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

Short title: Haemodynamic Response to Cannabidiol in Men

Salahaden R Sultan[1,2] MSc, Saoirse E O`Sullivan[1†]PhD, Timothy J England[1*†] PhD FRCP

Affiliations

[1]Vascular Medicine, Division of Medical Sciences & Graduate Entry Medicine, School of Medicine, University of Nottingham, Royal Derby Hospital, Derby, UK

[2]Diagnostic Radiology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia.

*Author for Correspondence: Timothy J England, Vascular Medicine, Division of Medical Sciences & Graduate Entry Medicine, School of Medicine, University of Nottingham, Royal Derby Hospital, Uttoxeter Road Derby, DE22 3DT, UK; timothy.england@nottingham.ac.uk

† Joint last authors

Principal Investigator statement

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

Key words: cannabidiol, cardiovascular system, haemodynamics, blood pressure, blood flow.

Abstract

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: 26 healthy males were given CBD (600mg) 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=0.04, 2-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=0.001, 2-way ANOVA, MD -6 mmHg, 95% CI -10 to -1) and repeated (p=0.02, 2-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=0.01). Within the CBD group, repeated dosing reduced arterial stiffness by day seven (pulse wave velocity; MD -0.44 m/s, p=0.05) and improved endothelial function (flow mediation dilatation, MD +3.5%, p=0.02, n=6 per group), compared to day one.

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 population with vascular diseases.

What is already known:

- Pre-clinical 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 the 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.

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 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 non-cannabinoids 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 PPARγ and $5HT_{1A}$ [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 the blood pressure, heart rate and blood flow in animals; CBD increased heart rate and mean blood pressure in anaesthetised dogs [13, 14], caused 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 has shown that acute and chronic dosing of CBD had no effect on haemodynamics under non-stress conditions, reduces the increase in BP and HR in response to stress, and increases 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.

METHODS

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.

Intervention

The placebo and CBD provided by Phivida Neutrafuels (Lot number 0717PV 0101).

Primary outcome and Sample size

In our previous study [19], CBD reduced resting systolic blood pressure by 6 mmHg in nine healthy male volunteers. The main aim of the study was to determine if the changes in blood pressure, 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 systolic blood pressure (primary outcome) after 1 week

between groups (assuming a standard deviation 4.5 mmHg and alpha 0.05). All other vascular endpoints assessed in this study are considered as secondary outcomes.

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].

Cardiovascular parameters

Haemodynamic parameters were assessed non-invasively after day one and day seven of receiving the drug. Blood pressure (BP) and other parameters, including heart rate (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®. Aortic blood pressure was measured from the brachial site by pulse wave analysis (PWA).

Arterial stiffness

Arterial stiffness was measured through pulse wave velocity (PWV) between the carotid and femoral anatomical sites using Vicorder®.

Blood flow and endothelial function

Duplex ultrasound and Cardiovascular suite Quipu 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 were assessed using a linear probe (L15-4 MHz) of high resolution ultrasound (Terason 3200T) [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 the angle in which the brachial artery dives in to cubital fossa. The diameter of 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) X area X 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 Sonora Transcranial Doppler System.

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 time (9 am – 11 pm), and every hour through the night (11pm – 8am) [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].

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.

The 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 one 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 three 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 two 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 three minutes [33] by using a dynamometer with their right hand while the Finometer® was continuously recording. The assessment on both study visits (day one and day seven) took place in the same assessment room at a temperature ~ 22° Celsius. On day seven (last day of receiving the drug), the same protocol was followed.

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

Statistical analysis

All statistical analysis was performed using IBM SPSS Statistics (Version 21.0. Armonk, NY: IBM Corp) 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 two-way ANOVA determined the effect of treatment on blood pressure and other cardiovascular parameters measured with 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.

RESULTS

Twenty-six healthy men were recruited (13 subjects in each group). 11 of 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 pre-study. The age, weight, height and 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 participants who received placebo: 27 ± 6 years, 82.1 ± 9.8 kg, 1.8 ± 0.1 m and 25.7 ± 2.9 (mean ± SD).

Blood pressure and other cardiovascular parameters

Acute dosing of CBD treatment at rest did not significantly reduce systolic BP (SBP p=0.08) or diastolic BP (DBP p=0.09) (Figure 1A, B), but significantly reduced mean arterial pressure (MAP) (p=0.04, 2-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 day one and day seven are presented in Suppl. Table 1 and Suppl. Figure 1.

Isometric exercise caused a significant increase in BP in the two treatment groups (Figures 3 and 4). SBP was significantly lower in CBD treated group compared to placebo group after single (p=0.001, 2-way ANOVA, Figure 3A; MD -6 mmHg, 95% CI -10 to -1) or repeated CBD dosing (p=0.02, 2-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' two (MD -6.6 mmHg, 95% CI -12.7 to -0.4, p=0.02, Figure 3A) and three (MD -6.5 mmHg, 95% CI -12.7 to -0.3, p=0.03, Figure 3A) during stress. After seven days of CBD dosing, the mean difference in

SBP compared to placebo was -8 mmHg (95% CI -15.7 to -0.57, p=0.02, Figure 4A) at the first minute of stress.

24-hour Ambulatory Blood Pressure Monitoring

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

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. 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=0.05, Figure 6C) compared to a single CBD dosing received on day one.

Blood flow and endothelial function

CBD dosing for seven days increased ICA-diameter (MD +0.55 mm, 95% CI 0.1 to 0.9, p=0.01, Figure 7B) without causing a significant increase in ICA-BFV (p=0.07) compared to placebo, at rest. Repeated CBD dosing also significantly increased ICA-diameter (MD +0.43 mm, 95% CI -0.8 to 0, p=0.05, Figure 7B), and significantly increases FMD (MD +3.5%, 95% CI -6.2 to -0.6, p=0.02, n= 6 in each group, Figure 8C) compared to the same group treated with CBD after a single dose on day one. No differences were seen in MCA velocity, or CCA and BA parameters between the two groups post-treatment (see Suppl. Figure 2 A-G).

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 light-headedness on day 6 (n=1).

Discussion

The aim of this study was to investigate the effects of acute and repeating 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 was a lower SBP response after both acute and repeated dosing. We also found that repeated CBD dosing increases 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 [34]. 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 (e.g. reading versus watching the TV). Further, 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, 35] preventing the increase in BP which was seen in the placebo group on the first day of receiving the treatment (see Suppl Figure 3). 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 [36], 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 14-23 mg/kg/day. A CBD dose of 14 mg/kg/d would be equivalent to a dose of 1,120 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 pharmacodynamics of cannabis derived medicines [37], 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 pre-stress (Sidak`s post-hoc analyses p=0.8) compared to -1 minute (p=0.06). 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. Pre-clinical studies on animals models of stress showed that CBD reduces anxiety through the activation of $5HT_{1A}$ and CB_1 receptors [38, 39]. CBD activation of $5HT_{1A}$ in rats also reduces the increase in BP induced by stress [40, 41]. 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 pre-clinical studies [42, 43], 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 [43, 44]. These effects were inhibited with the administration of $5HT_{1A}$ antagonist [43, 45]. Another study in piglets with brain injury reported that $5HT_{1A}$ and CB_2 have a role in CBD induced neuroprotection [42]. 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, e.g. in social anxiety disorder [46]. 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 seven 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 versus 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 pre-treatment 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 [47], and a meta-analysis on the association of FMD and NO demonstrates that FMD is significantly mediated by NO [48]. 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% [49]. This signal of reduction in arterial stiffness and the improvement 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 seven days is 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.

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.

Data availability statement

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

Funding

SRS's PhD fees and stipend were supported by King Abdulaziz University. There were no other funds for the trial. CBD and placebo were provided for free by Phivida Neutrafuels.

Conflict of Interest

None declared.

