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Association of Endothelial Dysfunction With Chronic Marijuana Smoking and THC-Edible Use

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IMPORTANCE Recreational and medicinal cannabis legalization has led to increased cannabis use. To understand the consequences for vascular health, we initiated the CANnabis: Does It Damage Endothelium (CANDIDE) study.

OBJECTIVE To investigate whether cannabis use is associated with vascular endothelial dysfunction.

DESIGN, SETTING, AND PARTICIPANTS In this cross-sectional study, sex- and age- matched healthy adults, aged 18 to 50 years, living in the San Francisco Bay Area, California, who neither smoke tobacco nor vape and were not frequently exposed to secondhand smoke were recruited into 3 cohorts: 2 chronic cannabis user groups (marijuana smokers and tetrahydrocannabinol [THC]–edible users) and 1 nonuser group. Participants were recruited from October 25, 2021, through August 1, 2024; analysis was completed September 2024. Participants' arterial flow-mediated dilation (FMD) and carotid-femoral pulse wave velocity (PWV) were measured. Human umbilical vein endothelial cells (HUVECs) were exposed to participant sera with and without vascular endothelial growth factor (VEGF) to assess the effects of user serum on endothelial nitric oxide production.

MAIN OUTCOMES AND MEASURES FMD and PWV were direct physiological measurements, and VEGF-stimulated nitric oxide production was measured from HUVECs incubated in user serum samples.

RESULTS Among 55 participants (20 female [37%]; 35 male [63%], mean age, 31.3 [SD, 8.4] years) arterial FMD was significantly lower among the marijuana smokers (mean, 6.0% [SD, 2.6%]; $P = .004$) and lower among THC-edible users (mean, 4.6% [SD, 3.7%]; $P = .003$) than among nonusers (mean, 10.4% [SD, 5.2%]). VEGF-stimulated nitric oxide levels in endothelial cells treated with participants' sera were significantly lower for the marijuana smoker group (mean, 1.1 nmol/L [SD, 0.3 nmol/L]) than for the nonuser group (mean, 1.5 nmol/L [SD, 0.3 nmol/L]; $P = .004$) but were unaffected among the THC-edible users group compared with the nonusers (mean, 1.5 nmol/L [SD, 0.3 nmol/L]; $P = .81$). FMD was inversely correlated with smoking frequency ($r = -0.7$; $P < .001$) and the amount of THC ingested ($r = -0.7$; $P = .03$). Other vascular properties showed no differences.

CONCLUSIONS This cross-sectional study found that chronic cannabis smoking and THC ingestion were associated with endothelial dysfunction similar to that observed in tobacco smokers, although apparently occurring via distinct mechanisms.

Supplemental content

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There is a popular belief that marijuana smoke is harmless. However, marijuana smoke contains many of the thousands of chemicals contained in tobacco smoke, along with fine particles that contribute to cardiovascular morbidity and mortality.[1,2] As cannabis legalization increases, it is crucial to understand the public health and clinical implications of marijuana use.[3]

Vascular health can be evaluated by measuring endothelial function as arterial flow-mediated dilation (FMD), the vasodilation of arteries in response to increased blood flow and resulting endothelial release of vasodilatory factors including nitric oxide.[4] FMD is a well-established clinical measure of endothelial function that is considered a predictor of later vascular disease.[5]

This study aimed to determine whether cannabis use is associated with vascular endothelial dysfunction.

Key Points

Question Is chronic cannabis use associated with endothelial dysfunction?

Findings In this cross-sectional study of 55 participants, vascular endothelial function was impaired in both chronic marijuana smokers and tetrahydrocannabinol (THC)–edible users, whereas serum from marijuana smokers but not THC-edible users blunted nitric oxide production in cultured endothelial cells.

Meanings Endothelial dysfunction was observed in otherwise healthy cannabis users, suggesting an increased risk of early development of vascular disease.

