Implications of Plasma Δ⁹-Tetrahydrocannabinol, 11-Hydroxy-THC, and 11-nor-9-Carboxy-THC Concentrations in Chronic Cannabis Smokers

Erin L. Karschner[1], **Eugene W. Schwilke**[1], **Ross H. Lowe**[1], **W. David Darwin**[1], **Ronald I. Herning**[2], **Jean Lud Cadet**[2], and **Marilyn A. Huestis**[*]

[1]Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, 251 Bayview Blvd., Suite 200, Baltimore, Maryland 21224

[2]Molecular Neuropsychiatry, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, 251 Bayview Blvd., Suite 200, Baltimore, Maryland 21224

Abstract

Δ^9-Tetrahydrocannabinol (THC) is commonly found in toxicological specimens from driving under the influence and accident investigations. Plasma cannabinoid concentrations were determined in 18 long-term heavy cannabis smokers residing on an in-patient research unit for seven days of monitored abstinence. THC, 11-hydroxy-THC, and 11-nor-9-carboxy-THC (THCCOOH) were quantified by two-dimensional gas chromatography–mass spectrometry with cryofocusing. THC concentrations were > 1 ng/mL in nine (50.0%) participants (1.2–5.5 ng/mL) on abstinence day 7. THCCOOH was detected (2.8–45.6 ng/mL) in all participants on study day 7. THC and THCCOOH median percent concentration decreases ($n = 18$) were 39.5% and 72.9% from day 1 to 7, respectively. Most (88.9%) of the participants had at least one specimen with increased THC compared to the previous day. Cannabis use duration and plasma THCCOOH concentrations were positively correlated on days 1–3 ($R = 0.584$–0.610; $p = 0.007$–0.011). There were no significant correlations between THC concentrations > 0.25 ng/mL and body mass index on days 1–7 ($R = -0.234$–0.092; $p = 0.350$–0.766). Measurable THC concentrations after seven days of abstinence indicate a potential mechanism for residual neurocognitive impairment observed in chronic cannabis users. THC's presence in plasma for seven days of abstinence suggests its detection may not indicate recent use in daily cannabis users.

Introduction

Δ^9-Tetrahydrocannabinol (THC) is the primary chemical component responsible for cannabis' euphoric and cardiovascular effects. Impaired psychomotor and neurocognitive performance are other acute pharmacodynamic effects of THC and its psychoactive metabolite, 11-hydroxy-THC (11-OH-THC). Neuropsychological decrements may persist in chronic cannabis users during multiple days of abstinence (1–6). Significantly larger deficits were observed in 77 chronic cannabis users on abstinence days 0, 1, and 7 but not 28 as compared to former cannabis users (2,7). In contrast, others reported decreases in cortical motor activity (8) and neurocognitive impairment (4) after 28 days or more of documented abstinence in chronic, heavy cannabis users. Additionally, duration rather than frequency of cannabis use has been suggested as the key factor in performance impairment (5).

[*]Author to whom correspondence should be addressed: Marilyn A. Huestis, PhD, Chief, Chemistry and Drug Metabolism, Intramural Research Program, NIDA, NIH, Biomedical Research Center Suite 200, 251 Bayview Blvd., Room 05A-721, Baltimore, MD 21224. mhuestis@intra.nida.nih.gov.

THC is a highly lipophilic compound that is extensively stored in adipose tissue (9,10) following chronic exposure with slow release over time back into the blood (11,12). This could lead to differences in THC detection windows in blood of occasional and frequent cannabis users. The rate-limiting step in the terminal elimination half-life of THC after chronic exposure was demonstrated in dogs to be the slow release of THC from tissue depots (11). Forensic toxicologists rely on drug detection windows, the time between first and last measurable concentrations of a drug in a biological fluid, to corroborate evidence of driving under the influence of drugs. As drug concentrations in brain are not accessible in living individuals, blood or plasma levels most closely reflect drug exposure at its sites of action.

Acute smoked administration of 1.75 or 3.55% THC cigarettes to six occasional cannabis users yielded mean ± SD plasma THC detection times of 7.2 ± 1.6 h (range 3–12 h) and 12.5 ± 3.1 h (range 6–27 h) (13) with a limit of quantification (LOQ) of 0.5 ng/mL THC. Limited data are available for interpreting THC concentrations after chronic cannabis exposure for two reasons: ethical and safety concerns limiting administration of chronic smoked doses and the high cost and difficulty of continuous monitoring, collection, and analysis of specimens during extended cannabinoid excretion. An early report indicated that mean plasma concentrations were only 0.86 ± 0.22 ng/mL THC, 0.46 ± 0.17 ng/mL 11-OH-THC, and 45.8 ± 13.1 ng/mL THCCOOH in frequent cannabis users 12 h after last cannabis use (14). However, a more recent study suggested THC serum concentrations 24–48 h after last cannabis use could be 1.2–6.4 ng/mL in 16 frequent, daily cannabis users who resided on a detoxification ward (15). After chronic cannabis exposure, THC's plasma half-life was estimated to be 4.1 days in two men (12).

