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RESEARCH ARTICLE

Habitual cannabis use is associated with altered cardiac mechanics and arterial stiffness, but not endothelial function in young healthy smokers

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Abstract

Cigarette smoking is among the most detrimental behaviors to cardiovascular health, resulting in arterial stiffening, endothelial dysfunction, and structural/functional alterations to the myocardium. Similar to cigarettes, cannabis is commonly smoked, and next to alcohol, is the most commonly used recreational substance in the world. Despite this, little is known about the long-term cardiovascular effects of smoking cannabis. This study explored the associations of cardiovascular structure and function with cannabis use in ostensibly healthy young participants (n = 35). Using echocardiography, carotid-femoral pulse wave velocity (cfPWV), and brachial flow-mediated dilation (FMD), we performed a cross-sectional assessment of cardiovascular function in cannabis users (n = 18) and controls (n = 17). There were no differences in cardiac morphology or traditional resting measures of systolic or diastolic function between cannabis users and controls (all $P > 0.05$), whereas cannabis users demonstrated reduced peak apical rotation compared with controls (cannabis users: 5.5 ± 3.8, controls: 9.6 ± 1.5; P = 0.02). Cannabis users had higher cfPWV compared with controls (cannabis users: 5.8 ± 0.6 m/s, controls: 5.3 ± 0.7 m/s; P = 0.05), whereas FMD was similar between cannabis users and controls (cannabis users: 8.3 ± 3.3%, controls: 6.8 ± 3.6%; P = 0.7). Young, healthy, and cannabis users demonstrate altered cardiac mechanics and greater aortic stiffness. Further studies should explore causal links between cannabis smoking and altered cardiovascular function.

NEW & NOTEWORTHY Recreational cannabis is the most widely used substance in the world, other than alcohol. Yet, the effects of cannabis use on cardiovascular function and health are not well understood. Our cross-sectional data demonstrate that young, ostensibly healthy cannabis users have greater arterial stiffness and altered cardiac mechanics compared to nonusers. These findings suggest that cannabis users may be at greater risk of the development of future cardiovascular disease.

echocardiography; flow-mediated dilation; marijuana; pulse wave velocity; smoking

INTRODUCTION

Cardiovascular disease (CVD) remains the world's leading cause of global mortality (1) despite global public health efforts to discourage lifestyle factors that increase CVD risk. Tobacco use and cigarette smoking are among the most harmful behaviors to cardiovascular health and CVD risk (2), as before the development of CVD, cigarette smokers develop detrimental structural and functional adaptations in both the heart and vasculature. Vascular consequences of cigarette smoking include increases in arterial stiffness (3, 4) and reductions in vascular endothelial function (5–8), whereas cardiac consequences include increased left ventricular mass, reduced left ventricular diastolic function, and reduced right ventricular systolic function (9–12).

With the exception of alcohol, cannabis is the most commonly used recreational substance in the world (13). However, long-standing global prohibition has introduced barriers to targeted scientific inquiry (14, 15). Cigarette smoking and recreational cannabis use share a number of important similarities, particularly in their methods of consumption, which require the inhalation of combusted material for chemical absorption through the lung membrane into the bloodstream. Although the chemical profile of cannabis differs from that of cigarettes, substantial overlap exists in the chemical components of the smoke inhaled from each product (16) including polycyclic aromatic hydrocarbons, carbon monoxide, and tar; each of which has been identified as a hazard to cardiovascular health (17–19). Despite these similarities, there are also some notable differences in the characteristic smoking behaviors associated with cannabis use. Cannabis smoking typically involves greater inhalation volumes and prolonged breath holds, both of which augment the deposition of tar and absorption of carbon monoxide (20–22). These factors alone suggest that cannabis could present a greater threat to cardiovascular health than cigarettes. However, it is notable that cannabis is typically smoked less frequently (22), and certain cannabinoids possess notable anti-inflammatory properties (23, 24). Thus, how each of these factors associated with cannabis smoking interact to impact cardiovascular function warrants investigation.

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There are limited investigations that directly examine the effects of cannabis use on human cardiovascular function, or cardiovascular risk factors that precede the development of CVD. Recent work has identified greater cardiac chamber volumes and reduced global circumferential strain in cannabis users (25). A single study performed in a primary care opioid addictions clinic has demonstrated that cannabis users may experience an accelerated loss of arterial compliance compared to concomitant users of tobacco and cannabis, and nonusers (26). In an animal model, acute exposure to second-hand cannabis smoke has also been demonstrated to reduce endothelial function (27). At present, there are no studies that directly compare measures of arterial or cardiac structure and function in cannabis users in a controlled laboratory setting, and at present, the degree to which habitual cannabis smoking impacts the cardiovascular system remains unclear.

The aim of the present study was to examine the relationship of chronic cannabis use with measures of cardiac morphology and function, arterial stiffness, and endothelial function in young, healthy cannabis smokers. We hypothesized that cannabis users would demonstrate altered cardiac function, greater arterial stiffness, and impairments in endothelial function compared to young, healthy, and nonusers of cannabis.

METHODS

Subjects

The present study employed a cross-sectional design, comparing cardiovascular variables of interest in cannabis users and nonusers. Participants were recruited by print advertisement and word of mouth in and around the university campus. Inclusion criteria captured those between 19 and 45 yr of age and required participants to have either smoked cannabis <5 times in their lifetime (controls) or have smoked cannabis for a minimum of 3 "cannabis years" (cannabis users). One cannabis year was defined as a year where cannabis was smoked once per week or an equivalent usage (e.g., 6 mo where cannabis was smoked twice per week). This definition was adapted from "pack years" traditionally used in cigarette literature, with consideration given to the aforementioned differences between typical frequencies of cannabis and cigarette smoking. Exclusion criteria comprised stage ≥ 1 hypertension (28), diagnoses of chronic cardiovascular or metabolic diseases, engagement in $\geq$150 min of physical activity per week in the preceding year, active use of prescription medication exclusive of oral contraceptives, previous use of recreational drugs other than cannabis, cigarette smoking, and a body mass index (BMI) $> 30\ kg/m^2$. Testing of female participants occurred during the early follicular phase or during the placebo phase for participants taking oral contraceptives.

General Procedure

Participants visited the laboratory on two separate occasions. In *visit 1*, participants provided written informed consent and completed an investigator-generated questionnaire to confirm eligibility. Information was also collected regarding physical activity, frequency of cannabis use, duration of cannabis use, and total lifetime cannabis use. Participants were then classified as either cannabis users or controls using stated criteria.

Prior to the second laboratory visit, participants were asked to abstain from: cannabis use (48 h), alcohol, vitamins, caffeine, and exercise (24 h), as well as any food or calorie-containing beverages (minimum 4 h). Participant height and weight were measured using a standard stadiometer (SECA; Hamburg, Germany) and a digital scale (Tanita; Tokyo, Japan) calibrated before each measurement. Brachial blood pressure was measured in triplicate, with subjects in the seated position using an automated sphygmomanometer (BpTRU; Coquitlam, Canada) according to the guidelines of the American Heart Association (29). Participants were then transitioned to an examination table where they rested awake in the supine position for a 10-min period, after which brachial blood pressure was again measured in triplicate. Arterial stiffness and vascular endothelial function were then assessed using noninvasive measures of carotid-femoral pulse wave velocity (cfPWV) and brachial artery reactive hyperemia flow-mediated dilation (FMD). Assessments of cfPWV and FMD were performed before a resting echocardiographic scan with and without the added stress of isometric handgrip exercise. Although this test is not the same as stress-echocardiography, isometric handgrip exercise has been demonstrated to induce a pressor response, and increase sympathetic activity (30). All testing was performed in a private, thermoneutral, and humidity-controlled laboratory environment.

Echocardiography

Resting transthoracic echocardiographic scans were performed with the participant in the left lateral decubitus position. Images were captured from the apical four-chamber view, parasternal long-axis view, and the parasternal short-axis view at the basal, mid-papillary, and apical planes in accordance with current recommendations from the American Society of Echocardiography (31). All images were collected using an ultrasound device (Vivid-Q, General Electric; Boston), and a 1.5–3.6-MHz phased array transducer. Two-dimensional B-mode imaging and tissue Doppler imaging was performed at a minimum frame rate of 50 and 100 frames per second, respectively.

After completion of resting echocardiographic scans, participants performed two maximal voluntary contractions (MVC) using a handgrip dynamometer (Baseline; White Plains). Each MVC was separated by 5 min. In an identical setting to the resting echocardiographic scans, subjects were required to perform an isometric contraction targeting 30% of MVC with the left hand for a period of 3 min while images were collected from the apical four-chamber, and the parasternal short-axis view at the basal and apical planes. Brachial blood pressures were measured using an automated sphygmomanometer placed around the right arm. The sphygmomanometer logged brachial blood pressures and heart rate at each minute during the stress test with the first measurement occurring at the start of the test, and the final measurement occurring at the end of the 3-min test. All images collected during rest and during the isometric handgrip stress test were saved and stored for offline analysis.

