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Special Issue

Plasma Cannabinoid Pharmacokinetics After Controlled Smoking and *Ad libitum* Cannabis Smoking in Chronic Frequent Users

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More Americans are dependent on cannabis than any other illicit drug. The main analytes for cannabis testing include the primary psychoactive constituent, Δ^9-tetrahydrocannabinol (THC), equipotent 11-hydroxy-THC (11-OH-THC) and inactive 11-nor-9-carboxy-THC (THCCOOH). Eleven adult chronic frequent cannabis smokers resided on a closed research unit with unlimited access to 5.9% THC cannabis cigarettes from 12:00 to 23:00 during two *ad libitum* smoking phases, followed by a 5-day abstinence period in seven participants. A single cigarette was smoked under controlled topography on the last day of the smoking and abstinence phases. Plasma cannabinoids were quantified by two-dimensional gas chromatography–mass spectrometry. Median plasma maximum concentrations (C_{max}) were 28.3 (THC), 3.9 (11-OH-THC) and 47.0 μg/L (THCCOOH) 0.5 h after controlled single cannabis smoking. Median C_{max} 0.2–0.5 h after *ad libitum* smoking was higher for all analytes: 83.5 (THC), 14.2 (11-OH-THC) and 155 μg/L (THCCOOH). All 11 participants' plasma samples were THC and THCCOOH-positive, 58.3% had THC ≥5 μg/L and 79.2% were 11-OH-THC-positive 8.1–14 h after last cannabis smoking. Cannabinoid detection rates in seven participants 106–112 h (4–5 days) after last smoking were 92.9 (THC), 35.7 (11-OH-THC) and 100% (THCCOOH), with limits of quantification of 0.5 μg/L for THC and THCCOOH, and 1.0 μg/L for 11-OH-THC. These data greatly expand prior research findings on cannabinoid excretion profiles in chronic frequent cannabis smokers during *ad libitum* smoking. Smoking multiple cannabis cigarettes led to higher C_{max} and AUC compared with smoking a single cigarette. The chronic frequent cannabis smokers exhibited extended detection windows for plasma cannabinoids, reflecting a large cannabinoid body burden.

Introduction

Cannabis is the most widely used illicit drug worldwide (1), with an estimated 19.8 million Americans aged 12 years or older smoking cannabis past month in 2013 and about 6,600 new initiates daily (2). Controlled laboratory research indicates that acute cannabis intoxication can impair driving performance. Cannabis is also the most prevalent illicit drug detected in motor vehicle accidents and fatalities (3), suggesting that cannabis-impaired driving significantly impacts public safety. In 2013, 10.6% of the US young adults (ages 18–25 years) reported driving under the influence of illicit drugs (2), with more drivers testing positive for drugs (22.5%) than for alcohol (8.3%) in 2013–2014 (4). Cannabinoids were detected in 7.4% oral fluid samples of California weekend nighttime drivers in random traffic stops (5). Nationally, 12.6% of weekend nighttime drivers providing oral fluid and/or blood in random traffic stops were cannabinoid-positive in the 2013–2014 roadside survey, and 48% increase from the 2007 survey (4); blood alcohol concentrations ≥0.08 g/dL were found in only 1.5% of drivers. Cannabis-impaired driving was responsible for 14% of fatally injured and 19% of non-fatally injured US drivers in 2003 (6). The major difficulty in interpreting roadside testing with respect to cannabis use is that the window of Δ^9-tetrahydrocannabinol (THC) detection outlasts the acute effects associated with driving impairment in chronic frequent cannabis smokers.

THC is the primary psychoactive component of cannabis, with bioavailability of ~25% via the smoked route, and a plasma terminal half-life of ~4 days (7). THC is metabolized via CYP2C9 and 2C19, producing the equipotent metabolite, 11-hydroxy-THC (11-OH-THC), and after further oxidation, the non-psychoactive metabolite 11-nor-9-carboxy-THC (THCCOOH) (8). We recently investigated plasma THC phase I and II metabolites following single controlled cannabis smoking in chronic frequent cannabis smokers (9). We now evaluate cannabinoid pharmacokinetics during *ad libitum* cannabis smoking and during 5 days cannabis abstinence in the same population. The primary aim of the study was to elucidate plasma cannabinoid disposition during *ad libitum* cannabis smoking, a more realistic smoking scenario for chronic smokers, and compare with that after a single smoked cannabis cigarette. Furthermore, different THC cutoff concentrations were examined that affect detection rates and windows of detection for positive cannabinoid samples.

Methods

Participants

Chronic frequent cannabis smokers were recruited from the community via advertisements. Eligibility criteria included (i) being at least 18 years old, (ii) being physically and psychologically healthy based on comprehensive medical and psychological evaluations, (iii) negative urine test for drugs other than cannabis, (iv) negative breath alcohol test at admission, (v) not meeting diagnostic criteria for Axis I psychiatric disorders (DSM-IV-TR) other than nicotine or cannabis dependence, (v) self-reported cannabis smoking at least 25 days per month for the prior 3 months, (vi) reported experiencing cannabis withdrawal symptoms of at least moderate severity, (vii) not seeking treatment for cannabis use disorder or using medical cannabis and (viii) a negative urine pregnancy test if female. All participants provided written informed consent to take part in this John Hopkins Bayview Medical Center Institutional Review Board-approved study. Participants resided on a residential research unit at the Johns Hopkins Bayview Medical Center for 51 days under constant 24-h medical surveillance. There were no dietary or physical activity restrictions.