Methods

Population

We recruited 55 healthy adults, aged 18 through 50 years, who neither smoke tobacco nor vape and who were not frequently exposed to secondhand smoke. Participants were age and sex matched in 3 groups based on chronic cannabis use: marijuana smokers (≥3 smoking sessions per week for ≥1 year), tetrahydrocannabinol (THC)-edible users (≥3 edibles per week for ≥1 year), and nonusers (see eMethods in Supplement 1 for inclusion and exclusion criteria). Protocols were approved by the University of California, San Francisco, institutional review board (19-27925). Informed consent was obtained from all participants by our study team upon arrival to the clinic. Serum and urine metabolites of THC, cannabidiol (CBD), nicotine, cholesterol levels, triglyceride levels, and body mass index were measured. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines were followed.

FMD Measurement

The right brachial artery was scanned 1 cm distal to the antecubital fossa, using a 13-6 MHz linear array probe and Sonosite SII (Fujifilm).[6] Baseline brachial artery images were recorded, and spectral Doppler images of flow velocity were obtained. To induce transient ischemia, a forearm cuff was inflated to 200 mm Hg for 5 minutes. Following cuff deflation, Doppler images were taken to measure reactive hyperemia. The digital images for FMD were analyzed (blinded) using specialized software (Information Integrity Inc).

Measurement of Vascular Stiffness and Tonometry

To assess vascular wall stiffness, we measured pulse wave velocity (PWV) and pulse wave analysis (PWA), including the augmentation index and Buckberg subendocardial viability ratio, using a SphygmoCor Xcel system (Atcor Medical), as well as arterial blood pressure. For PWV, the participant lay supine, and pulse transit distance and time were determined using carotid and femoral pulse waveforms.

HUVEC Culture

Human umbilical vein endothelial cells (HUVECs) at passages 3 through 6 were cultured in endothelial cell growth medium (EGM) at 37 °C in 5% carbon dioxide. Cell viability was assessed with trypan blue.

Nitric Oxide Production Measurements

Participant serum and EGM-2 medium were added 1:1 to HUVECs (20 000 cells/well) and incubated for 12 hours. Supernatants were collected for basal nitric oxide measurement. After washing, cells were stimulated with 50 ng/mL VEGF for 30 minutes for stimulated nitric oxide measurement. Nitric oxide was measured by chemiluminescence (ECO PHYSICS) and normalized to cell count.[6,7]

Statistical Analysis

Descriptive statistics (mean, SD, frequency, percentage) were used to summarize participant characteristics. Normality of data was checked using histograms and the Shapiro-Wilk test. One-way analysis of variance or Kruskal-Wallis tests were used for overall group comparisons, with 2-group comparisons performed using independent *t* tests or Wilcoxon rank sum tests. *P* values were adjusted for multiple comparisons using the Holm step-down approach to control the family-wise type I error rate; adjusted $P < .05$ was considered significant.

Results

Participant characteristics (20 female [37%], mean age, 31.3 [SD, 8.4] years) are shown in the **Table**.

Cannabis Use Impaired FMD but Not Arterial Stiffness

Compared with nonusers, FMD was significantly lower in marijuana smokers (mean, 6.0% [SD, 2.6%]; $P = .004$) and THC-edible users (mean, 4.6% [SD, 3.7%]; $P = .003$) than nonusers (mean, 10.4% [SD, 5.2%]; **Figure 1**A). There were no significant differences between groups in PWV or other measures of arterial pressure and vascular tone ($P > .12$); Figure 1B and the eFigure in Supplement 1). FMD in user groups was inversely correlated with the weekly number of smoking sessions ($r = -0.7$; $P < .001$) and the amount of edible THC used ($r = -0.7$; $P = .03$; Figure 1C and D).