Author contributions

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

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Figure Legends

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 Finometer (n=13). Closed circle is CBD group and opened square is placebo group. Repeated measure two-way ANOVA (* p≤0.05); data is presented as mean±SEM.

Figure 2 Cardiovascular parameters at rest after 7 days 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 Finometer on day 7 (n=13). Closed circle is CBD group and opened square is 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 Finometer on day 1; pre, during and post-isometric exercise stress (n=13). Closed circle is CBD group and opened square is placebo group. Repeated measure two-way ANOVA (* p≤0.05); mean± SEM (# p≤0.05 using Sidak post-hoc analysis between CBD and placebo groups).

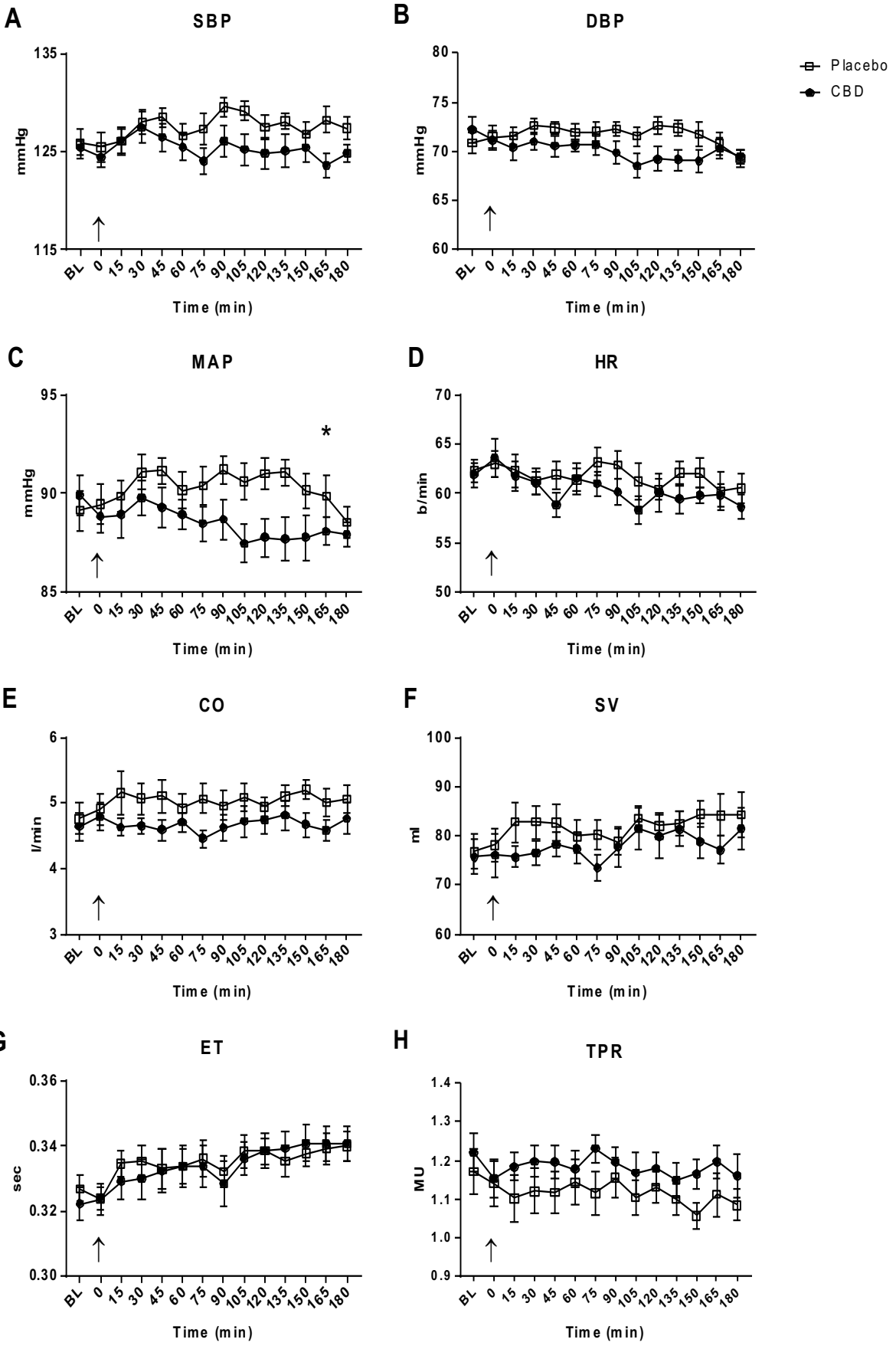
Figure 4 Cardiovascular parameters in response to exercise stress after 7 days CBD treatment. Systolic blood pressure (SBP) **(A)** , diastolic blood pressure (DBP) **(B)**, mean arterial pressure (MAP) **(C)**, heart rate (HR) **(D)**, cardiac output (CO) **(E)**, stroke volume (SV) **(F)**, ejection time (ET) **(G)**, and total peripheral resistance (TPR) **(H)** measured continuously using Finometer on day 7; pre, during and post-isometric exercise stress (n=13). Closed circle is CBD group and opened square is placebo group. Repeated measure two-way ANOVA (* p≤0.05); mean±SEM (# p≤0.05 using Sidak post-hoc analysis between CBD and placebo groups).

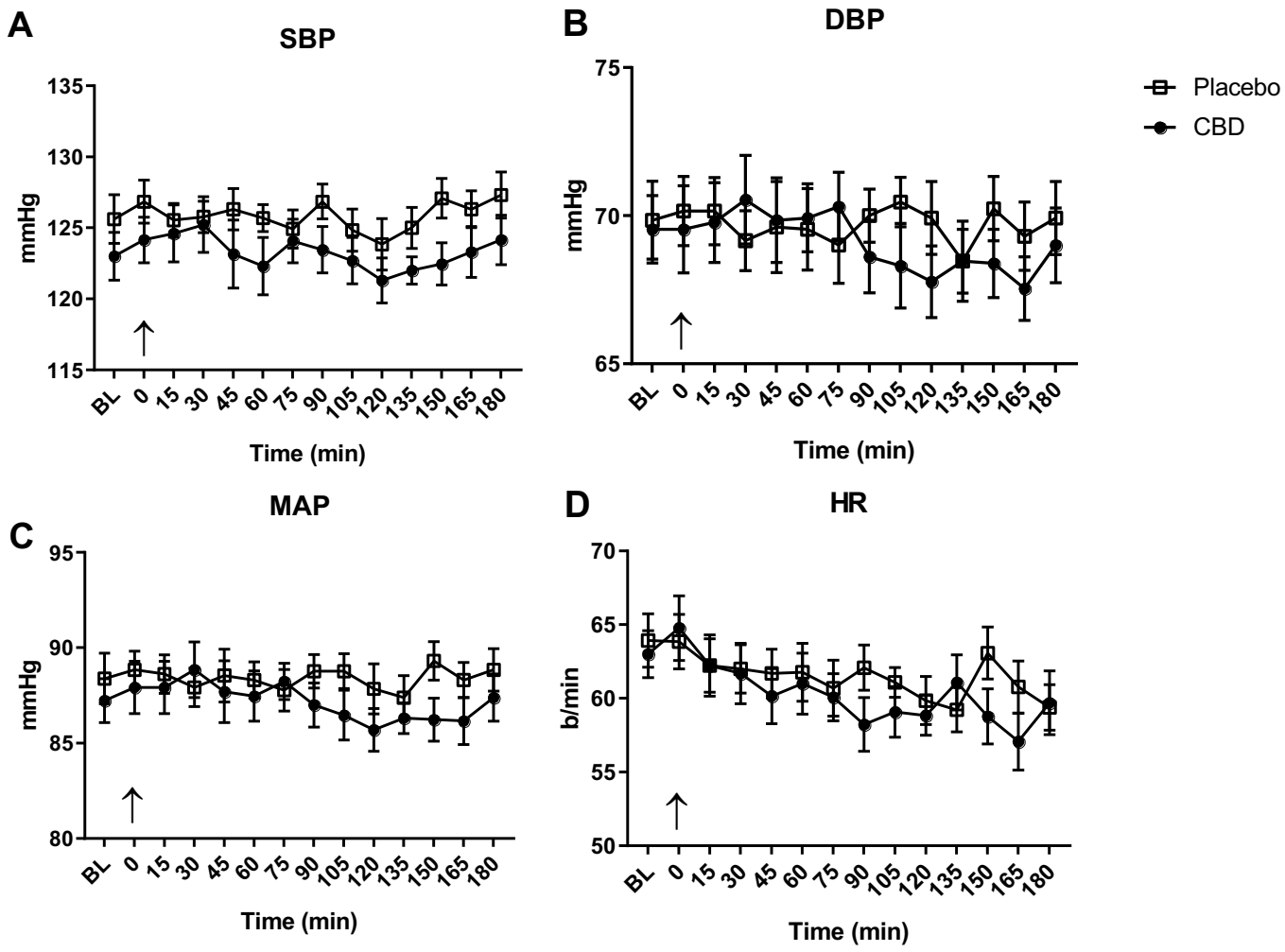
Figure 5 24-hour Ambulatory Blood Pressure Monitoring at rest after 6 days CBD treatment. Systolic blood pressure (SBP) (A), diastolic blood pressure (DBP) (B) and Mean arterial pressure (MAP) (C) were measured using an Ambulatory Blood Pressure device (AMBP) for 24 hours on day 6 of receiving the treatment (CBD group n=10 and placebo group n=7). Closed circle is CBD group and opened square is placebo group; mean ± SEM. No significant difference between the treatment groups.