Study design

Plasma cannabinoid concentrations after *ad libitum* cannabis smoking were compared with thrice-daily dronabinol doses (0, 10, 20 and 40 mg/3× day; counterbalanced order) for 5 days in a within-subjects crossover design (Figure 1). Data were obtained as part of a 51-day study investigating cannabis withdrawal symptoms, cognitive performance and physiological assessments during dronabinol administration interspersed with *ad libitum* cannabis smoking (11). The present report focuses on *ad libitum* cannabis smoking and abstinence (placebo dronabinol) phases. Following admission to the clinical research unit, participants had a 4-day *ad libitum* cannabis smoking phase (from 12:00 to 23:00 each day) for laboratory acclimation, with blood collection on the fourth day; followed by a 5-day smoked cannabis abstinence [when placebo or one of three doses of dronabinol (30, 60 or 120 mg/day) was given] phase with blood collection on the first and last days. Controlled smoking of a single cannabis cigarette started at approximately 11:30 on the last day of both abstinence and *ad libitum* cannabis smoking phases. The dronabinol administration phase was separated by a 9-day *ad libitum* cannabis smoking phase during which participants could self-administer smoked cannabis between 12:00 and 23:00 each day, with blood collection on the ninth day.

Sample collection

Ad libitum cannabis smoking blood collection was performed until the second *ad libitum* phase due to limited blood collection during the entire study. Specimens were collected at 9:00, 11:00, 11:45 (before), and 12:30, 14:00, 15:30, 17:00, 19:00, 20:30 and 22:00 during the last day of *ad libitum* cannabis smoking (Study Days 4 and 18); at 9:00, 14:00, 19:00 and 22:00 on the first day of the abstinence phase and at 9:00, 11:00, 11:45, 12:30, 14:00, 15:30, 17:00, 19:00, 20:30 and 22:00 on the fifth day of the abstinence phase. Six milliliters of blood specimens were collected into sodium heparin-containing tubes (BD Vacutainer® Becton Dickinson, Franklin Lakes, NJ, USA) at each time point through an indwelling peripheral intravenous catheter and plasma separated within 2 h. All samples were stored at −20°C in cryogenic tubes until analysis.

Cannabis cigarettes

Cannabis cigarettes (mean weight 0.9 g) were supplied by the National Institute on Drug Abuse and contained 5.9 ± 0.3% THC, yielding ~53.1 mg THC per cigarette. Controlled cannabis smoking consisted of five puffs with 5-s inhalations, 10-s breath holds and 40-s interpuff intervals. During the *ad libitum* cannabis smoking period, participants had free access to cannabis cigarettes from 12:00 to 23:00, with no restrictions on smoking topography or amount of cigarettes smoked, i.e., participants were not obligated to smoke an entire cannabis cigarette before proceeding to the next one. Each cannabis cigarette requested by participants was recorded in an inventory log and smoked under direct observation of study staff after documentation of the smoking time.

Plasma cannabinoid analysis

Plasma cannabinoid analysis was performed according to a previously published validated method (12) using two-dimensional gas chromatography–mass spectrometry, with minor improvements including an extended linear range due to expected high cannabinoid concentrations; d^9-THCCOOH as THCCOOH internal standard; and injection port and oven temperature program modifications to separate chromatographic interferences in plasma. The limits of quantification (LOQs) were 0.5 μg/L for THC and THCCOOH, and 1.0 μg/L for 11-OH-THC. Calibration curves were linear in the range of 0.5–100 μg/L for THC, 1.0–50 μg/L for 11-OH-THC and 0.5–200 μg/L for THCCOOH. Three quality control samples were analyzed in each batch across the linear range of the assay. Intra- and interassay imprecision were <5.2%, and analytical bias within 91.3–110.3%.

Data analysis

Cannabinoid plasma detection rates were determined based on the method's LOQs; samples were considered positive when cannabinoid concentrations were above LOQs unless otherwise specified. THC detection rates were additionally evaluated at 2 and 5 μg/L where impairment began to be observed in cognition and motor control tasks (13), and at 12.5 μg/L, the approximate plasma THC concentration corresponding to 5.0 μg/L blood THC concentration recently adopted in the state of Washington after legalizing cannabis. The latter cutoff was based on studies showing an increased risk for traffic accidents at 5.0 μg/L (14) and that even chronic heavy cannabis smokers had blood THC concentrations below 5.0 μg/L after a few hours (9, 15).

Preliminary non-parametric statistical analysis using the Kruskal–Wallis test showed no significant difference in cannabinoid concentration between the two *ad libitum* smoking phases. As a result, data from both *ad libitum* smoking phases were merged. Data analysis was conducted with SPSS Statistics version 19.0 (IBM, Chicago, IL, USA) and considered significant if two-tailed $P < 0.05$. Plasma cannabinoid concentrations during *ad libitum* smoking and abstinence were grouped by time from last cigarette

Study day	1–4	5–9	10–18	19–23	24–32	33–37	38–46	47–51
Treatment[a]	*Ad libitum* cannabis smoking	Dronabinol: 0, 30, 60 or 120 mg	*Ad libitum* cannabis smoking	Dronabinol: 0, 30, 60 or 120 mg	*Ad libitum* cannabis smoking	Dronabinol: 0, 30, 60 or 120 mg	*Ad libitum* cannabis smoking	Dronabinol: 0, 30, 60 or 120 mg
Plasma collection[b]	D4, 9–22 h	D5, 9–22 h D9, 9–22 h	D18, 9–22 h	D19, 9–22 h D23, 9–22 h		D33, 9–22 h D37, 9–22 h		D47, 9–22 h D51, 9–22 h

