in endothelial function after repeated CBD dosing are markers indicating a positive effect on vascular function. However, structural arterial changes and improvements in vascular stiffness after just 7 days are unexpected and further studies should also assess later time points and biomarkers of collagen and elastin turnover.

Our study has many strengths, including a robust study design, randomisation, concealment of allocation and blinding both the volunteers and the investigator. We considered a cross-over design as we did in our initial acute dosing study (rather than parallel group) but felt a prolonged length of involvement for each participant would have limited recruitment sufficiently to impair trial accrual. We should also accept some other limitations: (i) data should be interpreted with caution due to the small sample size and (ii) CBD effects on ICA diameter, endothelial function and arterial stiffness were only seen when compared to values after a single CBD dose, not when compared to placebo. Further investigation in larger sample sizes, in women and diseased populations are required.

5 | CONCLUSION

CBD reduced MAP after acute dosing at rest, and reduced SBP after acute and repeated dosing in response to stress-induced hypertension in healthy male volunteers. Seven days of CBD increased ICA diameter appeared to improve endothelial function and arterial stiffness. These findings suggest that CBD may be a potential treatment for cardiovascular disease and further studies are warranted.

ACKNOWLEDGEMENTS

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COMPETING INTERESTS

There are no competing interests to declare.

CONTRIBUTORS

TJE and SOS conceived and designed the experiments. SRS helped with the design of the experiments, and collected and analysed the data. All authors: wrote and revised the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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

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