Pulse Wave Velocity

Aortic stiffness was measured using cfPWV, which represents the propagation velocity of a systolic pressure wave through the aorta by measuring the time delay between systolic ejection and pulse arrival times at peripheral sites of known distances from the heart (32). cfPWV is a noninvasive surrogate of central artery stiffness given the relationship between stiffness and propagation velocity (33). cfPWV assessments were completed using a commercially available tonometry system (Sphygmocor CPVH, Atcor Medical; Sydney, Australia). cfPWV was measured with subjects in the supine position. Subjects were instrumented with a single-lead (Lead II) electrocardiogram, and the carotid and femoral (at the inguinal ligament) arteries were palpated to find the strongest pulse, which was marked with an indelible marker for repeat measures. A high-fidelity tonometer (Millar Instruments; Houston) was placed at each arterial pulse site until 10 high-quality pulse waves were recorded. The time of arrival of the pulse at the carotid site and the femoral site from each cardiac cycle was used to determine a foot-to-foot pulse transit time for each pulse pressure waveform via the intersecting tangent method. If heart rate differed by greater than 5 beats per minute between sites the measurement was discarded, in accordance with expert consensus (32). For velocity calculations, distance was measured with a standard anthropometric tape using a straight line above the body from the carotid to femoral sites, with an adjustment for the distance from the carotid site to the supra-sternal notch. Pulse transit distance was calculated by subtracting the distance from the carotid site to the suprasternal notch from the distance between the carotid site and the femoral site (33). cfPWV was determined as the average quotient of pulse transit distance and pulse transit time of all measured cycles. Three measures of cfPWV were collected and the mean cfPWV was taken as the value for each participant.

Reactive Hyperemia Flow-Mediated Dilation

Endothelial function was assessed using a brachial artery reactive hyperemia FMD test, which measures the vasodilatory response to reactive hyperemia. The right arm was supinated and abducted 90° at the shoulder while supported in a custom-made foam block at the level of the heart. A manual sphygmomanometer was placed around the subjects' forearm immediately distal to the antecubital fossa. An ultrasound device (Vivid-Q, General Electric; Boston) with a 6–13-MHz linear array transducer was used to image the brachial artery ~5–10 cm proximal to the edge of the tourniquet. Duplex ultrasound with an insonation angle of 60° and a sample volume spanning arterial diameter was used to simultaneously measure blood velocity and arterial diameter throughout the reactive hyperemia FMD test using continuous edge detection software (Quipu Medical; Pisa, Italy). After a satisfactory image was obtained, wherein the blood velocity signal was optimized and the arterial luminal border could be clearly defined, reactive hyperemia was assessed, starting with a 1 min baseline imaging period, after which the sphygmomanometer was inflated to a pressure of 250 mmHg for 5 min. Upon tourniquet, deflation imaging was continued for a 3-min recovery period. The selected durations of each phase of the reactive hyperemia FMD test were selected to match frequently used protocols, which adequately capture baseline diameter, occlude blood flow, and FMD (34).

Data Analysis

Cardiac structure and function.

All cardiac analyses were performed using EchoPac software (General Electric Healthcare; Boston) by one investigator blinded to group and all identifying information. Every echocardiographic image collected included five cardiac cycles, from which three consecutive cycles of sufficient image quality for analysis were selected. Each cardiac measurement reported was determined as the average derived from these three consecutive cardiac cycles. Left ventricular (LV) volumes were determined using the modified Simpson's Biplane analysis, and LV mass was calculated using the area-length method (35). Fractional shortening, LV internal diameter (LVIDd), and both interventricular septal and posterior wall thickness (LVPWd) at end diastole were measured in the parasternal long-axis view. Relative wall thickness was determined as $2 \bullet (\text{LVPWd} \bullet \text{LVIDd}^{-1})$. LV length was determined as the distance between the mitral plane and the most distal endocardial border observable in the four-chamber view. Peak early transmitral filling velocity (E) and peak late transmitral filling velocity (A) were measured with Doppler sampling, distal to the mitral valve at the point where the mitral valve leaflets no longer entered the sample volume. Mean mitral annular peak early velocity (E′), mean mitral annular peak late velocity (A′), mean mitral annular peak systolic velocity (S′), and isovolumetric contraction and relaxation times were obtained via tissue Doppler imaging and were determined as averages recorded from the interventricular septum and LV lateral wall at the mitral annular plane. Right ventricular (RV) areas were measured in the four-chamber view, at end systole and end diastole, and right ventricular fractional area change was calculated as $((\text{RV end diastolic area} - \text{RV end systolic area}) \bullet \text{RV end diastolic area}^{-1} \bullet 100)$. Arterial elastance was calculated by dividing end systolic pressure (brachial systolic blood pressure • 0.9) by stroke volume. Ventricular elastance was calculated by dividing end systolic pressure by end systolic volume. Vascular ventricular coupling was determined as the ratio of arterial elastance to ventricular elastance.

LV mechanics.

Parasternal short-axis views at the basal and apical levels were used to capture two-dimensional (2D) images for speckle tracking analysis of LV twist. Rotation and rotation rates were calculated as the average values of six individual myocardial wall segments. Rotation in the counterclockwise direction was defined as positive, whereas rotation in the clockwise direction was defined as negative. LV twist was defined as the difference between the peak rotation values at the basal and apical levels. LV peak twisting rate and untwisting rate were defined as the peak rotational velocity during systole and diastole, respectively.

LV strain was assessed via speckle tracking analysis of apical four-chamber and parasternal short-axis views. Specifically, radial and circumferential strain and strain rates were assessed from parasternal short-axis views at the mid-papillary level, and longitudinal strain was

assessed from the apical four-chamber view. Strain in each plane was determined as the average of strain of six individual myocardial wall segments.

All speckle tracking analysis was performed for three cardiac cycles for a given measure. If an individual myocardial wall segment was not imaged with significant quality for speckle tracking analysis or could not be accurately tracked as determined by the automated software, the segment was not included in the calculation of the average value for that particular cardiac cycle.

Pulse wave analysis.

To evaluate central wave reflection characteristics, a transfer function was applied to the carotid pulse waveforms collected during the measurement of pulse wave velocity. This analysis was performed post hoc using a transfer function native to the SphygmoCor software. This analysis generated measures of aortic systolic blood pressure (aSBP), aortic diastolic blood pressure (aDBP), augmentation index (AIx), AIx normalized to a heart rate of 75 beats per minute (AIx75), and Buckberg subendocardial viability ration (SEVR). Measurements were only included in this analysis if the software indicated an operator index greater than 75.

FMD.

Each reactive hyperemia FMD test was analyzed using continuous edge detection software by one investigator blinded to group and all identifying information. Arterial diameter was averaged into 3 s bins throughout the test, and missing data from tracking errors were interpolated from surrounding time bins. Antegrade and retrograde blood velocity was averaged to determine mean blood velocity and similarly expressed in 3 s time bins. Shear rate (SR) was calculated as the quotient of mean blood velocity and arterial diameter • 4. Shear rate area under the curve (SR AUC) was calculated as the sum of shear rate 3 s time bins in the first 60 s of recovery and up to the time of peak diameter change for each individual. Peak FMD% was determined as the percentage increase in diameter from baseline during the recovery period of the reactive hyperemia FMD test. To further account for potential confounds of between group differences by baseline diameter, allometric scaling was applied according to previously published methods (36). Peak FMD% and allometrically scaled peak FMD% are presented separately.

Statistical Analysis

A Fisher exact test was used to compare sex distribution in each group. For nonrepeated measures, independent samples *t* tests were used to compare group means of a given outcome between cannabis users and nonusers. This applied to all measures of anthropometrics, resting hemodynamics, resting measures of cardiac structure and function, cfPWV, and variables measured during the reactive hyperemia FMD test. Allometric scaling of each FMD test was performed by first calculating the natural logarithm of the absolute diameter change and baseline diameter. An analysis of covariance was performed with group as a fixed factor, log-transformed absolute diameter change as the outcome variable, and log-transformed baseline diameter as a covariate. Estimated marginal means were calculated for each group and were exponentiated and converted from a ratio statistic to a percentage change. To evaluate group differences during the isometric hand-grip stress test, a 2 (group) × 2 (rest-exercise) repeated-measures analysis of variance with Bonferroni corrected post hoc comparisons were performed with each cardiac measure as the outcome variable. For all tests, a two-sided $P \leq 0.05$ was defined as significant a priori. Same-day within-subject intraclass correlation coefficients for PWV, LV end diastolic volume, RV end systolic area, and E:A were 0.96, 0.87, 0.97, and 0.95, respectively.