Figure 1. Study design and plasma sample collection schedule. [a]Controlled single cannabis smoking occurred approximately 11:30 on the last day of each treatment phase. [b]This study evaluated plasma data collected in the first two *ad libitum* smoking phases and the abstinence (placebo dronabinol) phase; the plasma data during the active dronabinol phases were presented in a previously published paper (10).

smoked; time since last smoking was calculated by subtraction of last smoking times from sample collection times. Because of the complex study design and the use of *ad libitum* smoking conditions, plasma samples were collected at scheduled time points rather than relative time points from last smoking. Hence, figures illustrate cannabinoid concentrations plotted in time intervals instead of specific time points. Noncompartmental pharmacokinetics analyses were performed with WinNonlin Professional 5.2 for Windows (Pharsight Software).

Results

Participants

Participants' demographic characteristics are detailed in Table I. Seven participants (six males and one female) had blood collected during *ad libitum* cannabis smoking prior to the 5-day abstinence phase; four had *ad libitum* cannabis smoking followed by 5-day abstinence without blood collection due to a restriction on blood collection volumes during the 51-day study. All participants were African-Americans.

Table I
Demographic Characteristics and Self-Reported Cannabis Use History for 11 Participants

Subject	Sex	Age	BMI	Self-reported mean times smoked/day	Age at first use (years)	Lifetime duration of cannabis smoking (years)	# Cannabis cigarettes smoked	
							Study Day 4	Study Day 18
A	M	47	19.4	2	13	27	2	1
B	M	36	28.5	6	17	16	16	8
C	M	29	30.2	5	9	17	20	20
D	F	31	36.0	5	18	13	8	14
E	M	30	21.0	6	14	14	15	24
F	M	52	26.1	2	14	38	13	17
G	M	39	48.2	3	13	26	25	25
H	M	37	27.7	2	16	21	7	6
I	M	30	32.4	3	14	16	17	14
J	M	25	22.0	3	14	11	18	25
K	M	30	23.1	4	16	14	20	30
Mean		35.1	28.6	3.7	14.4	19.4	14.6	16.7
SD		8.3	8.2	1.4	2.4	8.1	6.7	9.1

SD, standard deviation; BMI, body mass index calculated as weight (kg)/height^2 (m^2).

Plasma cannabinoids during ad libitum *cannabis smoking*

Cannabinoid concentrations on the last days of both *ad libitum* cannabis smoking phases were grouped by time after the last cannabis cigarette (Table II, bottom). Median (range) plasma concentrations in 11 participants 0.1–0.5 h prior to *ad libitum* cannabis smoking initiation were 7.6 (0.8–18.7) μg/L THC, 2.7 (<LOQ–6.8) μg/L 11-OH-THC and 67.0 (20.1–295) μg/L THCCOOH. Time since last smoking for plasma samples during *ad libitum* smoking phases ranged from 0.0 to 13.7 h. Median (range) times of maximum THC, 11-OH-THC and THCCOOH concentrations (T_{max}) in positive samples were 0.2 (0.0–0.5) h THC, 0.3 (0.1–0.9) h 11-OH-THC and 0.5 (0.1–10.4) h THCCOOH after smoking, with concentrations of 83.5 (31.6–271) μg/L THC, 14.2 (5.8–41.3) μg/L 11-OH-THC and 155 (57.8–348) μg/L THCCOOH. Three participants had THCCOOH T_{max} 10 h after last smoking, with concentrations ≥255 μg/L. THC concentrations decreased rapidly after smoking and 11-OH-THC more slowly; THCCOOH concentrations remained more constant over time.

Figure 2 illustrates the medians of cannabinoid concentrations during *ad libitum* smoking divided into eight time intervals (from −0.5 to −0.1, 0–0.25, 0.26–0.5, 0.6–1, 1.1–2, 2.1–4, 4.1–8 and 8.1–14 h) with respect to time since last smoking. During the six intervals from 0 to 8 h, median (range) THC concentrations were 111 (35.1–271), 40.5 (6.7–169), 33.7 (5.7–136), 17.6 (2.7–60.2), 13.6 (2.2–33.1) and 3.6 (1.8–16.0) μg/L; 11-OH-THC concentrations were 11.6 (4.1–41.3), 8.9 (2.7–27.3), 8.2 (<LOQ–26.3), 5.8 (<LOQ–26.2), 5.5 (<LOQ–13.4) and 3.0 (<LOQ-9.9) μg/L; and THCCOOH concentrations were

Table II
Median (Range) Plasma Pharmacokinetic Parameters in Chronic Frequent Cannabis Smokers Following Controlled Smoking of a Single 5.9 ± 0.3% THC Cigarette at a Controlled Pace and During the Last Days of the 4- and 9-Day *Ad libitum* Cannabis Smoking Phases from 9:00 to 22:00 h

