Supplemental Online Content

Mohammadi L, Navabzadeh M, Jiménez-Téllez N, et al. Association of endothelial dysfunction with chronic marijuana smoking and THC-edible use. *JAMA Cardiol*. Published online May 25, 2025. doi:10.1001/jamacardio.2025.1399

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]

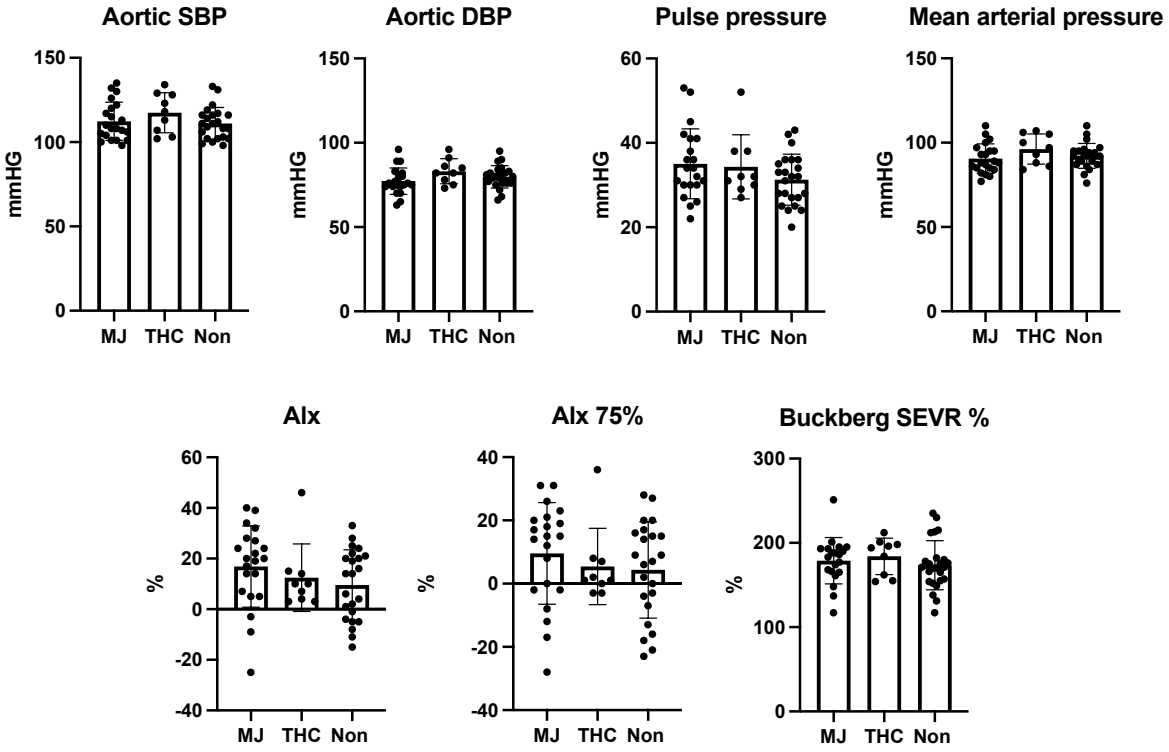
Human sample collection and analysis

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