Our research examines THC, 11-OH-THC, and THCCOOH disposition in plasma of 18 frequent cannabis users during seven days of continuously monitored abstinence on a secure research unit. These plasma THC detection times following chronic cannabis exposure strongly impact interpretation of cannabinoid concentrations and driving under the influence of drugs (DUID) legislation. We hypothesize that residual THC concentrations in brain after chronic THC exposure may be the source of observed neurocognitive impairment in abstinent frequent users and now provide pharmacokinetic data to support this hypothesis.

Methods

Participants

Cannabis users (21–45 years of age) with multiple years of frequent self-reported use were recruited into this National Institute on Drug Abuse (NIDA) Institutional Review Board-approved study. Participants were required to have a positive urine cannabinoid immunoassay test greater than 100 ng/mL. Exclusion criteria included clinically significant cardiovascular, pulmonary, endocrine, hematologic, and hepatic abnormalities. Participants were excluded if they presented with a history of past or current psychiatric disorder (i.e., post-traumatic stress, anxiety disorder, or major depression) as defined by the Diagnostic and Statistical Manual of Mental Disorders IV. Additionally, any history of neurologic illness, including head trauma resulting in loss of consciousness, seizure disorder, and stroke, were exclusionary. Females of child-bearing potential could not be pregnant or nursing and were required to use a medically accepted method of birth control or abstain from sexual intercourse throughout the study. Participants provided voluntary written informed consent. Medical (physical examination, ECG, blood, and urine chemistries) and psychological evaluations including self-reported drug use history were conducted.

Participants resided on the NIDA Intramural Research Program's (IRP) secure clinical research unit under 24 h medical surveillance throughout the study to ensure cannabis

abstinence. Upon admission, all participants' belongings were searched for drugs. Participants were constantly monitored, and no visitors were permitted. Subjects had access to a universal gym, exercise bike, and a secure courtyard area for recreation. Financial compensation for participation also was provided. Meals were ordered from the hospital cafeteria, and no diet or liquid restrictions were imposed.

Plasma specimen collection

Five-milliliter whole blood specimens were collected from an indwelling venous catheter into sodium heparin tubes at the time of admission and generally at 9 a.m. each day there-after for seven days. Indwelling venous catheters were replaced at least every 72 h. Blood was collected, stored on ice, centrifuged, and plasma separated within 2 h. Plasma was transferred to cryotubes and stored at −20°C until analysis.

Specimen analysis

Plasma specimens were analyzed for cannabinoids by a validated two-dimensional gas chromatography–mass spectrometry (2D-GC–MS) method for the simultaneous quantification of THC, 11-OH-THC, and THCCOOH (16). Briefly, proteins in 1 mL plasma were precipitated by the addition of 2 mL cold acetonitrile while vortex mixing. After centrifugation, supernatants were decanted into 3 mL sodium acetate buffer (pH 4.0), mixed, and applied to conditioned 200-mg ZSTHC020® solid-phase extraction (SPE) columns (United Chemical Technologies, Bristol, PA). Columns were washed with 3 mL deionized water, 2 mL 0.1 N hydrochloric acid/acetonitrile (70:30, v/v), and dried by full vacuum for 20 min. Analytes were eluted with 1×3 mL and 1×2 mL hexane/ethyl acetate (80:20, v/v). Eluents were dried under nitrogen and derivatized with 25 µL N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) at 70°C for 30 min.

Trimethylsilyl derivatized extracts were injected (3 µL) onto an Agilent 6890/5973 2D-GC–MS (Santa Clara, CA) with cry-ofocusing operated in electron impact (EI)/selected ion monitoring (SIM) mode. 2D-GC–MS effectively separated analytes from matrix while cryofocusing significantly enhanced signal. THC ions were m/z 386 (quantitative ion) and three qualifier ions, 371, 303, and 387. The method was fully validated for all ions; occasional endogenous interference required use of the 387 ion as qualifier ion 2 for identification in place of 303. If this was necessary, all calibrators and quality control samples were evaluated similarly. Split calibration curves (low, 0.25–25, and high, 25–100 ng/mL) had $r^2 \geq 0.990$. LOQ were 0.25 ng/mL for THC and THCCOOH and 0.50 ng/mL for 11-OH-THC. Intraassay imprecision ($n = 5$; assays = 4) was 1.5–13.6% for all analytes, and interassay imprecision ($n = 20$) was less than 10.7%.

Data analysis

Statistical analyses were performed with SPSS for Windows, version 13.0 (Chicago, IL) and Microsoft Excel 2002 for Windows. Body mass index (BMI) is a statistical approximation of total body fat calculated using an individual's height and weight. BMI was calculated as BMI = $703 \times$ [weight (lb)/height^2 (in.^{2})]. When statistically evaluating amount of cannabis used with cannabinoid concentrations, one blunt was considered equivalent to four joints as per Bolla et al. (4). Correlation coefficient (R) and p value (p) are statistical calculations that, when combined, can help to determine if a significant relationship exists between variables. If the p value is < 0.05, the relationship is considered statistically significant.