RESULTS

Subjects

A total of 35 participants we recruited for this study, 18 cannabis users (12 M, 6 F) and 17 controls (10 M, 7 F). Descriptive participant statistics for each group are presented in Table 1. There were no differences in anthropometric measurements between groups and neither blood pressure nor heart rate differed at rest. Cannabis users reported smoking cannabis 4.9 ± 4.4 times/wk for 5.5 ± 1.9 yr. Echocardiographic data at rest and during isometric hand-grip are presented for a subset of 28 (14 cannabis users, 14 controls) subjects matched for height, weight, age, sex, body surface area (BSA), and ethnicity. Full LV twist and LV strain data are presented for 16 matched subjects (8 cannabis users, 8 controls). Matching and selection of this sample was performed before echocardiographic analysis. None of age, height, weight, sex, and BSA differed between groups in the

Table 1. *Participant characteristics and absolute values of brachial artery diameter and shear rate during a reactive hyperemia FMD test in controls (n = 17) and cannabis users (n = 18)*

	Controls	Cannabis Users	*P*
Sex (M/F)	10/7	12/6	0.9
Age, yr	21 ± 3	22 ± 2	0.8
Height, cm	174 ± 8	174 ± 6	0.9
Weight, kg	71 ± 10	72 ± 12	0.8
BMI, kg/m^2	23.4 ± 2.3	24.7 ± 3.8	0.8
BSA, m^2	1.9 ± 0.2	1.9 ± 0.2	0.9
Heart rate, beats/min	68 ± 8	64 ± 8	0.2
SBP, mmHg	111 ± 8	110 ± 8	0.6
DBP, mmHg	69 ± 8	66 ± 5	0.3
MAP, mmHg	83 ± 7	81 ± 5	0.3
aSBP, mmHg	118 ± 8	115 ± 9	0.5
aDBP, mmHg	68 ± 7	65 ± 5	0.2
Aix	-4 ± 12	2 ± 10	0.2
AIx75	-9 ± 15	-5 ± 12	0.4
Buckberg SEVR	160 ± 20	172 ± 23	0.1
Baseline diameter, mm	3.77 ± 0.64	3.96 ± 0.70	0.4
Occlusion diameter, mm	3.77 ± 0.60	3.97 ± 0.65	0.4
Peak diameter, mm	4.00 ± 0.60	4.24 ± 0.70	0.3
Baseline SR, s^{-1}	61.5 ± 31.8	62.1 ± 37.5	0.9
Peak SR, s^{-1}	477.8 ± 163.3	417.8 ± 165.9	0.3

Heart rate, blood pressure, and FMD were measured in the supine position. Sex distribution between groups was compared using Fisher's exact test. All other means were compared using independent samples *t* tests. Data are presented as means ± SD. aSBP, aortic systolic blood pressure; aDBP, aortic diastolic blood pressure; Aix, augmentation index; AIx75, augmentation index @75bpm; BMI, body mass index; BSA, body surface area; DBP, diastolic blood pressure; FMD, flow-mediated dilation; MAP, mean arterial pressure; SBP, systolic blood pressure SEVR, subendocardial viability ratio; SR, shear rate.

speckle tracking subset nor were there differences in brachial blood pressure or heart rate between groups.

Echocardiography

Measures of cardiac structure and function collected at rest, and their change in response to sustained isometric handgrip exercise, are displayed in Table 2. Brachial systolic blood pressure and diastolic blood pressure increased during isometric handgrip exercise in cannabis users and controls (all $P < 0.05$, Supplemental Figure S1, https://doi.org/10.6084/m9.figshare.13046132.v1). Traditional 2D imaging, Doppler, and tissue Doppler imaging did not reveal any differences between cannabis users and controls at rest. Each group demonstrated similar LV and RV structural characteristics. Traditional measures of systolic function were similar between groups at rest and during handgrip exercise. Diastolic function also appeared similar between groups at rest and during handgrip exercise. There were no differences in atrial measurements at rest, but right atrial area at end systole was decreased during handgrip exercise irrespective of group ($P = 0.01$). Handgrip exercise also resulted in increases in A, A', and E:E', and reductions in E:A irrespective of group (all $P < 0.05$).

Measures of LV mechanics at rest, and their change in response to sustained isometric handgrip exercise, are displayed in Table 3. Peak global longitudinal, circumferential, and radial strain were similar between groups at rest. Peak systolic and diastolic longitudinal, circumferential, and radial strain rates were also similar between groups with the exception of peak longitudinal diastolic strain rate, which was greater in cannabis users at rest. At rest, peak global LV twist and peak basal rotation were similar between groups. Peak systolic twisting and diastolic untwisting velocities were similar between groups, as were basal systolic and diastolic rotation velocities. Peak LV twist and peak systolic twist velocity were increased during handgrip exercise irrespective of group (both $P = 0.04$). Despite no observed difference in peak global LV twist, peak apical rotation was lower in cannabis users compared to controls at rest. Peak apical rotation velocities also differed between groups, as cannabis users demonstrated lower peak systolic rotation velocity and lower peak diastolic rotation velocity than controls at rest.

Table 2. *Indices of cardiac structure and function at rest and changes with isometric handgrip exercise in controls (n = 14) and cannabis users (n = 14)*

	Rest			ΔHGEX		
	Controls	Cannabis Users	*P*	Controls	Cannabis Users	*P*
LV structure						
LV internal diameter, cm	4.6 ± 0.3	4.7 ± 0.4	0.7	—	—	—
LV end diastolic volume, mL	98 ± 14	98 ± 33	0.9	8 ± 14	−0.4 ± 11	0.7
LV length, cm	7.0 ± 0.8	7.0 ± 0.5	0.8	—	—	—
LV mass, g	105 ± 29	98 ± 22	0.5	—	—	—
LV mass index, $g \cdot m^{-2}$	57 ± 12	53 ± 8	0.8	—	—	—
Relative wall thickness	0.41 ± 0.04	0.43 ± 0.07	0.6	—	—	—
LV systolic function						
S', $cm \cdot s^{-1}$	0.09 ± 0.02	0.08 ± 0.01	0.3	0.02 ± 0.03	−0.01 ± 0.01	0.1
Isovolumetric contraction time, ms	72 ± 8	67 ± 11	0.2	4 ± 17	0.05 ± 10	0.9
Ejection fraction, %	61 ± 6	61 ± 5	0.8	−1 ± 5	0.1 ± 7	0.6
Fractional shortening, %	33 ± 3	34 ± 4	0.7	—	—	—
Stroke volume, mL	59 ± 7	63 ± 12	0.4	4 ± 11	—	0.4
Cardiac output, L/min	2.5 ± 0.3	2.6 ± 0.4	0.6	2.2 ± 1.0	-0.4 ± 11	0.5
Arterial elastance, mmHg/mL	2.4 ± 0.4	2.4 ± 0.5	0.9	—	2.0 ± 0.9	—
Ventricular elastance, mmHg/mL	2.9 ± 0.8	2.6 ± 0.5	0.4	—	—	—
Vascular ventricular coupling	0.91 ± 0.2	0.97 ± 0.2	0.4	—	—	—
LV diastolic function						
E', cm/s	0.14 ± 0.02	0.13 ± 0.02	0.6	−0.02 ± 0.03	−0.01 ± 0.02	0.2
A', cm/s	0.06 ± 0.01	0.06 ± 0.01	0.2	0.02 ± 0.02	0.02 ± 0.03	0.9
E, cm/s	0.84 ± 0.14	0.82 ± 0.17	0.7	−0.002 ± 0.1	0.01 ± 0.1	0.9
A, cm/s	0.41 ± 0.09	0.39 ± 0.08	0.5	0.20 ± 0.16	0.11 ± 0.13	0.1
E:A	2.2 ± 0.6	2.2 ± 0.4	0.9	−0.70 ± 0.55	−0.45 ± 0.53	0.2
E:E'	6.1 ± 1.0	6.3 ± 1.7	0.7	1.6 ± 1.9	0.35 ± 1.7	0.1
Isovolumetric relaxation time, ms	77 ± 15	74 ± 8	0.5	4 ± 21	−2 ± 12	0.7
RV structure						
End diastolic area, cm^2	16.6 ± 2.9	17.5 ± 3.9	0.5	−0.71 ± 1.9	1.0 ± 5.4	0.9
End systolic area, cm^2	8.9 ± 1.9	9.5 ± 2.6	0.5	0.3 ± 1.6	−1.0 ± 1.7	0.3
RV fractional area change, %	46.8 ± 5.5	45.8 ± 5.4	0.7	−3.8 ± 7.3	2.8 ± 10.2	0.3
TAPSE, cm	2.5 ± 0.4	2.4 ± 0.1	0.8	—	—	—
Atria						
Left atrial area, cm^2	15.5 ± 3.4	16.1 ± 3.7	0.5	-1.1 ± 1.8	-0.8 ± 2.5	0.6
Right atrial area, cm^2	13.4 ± 3.1	14.0 ± 3.3	0.7	-0.9 ± 1.6	-1.5 ± 1.2	0.5

Resting means were compared using independent samples t tests. The effect of isometric handgrip exercise was examined using a 2 × (group) 2 (rest-exercise) repeated-measures analysis of variance with Bonferonni corrected post hoc comparisons. Data are presented as mean ± SD. A′, mean mitral annular peak late velocity; a, peak late transmitral filling velocity; E, peak early transmitral filling velocity; E′, mean mitral annular peak early velocity; FMD, flow-mediated dilation; ΔHGEX, change with isometric handgrip exercise; LV, left ventricle; RV, right ventricle; S′, mean mitral annular peak systolic velocity; TAPSE, tricuspid annular plane systolic excursion.