Table. Characteristics of Research Participants in Each Group

Variable	Marijuana smokers (n = 20)	THC-edible users (n = 9)	Nonusers (n = 26)	*P* value
Age, mean (SD), y	30.4 (8.4)	30.8 (9.9)	28 (6.4)	Overall, .76
Sex, No. (%)				
Male	13 (65.0)	8 (88.9)	14 (53.8)	Overall, .38
Female	7 (35.0)	1 (11.1)	12 (46.2)	
Race, No. (%)[a]				
Asian	3 (15.0)	1 (11.1)	7 (26.9)	
Black or African American	1 (5.0)	1 (11.1)	1 (3.8)	
Hispanic	7 (35.0)	2 (22.2)	4 (15.4)	
White	9 (45.0)	4 (44.5)	14 (53.8)	
Other	0	1 (11.1)	0	
BMI, mean (SD)	25.3 (3.6)	26.5 (3.8)	23.7 (2.6)	Overall, .06
Biological measures, mean (SD)				
Systolic blood pressure, mm Hg	117.3 (11)	124.2 (10)	117.1 (12)	Overall, .23
Diastolic blood pressure, mm Hg	69.5 (7.6)	75.9 (9.9)	69 (11)	Overall, .17
Heart rate, bpm	62.5 (13)	64.1 (7)	68 (13)	Overall, .29
Buckberg subendocardial viability ratio	178.8 (27.4)	184 (21.6)	173.5 (29.1)	Overall, .58
Duration of product use, y	10 (9)	5 (4)	NA	.12
Frequency of product use, per wk	6.3 (3.5)	4 (2)	NA	.07
Urine 11-carboxy-THC, ng/mL	114.2 (234.1)	40.26 (56.1)	BLQ	.42
Urine 7-carboxy-CBD, ng/mL	BLQ	BLQ	BLQ	
Serum THC metabolites, ng/mL	78 (150.2)	36.1 (62.5)	BLQ	.73
Urine nicotine, ng/mL	BLQ	BLQ	BLQ	
Urine cotinine, ng/mL	BLQ	BLQ	BLQ	
Total cholesterol, mg/dL	186.2 (24.6)	187.1 (24.5)	196 (23.2)	Overall, .37
Triglyceride, mg/dL	70.5 (25.7)	74.1 (42.5)	74.3 (34.6)	Overall, .92

Abbreviations: BLQ, below limit of quantification; BMI, body mass index, calculated as weight in kilograms divided by height in meters squared; CBD, cannabidiol; NA, not applicable; THC, tetrahydrocannabinol.

SI conversion factors: To convert cholesterol from mg/dL to mmol/L, multiply by 0.0259; triglyceride from mg/dL to mmol/L, 0.0113.

[a] Race was determined by self-report. Other includes mixed race.

Serum From Marijuana Smokers Reduced VEGF-Stimulated Nitric Oxide Production by HUVECs

Nitric oxide release from VEGF-stimulated HUVECs was reduced by serum from marijuana smokers (mean, 1.1 nmol/L [SD, 0.3 nmol/L] vs 1.5 nmol/L [SD, 0.3 nmol/L]; *P* = .004) but not from THC-edible users (mean, 1.5 nmol/L [SD, 0.3 nmol/L]; *P* = .81; **Figure 2**).