Results

Eighteen frequent cannabis smokers (6 males, 12 females) completed the study. Participant demographic data and self-reported cannabis use are detailed in Table I. The World Health Organization defines persons with BMI values < 18.5 as underweight, 18.6–24.9 as normal, 25–29.9 as overweight, and ≥ 30 as obese (17). Participant BMI ranged from 21.2 to 42.5. Median age of first cannabis use was 14.5 years (range 9–21 years) with 2–22 years of cannabis smoking experience. Most reported daily cannabis smoking in the two weeks prior to study initiation with over half smoking on the day of admission. Only one participant reported smoking cannabis less than 7 of the last 14 days. The reported amounts of cannabis smoked were variably described as joints (a cannabis cigarette), blunts (cannabis plant material rolled into a cigar), ounces, or a dime (i.e., amount of cannabis available for $10). All participants drank alcohol in the month prior to admission, and all but two (participants L and Q) smoked tobacco. None reported other illicit drug use during the two weeks prior to admission.

A total of 126 plasma specimens were analyzed for THC, 11-OH-THC, and THCCOOH by 2D-GC–MS. It was necessary to use the 387 ion to obtain acceptable results for 4 (or 3.2%) of specimens because of endogenous interferences with the 303 ion. All quality control samples and authentic specimens' ion ratios were acceptable based on criteria established by calibrators assayed in the batch for quantifier and qualifier ion ratios.

Median cannabinoid plasma concentrations from admission through seven days of abstinence are presented in Table II. Median ($n = 18$) THC concentration on admission (day 1) was 1.9 ng/mL with a range of concentrations between 0.5 and 9.0 ng/mL (Table II and Figure 1). THC was still detectable in 16 cannabis smokers on day 7 at a median concentration of 1.1 ng/mL. Participant L had the highest THC on six of the seven days with concentrations more than 1.5 times the interquartile range (IQR) on days 1, 2, 3, and 6 and more than 3 times the IQR on days 4 and 7 (Figures 1 and 2). 11-OH-THC was only detected in 17 (13.5%) specimens. Median 11-OH-THC concentration in the 18 participants on admission was none detected (ND) with concentrations ranging between ND and 7.0 ng/mL. Only one participant was positive for the hydroxylated metabolite on day 5; there were no further 11-OH-THC specimens above the 0.5 ng/mL LOQ after this time. THCCOOH was quantifiable in all participants for seven days of abstinence. Concentrations of THCCOOH ranged from 12.1 to 205.4 ng/mL on day 1 and decreased to 2.8–45.6 ng/mL on the final study day. All but three participants had THCCOOH concentrations greater than 5.0 ng/mL on day 7

Plasma cannabinoid concentrations generally decreased over time (Table III) with median decreases from day 1 to day 2 of 24.6% for THC and 23.6% for THCCOOH. The median percent decrease in concentration from day 1 to 7 was 39.5% for THC ($n = 11$) and 72.9% for THCCOOH ($n = 18$). THC median percent decreases from day 1 to 7 ranged between 2.7 and 75.4%. Decrease in analyte concentration was variable from day to day in participants for THC [coefficient of variation (CV) 33.9–125.6%] and THCCOOH (CV 27.9–100.2%). THC concentrations sometimes increased on consecutive days; 88.9% of participants had an increase on at least one day, and a minimum of four participants had THC increases each day. THC median percent increases were between 3.7 and 27.5%. Few THCCOOH increases were observed with median increases between 4.5 and 60.9%. Median changes in THC and THCCOOH concentrations were greater in participants that smoked the day of admission ($n = 10$) than in those who last smoked the previous day ($n = 8$), although there were no significant differences in mean changes. The median change in THC concentrations in individuals smoking the day of admission was −0.6 ng/mL with increases of 1.3 ng/mL ($n = 2$) and decreases of 0.2–3.7 ng/mL ($n = 8$), and for THCCOOH −7.4 ng/mL with increases

of 2.5–25.1 ng/mL ($n = 2$) and decreases of 1.4–47.2 ng/mL ($n = 8$). The median change in THC concentrations in individuals smoking the day prior to admission was +0.1 ng/mL with increases from 0.1 to 0.4 ng/mL ($n = 5$) and decreases of 0.0–0.2 ng/mL ($n = 3$), and for THCCOOH −6.0 ng/mL with increases of 4.7 ng/mL ($n = 1$) and decreases of 0.0–16.7 ng/mL ($n = 7$).

Variable cannabinoid concentrations were observed between participants. Participant A, a normal weight 36-year-old male, had the longest duration (22 years) of cannabis smoking. On day 1, THC concentrations in Participant A's plasma were 4.9 ng/mL and decreased steadily to 1.2 ng/mL on day 7 (Figure 2A). 11-OH-THC was only measurable on admission for this subject. He had the highest THCCOOH concentrations on admission (205.4 ng/mL) through day 3 (111.1 ng/mL) followed by a slow decrease to 32.0 ng/mL on day 7. Participant K, an overweight 26-year-old female, had the shortest duration of use at 2 years. She first smoked cannabis at 21 years of age and reported an average use of 7 cannabis cigarettes per day. Participant K's plasma THC and 11-OH-THC concentrations were 5.3 and 4.0 ng/mL, respectively, on admission and decreased to 1.6 ng/mL. None was detected on day 2 (Figure 2B). She remained THC positive for each of the seven days. Concentrations of THCCOOH in Participant K's plasma ranged from 59.6 ng/mL on day 1 to 9.0 ng/mL on day 7. Participant L, a normal weight 21-year-old female, was the participant with the highest THC concentrations during six of seven study days (Figures 1 and 2C). THC concentrations decreased from 9.0 ng/mL on day 1 to 3.8 ng/mL on day 5 and were higher (5.5 ng/mL) on day 7. 11-OH-THC was detected for the first four days, the longest duration of detection for this cannabinoid. Participant L's THCCOOH concentrations were between the third and fifth highest during the seven days. THC decreases from day 1 to 7 were 75.4, 83.1, and 38.9% for Participants A, K, and L, respectively, and 84.4, 84.9, and 65.8%, respectively for THCCOOH. Mean ± SD plasma cannabinoid concentrations are found in Figure 2D.