Table 3. *LV twist and LV strain at rest and changes with sustained isometric handgrip exercise in controls (n = 8) and cannabis users (n = 8)*

	Rest			ΔHGEX		
	Controls	Cannabis Users	P	Controls	Cannabis Users	P
LV twist						
Global						
Peak twist, °	10.0 ± 4.4	7.1 ± 5.7	0.3	4.8 ± 5.3	6.0 ± 5.5	0.8
Peak systolic twist velocity, °/s	73.0 ± 21.1	59.3 ± 31.9	0.3	33.5 ± 38.0	34.7 ± 30.6	0.9
Peak untwisting velocity, °/s	−77.6 ± 24.7	−61.9 ± 25.3	0.2	−15.7 ± 30.6	−22.8 ± 29.6	0.8
Time to peak twist, ms	338.1 ± 30.2	481.7 ± 194.2	0.08	−9.2 ± 35.8	−164.6 ± 184.4	0.2
Basal						
Peak rotation, °	−3.1 ± 2.3	−3.0 ± 1.7	0.9	−3.5 ± 5.9	−2.9 ± 3.1	0.9
Peak systolic rotation velocity, °/s	−49.0 ± 14.8	−50.0 ± 17.3	0.9	−2.5 ± 63.7	−19.9 ± 26.6	0.6
Peak diastolic rotation velocity, °/s	30.5 ± 12.3	33.3 ± 17.9	0.7	16.1 ± 35.4	14.6 ± 19.2	0.9
Time to peak rotation, ms	444.8 ± 245.2	409.0 ± 137.8	0.7	−145.3 ± 193.3	−76.1 ± 138.0	0.5
Apical						
Peak rotation, °	9.6 ± 1.5	5.5 ± 3.8	**0.02**	−0.4 ± 2.0	2.3 ± 5.1	0.3
Peak systolic rotation velocity, °/s	71.8 ± 15.5	45.2 ± 20.7	**0.01**	10.7 ± 38.0	31.1 ± 28.0	0.4
Peak diastolic rotation velocity, °/s	−68.5 ± 19.0	−47.9 ± 13.8	**0.01**	2.8 ± 25.6	−7.4 ± 12.1	0.3
Time to rotation, ms	317.4 ± 67.4	279.3 ± 139.1	0.5	−28.9 ± 59.5	−23.2 ± 148.3	0.9
LV strain						
Radial						
Peak strain, %	32.5 ± 16.7	35.7 ± 24.1	0.8	—	—	—
Peak systolic strain rate, %/s	1.6 ± 0.4	1.8 ± 1.0	0.6	—	—	—
Peak diastolic strain rate, %/s	−1.9 ± 0.6	−1.8 ± 0.8	0.9	—	—	—
Time to peak strain, ms	436.6 ± 179.9	413.4 ± 52.9	0.7	—	—	—
Circumferential						
Peak strain, %	−15.2 ± 3.9	−16.4 ± 4.5	0.6	—	—	—
Peak systolic strain rate, %/s	−0.9 ± 0.2	−1.0 ± 0.2	0.2	—	—	—
Peak diastolic strain rate, %/s	1.1 ± 0.3	1.2 ± 0.4	0.4	—	—	—
Time to peak strain, ms	387.4 ± 49.7	381.5 ± 32.1	0.8	—	—	—
Longitudinal						
Peak strain, %	−17.4 ± 3.2	−18.9 ± 3.8	0.4	−0.2 ± 3.0	1.2 ± 4.1	0.6
Peak systolic strain rate, %/s	−0.9 ± 0.1	−1.0 ± 0.1	0.7	−0.04 ± 0.3	−0.05 ± 0.2	0.9
Peak diastolic strain rate, %/s	1.3 ± 0.4	1.7 ± 0.3	**0.02**	0.06 ± 0.3	−0.2 ± 0.6	0.3
Time to peak strain, ms	391.8 ± 20.4	396.5 ± 34.3	0.7	−20.9 ± 34.7	−4.7 ± 21.4	0.3

Resting means were compared using independent samples *t* tests. The effect isometric handgrip exercise was examined using a 2 × (group) 2(rest-exercise) repeated-measures analysis of variance with Bonferonni corrected post hoc comparisons. Data are presented as means ± SD. Bold text indicates statistical significance. ΔHGEX, change with isometric handgrip exercise; LV, left ventricle.

Differences between groups in apical rotation and systolic/diastolic apical rotation velocities were not observed during handgrip exercise.

Pulse Wave Velocity and Pulse Wave Analysis

cfPWV was recorded in all but one male cannabis user, due to an immeasurably low femoral pulse. cfPWV was found to be greater in cannabis users compared to controls (cannabis users: 5.8 ± 0.6m/s, controls: 5.3 ± 0.7m/s; $P = 0.05$; Fig. 1). Data obtained from pulse wave analysis are displayed in Table 1. Pulse wave analysis of carotid waveforms revealed that aSBP, aDBP, AIx, AIx75, and Buckberg SEVR were similar between cannabis users and controls.

Reactive Hyperemia Flow-Mediated Dilation

Absolute artery diameters and both baseline and peak SR during the reactive hyperemia FMD test are displayed in Table 1. Brachial artery diameter and SR during the baseline period of the test were not significantly different between groups. Artery diameter during the last minute of the 5-min occlusion period was also similar between groups, and there was no difference in peak artery diameter or peak SR during the recovery portion of the test. Peak FMD (cannabis users: 8.3 ± 3.3%, controls: 6.8 ± 3.6%; $P = 0.7$; Fig. 2*A*) did not differ between groups. SR AUC in the first 60 s of the recovery period and SR AUC before peak diameter change are displayed in Fig. 2, *B* and *C*, respectively. SR AUC in the first 60 s of the recovery period (cannabis users: 4,432 ± 1,630 a.u., controls: 4,900 ± 2,222 a.u.; $P = 0.7$; Fig. 2*B*) and SR AUC to the time of peak diameter change (cannabis users: 4,177 ± 1,808, controls: 4,179 ± 1,752; $P = 0.8$; Fig. 2*C*) were

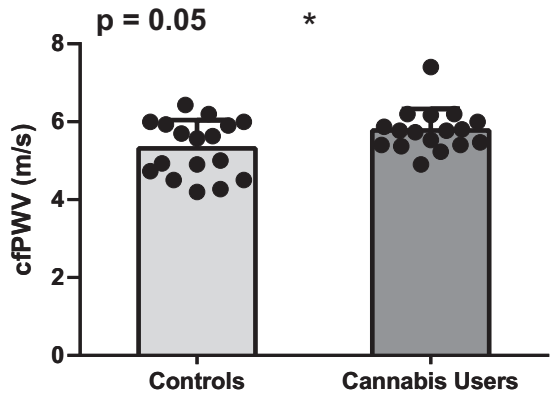

Figure 1. cfPWV measured in controls (*n* = 17) and cannabis users (*n* = 17). Means were compared using an independent samples *t* test. Bars and error bars represent mean and SD, respectively. Circles represent individual values. cfPWV, carotid-femoral pulse wave velocity.