Analyte	*n*	T_{max}, (h)	Apparent C_{max} (μg/L)	T_{last}[a] (h)	C_{last} (μg/L)	AUC_{last} (h μg/L)
Single cannabis smoking						
THC	11	0.50 (0.40–0.60)	28.3 (1.9–43.6)	10.7 (9.3–10.8)	4.8 (0.7–5.9)	89.3 (9.4–124)
11-OH-THC	10[b]	0.50 (0.40–0.60)	3.9 (2.6–9.2)	9.9 (1.2–10.8)	1.1 (1.0–1.9)	16.5 (3.7–32.1)
THCCOOH	11	0.50 (0.40–0.60)	47.0 (12.6–117)	10.7 (10.5–10.8)	30.5 (8.9–63.9)	352 (113–805)
Ad libitum cannabis smoking[c]						
THC	11	0.20 (0.03–0.52)	83.5 (31.6–271)	10.5 (10.3–13.7)	7.3 (1.0–14.0)	170 (57.7–310)
11-OH-THC	11	0.28 (0.07–1.15)	14.2 (5.8–41.3)	10.4 (10.3–13.4)	3.3 (1.4–7.0)	59.1 (9.6–128)
THCCOOH	11	0.52 (0.07–10.43)	155 (57.8–348)	10.5 (10.3–13.7)	93.7 (22.1–348)	1,034 (351–3,019)

Time indicates difference between the last cannabis cigarette smoked and sample collection.

n, number of participants included for calculations; T_{max}, time for maximum concentration; C_{max}, maximum concentration; T_{last}, time of last positive sample; C_{last}, last positive concentration; AUC_{last}, area under the curve from 0 to T_{last}.

[a]Time of last positive sample mostly coincided with time of last collection (range 10.5–10.8 and 10.3–13.7 h since last smoking for the single and *ad libitum* cannabis smoking samples, respectively). Hence, these times are shorter than true times of last detection.

[b]One participant's 11-OH-THC concentrations were never positive during the session and consequently his data could not be included in the analysis.

[c]Values from the two *ad libitum* cannabis smoking phases were combined for each participant as the Kruskal–Wallis test showed no significant difference in cannabinoid concentration between the phases.

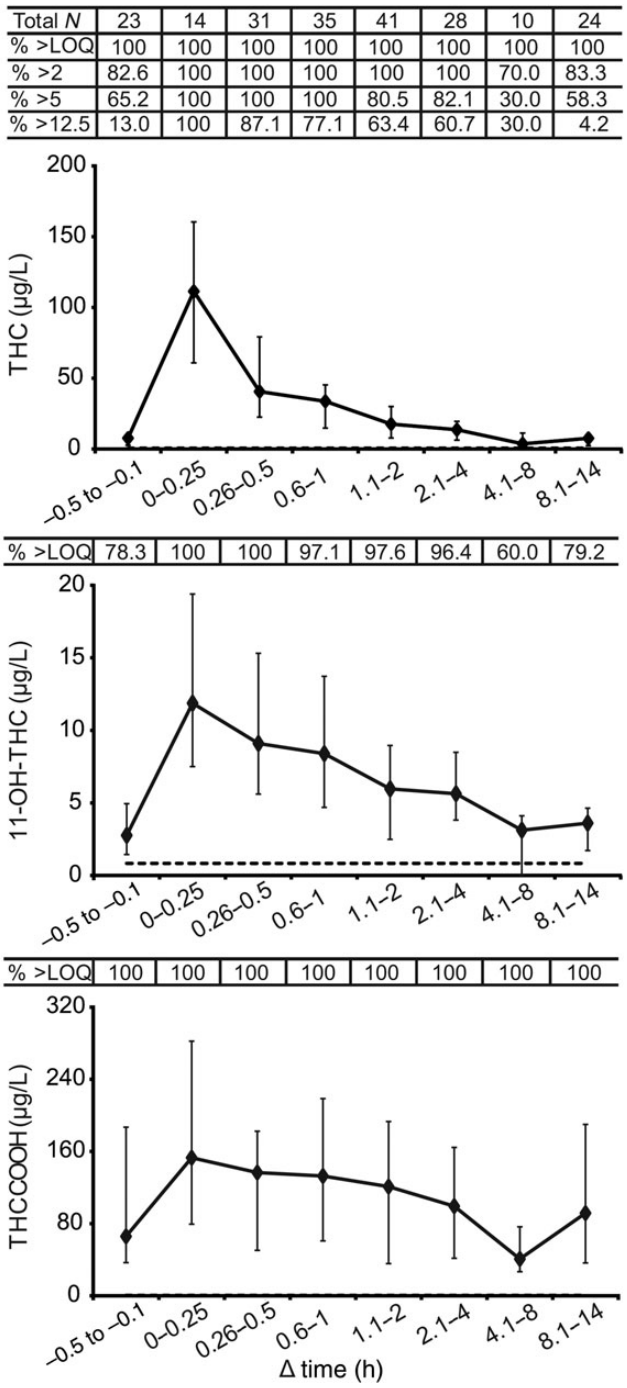

Figure 2. Median plasma cannabinoid concentrations in 11 participants collected during *ad libitum* cannabis smoking. *X*-axis specifies eight time intervals equaling the time difference between the last cigarette smoked and sample collection time (Δtime) during *ad libitum* smoking. Error bars represent interquartile range. Dotted lines indicate LOQ (0.5 µg/L for THC and THCCOOH and 1.0 µg/L for 11-OH-THC). *N*, number of samples; LOQ, limit of quantification; % >2, % of plasma samples positive for THC at 2 µg/L cutoff; % >5, % of plasma samples positive for THC at 5 µg/L cutoff; % >12.5, % of plasma samples positive for THC at 12.5 µg/L cutoff, approximately equivalent to 5 µg/L in whole blood.