No statistically significant differences in THC or THCCOOH concentrations on any study day were observed between normal ($n = 7$), overweight ($n = 6$), or obese ($n = 5$) subjects based on BMI calculations. Also, no correlations were found between THC concentrations greater than LOQ on days 1–7 and BMI ($R = −0.234–0.092$; $p = 0.350–0.766$). In addition, there were no significant correlations in percent decrease in THC ($R = –0.315$, $p = 0.203$), or THCCOOH ($R = −0.308$; $p = 0.213$) concentrations from day 1 to 7 and BMI.

The relationship between years of cannabis use and cannabinoid concentrations throughout seven days of abstinence was examined. There was no statistically significant difference in THC concentration on day 7 based on cannabis use of ≥ 10 years (group 1) or < 10 years (group 2) ($p = 0.961$). No correlation was found between years of use and THC concentration on days 1–7 ($R = −0.211–0.063$; $p = 0.401–0.968$). There also were no relationships between THC or THCCOOH concentrations on days 1 and 7 and frequency of cannabis use ($R = −0.375–0.042$; $p = 0.125–0.986$) or amount smoked daily ($R = 0.044–0.260$; $p = 0.350–0.876$). Additionally, there was no relationship between duration of use and percent decrease in THC ($R = −0.208$; $p = 0.407$) or THCCOOH (R = 0.051; $p = 0.840$) from days 1 to 7. However, plasma THCCOOH concentrations on abstinence days 1–3 were significantly correlated with duration of cannabis use ($R = 0.584–0.610$; $p = 0.007–0.011$).

THCCOOH/THC ratios were evaluated over seven days of abstinence. Median THCCOOH/THC ratios were 16.0 on day 1 ($n = 11$; range 11.2–42.1), generally decreasing between days 1 and 3 and stabilizing on days 4–7. By day 7, the median THCCOOH/THC ratio was 7.7 ($n = 11$; range 4.4–26.7). Median THCCOOH/THC ratios for 11 participants with quantifiable THC and THCOOH for each of seven days are shown in Figure 3.

Discussion

This study presents for the first time plasma cannabinoid concentrations during seven days of continuously monitored abstinence in chronic cannabis smokers. Plasma THC concentrations exceeded 1 ng/mL for seven days in nine frequent cannabis users, and THCCOOH was measurable in all participants each day. Cannabis is the most commonly used drug worldwide and higher cannabis potency in recent years (18) has led to increased cannabis dependence (19) and markedly higher demand for drug treatment (20).

Heavy chronic cannabis use may lead to impaired cognitive performance. There are two primary hypotheses for impairment noted after long-term heavy cannabis use. In one, there is partial recovery of performance after chronic exposure. Solowij et al. (21) reported that individuals smoking cannabis an average of 9.0 ± 3.8 years only partially recovered brain function following extended abstinence. A second hypothesis suggests that cognitive deficits in heavy frequent users can return to normal performance after extended abstinence (22). Current heavy cannabis smokers with more than 5000 lifetime uses had impaired performance on a memory task at baseline and after 1 and 7 days of abstinence, but no significant differences were observed on day 28 as compared to control subjects with less than 50 lifetime cannabis use occurrences (7,22). In their study, a significant relationship was noted between baseline urinary creatinine-normalized THCCOOH concentrations and verbal learning and memory performance on cannabis abstinence day 1. Other investigators documented neurocognitive performance decrements in chronic cannabis users on day 28 of abstinence (4). Thus, studies are needed to determine if decrements in performance found after long-term cannabis exposure can be reversed with extended abstinence and if the impairment and reversal correlate with plasma THC concentrations.

We hypothesize that decreasing THC concentrations in brain during abstinence correlate with improved neuropsychological performance. In our study, THC was detected in plasma on abstinence day 7 in 88.9% of participants. There are two pieces of evidence to date that suggest THC may be present in brain longer than in blood. Brunet et al. (23) demonstrated that blood THC concentrations decreased faster than brain THC when modeled in the large white pig. In addition, THC was detected longer in human brain than in blood in 12 post-mortem cases positive for the inactive THCCOOH metabolite (24).