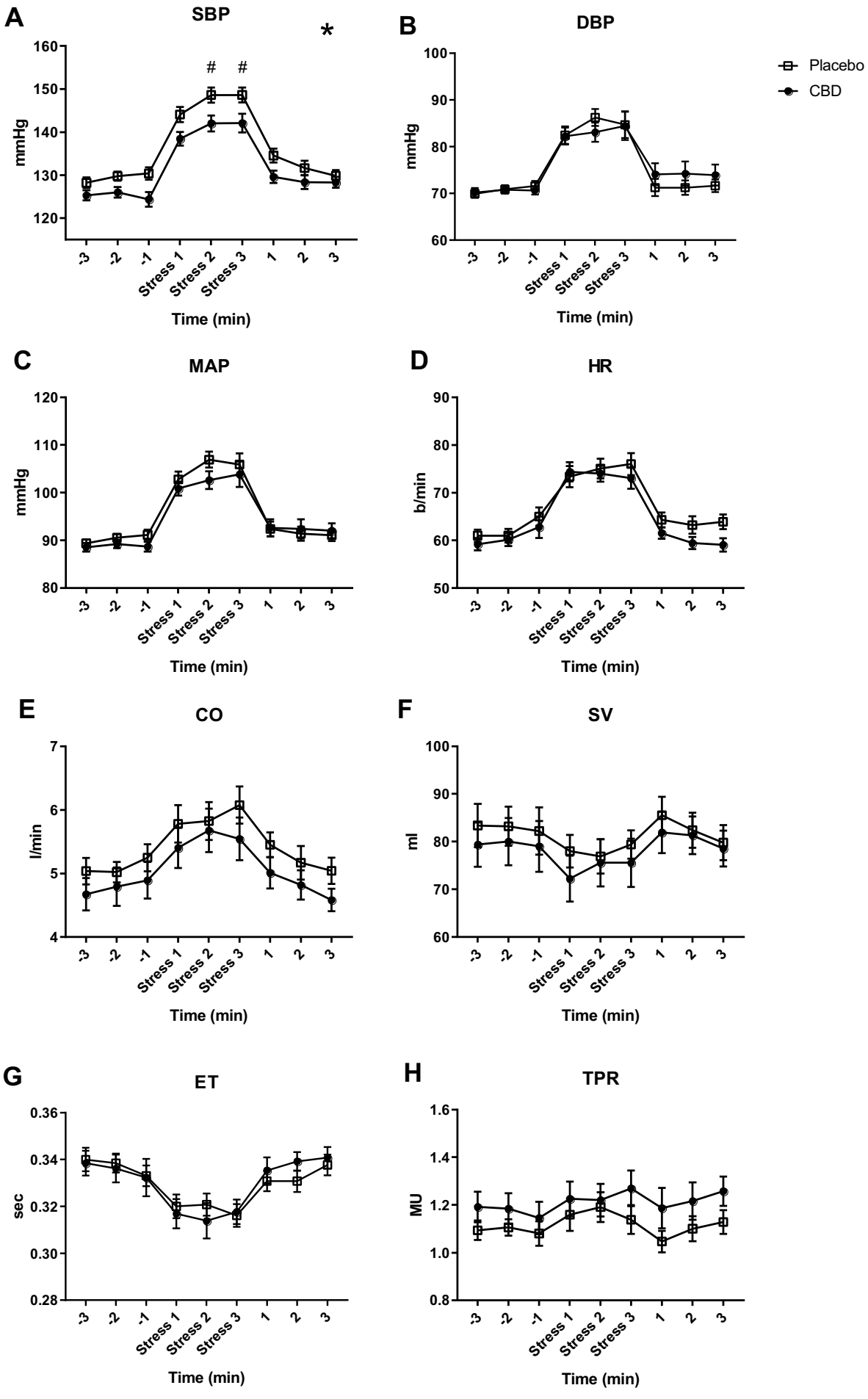
Figure 6 Aortic blood pressure and arterial stiffness after the first (day 1) and seventh dose of CBD. Aortic systolic blood pressure (Ao-SBP) **(A),** aortic diastolic blood pressure (Ao-DBP) **(B)** (n=9 in CBD group, and n=8 and 9 in placebo group at day 1 and 7, respectively), and pulse wave velocity (PWV) **(D)** (n=10) measured using Vicorder 2 hours after acute dosing on day 1 (D1) and chronic dosing on day 7 (D7); mean±SEM (* p≤0.05 using Paired t-test between related groups post-drug ingestion).