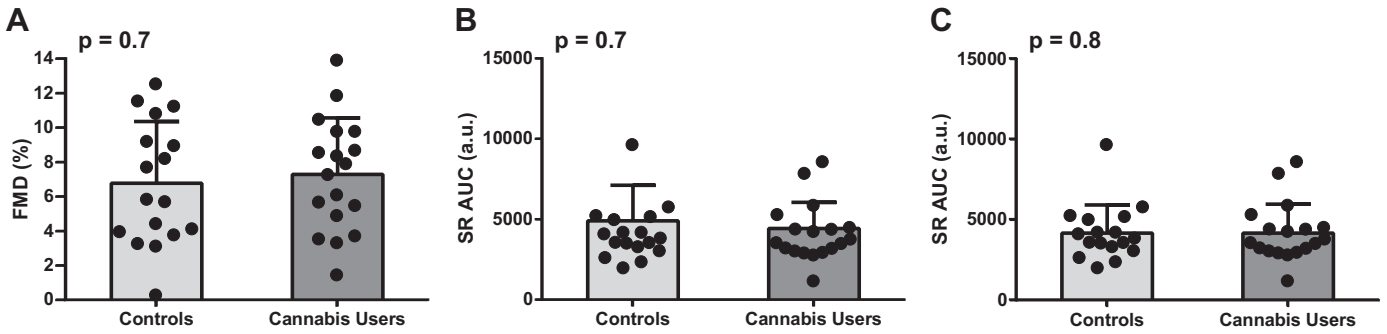

Figure 2. *A*: peak FMD expressed as a percentage of baseline diameter in controls (n = 17) and cannabis users (n = 17). *B*: SR AUC in the first 60 s of the recovery period of the reactive hyperemia FMD test in controls and cannabis users. *C*: total SR AUC to the time of peak diameter change during the recovery period of the reactive hyperemia FMD test in controls and cannabis users. All means were compared using an independent samples t test. Bars and error bars represent mean and SD, respectively. Circles represent individual values. FMD, flow-mediated dilation; SR AUC, shear rate area under the curve.

similar between groups. When an allometric scaling approach was applied, peak FMD% still remained similar between groups (cannabis users: 7.5 ± 0.6%, controls: 6.4 ± 0.6%; P = 0.2).

DISCUSSION

The major novel finding of this investigation is that young, apparently healthy, cannabis users demonstrate lower apical rotation and greater aortic stiffness compared with healthy age-matched controls. In contrast, endothelial function and cardiac responses to isometric handgrip exercise did not differ between cannabis users and nonusers. Collectively, these findings suggest there exist subclinical differences in cardiovascular physiology in cannabis users, which may be indicative of greater risk of the development of future CVD.

Cannabis users demonstrated lower peak apical rotation, peak apical systolic rotation velocity, and peak apical diastolic rotation velocity compared with controls. These differences were observed despite similar resting heart rates and blood pressure. While the precise functional significance of these alterations of cardiac mechanics among cannabis users remains unclear, peak apical rotation is a strong indicator of LV contractility (37). Studies on the effects of cannabis use have revealed that acute cannabis consumption alters systolic time intervals and tissue velocity (38, 39) and reduces both stroke index and ejection fraction (40). Experimental data also support the potential negative effects of cannabis use on systolic function, as reduced contractility in human tissue has been demonstrated in vitro with cannabinoid receptor 1 (CB1) activity (41). The most abundant cannabinoid in recreational cannabis products is typically delta-9-tetrahydrocannabinol (THC), a partial CB1 agonist. Thus, acute reductions in contractility, and consequently systolic function associated with cannabis use, may be CB1 mediated. Whether these acute effects translate to chronic effects that explain the reduced peak apical rotation and rotation velocities in cannabis users in our study requires further investigation.

Similar to our study, a previous investigation of the chronic effects of cannabis use also found an association between altered cardiac mechanics with normal systolic function and cannabis use. Khanji and colleagues (25) used cardiac MRI to examine cardiac function in cannabis users and reported no differences in ejection fraction between regular cannabis users and abstinent controls. They did, however, identify reduced global circumferential strain in cannabis users. While our ejection fraction data agree with these findings, we did not identify regional impairments in left ventricular strain that has been identified as an indicator of subclinical cardiac dysfunction (42). However, our finding of reduced peak apical rotation aligns with the notion that traditional measures of systolic function may not be sensitive to the potential early effects of regular cannabis use on the myocardium. Whether these subtle alterations to myocardial mechanics eventually manifest as clinically significant reductions in cardiac function is currently unknown. It should be noted that both groups demonstrated LV twist and rotation within age-expected ranges (43). Future work with larger sample sizes is necessary to extrapolate this finding to the general population. An important null finding in both our investigation and past work is the lack of difference between cannabis users and nonusers in LV mass, an independent predictor of increased CVD (44), which has been demonstrated to be elevated in cigarette smokers (11). This discrepancy between smoking populations may be explained by the notion that greater LV mass is related to higher blood pressure in cigarette smokers (45), whereas cannabis smokers may not experience chronically elevated blood pressure (46). It is also important to note that we did not observe any impairment in the cardiac response to isometric exercise in cannabis users. Although sustained isometric exercise and dynamic exercise present different hemodynamic challenges (47, 48), this finding may be considered preliminary evidence supporting preserved cardiac function during exercise in young healthy cannabis users. This is important given recent reports that suggest cannabis use with exercise is popular within certain populations, including athletes in competition (49). Further research should seek to understand how acute cannabis consumption affects cardiac function both at rest and during dynamic exercise.

Although not a universal finding, cigarette users have been reported to have advanced arterial stiffening compared to age-matched nonsmokers, independent of other risk factors such as hypertension (50, 51). Cigarette smoking has far more consistently been shown to transiently increase cfPWV; however, no study has examined whether the acute effects of cannabis use parallels the acute effects of cigarette

use in this manner. One large study of patients in an opioid addiction care clinic conducted by Reece and colleagues (26), demonstrated that vascular aging (an age-adjusted metric of arterial stiffness) and central augmentation index measured by radial artery tonometry were greater in cannabis-only users compared to opioid-only users, tobacco users, and users of both tobacco and cannabis. The present study differs from this study in that we demonstrate a much lower degree of stiffness in cannabis users, but it is important to acknowledge differences between these two studies that may explain these results. The present study was performed in a laboratory with healthy individuals, rather than a clinic, and thus could regulate participant behaviors that may have confounded outcomes before measurement (cannabis use, smoking, eating, etc.). In contrast, Reece and colleagues recorded measurements in opioid users upon intake to their clinic, making such premeasurement controls impossible. Additionally, the relatively low degree of stiffness we report may be explained by participant age and cumulative cannabis exposure. Volunteers in our study were young (22 ± 2 yr), as opposed to the middle-aged (40 ± 2 yr) adults studied in previous work. The younger population may not yet manifest changes in stiffness that are hypothesized to occur with cannabis use. Additionally, as a result of their age, demographic, and reported behaviors, we can infer that our population had less cannabis exposure. Cannabis users in the previous study had used cannabis for an average of 37.67 g-yr, where 1 g-year is the product of self-reported grams of cannabis consumed daily and years of use (52). Although we quantified "cannabis years" as opposed to gram-years, it is highly unlikely our population had reached similar exposure owing to their young age. The study of Reece and colleagues also had notable sample size imbalances, with only 11 cannabis-only users, and between 100 and 500 individuals in all other groups. These key differences may explain the differing degree of arterial stiffness we observed in our population of cannabis users.

It is also important to note the absolute cfPWV measured in each of our groups (cannabis users: 5.8 ± 0.6m/s, controls: 5.3 ± 0.7m/s) were within the expected range for individuals <30 yr of age with normal blood pressure (53). This, and the difference of 0.5 m/s between groups, suggests that the degree of aortic stiffening in young healthy cannabis users is relatively small and thus may not result in clinically meaningful differences. Future studies should include larger and more diverse samples to determine whether our results can be extrapolated to the general population. We must acknowledge that cfPWV was not significantly different between groups (P = 0.09) with the omission of one cannabis user who exhibited a cfPWV > 2SD from the group mean. Despite the loss of significance, it should be noted that this individual's cfPWV was still within expected population range (7.4 m/s) and thus was still included. Elevated cfPWV in cannabis users could represent an early cardiovascular maladaptation to cannabis smoking that worsens with more cannabis exposure. Studies directly comparing cfPWV in cannabis users with varying degrees of cannabis exposure or users of different age groups are needed to address this hypothesis. It is important to acknowledge that despite the relatively small difference in cfPWV in cannabis users that we report (0.5 m/s), even a modest increase in cfPWV of 1 m/s indicates a 15% greater risk of cardiovascular mortality (54). Thus, our findings indicate adverse vascular characteristics in cannabis users.