Discussion

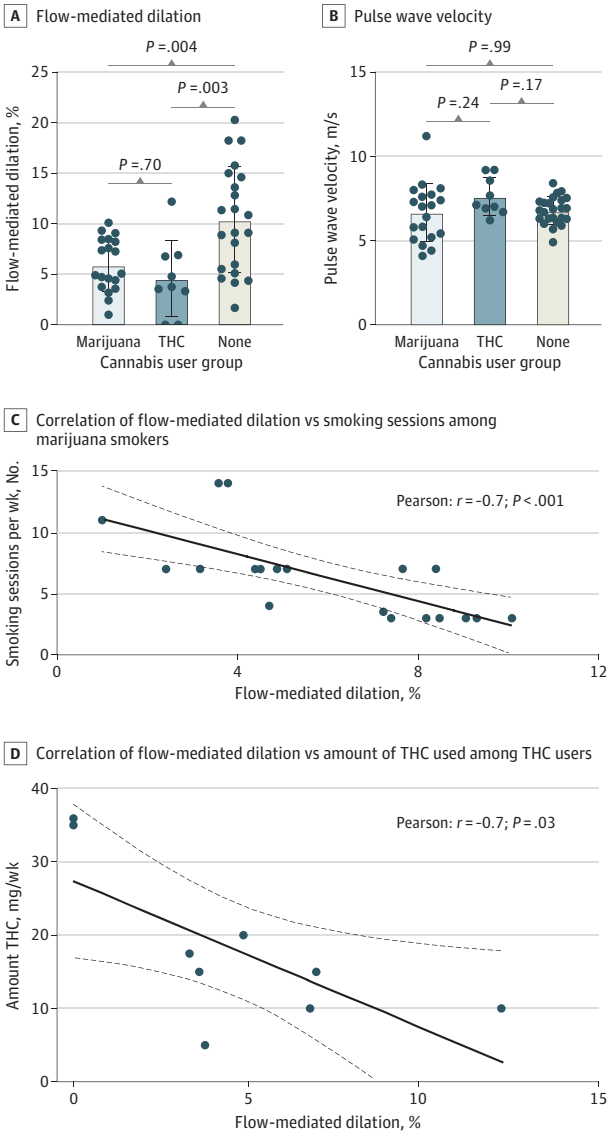
The cardiovascular consequences of cannabis use are poorly understood but are becoming increasingly appreciated. Studying effects of cannabis use can be difficult due to the uncertainty and variability caused by other product use and exposures. Using strict inclusion and exclusion criteria to avoid confounding effects of other potential exposures, we found that chronic marijuana smoking and THC-edible use were both associated with reductions in FMD compared with nonuser controls. The reduction in FMD was similar to what we have reported previously for chronic tobacco smokers.[6] Specifically, the mean FMD in the marijuana smokers, THC-edible users, and nonuser controls was 6.0% [SD, 2.6%], 4.6% [SD, 3.7%], and 10.4% [SD, 5.2%], respectively.

The percent reduction (27%) in cultured endothelial cell nitric oxide release caused by incubation in marijuana smoker serum (relative to nonuser serum) was similar to that what we reported in tobacco smokers (39%).[6] Methodological differences in nitric oxide measurement techniques do not allow direct between-study comparisons of nitric oxide levels. The variable effects of participants' serum on endothelial nitric oxide production indicate that despite the similar physiological effects, marijuana smoking and THC-edible use trigger distinct molecular responses. Differences between the 2 types of cannabis use include overall inhalational vs oral exposure and the many other chemicals contained in smoke.

Epidemiological studies have found mixed results regarding cannabis use and cardiovascular effects.[8,9] A 2017 National Academies of Sciences, Engineering, and Medicine report[10] found limited evidence for increased risk of stroke and myocardial infarction, with inconclusive evidence for long-term risk. However, many studies have emerged since 2017 that indicate cardiovascular risk may be elevated by marijuana, and the American Heart Association has concluded that there are negative health implications of cannabis use that require further research.[11] Notably, this current study shows a clear increase in early indicators of vascular dysfunction in otherwise healthy cannabis users.