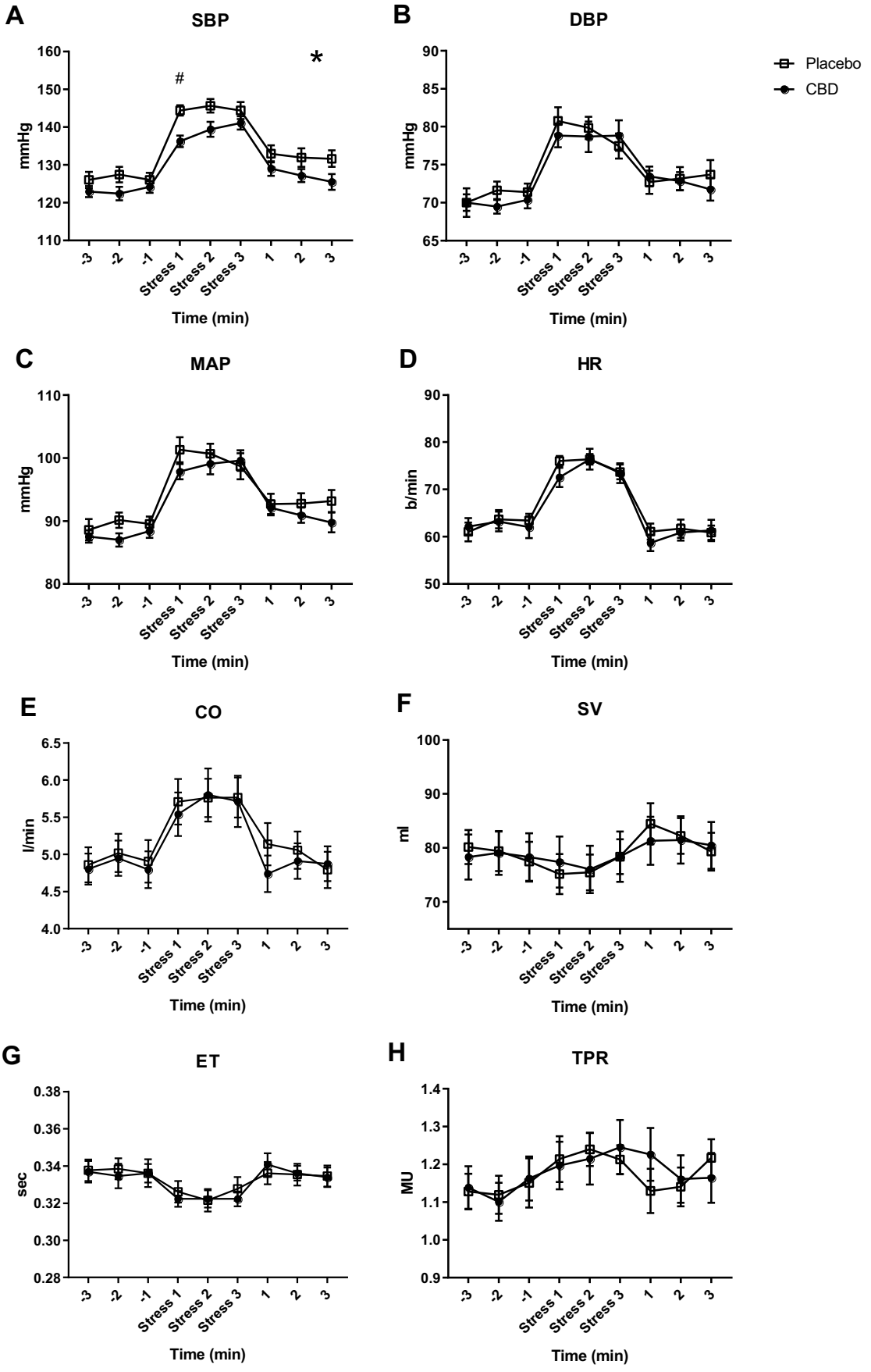
Figure 7 Internal carotid artery response after the first (day 1) and seventh dose of CBD. Internal carotid artery blood flow volume (ICA-BFV) **(A)**, diameter (ICA-D) **(B)** and peak systolic velocity (ICA-PSV) **(C)** (n=13) measured using ultrasound 2 hours after acute dosing on day 1 (D1) and chronic dosing on day 7 (D7); mean±SEM (*p≤0.05 using Paired t-test between related groups, and Unpaired t-test between independent groups post-drug ingestion).

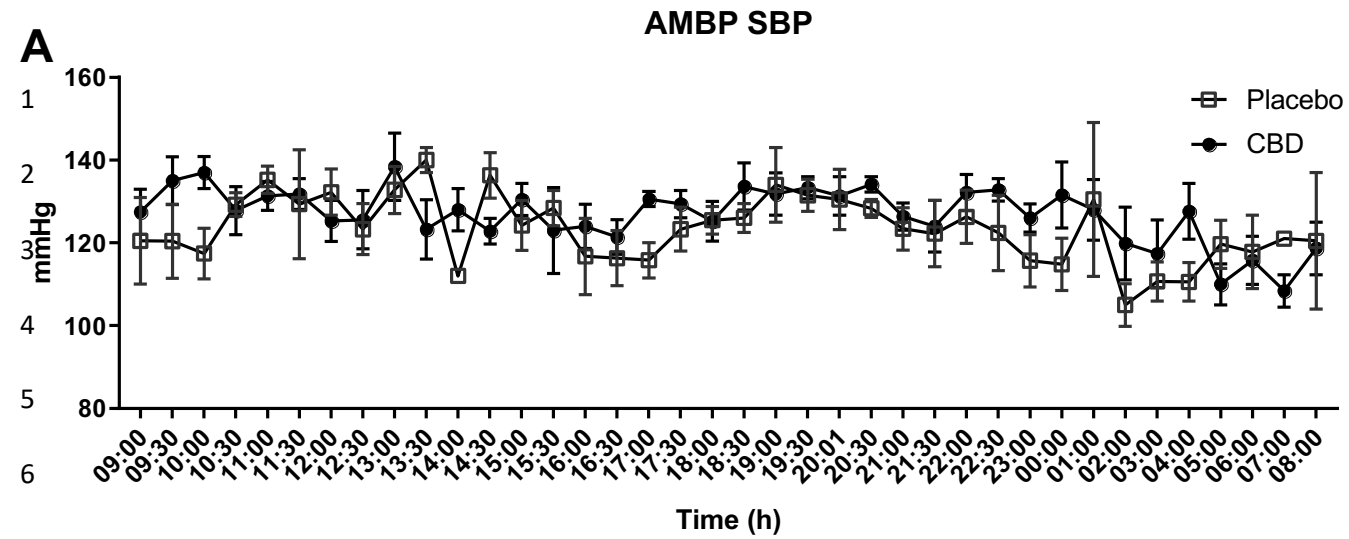
Figure 8 Endothelial function response after the first (day 1) and seventh dose of CBD. Brachial artery diameter (BA-D) at baseline (BL) **(A)** and post-occlusion (PO) **(B)** and Flow mediation dilatation (FMD) **(C)** (n=6) measured using ultrasound 2 hours after acute dosing on day 1 (D1) and chronic dosing on day 7 (D7); mean±SEM (* p≤0.05 using Paired t-test between related groups post-drug ingestion).


A
SBP
mmHg
135
125
115
B
DBP
mmHg
80
75
70
65
60
Placebo
CBD
C
MAP
mmHg
95
90
85
*
D
HR
b/min
70
65
60
55
50
E
CO
l/min
6
5
4
3
F
SV
ml
100
90
80
70
60
G
ET
sec
0.36
0.34
0.32
0.30
H
TPR
MU
1.4
1.3
1.2
1.1
1.0
0.9
BL 0 15 30 45 60 75 90 105 120 135 150 165 180
Time (min)


A
SBP
mmHg
135
130
125
120
115
BL 0 15 30 45 60 75 90 105 120 135 150 165 180
Time (min)
B
DBP
mmHg
75
70
65
BL 0 15 30 45 60 75 90 105 120 135 150 165 180
Time (min)
Placebo
CBD
C
MAP
mmHg
95
90
85
80
BL 0 15 30 45 60 75 90 105 120 135 150 165 180
Time (min)
D
HR
b/min
70
65
60
55
50
BL 0 15 30 45 60 75 90 105 120 135 150 165 180
Time (min)


A
SBP
*
#
#
mmHg
160
150
140
130
120
-3
-2
-1
Stress 1
Stress 2
Stress 3
1
2
3
Time (min)
B
DBP
mmHg
100
90
80
70
60
Time (min)
Placebo
CBD
C
MAP
mmHg
120
110
100
90
80
Time (min)
D
HR
b/min
90
80
70
60
50
Time (min)
E
CO
l/min
7
6
5
4
Time (min)
F
SV
ml
100
90
80
70
60
Time (min)
G
ET
sec
0.36
0.34
0.32
0.30
0.28
Time (min)
H
TPR
MU
1.6
1.4
1.2
1.0
0.8
Time (min)


A
SBP
mmHg
160
150
140
130
120
110
#
*
B
DBP
mmHg
90
85
80
75
70
65
Placebo
CBD
C
MAP
mmHg
110
100
90
80
D
HR
b/min
90
80
70
60
50
E
CO
l/min
6.5
6.0
5.5
5.0
4.5
4.0
F
SV
ml
100
90
80
70
G
ET
sec
0.38
0.36
0.34
0.32
0.30
0.28
H
TPR
MU
1.4
1.3
1.2
1.1
1.0
0.9
-3
-2
-1
Stress 1
Stress 2
Stress 3
1
2
3
Time (min)