149 (40.8–292), 134 (30.4–302), 130 (27.2–344), 119 (25.7–284), 98.6 (26.1–261) and 43.4 (26.4–207) µg/L, respectively. From 8.1 to 14 h after smoking, all samples were still positive for THC and THCCOOH at LOQ, while only 19 of 24 (79.2%) were 11-OH-THC-positive, with median concentrations of 7.5 (1.0–14.0) µg/L THC, 3.5 (<LOQ–7.7) µg/L 11-OH-THC and 91.4 (22.1–348) µg/L THCCOOH. Increasing the THC cutoff concentration decreased its detection rates. All samples were positive 0–4 h at a 2-µg/L THC cutoff and 0–1 h at a 5-µg/L THC cutoff. In the 8.1- to 14-h interval, 83 and 58% were >2 and 5 µg/L, respectively. At a 12.5-µg/L THC cutoff, all samples collected within 0.25 h post smoking were THC-positive. The plasma THC detection rates rapidly decreased to 63, 30 and 4% at 1.1–2, 4.1–8 and 8.1–14 h intervals, respectively, with no sample having THC ≥12.5 µg/L beyond 10.3 h.

Plasma cannabinoids after smoking a single 5.9% cannabis cigarette

Median (range) plasma concentrations 0.3 (0.1–0.4) h prior to smoking initiation were 4.6 (0.5–6.9) µg/L THC, <LOQ (<LOQ–1.2) µg/L 11-OH-THC and 27.4 (4.9–89.0) µg/L THCCOOH; all participants' ($n = 11$) plasma samples were THC- and THCCOOH-positive at LOQ prior to smoking, while three were 11-OH-THC-positive. Median (range) T_{max} of THC, 11-OH-THC and THCCOOH concentrations were at the time of first blood collection, 0.5 (0.4–0.6) h after smoking (Table II, top). THC and 11-OH-THC concentrations decreased rapidly after smoking, whereas THCCOOH remained more constant. Median (range) concentrations after 1.2 (1.1–1.4), 2.7 (2.6–2.9), 4.2 (4.1–4.4), 5.7 (5.6–5.9), 7.7 (7.5–7.9) and 9.2 (9.0–9.3) h were 13.6 (1.3–18.5), 7.1 (1.0–10.3), 6.2 (0.7–8.0), 4.8 (0.9–6.6), 4.6 (0.7–6.2) and 5.2 (0.7–6.6) µg/L THC; 2.2 (<LOQ–6.1), 1.7 (<LOQ–3.1), 1.4 (<LOQ–1.7), 1.2 (<LOQ–1.5), 1.0 (<LOQ–1.5) and 1.0 (<LOQ–1.4) µg/L 11-OH-THC; and 42.2 (11.4–98.6), 36.5 (11.8–72.8), 29.7 (9.6–69.4), 25.1 (10.0–60.7), 27.2 (9.2–67.6) and 30.1 (8.7–72.2) µg/L THCCOOH, respectively.

One participant's plasma became THC-negative in the last sample 10.7 (10.5–10.8) h after smoking, whereas all other participants samples remained positive at LOQ. 11-OH-THC detection rate decreased over time, with 5 of 11 participants 11-OH-THC-positive 10.7 h after smoking; one participant was never positive for 11-OH-THC. All participants were THCCOOH-positive throughout the day. Eleven hours after smoking, median residual cannabinoid concentrations were 4.8 (<LOQ–5.9) µg/L THC, <LOQ (<LOQ–1.4) µg/L 11-OH-THC and 30.5 (8.9–63.9) µg/L THCCOOH. All but one participant were positive at 2 and 5 µg/L THC cutoffs 0.5 h after smoking; 73 and 45% were positive, respectively, 10.7 h after smoking. At 12.5 µg/L THC, 82 and 64% of plasma samples were positive 0.5 and 1.2 h after smoking, respectively, but no sample was positive beyond 1.2 h. Figure 3 illustrates cannabinoid median concentrations after smoking. There was large intersubject variability in cannabinoid concentrations, especially for THCCOOH.

Ad libitum cannabis smoking followed by an abstinence phase

Seven of 11 participants had an *ad libitum* smoking phase (with plasma collections) followed by 5-day abstinence. Cannabinoid concentrations on the last day of the *ad libitum* cannabis smoking phase and on the first and last days of the abstinence phase