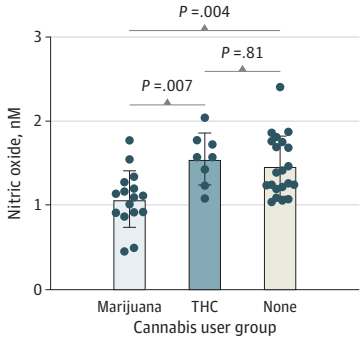
Tobacco smokers are known to have impaired FMD.[12,13] Although we have previously shown that acute marijuana smoking reduces FMD in rats, regardless of the presence or absence of THC in the smoke,[14] this study's current results show that chronic marijuana smoking and THC-edible use

Figure 1. Worsening of Vascular Endothelial Function But Not Arterial Stiffness



A, Flow-mediated dilation was significantly lower in both cannabis user groups. B, No differences in pulse wave velocity were observed. C and D, Dose-response associations were observed between flow-mediated dilation and amount of cannabis use. All analyses were performed using analysis of variance. Error bars indicate SD; data points, study participants. In panels C and D, solid lines indicate the line of best fit; dashed lines, 95% CIs; THC, tetrahydrocannabinol.

Figure 2. Lower Production of Cellular Nitric Oxide



Nitric oxide production was significantly lower only in the human umbilical vein endothelial cells that were treated with marijuana smokers' serum. All analyses were performed using analysis of variance. Error bars indicate SD; data points, study participants; THC, tetrahydrocannabinol.

were associated with reduced FMD in human volunteers. In contrast, Cheung et al[15] did not find a reduction in FMD among chronic cannabis users, possibly due to less frequent use. It is important to note that our findings indicate a significant correlation between the frequency of marijuana smoking or the amount of THC edible use and worsening of endothelial function.

Although previous studies have indicated that cannabis use can lead to an increase in PWV,[15] this effect was not observed in the current study, despite using the same methods of measurement. Other measures of arterial pressure and vascular tone also did not differ among groups. These discrepancies may be due to differences in characteristics of the study populations or variations in cannabis types and consumption patterns.

Limitations

This study has several limitations. Variability in cannabis strains complicates standardization. Self-reported cannabis use may introduce recall bias; thus, participants were queried at multiple points: in the online survey, at the eligibility interview, and before each visit. Interpreting physiological end points like FMD, arterial stiffness, or blood pressure can be challenging due to variability, lifestyle factors (eg, stress, caffeine, secondhand smoke exposure), and the cross-sectional rather than longitudinal nature of the study. These potential confounding effects were minimized by strict inclusion and exclusion criteria and validation by measuring circulating cannabinoid and nicotine metabolites.

Conclusions

Chronic cannabis smoking and THC ingestion were associated with endothelial dysfunction similar to that observed in tobacco smokers, although apparently occurring via distinct mechanisms. This study enhances the understanding of the potential risks to vascular health linked to cannabis use and provides more evidence that cannabis use is not benign.

ARTICLE INFORMATION

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Statistical analysis: Mohammadi, Navabzadeh, Jiménez-Téllez, Naughton, Park, Goyal, Cheng.
Obtained funding: Springer.
Administrative, technical, or material support: Reagan, Almeida, Castaneda, Uyemura, Onder, Hellman, Wu.
Supervision: Mohammadi, Springer.

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Supplemental Online Content

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eMethods.
eFigure. PWA measurements between the groups
eReference

This supplemental material has been provided by the authors to give readers additional information about their work.

eMethods

Inclusion and exclusion criteria:

Inclusion Criteria for all participants:

- 18-50 years old and healthy based on medical history (normal blood pressure, fasting lipid profile, and glucose level)
- Lifetime non-use of tobacco (based on oral interview and nicotine/cotinine tests)

Exclusion Criteria for all participants:

- Tested positive for nicotine or cotinine
- Recent use of cannabis, caffeine, or alcohol products within the last 12 hours (based on oral interview)
- Frequent* exposure to secondhand tobacco or marijuana smoke (based on oral interview: not living, working, or congregating with people who are smoking)
- Dual or poly use of cigarette/cannabis and vape products (based on oral interview)
- Did not fast within the last 12 hours (based on oral interview)
- Exercise <12 hours before the visit
- Physician diagnosis of asthma, heart disease, hypertension, dyslipidemia, thyroid disease, diabetes, renal or liver impairment, or glaucoma (based on oral interview)
- Pregnancy or breastfeeding (by oral history and urine pregnancy tests)
- Female participants of childbearing potential who are 8 days past the first day of their menstrual cycle
- Women who are post-menopausal and on hormone replacement therapy, or premenopausal and on birth control pills (premenopausal women will be screened verbally about their menstrual cycle, to reduce variability) (based on oral interview)
- Hypertension at screening defined by systolic blood pressure >140 and/or diastolic blood pressure >90
- Currently taking Viagra, Levitra, or Cialis (based on oral interview)
- Currently taking any kind of hormone replacement therapy (based on oral interview)
- Alcohol, opiate, cocaine, amphetamine, or methamphetamine dependence within the past 5 years (based on oral interview)
- Current opiate, cocaine, amphetamine, or methamphetamine use (based on oral interview and toxicology test)
- BMI >35 or <18 kg/m^2 measured at screening
- On anticoagulant therapy (warfarin, direct thrombin inhibitors, factor Xa inhibitors)
- Occupational exposure to smoke, dust, and fumes (based on oral interview)
- Unable to communicate in English
- Known history of infection in last 6 months (based on oral interview)
- Any other condition(s) that would compromise the safety of the subject or compromise the quality of the clinical study, as judged by the Investigator

* We asked all potential study participants about their exposure to secondhand smoke. If their exposure was limited to passing by smokers on the street occasionally, we considered it infrequent and included them in the study. However, individuals who live, work, or frequently spend time around people who smoke were not eligible to participate.

Additional Inclusion Criteria for Active Marijuana Smokers:
Currently smoke >3 sessions per week (items allowed for smoking in this group included joints, bongs, blunts, and pipes)
≥ 1 year

Additional Inclusion Criteria for THC-Edible Users:
Currently use THC-edible items > 3 times a week (minimum 2.5 mg per use)
≥ 1 year

Additional Exclusion Criteria for THC-Edible Users:
Ever vaped or smoked any marijuana products

Additional Exclusion Criteria for Nonusers:
Ever vaped or smoked any tobacco or marijuana products or used edible THC products
Tested positive for THC

Recruitment and population

For this cross-sectional study, participants were recruited through paid advertisements on Meta platforms and IRB-approved flyers distributed in the greater San Francisco Bay Area. Interested individuals could either scan a QR code on the flyer to complete a Qualtrics questionnaire or contact the study team via email or social media message. A designated study team member then contacted potential participants by phone to assess their eligibility based on inclusion and exclusion criteria. During this screening, the participant's medical history, age, smoking status, and exposure to secondhand smoke were reviewed to determine eligibility. We divided our study participants into 3 groups based on cannabis use: marijuana smokers (≥3 smoking sessions/week for at least 1 year), THC-edible users (≥3 edibles/week for at least 1 year), and nonusers. Protocols were approved by the UCSF IRB (#19-27925). Informed consent was obtained from all participants. THC, nicotine, and cotinine levels were measured in urine and saliva, and participants were instructed to avoid exercise, caffeine, or using cannabis products for 12 hours before the study. To minimize variation in FMD during the ovarian cycle, menstruating women were tested during the first 7 days of their menstrual period.[1]