A
AMBP SBP
Placebo
CBD
mmHg
160
140
120
100
80
09:00
09:30
10:00
10:30
11:00
11:30
12:00
12:30
13:00
13:30
14:00
14:30
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15:30
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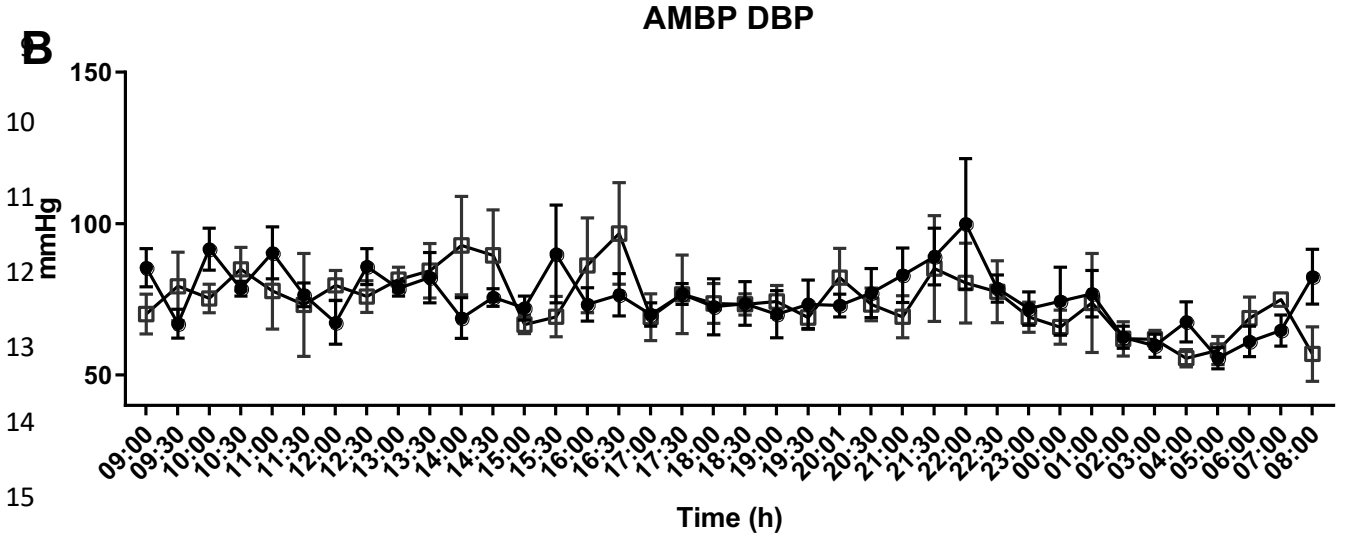
05:00
06:00
07:00
08:00
Time (h)


B
AMBP DBP
mmHg
150
100
50
09:00
09:30
10:00
10:30
11:00
11:30
12:00
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13:00
13:30
14:00
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22:00
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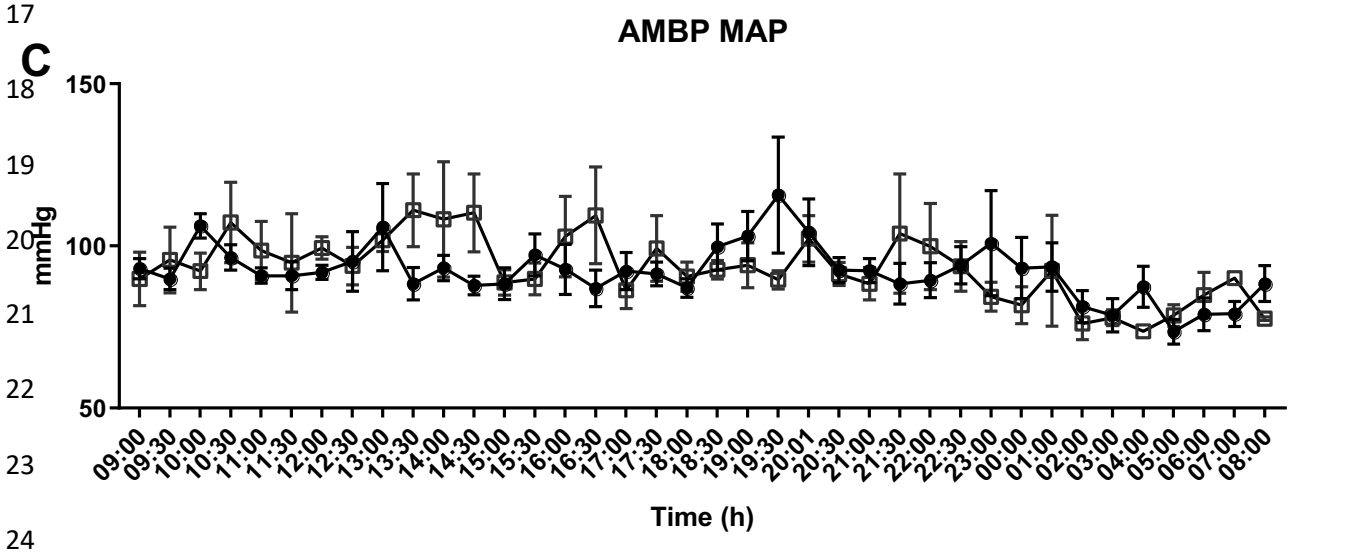
05:00
06:00
07:00
08:00
Time (h)