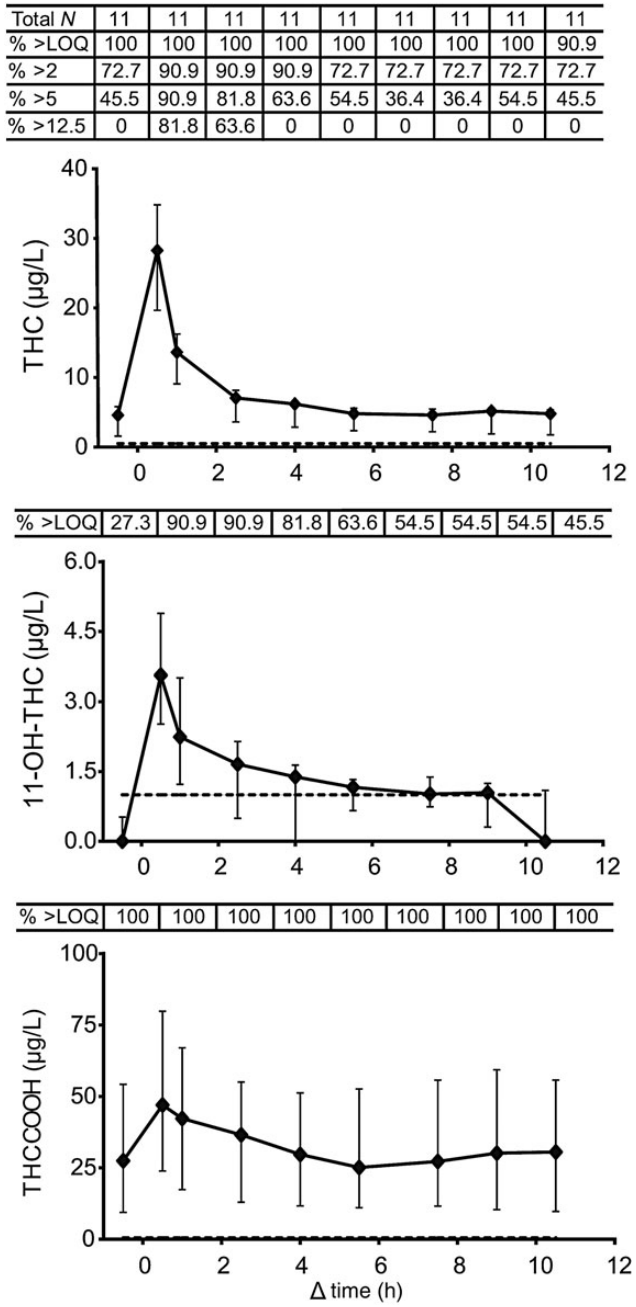

Figure 3. Median plasma cannabinoid concentrations in 11 participants following controlled paced smoking of one cannabis cigarette on the last day of the abstinence phase. Error bars represent interquartile range. Δtime indicates time between the controlled cannabis cigarette smoking and sample collection. Dotted lines indicate LOQ (0.5 µg/L for THC and THCCOOH and 1.0 µg/L for 11-OH-THC). *N*, number of samples; LOQ, limit of quantification; % >2, % of plasma samples positive for THC at 2 µg/L cutoff; % >5, % of plasma samples positive for THC at 5 µg/L cutoff; % >12.5, % of plasma samples positive for THC at 12.5 µg/L cutoff, approximately equivalent to 5 µg/L in whole blood.

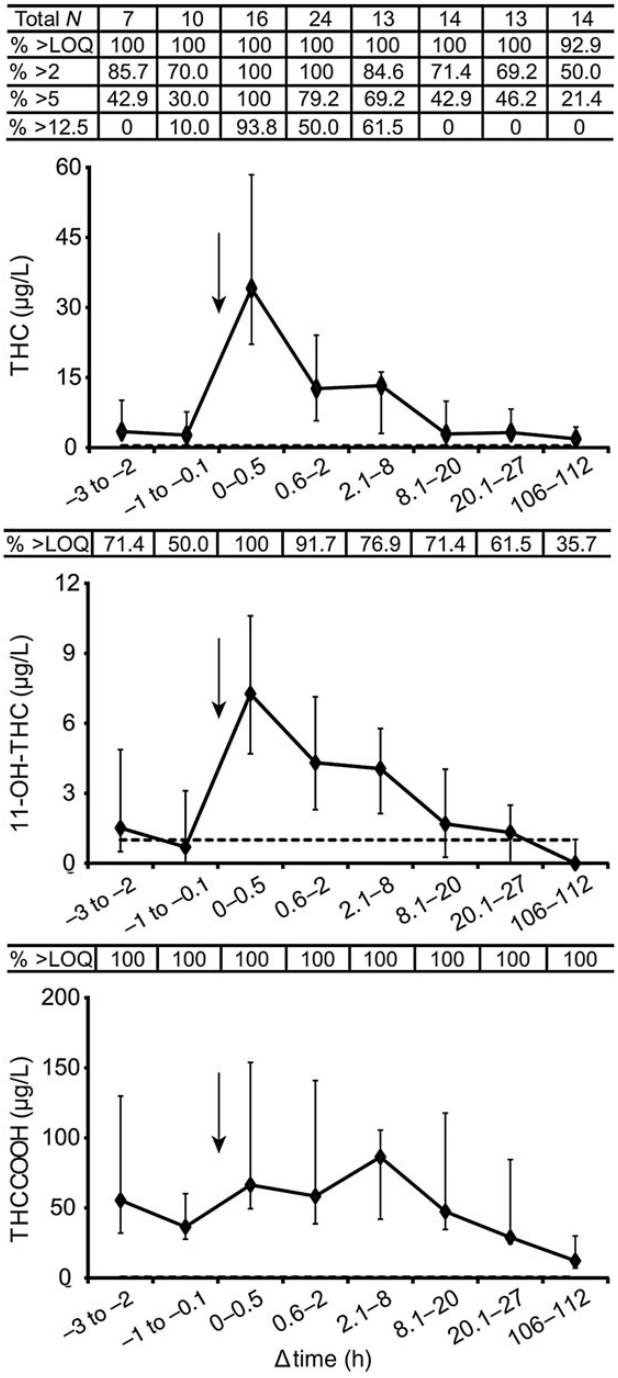

Figure 4. Median plasma cannabinoid concentrations in seven participants during *ad libitum* cannabis smoking followed by the 5-day abstinence phase. *X*-axis specifies eight time intervals equaling the time difference between the last cigarette smoked and sample collection time (Δtime) during *ad libitum* smoking. Error bars represent interquartile range. Dotted lines indicate LOQ (0.5 µg/L for THC and THCCOOH and 1.0 µg/L for 11-OH-THC). *N*, number of samples; LOQ, limit of quantification; % >2, % of plasma samples positive for THC at 2 µg/L cutoff; % >5, % of plasma samples positive for THC at 5 µg/L cutoff; % >12.5, % of plasma samples positive for THC at 12.5 µg/L cutoff, approximately equivalent to 5 µg/L in whole blood. Arrows represent start of the *ad libitum* smoking phase.