Unfortunately, in the present study, blood could only be collected for seven days, an insufficient period for total THC elimination in nine of 18 heavy cannabis users participating in this in-patient research study. At the time, it was difficult to justify seven days of blood collection to the ethical review committees due to the belief that THC would be rapidly eliminated, as occurs within hours of acute exposure in occasional cannabis users. Past research conducted in our laboratory showed that THC concentrations decreased rapidly and fell below quantification limits in approximately 12.5 ± 3.1 h after a 3.55% THC cigarette (13). Also, mean THC concentrations were only 0.86 ± 0.22 ng/mL in chronic users 12 h after last cannabis use in a study by Peat et al. (14). A 13-day THC detection period was described for three chronic cannabis smokers administered 60 mg THC over a 2-day period (12). However, the analytical method did not report qualifier ions, LOQ, or other method validation parameters for the few specimens analyzed by GC–MS. More recently, participants resided on the closed research unit for as long as 30 days of abstinence with collection of all urine specimens. Urine specimens were specially hydrolyzed with *E. coli* β-glucuronidase to analyze free THC and 11-OH-THC (25). We found measurable THC ($\geq$ 2.5 ng/mL) in urine up to 24 days after initiation of abstinence on a closed research unit with 24 h monitoring (26). These urine results in conjunction with our plasma THC data suggest that residual THC in the brain could be responsible for noted neurocognitive impairment following heavy, frequent cannabis use.

THC has a high octanol/water partition coefficient (log P = 6.97) (27) and rapidly distributes into lipophilic tissue. A heavy, frequent cannabis user with a greater amount of fat would be expected to have a larger THC body burden compared to a person with less adipose tissue. However, in the present study, plasma cannabinoid concentrations in 18 chronic cannabis users were not correlated with participant BMI values on any study day. Also, there were no significant THC or THCCOOH concentration differences between normal, overweight, and obese participants. Furthermore, no significant correlation was found between THC percent decrease from day 1 to 7 and BMI. BMI as a statistical function of height and weight may not be the strongest measure of adipose content; future studies should consider body fat percent measurements.

Solowij et al. suggested that cannabis use duration was related to cognitive impairment (5). In the present study, no significant correlations were found between THC concentrations and BMI or cannabis use history. However, THCCOOH concentrations were positively associated with use duration on days 1–3. This might indicate that the longer an individual used cannabis, the higher the THCCOOH concentrations for the first three days of abstinence. The best estimate for THCCOOH half-life is approximately 3–4 days (12,28). The average decrease in THCCOOH concentration between days 1 and 5 was 58.7%, indicating that the terminal elimination phase may not have been reached.

Metabolite/parent drug ratios were calculated to further evaluate cannabinoid excretion in this unique cohort. THCCOOH/THC median ratios generally decreased from days 1–4 with a plateau from days 4–7. Median THC and THCCOOH concentrations decreased 39.5% and 72.9% from day 1 to 7. These data suggest that residual and newly formed THCCOOH from THC metabolism might be excreted days 1 to 4. This is followed by THCCOOH formation that is rate-limited by THC release from tissues and subsequent metabolism, indicated by stable THCCOOH/THC ratios from days 4 to 7. One might expect in this case that 11-OH-THC concentrations would be equal to THCCOOH; however, the shorter half-life of this metabolite (29), higher analytical LOQ of 0.5 ng/mL, and much lower residual concentrations of 11-OH-THC after frequent smoking could result in lower 11-OH-THC concentrations. Only 44.4% of participants had measurable 11-OH-THC concentrations upon admission; this number decreased to 16.7% by day 2. After smoked administration, low 11-OH-THC concentrations are formed by THC hepatic microsomal oxidation (approximately 5–10% of THC) (13,29). In contrast, oral THC first pass metabolism yields equivalent or sometimes higher 11-OH-THC concentrations (13,29).

Plasma cannabinoid excretion was variable between individuals as evidenced in three individuals' excretion profiles in Figure 2. The participant with the longest cannabis exposure history (A) had measurable THC concentrations throughout the study, and by day 3, THC and THCCOOH concentrations decreased in a parallel fashion. The participant who most recently became a cannabis user (K) had large decreases in THC and THCCOOH on the first day and sustained low level THC for the next six days. Additionally, Participant K last smoked cannabis the day of admission, which may explain the large drop in THC and THCCOOH concentrations observed between days 1 and 2. 11-OH-THC concentrations were greater than LOQ for four days in Participant L's plasma, the participant with the highest THC concentrations for six of seven days.

Most participants (88.9%) had at least one day where THC concentrations increased from the previous day. Possible reasons for an increase in release might include differences in exercise, diet, or hydration. Although THC and THCCOOH median percent decreases were similar on most days, the most dramatic difference occurred between days 2 and 3. Almost five-fold higher THCCOOH decreases may be explained by excretion of high concentrations of residual THCCOOH between days 2 and 3 with similar decreases in THC and

THCCOOH from days 4–7. This could be due to THC release from the tissues and rapid metabolism. Importantly, reported increases and decreases are based on median data, thus decreasing the variability between participants.