C
AMBP MAP
mmHg
150
100
50
09:00
09:30
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12:00
12:30
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13:30
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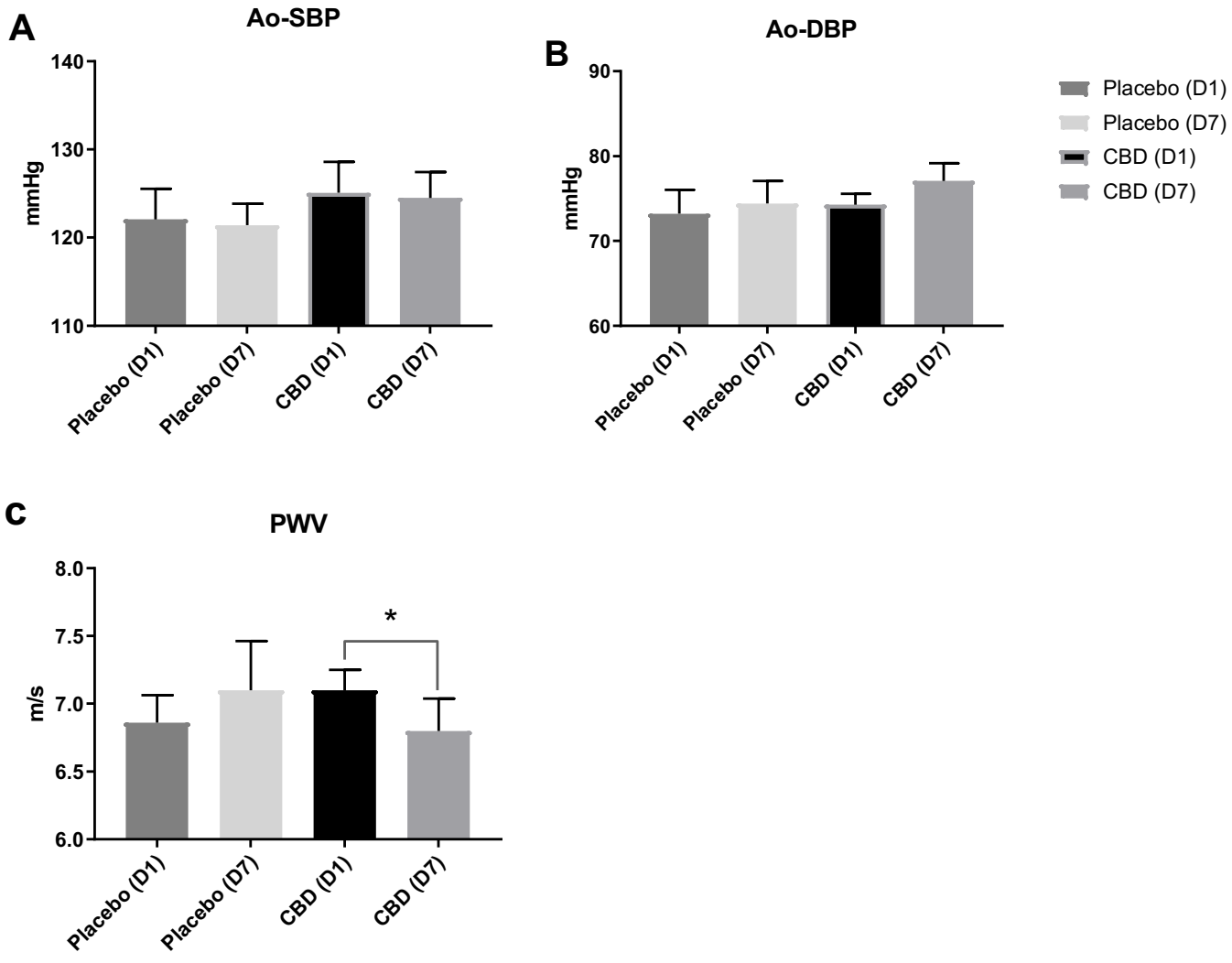
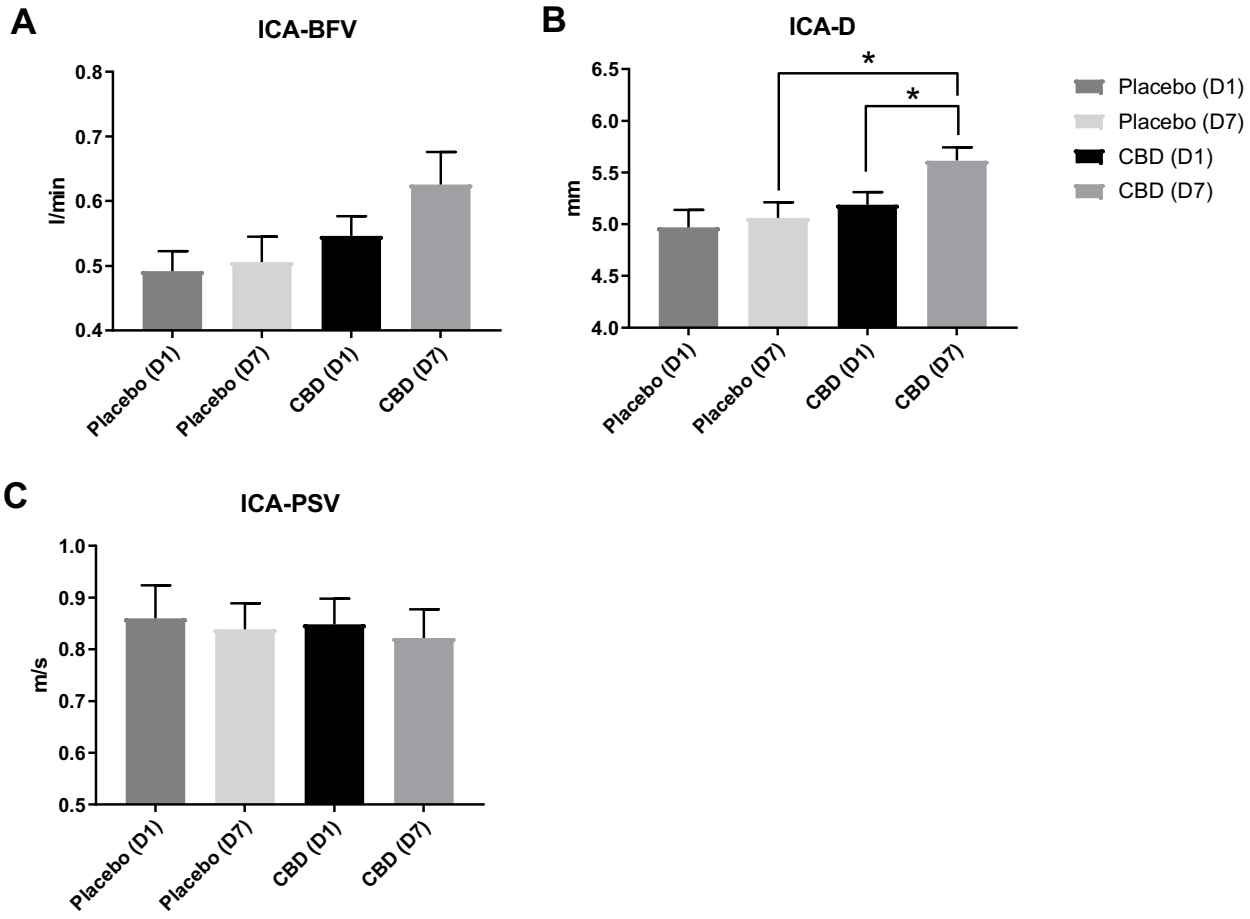
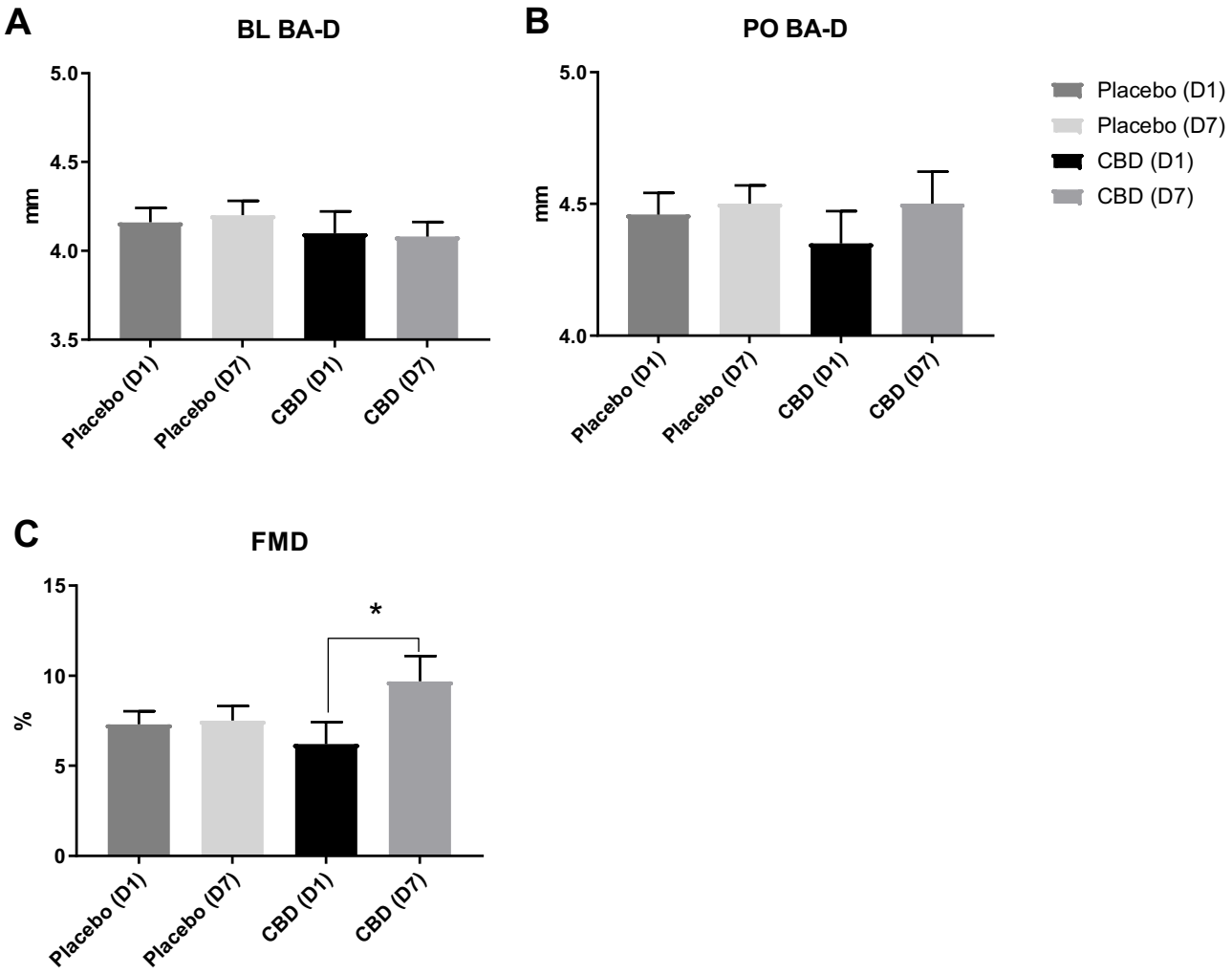
05:00
06:00
07:00
08:00
Time (h)