Contrary to our hypotheses, we did not observe impairments in endothelial function measured by reactive-hyperemia FMD in cannabis users. Given the similarities between cannabis and cigarettes, the effects of cigarette use on FMD, and evidence that implicates cannabinoid receptor-mediated pathways in impaired endothelial function we had expected that such changes would occur. Studies of cigarette smokers have revealed impairments in reactive hyperemia FMD compared to age-matched controls (5–8). Carbon monoxide and hydrocarbons, both of which are present in both cigarette and cannabis smoke, have also been shown to induce damage to the endothelium (55). The negative effects of cigarette smoke on FMD are strongly related to the generation of reactive oxygen species (ROS) and consequent reductions in nitric oxide availability (56); cannabis smoke has also been shown to produce ROS (57). Endothelial cells also express CB1, the cannabinoid receptor upon which THC acts (58). CB1 activation may negatively affect endothelial function as CB1 activation has been demonstrated to increase ROS generation by both macrophages (59) and endothelial cells (60). Thus, reduced nitric oxide availability due to generation of intravascular or endothelial ROS provides a potential mechanism for CB1-mediated impairments in FMD. Additionally, second-hand cannabis smoke has been demonstrated to reduce FMD in a rodent model, independent of THC content (61), making similar FMD between groups in our study a notable null finding.

Although FMD was not different between groups, it is notable that nonusers had greater SR and smaller baseline artery diameter, which would not be explained by healthier lifestyle choices (such as exercise). By conventional knowledge, these conditions would predispose the control group to greater FMD than cannabis users in the current study. Despite these conditions, FMD remained similar between groups, differing by 1.5%. For this difference to attain significance, a sample of 168 participants would be needed (power = 0.8 and α = 0.05). One speculative explanation for this observation may be that cannabis users have greater shear stress than nonusers due to greater blood viscosity. Therefore, the similarities in SR between groups in the present study do not represent similar stimuli for FMD, as blood viscosity is typically assumed to be equivalent, and not accounted for with SR. We did not measure blood viscosity; however, cigarette smokers have been reported to have greater blood viscosity than nonsmokers, which is ameliorated with smoking cessation (62). The similarity in FMD between cannabis users and nonusers may also be explained by the relatively young age of our participants, and insufficient lifetime exposure to cannabis smoking to meaningfully impair endothelial function. We believe this is the most likely explanation, as unlike their middle-aged and older counterparts, young healthy cigarette smokers do not always present with measurably impaired reactive hyperemia FMD compared to nonsmokers (63–65).

Limitations

Owing to the use of a cross-sectional design, we cannot establish causality between cannabis smoking and the observed

differences in cardiovascular indices. Interventional studies and/or studies employing longitudinal designs are needed to assess cause and effect relationships. Additionally, we employed self-reported cannabis use and did not implement objective screening tests to confirm recent cannabis use in our population, although there is no apparent reason for us to believe patterns of use were misrepresented by either group. Additionally, we did not sample blood to assess the inflammatory or oxidative stress profiles of participants. Hypothetically, greater levels of inflammatory markers in cannabis users compared to nonusers could help to mechanistically explain the observed differences in arterial stiffness, as a relationship between chronic inflammation and both atherosclerosis and cfPWV has been reported previously (66). Indeed, a recent study identified that cannabis users are at greater risk of CVD compared to nonusers based on circulating levels of c-reactive protein (67). This hypothesis should, however, be approached cautiously, as other studies have found contradictory results (68), and the relationship between cannabinoid receptor signaling and inflammation is multifactorial (69).

CONCLUSIONS

Here, we present evidence of differences in both cardiac mechanics and vascular wall properties between cannabis users and nonusers. Specifically, cannabis users demonstrated suboptimal apical cardiac function and higher aortic stiffness that are hypothesized to represent cardiovascular maladaptation to cannabis smoking. These differences in cardiac function and vascular structure suggest that cannabis users may be at greater risk of the development of CVD. Future work should prospectively explore causal links between cannabis smoking and altered cardiovascular function, with a goal of characterizing the relationship between cannabis use and the development of CVD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.P.C. and J.F.B. conceived and designed research; C.P.C. and A.M.C. performed experiments; C.P.C. analyzed data; C.P.C., A.M.C., P.J.M., and J.F.B. interpreted results of experiments; C.P.C. prepared figures; C.P.C. drafted manuscript; C.P.C., A.M.C., P.J.M., and J.F.B. edited and revised manuscript; C.P.C., A.M.C., P.J.M., and J.F.B. approved final version of manuscript.

REFERENCES

1. **Roth GA**, **Abate D**, **Abate KH**, **Abay SM**, **Abbafati C**, **Abbasi N**, **et al.** Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 392: 1736–1788, 2018. doi:10.1016/S0140-6736(18)32203-7.
2. **Jonas MA**, **Oates JA**, **Ockene JK**, **Hennekens CH.** Statement on smoking and cardiovascular disease for health care professionals. American Heart Association. *Circulation* 86: 1664–1669, 1992. doi:10.1161/01.CIR.86.5.1664.
3. **Doonan RJ**, **Hausvater A**, **Scallan C**, **Mikhailidis DP**, **Pilote L**, **Daskalopoulou SS.** The effect of smoking on arterial stiffness. *Hypertens Res* 33: 398–410, 2010. doi:10.1038/hr.2010.25.
4. **Mahmud A**, **Feely J.** Effect of smoking on arterial stiffness and pulse pressure amplification. *Hypertension* 41: 183–187, 2003. doi:10.1161/01.HYP.0000047464.66901.60.
5. **Amato M**, **Frigerio B**, **Castelnuovo S**, **Ravani A**, **Sansaro D**, **Tremoli E**, **Squellerio I**, **Cavalca V**, **Veglia F**, **Sirtori CR**, **Werba JP**, **Baldassarre D.** Effects of smoking regular or light cigarettes on brachial artery flow-mediated dilation. *Atherosclerosis* 228: 153–160, 2013. doi:10.1016/j.atherosclerosis.2013.02.037.
6. **Barua RS**, **Ambrose JA**, **Eales-Reynolds L-J**, **DeVoe MC**, **Zervas JG**, **Saha DC.** Dysfunctional endothelial nitric oxide biosynthesis in healthy smokers with impaired endothelium-dependent vasodilatation. *Circulation* 104: 1905–1910, 2001. doi:10.1161/hc4101.097525.
7. **Celermajer DS**, **Sorensen KE**, **Georgakopoulos D**, **Bull C**, **Thomas O**, **Robinson J**, **Deanfield JE.** Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 88: 2149–2155, 1993. doi:10.1161/01.CIR.88.5.2149.
8. **Iwamoto E**, **Washio T.** Habitual cigarette smoking attenuates shear-mediated dilation in the brachial artery but not in the carotid artery in young adults. *Physiol Rep* 8: 1–9, 2020. doi:10.14814/phy2.14369.
9. **Farsalinos K**, **Tsiapras D**, **Kyrzopoulos S**, **Voudris V.** Acute and chronic effects of smoking on myocardial function in healthy heavy smokers: a study of Doppler Flow, Doppler Tissue Velocity, and two-dimensional speckle tracking echocardiography. *Echocardiography* 30: 285–292, 2013. doi:10.1111/echo.12052.
10. **Gidding SS**, **Xie X**, **Liu K**, **Manolio T**, **Flack JM**, **Gardin JM.** Cardiac function in smokers and nonsmokers: the CARDIA study. *J Am Coll Cardiol* 26: 211–216, 1995. doi:10.1016/0735-1097(95)00118-j.
11. **Leigh JA**, **Kaplan RC**, **Swett K**, **Balfour P**, **Kansal MM**, **Talavera GA**, **Perreira K**, **Blaha MJ**, **Benjamin EJ**, **Robertson R**, **Bhartnagar A**, **Rodriguez CJ.** Smoking intensity and duration is associated with cardiac structure and function: the ECHOcardiographic Study of Hispanics/Latinos. *Open Hear* 4: 1–12, 2017. doi:10.1136/openhrt-2017-000614.
12. **Lichodziejewska B**, **Kurnicka K**, **Grudzka K**, **Małysz J**, **Ciurzyński M**, **Liszewska-Pfejfer D.** Chronic and acute effects of smoking on left and right ventricular relaxation in young healthy smokers. *Chest* 131: 1142–1148, 2007. doi:10.1378/chest.06-2056.
13. **World Health Organization.** World Drug Report: Drug Use and Health Consequences [Online]. https://wdr.unodc.org/wdr2020/field/WDR20_Booklet_2.pdf.
14. **Wadman M.** Canadian cannabis research hits licensing logjam. *Science* 365: 729–730, 2019. doi:10.1126/science.365.6455.729.
15. **Zarrabi AJ**, **Frediani JK**, **Levy JM.** The state of cannabis research legislation in 2020. *N Engl J Med* 382: 1876–1877, 2020. doi:10.1056/NEJMp2003095.
16. **Moir D**, **Rickert WS**, **Levasseur G**, **Larose Y**, **Maertens R**, **White P**, **Desjardins S.** A comparison of mainstream and sidestream marijuana and tobacco cigarette smoke produced under two machine smoking conditions. *Chem Res Toxicol* 21: 494–502, 2008. doi:10.1021/tx700275p.
17. **Alshaarawy O**, **Elbaz HA**, **Andrew ME.** The association of urinary polycyclic aromatic hydrocarbon biomarkers and cardiovascular disease in the US Population. *Environ Int* 89–90: 174–178, 2016. doi:10.1016/j.envint.2016.02.006.
18. **Astrup P**, **Kjeldsen K.** Carbon monoxide, smoking, and atherosclerosis. *Med Clin North Am* 58: 323–350, 1974. doi:10.1016/s0025-7125(16)32161-7.
19. **Higenbottam T**, **Shipley MJ**, **Rose G.** Cigarettes, lung cancer, and coronary heart disease: the effects of inhalation and tar yield. *J Epidemiol Community Health* 36: 113–117, 1982. doi:10.1136/jech.36.2.113.