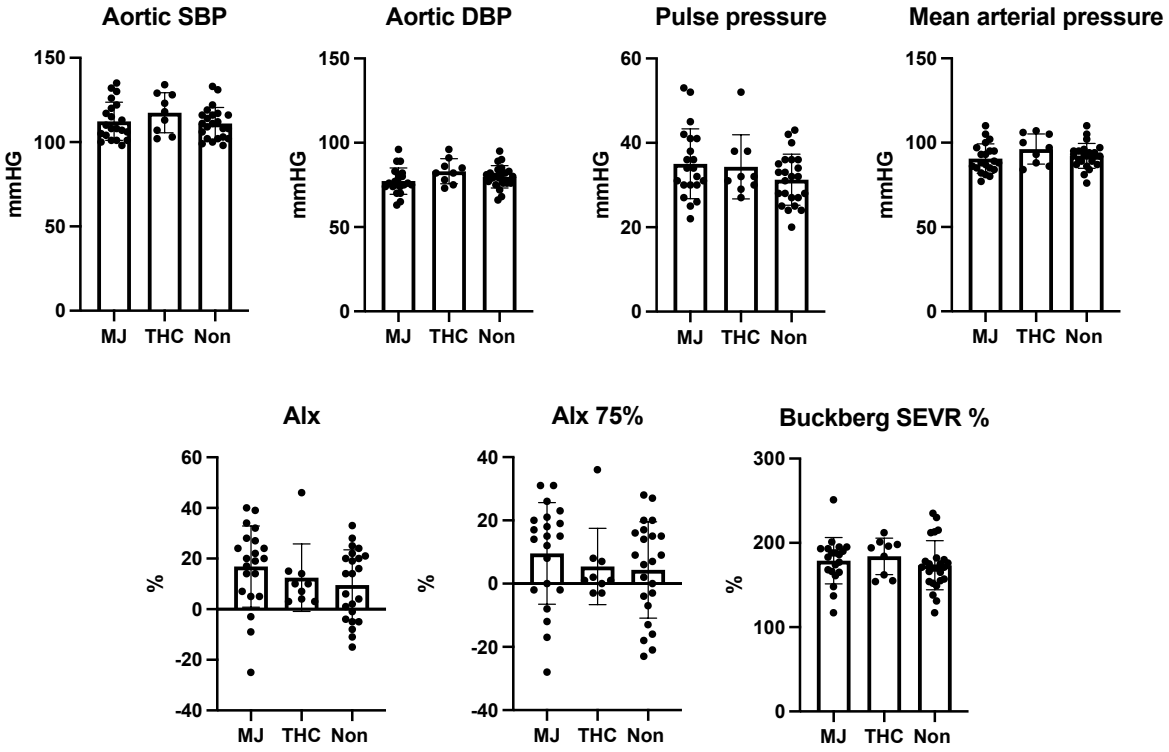
Human sample collection and analysis

Participants were asked to abstain from smoking marijuana, using any cannabis products, eating food, exercising, and consuming caffeine for 12 hours before the study visit. Blood was collected by venipuncture at fasting state from the antecubital area. Serum and plasma were prepared, using EDTA tubes for plasma collection and SST silicon-coated tubes for serum collection. Saliva and urine were collected as well. All the samples were aliquoted and immediately stored at −80°C for subsequent assays. The samples were sent to the appropriate core laboratory for the measurement of THC, CBD metabolites, nicotine, cotinine, and caffeine levels.

THC, CBD, nicotine, illicit drugs, and caffeine measurements

To exclude tobacco and other drug users from our study, we measured the levels of nicotine and cotinine immediately after obtaining consent from the study participants using a rapid saliva test and conducted a rapid urine toxicology test with the kit (14-panels First Sign). Subsequently, we sent the urine samples to the UCSF Helen Diller Family Comprehensive Cancer Center Tobacco Biomarker Core facility to measure the levels of THC and CBD metabolites, nicotine, and caffeine. We maintained strict inclusion criteria, and after receiving the data from the core lab, we had to exclude two study participants. Additionally, we measured the level of THC metabolites in the serum using the ELISA kit (Cayman).

eFigure. No significant changes were observed in PWA measurements between the groups.



p<.05 required for significance; all p values were >0.2. Group means were compared by ANOVA. Bars=SD.

eReference:

1. Williams MR, Westerman RA, Kingwell BA, et al. Variations in endothelial function and arterial compliance during the menstrual cycle. *J Clin Endocrinol Metab*. Nov 2001;86(11):5389-95. doi:10.1210/jcem.86.11.8013