are presented according to the time of last smoking divided into eight time intervals (Figure 4): 2–3 prior, 0.1–1 prior, 0–0.5, 0.6–2, 2.1–8, 8.1–20, 20.1–27 and 106–112 h. Median (range) cannabinoid concentrations 2–3 h prior to initiation of *ad libitum* cannabis smoking were 3.4 (0.9–11.2) µg/L THC, 1.5 (<LOQ–8.7) µg/L 11-OH-THC and 55.4 (29.2–317) µg/L THCCOOH. Cannabinoid concentrations 0.1–1 h before starting smoking were 2.7 (0.8–13.7) µg/L THC, <LOQ (<LOQ–6.3) µg/L 11-OH-THC and 36.4 (23.7–226) µg/L THCCOOH.

Cannabinoids reached T_{max} in positive samples 0–0.5 h (THC and 11-OH-THC), and 2.1–8 h (THCCOOH) since last smoking, with median concentrations of 33.7 (6.7–161) μg/L THC, 7.3 (2.7–41.3) μg/L 11-OH-THC and 86.4 (26.1–234) μg/L THCCOOH.

THC concentrations decreased rapidly after smoking, with a much slower decrease in 11-OH-THC concentrations; THCCOOH concentration remained more constant over time. During the four intervals from 0 to 20 h, median (range) THC concentrations were 33.7 (6.7–161), 12.5 (2.7–58.9), 13.1 (1.8–21.7) and 2.9 (0.8–10.7) μg/L; 11-OH-THC concentrations were 7.3 (2.7–41.3), 4.3 (<LOQ–26.2), 4.1 (<LOQ–12.7) and 1.7 (<LOQ–7.0) μg/L; and THCCOOH concentrations were 66.4 (37.4–271), 58.4 (26.6–307), 86.4 (26.1–234) and 47.4 (26.7–348) μg/L, respectively. From 20.1 to 27 h after last smoking, all samples were still positive at LOQ for THC and THCCOOH, and 8 of 13 (61.5%) were 11-OH-THC-positive, with median concentrations of 3.2 (0.8–9.9) μg/L THC, 1.3 (<LOQ–2.9) μg/L 11-OH-THC and 28.9 (24.4–178) μg/L THCCOOH. On the fifth day of abstinence (106–112 h post smoking), cannabinoid concentrations decreased to 1.9 (<LOQ–6.0) μg/L THC, <LOQ (<LOQ–1.2) μg/L 11-OH-THC and 12.2 (4.9–111) μg/L THCCOOH. All samples had THC >2 and >5 μg/L 0–2 and 0–0.5 h after smoking, respectively, and the detection rate decreased to 50 and 21% in the last 106–112 h interval, respectively. At 12.5 μg/L THC, 94% of plasma samples in the 0- to 0.5-h interval were positive, but only half of the samples were positive in the next interval, 0.6–2 h; the latest positive sample was obtained at 5.7 h post smoking.

Discussion

This study characterized for the first time cannabinoid pharmacokinetics during *ad libitum* cannabis smoking over multiple days and compared THC disposition in plasma after multiple and single cannabis smoking in chronic frequent cannabis smokers. Cannabidiol (CBD) concentrations were not reported because they were not detected in any specimen, as cannabis cigarettes contained only 0.01% CBD. The LOQ was 0.5 μg/L for CBD.

Schwope *et al.* (9) reported median observed maximum plasma concentrations (C_{max}) in chronic frequent cannabis smokers of 76 μg/L THC, 10 μg/L 11-OH-THC and 67 μg/L THCCOOH 0.25 h after starting smoking a 6.8% THC cigarette. Huestis *et al.* (16) reported plasma THC concentrations lower than 129 and 267 μg/L following smoking 1.75 or 3.55% THC cigarettes, respectively, with THC detectable for up to 12 h. Our observed C_{max} was lower, most likely due to later first sample collection at 0.5 h after smoking when THC concentrations had decreased. The use of a lower potency cigarette (5.9% THC) than in the Schwope *et al.*'s study (6.8% THC) (9), differences in smoking topography and physiological variability among participants could be additional contributing factors.

Given the difficulty of objectively measuring impairment, legislation for driving under the influence of drugs (DUID) favors a *per se* limit approach based on available scientific evidence demonstrating an increased risk of impaired driving with higher blood THC concentrations (17). At blood THC concentrations of 1–2 μg/L, odds ratios of being involved in traffic accidents were 1.5–2.5 (18, 19), increasing to 2.1–6.6 at ≥5 μg/L (14, 19). Impairment became evident in cognition and motor control performance measures at serum THC 2–5 μg/L with 75–90% observations exhibiting impairment in every performance task at 5–10 μg/L (13). Several European countries established blood THC thresholds of 0.3–3.0 μg/L; for those with zero tolerance legislation, the laboratory limit of detection becomes the legal limit (20). In the USA, Washington State adopted a *per se* 5-μg/L blood THC concentration (Colorado has a permissive inference law at 5 μg/L). While there is significant increased accident risk for drivers with blood THC concentration ≥5 μg/L or while driving within 2 h of using cannabis (21), there is concern that the threshold is too high. Considering that blood THC concentrations rapidly decrease within a few hours of smoking and time between roadside accident and blood sample collection may take 0.5–3 h (18, 22, 23), many impaired drivers would be below this detection limit (24). In Washington, an average of 56% of suspected DUID cases positive for THC had blood concentrations ≥5 μg/L in 2009–2012 (25). In Colorado, 52% of suspected DUID cases positive for THC in 2011–2014 had blood concentrations ≥5 μg/L (26). The present study similarly demonstrated that at 12.5 μg/L plasma THC (equivalent to 5 μg/L in blood), ≤61% of samples were positive beyond 2 h post smoking during *ad libitum* smoking phases. Following single controlled smoking of one 5.9% THC cigarette, all samples had plasma THC concentrations below 12.5 μg/L 2.6–2.9 h after last use. The possibility of false-negative plasma cannabinoid samples would be even greater in occasional smokers. When a single 6.8% THC cannabis cigarette was administered, plasma THC concentrations were significantly higher in frequent smokers than in occasional smokers at most time points from 0.5 to 30 h (median C_{max} 47.7 vs. 16.7 μg/L) (27). At blood THC >5 μg/L cutoff, median (range) time of last detection was 3.5 h (1.1 to >30 h) in frequent smokers and 1.0 h (0–2.1 h) in occasional smokers (27).