A
Ao-SBP
mmHg
140
130
120
110
Placebo (D1)
Placebo (D7)
CBD (D1)
CBD (D7)
B
Ao-DBP
mmHg
90
80
70
60
Placebo (D1)
Placebo (D7)
CBD (D1)
CBD (D7)
Placebo (D1)
Placebo (D7)
CBD (D1)
CBD (D7)
C
PWV
m/s
8.0
7.5
7.0
6.5
6.0
*
Placebo (D1)
Placebo (D7)
CBD (D1)
CBD (D7)


A
ICA-BFV
l/min
0.8
0.7
0.6
0.5
0.4
Placebo (D1)
Placebo (D7)
CBD (D1)
CBD (D7)
B
ICA-D
mm
6.5
6.0
5.5
5.0
4.5
4.0
*
*
Placebo (D1)
Placebo (D7)
CBD (D1)
CBD (D7)
C
ICA-PSV
m/s
1.0
0.9
0.8
0.7
0.6
0.5
Placebo (D1)
Placebo (D7)
CBD (D1)
CBD (D7)

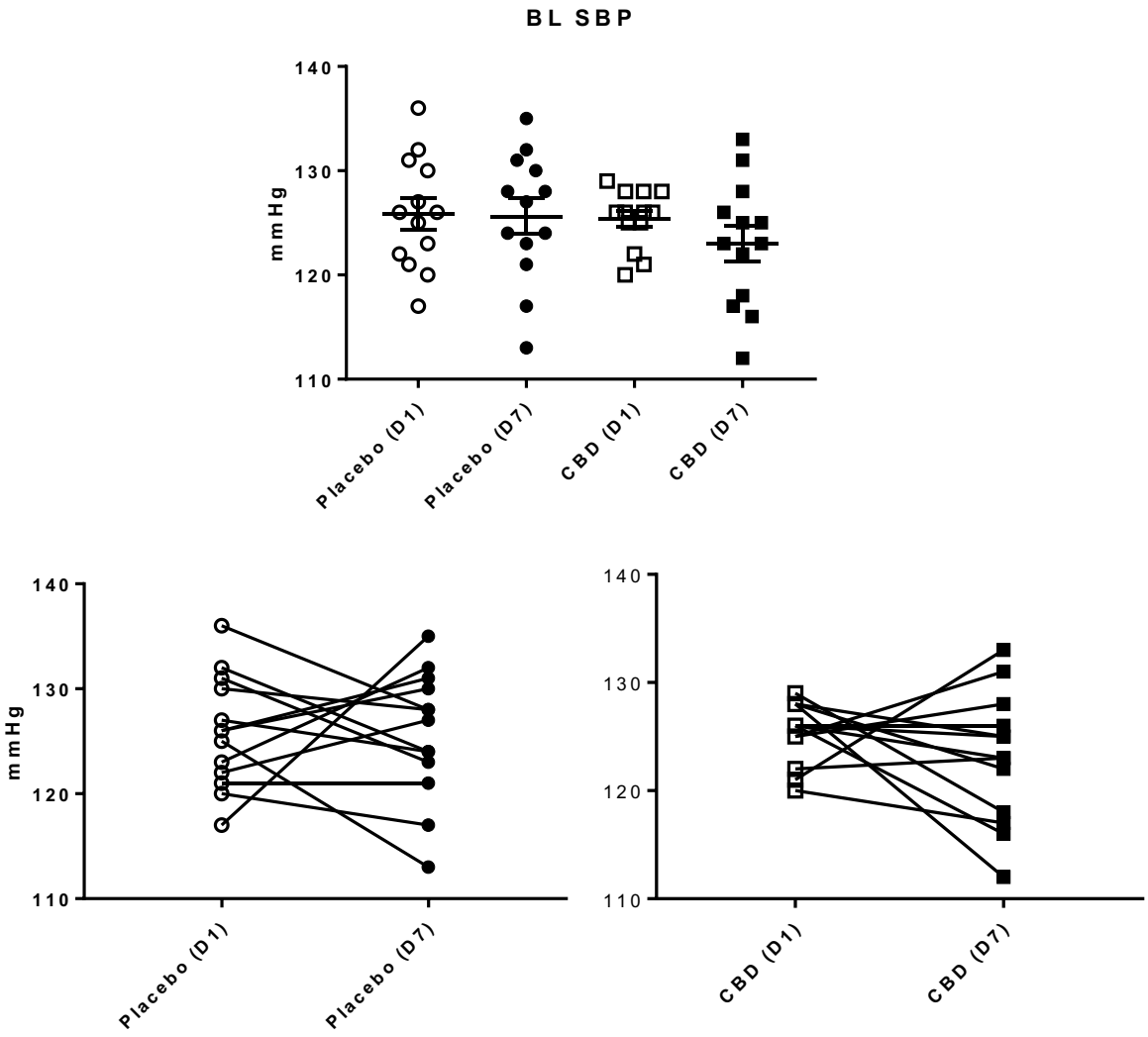

A
BL BA-D
mm
5.0
4.5
4.0
3.5
Placebo (D1)
Placebo (D7)
CBD (D1)
CBD (D7)
B
PO BA-D
mm
5.0
4.5
4.0
Placebo (D1)
Placebo (D7)
CBD (D1)
CBD (D7)
Placebo (D1)
Placebo (D7)
CBD (D1)
CBD (D7)
C
FMD
%
15
10
5
0
*
Placebo (D1)
Placebo (D7)
CBD (D1)
CBD (D7)