Study limitations included reliance on self-report as data analysis regressors, limited specimen volume, and long-term storage of clinical specimens. Participant L, who had the highest plasma THC concentrations, reported using only three of the last 14 days, which suggests inaccuracy in reporting. A highly sensitive 2D-GC–MS method was developed for cannabinoid analysis during residual excretion (16). LOQs of 0.25 ng/mL for THC and THCCOOH and 0.5 ng/mL for 11-OH-THC were achieved. Sometimes there was difficulty in obtaining an adequate blood volume or multiple analyses were required, which decreases available blood volume below the usual 1-mL specimen size. In these cases, specimen volume was decreased, resulting in a higher LOQ for that specimen. A third limitation involved specimen storage in polypropylene cryotubes for up to five years at −20°C. Cannabinoid concentration decreases have been observed when specimens were frozen for an extended period before analysis (30), although this could only have caused an under-, not over-, estimation of cannabinoid concentrations.

Forensic interpretation

There are several important implications of this research to forensic toxicology. Plasma THC concentrations were greater than 1 ($n = 9$) and 2 ($n = 4$) ng/mL for seven days in frequent cannabis smokers during continuously monitored abstinence. These concentrations are commonly thought to signify recent cannabis smoking and, in some jurisdictions, can imply performance impairment. In some U.S. states, per se DUID legislation may specify impairment based on the presence of measurable or low THCCOOH blood or plasma concentrations. THCCOOH is an inactive THC metabolite that does not affect driving performance. Some jurisdictions state "any measurable controlled substance or metabolite of a controlled substance in the person's body" (31) is illegal while driving. In the present study, THCCOOH was detected in all participants' plasma throughout the study with day 7 concentrations greater than 5.0 ng/mL in 83.3% of heavy, chronic cannabis smokers.

Although neuropsychological impairment (32–36) and increased motor vehicle accident risk (37) occur when under the influence of acute THC, it has been difficult to correlate these findings to specific blood or plasma concentrations. Accident risk attributed to cannabis use was assessed in 3398 Australian driving fatalities. Drivers with detectable blood THC concentrations were 2.7 times (95% CI 1.0–7.0) more likely to be culpable in an accident compared to drug-free drivers (37). The likelihood of culpability increased 6.6-fold (95% CI 1.5–28.0) when drivers had blood THC concentrations at or above 5.0 ng/mL (approximately 10 ng/mL plasma).

Recently, experimental research suggested cognitive impairment at serum THC concentrations of 2–5 ng/mL on a critical tracking task, a skill important to driving performance (38). At these limits, four of our participants would be judged impaired after seven days of cannabis abstinence. Other investigators performed a comprehensive meta-analysis of experimental data and proposed 7–10 ng/mL serum THC as per se limits of impairment (39). Based on this assessment, participant L would be deemed impaired on day 4 of our study, three days after last cannabis use. On the other hand, blood THC concentrations at the time of an accident are likely much higher than at the time blood is collected by clinical staff or police, which is generally 2–4 h after an incident. Thus, although per se limits may be useful in cases where impairment cannot be documented by field sobriety tests (40,41), drug recognition expert examinations, or other measures of performance, limits as high as 2–5 ng/mL would prevent conviction of many drugged drivers who had documented poor performance with much lower THC concentrations at the

time of blood collection. Jones et al. (42) clearly showed that 77–90% of Swedish motor vehicle operators from 1995 to 2004 who recently smoked cannabis would escape criminal charges if a 5 ng/mL whole blood limit were applied.

Future studies

Future cannabinoid excretion studies in chronic cannabis users should be conducted for longer than seven days and include psychomotor and cognitive performance measures to document the presence or absence of impairment. If measurable THC concentrations were accompanied by neuropsychological impairment, this would provide a justification for per se law implementation. An extended monitoring period would allow for more comprehensive data on cannabinoid last detection times in plasma of frequent, daily cannabis users and accompanying performance deficits. Multiple daily blood draws are also recommended, enabling plasma terminal half-life determination in this unique cannabis-using population. Furthermore, specimens should be analyzed after short-term storage, which facilitates the development of models to predict the time of last cannabis use. Although models estimating last cannabis use were developed for occasional cannabis users and validated with data from multiple smoked and oral administration studies (43–45), models have not been validated in heavy, long-term daily cannabis smokers.

Conclusions

This study is the first to determine plasma cannabinoid concentrations in chronic cannabis smokers during seven days of continuously monitored abstinence. Plasma THC concentrations greater than 2 ng/mL were observed in multiple specimens on the seventh day of cannabis abstinence, suggesting THC's presence in plasma may not necessarily indicate recent cannabis smoking in chronic cannabis users. These data may impact clinical cannabinoid pharmacotherapy, cannabis dependence treatment, and per se DUID legislation in the U.S. and abroad. It also suggests a mechanism for residual neurocognitive impairment observed in chronic cannabis smokers after multiple days of abstinence.

Acknowledgments

References

1. Pope HG, Yurgelun-Todd D. The residual cognitive effects of heavy marijuana use in college students. J. Am. Med. Assoc. 1996; 275:521–527.