On the other hand, residual cannabinoid concentrations in chronic frequent smokers who often use cannabis multiple times per day have significant cannabinoid body burdens that might produce positive plasma THC concentrations beyond the window of drug impairment. This also is an important consideration in establishing a blood THC cutoff concentration for DUID investigations. Our data better reflect THC and metabolite concentrations in plasma of chronic frequent cannabis smokers in the community than do single smoked cannabis data. Smoking multiple cigarettes increased C_{max}, T_{max} and area under the curve, compared with smoking a single cigarette. The large THC body burden (28) accumulated over a long period of smoking multiple cigarettes per day, led to increased cannabinoid excretion in chronic daily cannabis smokers. Karschner *et al.* (29) previously documented THC and THCCOOH concentrations after 1 week of monitored abstinence with LOQs of 0.25 μg/L after admission in chronic cannabis smokers' plasma. We recently documented THC and THCCOOH detection in chronic cannabis smokers' blood over 30 days of sustained abstinence (30). Considering a whole blood/plasma ratio around 0.4 (31), it would not be surprising that plasma cannabinoid concentrations were present in chronic cannabis smokers' plasma over 30 days of abstinence.

When THC is no longer present in blood, it may still be present in brain. Brunet *et al.* (32) showed that brain THC concentrations

decreased more slowly than blood THC. In addition, THC was present in the brain of motor vehicle fatalities when it was no longer detectable in blood (33). These pharmacokinetic characteristics make it difficult to identify a minimum plasma THC concentration consistently associated with impairment (23). Multiple studies showed prolonged cognitive and psychomotor impairment for at least several weeks after initiation of abstinence among daily cannabis users (34–39). However, additional research is warranted on the development of and dissipation of pharmacodynamic tolerance (40–43), the relationship between concentrations in blood and brain (the site of action of impairment) (33) and corresponding ability to operate an automobile. While implementation of a *per se* limit protects the public and eases DUID prosecution, determining a cutoff concentration above which impairment is unequivocally demonstrative in both chronic and occasional smokers is challenging, as it is for alcohol. Finally, it is an administrative decision based on laboratory, driving simulator and epidemiological data showing increased odds ratio for crashes and fatalities after cannabis intake. Another solution is a roadside evaluation of signs and symptoms of impairment sensitive to the effects of cannabis; the search for such an objective means to do so should continue.

We documented significant cannabinoid receptor type 1 (CB_1) downregulation or neuroadaptation in daily cannabis smokers compared with normal controls (39) that may provide a mechanism for the development of tolerance in this population. With sustained abstinence of 28 days, there no longer was a significant difference in the density of CB_1 receptors (39). Neuropsychological performance of chronic frequent cannabis smokers was significantly impaired at baseline and after 7 days of cannabis abstinence, compared with former heavy cannabis smokers and current occasional cannabis smokers; the difference was no longer significant at 28 days. Furthermore, psychomotor performance impairment validated to predict poor on-the-road driving behavior in chronic daily cannabis smokers (i.e., critical tracking and divided attention) remained significantly impaired after 7, 14 and 21 days of sustained abstinence (38). These findings suggest that different functions may require different periods of sustained abstinence to reduce effects of chronic cannabis exposure. This is yet another complication when establishing proof of impairment in chronic smokers who may exhibit less impairment after cannabis use than occasional smokers, potentially owing to behavioral tolerance or acquired compensatory driving performance (20, 44). In contrast, while occasional smokers more intensely experienced psychomotor, subjective and physiological effects of smoked cannabis, their blood THC concentrations were lower than chronic smokers, indicating a higher risk of false-negative results (45).

Strengths of this study include 4- and 9-day *ad libitum* smoking periods, generating plasma cannabinoid concentrations typical of chronic frequent cannabis smokers, and a 5-day abstinence phase to determine detection times and concentrations at different cutoff concentrations utilized in forensic drugged driving investigations. Study limitations include limited plasma collection due to safety restrictions on total volume of blood collected. Consequently, window of detection for plasma cannabinoids could not be fully evaluated. In conclusion, our results demonstrate for the first time, the impact of smoking multiple cannabis cigarettes on cannabinoid excretion profiles in chronic frequent cannabis smokers with known time since last smoking for evaluation of concentration–time course. The wide range of cannabinoid concentrations is likely due to interindividual variability and smoking topography. These data provide important information for cannabinoid concentration interpretation in forensic and clinical monitoring contexts and further our understanding of cannabinoid disposition after smoking multiple cannabis cigarettes.

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Conflict of interest

None declared.

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