20. **Azorlosa JL**, **Greenwald MK**, **Stitzer ML.** Marijuana smoking: effects of varying puff volume and breathhold duration. *J Pharmacol Exp Ther* 272: 560–569, 1995.
21. **Tashkin DP**, **Gliederer F**, **Rose J**, **Change P**, **Hui KK**, **Yu JL**, **Wu TC.** Effects of varying marijuana smoking profile on deposition of tar and absorption of CO and Delta-9-THC. *Pharmacol Biochem Behav* 40: 651–656, 1991. doi:10.1016/0091-3057(91)90377-E.
22. **Wu TV**, **Tashkin DP**, **Djahed B**, **Rose JE.** Pulmonary hazards of smoking marijuana as compared with tobacco. *N Engl J Med* 318: 347–351, 1988. doi:10.1056/NEJM198802113180603.
23. **Gallily R**, **Yekhtin Z**, **Hanuš LO.** The anti-inflammatory properties of terpenoids from cannabis. *Cannabis Cannabinoid Res* 3: 282–290, 2018. doi:10.1089/can.2018.0014.
24. **Klein TW.** Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol* 5: 400–411, 2005. doi:10.1038/nri1602.
25. **Khanji MY**, **Jensen MT**, **Kenawy AA**, **Raisi-Estabragh Z**, **Paiva JM**, **Aung N**, **Fung K**, **Lukaschuk E**, **Zemrak F**, **Lee AM**, **Barutcu A**, **Maclean E**, **Cooper J**, **Piechnik SK**, **Neubauer S**, **Petersen SE.** Association between recreational cannabis use and cardiac structure and function. *JACC Cardiovasc Imaging* 13: 886–888, 2020. doi:10.1016/j.jcmg.2019.10.012.
26. **Reece AS**, **Norman A**, **Hulse GK.** Cannabis exposure as an interactive cardiovascular risk factor and accelerant of organismal ageing: a longitudinal study. *BMJ Open* 6: e011891, 2016. doi:10.1136/bmjopen-2016-011891.
27. **Wang X**, **Derakhshandeh R**, **Liu J**, **Narayan S**, **Nabavizadeh P**, **Le S**, **Danforth OM**, **Pinnamaneni K**, **Rodriguez HJ**, **Luu E.** One minute of marijuana secondhand smoke exposure substantially impairs vascular endothelial function. *J Am Heart Assoc* 5: e003858, 2016.
28. **Reboussin DM**, **Allen NB**, **Griswold ME**, **Guallar E**, **Hong Y**, **Lackland DT**, **Miller EPR**, **Polonsky T**, **Thompson-Paul AM**, **Vupputuri S.** Systematic review for the 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association. *J Am Coll Cardiol* 71: 2176–2198, 2018. doi:10.1016/j.jacc.2017.11.004.
29. **Muntner P**, **Shimbo D**, **Carey RM**, **Charleston JB**, **Gaillard T**, **Misra S**, **Myers MG**, **Ogedegbe G**, **Schwartz JE**, **Townsend RR**, **Urbina EM**, **Viera AJ**, **White WB**, **Wright JT.** Measurement of blood pressure in humans: a scientific statement from the American Heart Association. *Hypertension* 73: E35–E66, 2019. doi:10.1161/HYP.0000000000000087.
30. **Fu Q**, **Levine BD**, **Pawelczyk JA**, **Ertl AC**, **Diedrich A**, **Cox JF**, **Zuckerman JH**, **Ray CA**, **Smith ML**, **Iwase S**, **Saito M**, **Sugiyama Y**, **Mano T**, **Zhang R**, **Iwasaki K**, **Lane LD**, **Buckeyjr JC**, **Cooke WH**, **Robertson RM**, **Baisch FJ**, **Blomqvist CG**, **Eckberg DL**, **Robertson D**, **Biaggioni I.** Cardiovascular and sympathetic neural responses to handgrip and cold pressor stimuli in humans before, during and after spaceflight. *J Physiol* 544: 653–664, 2002. doi:10.1113/jphysiol.2002.025098.
31. **Mitchell C**, **Rahko PS**, **Blauwet LA**, **Canaday B**, **Finstuen JA**, **Foster MC**, **Horton K**, **Ogunyankin KO**, **Palma RA**, **Velazquez EJ.** Guidelines for performing a comprehensive transthoracic echocardiographic examination in adults: recommendations from the American Society of Echocardiography. *J Am Soc Echocardiogr* 32: 1–64, 2019. doi:10.1016/j.echo.2018.06.004.
32. **Laurent S**, **Cockcroft J**, **Van Bortel L**, **Boutouyrie P**, **Giannattasio C**, **Hayoz D**, **Pannier B**, **Vlachopoulos C**, **Wilkinson I**, **Struijker-Boudier H**, **European Network for Non-invasive Investigation of Large Arteries.** Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 27: 2588–2605, 2006. doi:10.1093/eurheartj/ehl254.
33. **Chirinos JA.** Arterial stiffness: basic concepts and measurement techniques. *J Cardiovasc Transl Res* 5: 243–255, 2012. doi:10.1007/s12265-012-9359-6.
34. **Thijssen DHJ**, **Bruno RM**, **Van Mil ACCM**, **Holder SM**, **Faita F**, **Greyling A**, **Zock PL**, **Taddei S**, **Deanfield JE**, **Luscher T**, **Green DJ**, **Ghiadoni L.** Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J* 40: 2534–2547, 2019. doi:10.1093/eurheartj/ehz350.
35. **Lang RM**, **Badano LP**, **Victor MA**, **Afilalo J**, **Armstrong A**, **Ernande L**, **Flachskampf FA**, **Foster E**, **Goldstein SA**, **Kuznetsova T**, **Lancellotti P**, **Muraru D**, **Picard MH**, **Retzschel ER**, **Rudski L**, **Spencer KT**, **Tsang W**, **Voigt JU.** Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 28: 1–39.e14, 2015. doi:10.1016/j.echo.2014.10.003.
36. **Atkinson G**, **Batterham AM.** Allometric scaling of diameter change in the original flow-mediated dilation protocol. *Atherosclerosis* 226: 425–427, 2013. doi:10.1016/j.atherosclerosis.2012.11.027.
37. **Kim WJ**, **Lee BH**, **Kim YJ**, **Kang JH**, **Jung YJ**, **Song JM**, **Kang DH**, **Song JK.** Apical rotation assessed by speckle-tracking echocardiography as an index of global left ventricular contractility. *Circ Cardiovasc Imaging* 2: 123–131, 2009. doi:10.1161/CIRCIMAGING.108.794719.
38. **Kanakis C**, **Pouget JM**, **Rosen KM.** The effects of Delta-9-tetrahydrocannabinol (cannabis) on cardiac performance with and without beta blockade. *Circulation* 53: 703–707, 1976. doi:10.1161/01.CIR.53.4.703.
39. **Tashkin DP**, **Levisman JA**, **Abbasi AS**, **Shapiro BJ**, **Ellis NM.** Short term effects of smoked marihuana on left ventricular function in man. *Chest* 72: 20–26, 1977. doi:10.1378/chest.72.1.20.
40. **Prakash R**, **Aronow WS**, **Warren M**, **Laverty W**, **Gottschalk LA.** Effects of marihuana and placebo marihuana smoking on hemodynamics in coronary disease. *Clin Pharmacol Ther* 18: 90–95, 1975. doi:10.1002/cpt197518190.
41. **Bonz A**, **Laser M**, **Kullmer S**, **Kniesch S**, **Babin-Ebell J**, **Popp V**, **Ertl G**, **Wagner JA.** Cannabinoids acting on cb1 receptors decrease contractile performance in human atrial muscle. *J Cardiovasc Pharmacol* 41: 657–664, 2003. doi:10.1097/00005344-200304000-00020.
42. **Gunasekaran P**, **Panaich S**, **Briasoulis A**, **Cardozo S**, **Afonso L.** Incremental value of two dimensional speckle tracking echocardiography in the functional assessment and characterization of subclinical left ventricular dysfunction. *Curr Cardiol Rev* 13: 32–40, 2017. doi:10.2174/1573403x12666160712095938.
43. **Kormányos Á**, **Kalapos A**, **Domsik P**, **Lengyel C**, **Forster T**, **Nemes A.** Normal values of left ventricular rotational parameters in healthy adults—insights from the three-dimensional speckle tracking echocardiographic MAGYAR-Healthy Study. *Echocardiography* 36: 714–721, 2019. doi:10.1111/echo.14285.
44. **Levy D**, **Garrison RJ**, **Savage DD**, **Kannel WB**, **Castelli WP.** Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 322: 1561–1566, 1990. doi:10.1056/NEJM199005313222203.
45. **Majahalme S**, **Turjanmaa V**, **Weder A**, **Lu H**, **Tuomisto M**, **Virjo A**, **Uusitalo A.** Blood pressure levels and variability, smoking, and left ventricular structure in normotension and in borderline and mild hypertension. *Am J Hypertens* 9: 1110–1118, 1996. doi:10.1016/0895-7061(96)00197-5.
46. **Alshaarawy O**, **Elbaz HA.** Cannabis use and blood pressure levels: United States National Health and Nutrition Examination Survey, 2005–2012. *J Hypertens* 34: 1507–1502, 2016. doi:10.1016/j.physbeh.2017.03.040.
47. **Åstrand P-O**, **Cuddy TE**, **Saltin B**, **Stenberg J.** Cardiac output during submaximal and maximal work. *J Appl Physiol* 19: 268–274, 1964. doi:10.1152/jappl.1964.19.2.268.
48. **Schibye B**, **Mitchell JH**, **Payne FC**, **Saltin B.** Blood pressure and heart rate response to static exercise in relation to electromyographic activity and force development. *Acta Physiol Scand* 113: 61–66, 1981. doi:10.1111/j.1748-1716.1981.tb06862.x.
49. **Campian MD**, **Flis AE**, **Teramoto M**, **Cushman DM.** Self-reported use and attitudes toward performance-enhancing drugs in ultramarathon running. *Wilderness Environ Med* 29: 330–337, 2018. doi:10.1016/j.wem.2018.04.004.
50. **Liang YL**, **Shiel LM**, **Teede H**, **Kotsopoulos D**, **McNeil J**, **Cameron JD**, **McGrath BP.** Effects of blood pressure, smoking, and their interaction on carotid artery structure and function. *Hypertension* 37: 6–11, 2001. doi:10.1161/01.HYP.37.1.6.
51. **Stefanadis C**, **Tsiamis E**, **Vlachopoulos C**, **Stratos C**, **Toutouzas K**, **Pitsavos C**, **Marakas S**, **Boudoulas H**, **Toutouzas P.** Unfavorable effect of smoking on the elastic properties of the human aorta. *Circulation* 95: 31–38, 1997. doi:10.1161/01.CIR.95.1.31.
52. **Wetherill RR**, **Hager N**, **Guthier E**, **Franklin TR.** Gram years: a method to standardize and quantify lifetime cannabis consumption.