2. Pope H, Gruber A, Hudson J, Huestis M, Yurgelun-Todd D. Neuropsychological performance in long-term cannabis users. Arch. Gen. Psychiatry. 2001; 58:909–915. [PubMed: 11576028]

3. Block RI, Ghoneim MM. Effects of chronic marijuana use on human cognition. Psychopharmacology (Berl). 1993; 110:219–228. [PubMed: 7870889]

4. Bolla KI, Brown K, Eldreth D, Tate K, Cadet JL. Dose-related neurocognitive effects of marijuana use. Neurology. 2002; 59:1337–1343. [PubMed: 12427880]

5. Solowij N, Stephens RS, Roffman RA, Babor T, Kadden R, Miller M, Christiansen K, McRee B, Vendetti J. Cognitive functioning of long-term heavy cannabis users seeking treatment. J. Am. Med. Assoc. 2002; 287:1123–1131.

6. Fletcher JM, Page JB, Francis DJ, Copeland K, Naus MJ, Davis CM, Morris R, Krauskopf D, Satz P. Cognitive correlates of long-term cannabis use in Costa Rican men. Arch. Gen. Psychiatry. 1996; 53:1051–1057. [PubMed: 8911228]

7. Pope HG, Gruber AJ, Hudson JI, Huestis MA, Yurgelun-Todd D. Cognitive measures in long-term cannabis users. J. Clin. Pharmacol. 2002; 42:41S–47S. [PubMed: 12412835]

8. Pillay SS, Rogowska J, Kanayama G, Gruber S, Simpson N, Pope HG, Yurgelun-Todd DA. Cannabis and motor function: fMRI changes following 28 days of discontinuation. Exp. Clin. Psychopharmacol. 2008; 16:22–32. [PubMed: 18266549]

9. Kreuz DS, Axelrod J. Delta-9-tetrahydrocannabinol: Localization in body fat. Science. 1973; 179:391–393. [PubMed: 4682965]

10. Agurell S, Nilsson IM, Ohlsson A, Sandberg F. On the metabolism of tritium-labelled 1-tetrahydrocannabinol in the rabbit. Biochem. Pharmacol. 1970; 19:1333–1339. [PubMed: 5513923]

11. Garrett ER, Hunt CA. Pharmacokinetics of delta-9-tetrahydrocannabinol in dogs. J. Pharm. Sci. 1977; 66:395–406. [PubMed: 845807]

12. Johansson E, Agurell S, Hollister LE, Halldin MM. Prolonged apparent half-life of delta-1-tetrahydrocannabinol in plasma of chronic marijuana users. J. Pharm. Pharmacol. 1988; 40:374–375. [PubMed: 2899638]

13. Huestis MA, Henningfield JE, Cone EJ. Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. J. Anal. Toxicol. 1992; 16:276–282. [PubMed: 1338215]

14. Peat, MA. Distribution of delta-9-tetrahydrocannabinol and its metabolites. In: Baselt, RC., editor. Advances in Analytical Toxicology II. Chicago, IL: Year Book Medical Publishers; 1989. p. 186-217.

15. Skopp G, Potsch L. Cannabinoid concentrations in spot serum samples 24–48 hours after discontinuation of cannabis smoking. J. Anal. Toxicol. 2008; 32:160–164. [PubMed: 18334100]

16. Lowe RH, Karschner EL, Schwilke EW, Barnes AJ, Huestis MA. Simultaneous quantification of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) in human plasma using two-dimensional gas chromatography, cryofocusing, and electron impact-mass spectrometry. J. Chromatogr. A. 2007; 1163:318–327. [PubMed: 17640656]

17. World Health Organization. Physical Status: The Use and Interpretation of Anthropometry. Geneva, Switzerland: World Health Organization; 1995. p. 47

18. ElSohly MA, Ross SA, Mehmedic Z, Arafat R, Banahan BFI. Potency trends of Δ^9-THC and other cannabinoids in confiscated marijuana from 1980–1997. J. Forensic Sci. 2000; 45:24–30. [PubMed: 10641915]

19. Stinson FS, Ruan WJ, Pickering R, Grant BF. Cannabis use disorders in the USA: prevalence, correlates and co-morbidity. Psychol. Med. 2006; 36:1447–1460. [PubMed: 16854249]

20. Carpenter KM, McDowell D, Brooks DJ, Cheng WY, Levin FR. A preliminary trial: double-blind comparison of nefazodone, bupropion-SR, and placebo in the treatment of cannabis dependence. Am. J. Addict. 2009; 18:53–64. [PubMed: 19219666]

21. Solowij N. Do cognitive impairments recover following cessation of cannabis use? Life Sci. 1995; 56:2119–2126. [PubMed: 7776840]

22. Pope HG, Gruber AJ, Hudson JI, Huestis MA, Yurgelun-Todd D. Neuropsychological performance in long-term cannabis users. Arch. Gen. Psychiatry. 2001; 58:909–915. [PubMed: 11576028]

23. Brunet B, Doucet C, Venisse N, Hauet T, Hebrard W, Papet Y, Mauco G, Mura P. Validation of Large White Pig as an animal model for the study of cannabinoids metabolism: application to the study of THC distribution in tissues. Forensic Sci. Int. 2006; 161:169–174. [PubMed: 16859848]

24. Mura P, Kintz P, Dumestre V, Raul S, Hauet T. THC can be detected in brain while absent in blood. J. Anal. Toxicol. 2005; 29:842–843. [PubMed: 16356342]

25. Abraham TT, Lowe RH, Pirnay SO, Darwin WD, Huestis MA. Simultaneous GC–EI-MS determination of Δ^9-tetrahydrocannabinol, 11-hydroxy-Δ^9-tetrahydrocannabinol, and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol in human urine following tandem enzyme-alkaline hydrolysis. J. Anal. Toxicol. 2007; 31:477–485. [PubMed: 17988462]

26. Lowe RH, Abraham TT, Darwin WD, Herning R, Cadet JL, Huestis MA. Extended urinary Δ^9-tetrahydrocannabinol excretion in chronic cannabis users precludes use as a biomarker of new drug exposure. Drug Alcohol Depend. 2009 Jul 22. E pub ahead of print.