Supplementary figures

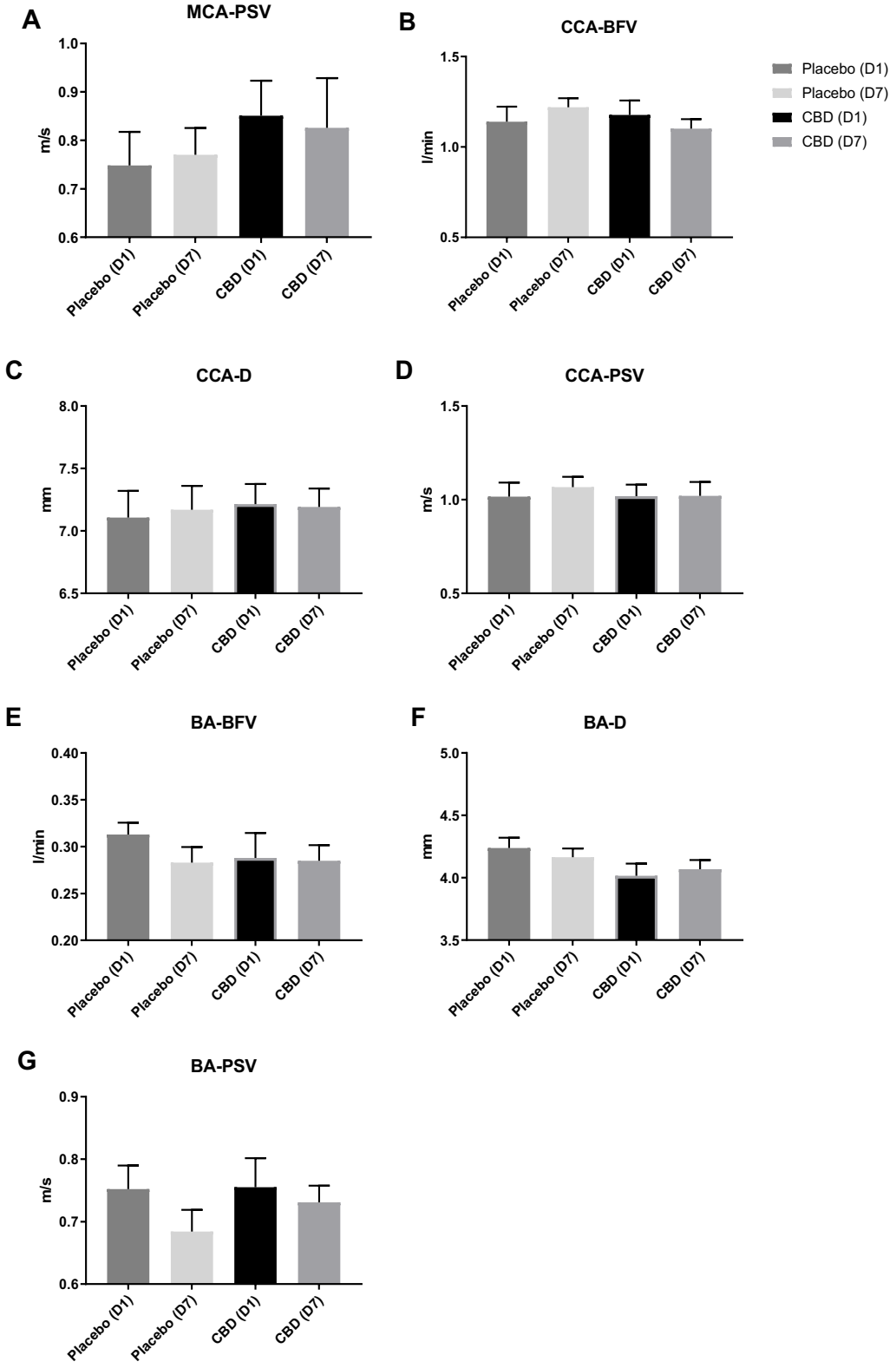
	Pre-CBD day 1	Pre-CBD day 7
Participant 1	128	112
Participant 2	128	125
Participant 3	125	128
Participant 4	120	117
Participant 5	126	123
Participant 6	126	126
Participant 7	126	116
Participant 8	126	125
Participant 9	125	131
Participant 10	121	133
Participant 11	128	122
Participant 12	122	123
Participant 13	129	118

	Pre-placebo day 1	Pre-placebo day 7
Participant 1	136	128
Participant 2	121	121
Participant 3	125	113
Participant 4	130	128
Participant 5	127	124
Participant 6	131	123
Participant 7	132	124
Participant 8	123	132
Participant 9	126	131
Participant 10	126	130
Participant 11	122	127
Participant 12	117	135
Participant 13	120	117

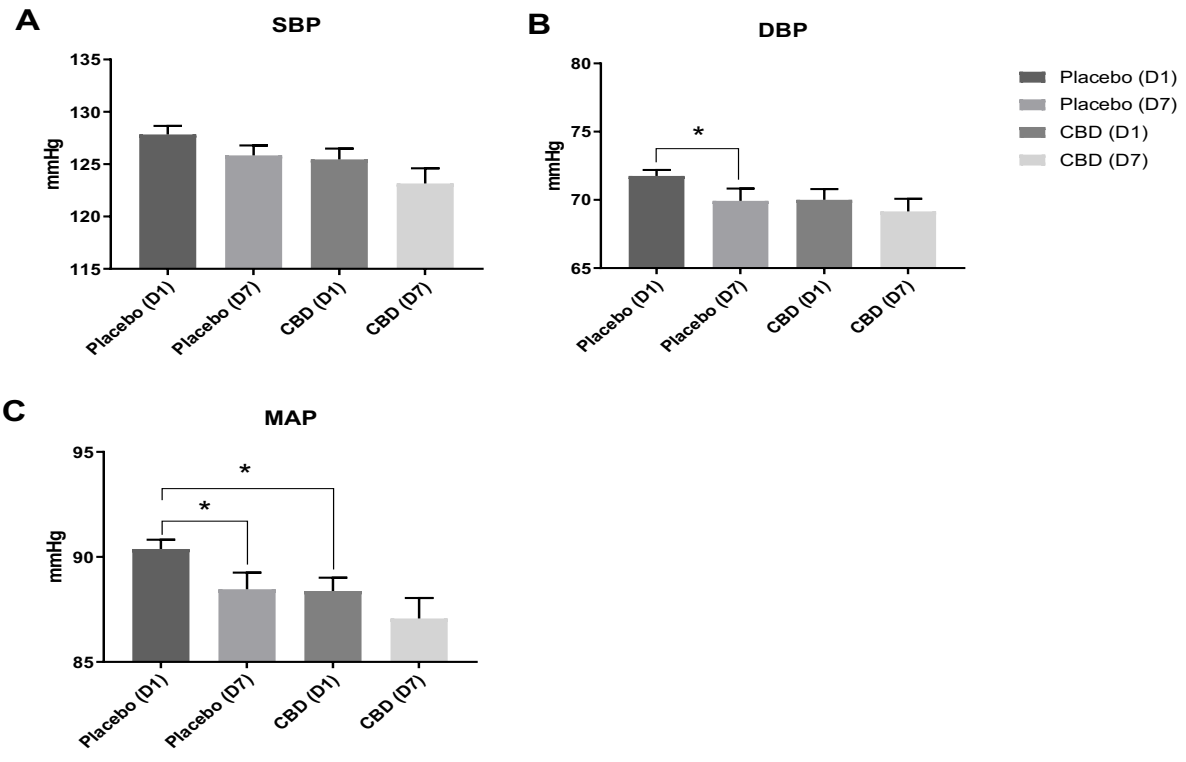
Suppl. Table 1 Participant pre-drug systolic blood pressure on day 1 and 7. Pre-CBD Systolic blood pressure (SBP) and Pre-placebo SBP on day 1 and day 7 at rest (n=13 in each group).



Suppl. Figure 1 Participant baseline (BL) pre-drug systolic blood pressure (SBP) of CBD and placebo on day 1 and 7 at rest (n=13 in each group); mean ± SEM.



Suppl. Figure 2 Cerebral and brachial artery blood flow after acute and 7 days CBD treatment. Middle cerebral artery blood flow peak systolic velocity (MCA-PSV) **(A),** common carotid artery blood flow volume (CCA-BFV) **(B)**, diameter (CCA-D) **(C)** and peak systolic velocity (CCA-PSV) **(D)**, brachial artery blood flow volume (BA-BFV) **(E)**, diameter (BA-D) **(F)** and peak systolic velocity (BA-PSV) **(G)** measured using ultrasound 2 hours after acute dosing on day 1 (D1) and chronic dosing on day 7 (D7) (n=13 in each group); mean±SEM.



Suppl. Figure 3 Blood pressure at rest after acute and chronic dosing. Systolic blood pressure (SBP) **(A)** , diastolic blood pressure (DBP) **(B)** and mean arterial pressure (MAP) **(C)** after single (D1) and repeated dosing for 7 days (D7) at rest (n=13 in each group); mean ± SEM (* p≤0.05 using additional exploratory Paired t-test between