Cannabis Cannabinoid Res 1: 216–217, 2016. doi:10.1089/can.2016.0025.

53. **Mattace-Raso FUS**, **Hofman A**, **Verwoert GC**, **Wittemana JCM**, **Wilkinson I**, **Cockcroft J**, **McEniery C**, **et al.** Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values.' *Eur Heart J* 31: 2338–2350, 2010. doi:10.1093/eurheartj/ehq165.
54. **Vlachopoulos C**, **Aznaouridis K**, **Stefanadis C.** Prediction of cardiovascular events and all-cause mortality with arterial stiffness. A systematic review and meta-analysis. *J Am Coll Cardiol* 55: 1318–1327, 2010. doi:10.1016/j.jacc.2009.10.061.
55. **Schneiderman G**, **Goldstick TK.** Carbon monoxide-induced arterial wall hypoxia and atherosclerosis. *Atherosclerosis* 30: 1–15, 1978. doi:10.1016/0021-9150(78)90148-x.
56. **Raij L**, **DeMaster EG**, **Jaimes EA.** Cigarette smoke-induced endothelium dysfunction: role of superoxide anion. *J Hypertens* 19: 891–897, 2001. doi:10.1097/00004872-200105000-00009.
57. **Sarafian TA**, **Antonio J**, **Magallanes M**, **Shau H**, **Tashkin D**, **Roth MD.** Oxidative stress produced by marijuana smoke an adverse effect enhanced by cannabinoids. *Am J Respir Cell Mol Biol* 20: 1286–1293, 1999. doi:10.1165/ajrcmb.20.6.3424.
58. **Liu J**, **Gao B**, **Mirshahi F**, **Sanyal AJ**, **Khanolkar AD**, **Makriyannis A**, **Kunos G.** Functional CB1 cannabinoid receptors in human vascular endothelial cells. *Biochem J* 346: 835–840, 2000. doi:10.1042/0264-6021:3460835.
59. **Han KH**, **Lim S**, **Ryu J**, **Lee CW**, **Kim Y**, **Kang JH**, **Kang SS**, **Ahn YK**, **Park CS**, **Kim JJ.** CB1 and CB2 cannabinoid receptors differentially regulate the production of reactive oxygen species by macrophages. *Cardiovasc Res* 84: 378–386, 2009. doi:10.1093/cvr/cvp240.
60. **Rajesh M**, **Mukhopadhyay P**, **Haskó G**, **Liaudet L**, **MacKie K**, **Pacher P.** Cannabinoid-1 receptor activation induces reactive oxygen species-dependent and -independent mitogen-activated protein kinase activation and cell death in human coronary artery endothelial cells. *Br J Pharmacol* 160: 688–700, 2010. doi:10.1111/j.1476-5381.2010.00712.x.
61. **Thomsen HK.** Carbon monoxide-induced atherosclerosis in primates. *Atherosclerosis* 20: 233–240, 1974. doi:10.1016/0021-9150(74)90008-2.
62. **Shimada S**, **Hasegawa K**, **Wada H**, **Terashima S**, **Satoh-Asahara N**, **Yamakage H**, **Kitaoka S**, **Akao M**, **Shimatsu A**, **Takahashi Y.** High blood viscosity is closely associated with cigarette smoking and markedly reduced by smoking cessation. *Circ J* 75: 185–189, 2011. doi:10.1253/circj.CJ-10-0335.
63. **Findlay BB**, **Gupta P**, **Szijgyarto IC**, **Pyke KE.** Impaired brachial artery flow-mediated vasodilation in response to handgrip exercise-induced increases in shear stress in young smokers. *Vasc Med* 18: 63–71, 2013. doi:10.1177/1358863X13480259.
64. **Rooks CR**, **McCully KK**, **Dishman RK.** Acute exercise improves endothelial function despite increasing vascular resistance during stress in smokers and nonsmokers. *Psychophysiology* 48: 1299–1308, 2011. doi:10.1111/j.1469-8986.2011.01194.x.
65. **Stoner L**, **Sabatier M**, **Edge K**, **McCully K.** Relationship between blood velocity and conduit artery diameter and the effects of smoking on vascular responsiveness. *J Appl Physiol (1985)* 96: 2139–2145, 2004. doi:10.1152/japplphysiol.01107.2003.
66. **Nagano M**, **Nakamura M**, **Sato K**, **Tanaka F**, **Segawa T**, **Hiramori K.** Association between serum C-reactive protein levels and pulse wave velocity: a population-based cross-sectional study in a general population. *Atherosclerosis* 180: 189–195, 2005. doi:10.1016/j.atherosclerosis.2004.11.019.
67. **Lisano JK**, **Kisiolek JN**, **Smoak P**, **Phillips KT**, **Stewart LK.** Chronic cannabis use and circulating biomarkers of neural health, stress, and inflammation in physically active individuals. *Appl Physiol Nutr Metab* 45: 258–263, 2020. doi:10.1139/apnm-2019-0300.
68. **Alshaarawy O**, **Anthony JC.** Cannabis smoking and serum c-reactive protein: a quantile regressions approach based on NHANES 2005-2010. *Drug Alcohol Depend* 147: 203–207, 2015. doi:10.1016/j.drugalcdep.2014.11.017.
69. **Pacher P**, **Steffens S**, **Haskó G**, **Schindler TH**, **Kunos G.** Cardiovascular effects of marijuana and synthetic cannabinoids: the good, the bad, and the ugly. *Nat Rev Cardiol* 15: 151–166, 2018. doi:10.1038/nrcardio.2017.130.