27. Thomas BF, Compton DR, Martin BR. Characterization of the lipophilicity of natural and synthetic analogs of delta 9-tetrahydrocannabinol and its relationship to pharmacological potency. J. Pharmacol. Exp. Ther. 1990; 255:624–630. [PubMed: 2173751]

28. Johansson E, Halldin MM. Urinary excretion half-life of delta1-tetrahydrocannabinol-7-oic acid in heavy marijuana users after smoking. J. Anal. Toxicol. 1989; 13:218–223. [PubMed: 2550702]

29. Wall ME, Sadler BM, Brine D, Taylor H, Perez-Reyes M. Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women. Clin. Pharmacol. Ther. 1983; 34:352–363. [PubMed: 6309462]

30. Schwilke EW, Karschner EL, Lowe RH, Gordon AM, Cadet JL, Herning R, Huestis MA. Intra- and Inter-subject whole blood/plasma cannabinoid ratios determined by 2-dimenJournal of Analytical Toxicology, Vol. 33, October 2009 sional, electron impact-gas chromatography, mass spectrometry with cryofocusing. Clin. Chem. 2009; 55(6):1188–1195. [PubMed: 19264857]

31. N. T. L. C. American Prosecutors Research Institute. American Prosecutors Research Institute; 2006. Appendix 2. Driving while under the influence of drugs, state law summary; p. 1-28.

32. Chait, LD.; Pierri, J. Effects of smoked marijuana on human performance: a critical review. Marijuana/Cannabinoids. In: Murphy, L.; Bartke, A., editors. Neurobiology and Neurophysiology. Boca Raton, FL: CRC Press; 1992. p. 387-423.

33. Kurzthaler I, Hummer M, Miller C, Sperner-Unterweger B, Gunther V, Wechdorn H, Battista HJ, Fleischhacker WW. Effect of cannabis use on cognitive functions and driving ability. J. Clin. Psychiatry. 1999; 60:395–399. [PubMed: 10401919]

34. Riedel, G.; Davies, SN. Cannabinoid function in learning, memory and plasticity. In: Pertwee, RG., editor. Handbook of Experimental Pharma–cology. New York, NY: Springer; 2005. p. 446-470.

35. Hall W, Solowiji N, Lemon J. The health and psychological consequences of cannabis use. National task force on cannabis. 1994

36. Curran HV, Brignell C, Fletcher S, Middleton P, Henry J. Cognitive and subjective dose-response effects of acute oral delta-9-tetrahydrocannabinol (THC) in infrequent cannabis users. Psychopharmacology (Berl). 2002; 164:61–70. [PubMed: 12373420]

37. Drummer OH, Gerostamoulos J, Batziris H, Chu M, Caplehorn J, Robertson MD, Swann P. The involvement of drugs in drivers of motor vehicles killed in Australian road traffic crashes. Accid. Anal. Prev. 2004; 36:239–248. [PubMed: 14642878]

38. Ramaekers JG, Moeller MR, van Ruitenbeek P, Theunissen EL, Schneider E, Kauert G. Cognition and motor control as a function of Δ^9-THC concentration in serum and oral fluid: limits of impairment. Drug Alcohol Depend. 2006; 85:114–122. [PubMed: 16723194]

39. Grotenhermen F, Leson G, Berghaus G, Drummer OH, Kruger HP, Longo M, Moskowitz H, Perrine B, Ramaekers JG, Smiley A, Tunbridge R. Developing limits for driving under cannabis. Addiction. 2007; 102:1910–1917. [PubMed: 17916224]

40. Papafotiou K, Carter JD, Stough C. An evaluation of the sensitivity of the Standardised Field Sobriety Tests (SFSTs) to detect impairment due to marijuana intoxication. Psychopharmacology (Berl). 2005; 180:107–114. [PubMed: 15619106]

41. Papafotiou K, Carter JD, Stough C. The relationship between performance on the standardised field sobriety tests, driving performance and the level of delta-9-tetrahydrocannabinol (THC) in blood. Forensic Sci. Int. 2005; 155:172–178. [PubMed: 16226154]

42. Jones AW, Holmgren A, Kugelberg FC. Driving under the influence of cannabis: a 10-year study of age and gender differences in the concentrations of tetrahydrocannabinol in blood. Addiction. 2008; 103:452–461. [PubMed: 18190663]

43. Huestis MA, Henningfield JE, Cone EJ. Blood cannabinoids. II. Models for the prediction of time of marijuana exposure from plasma concentrations of delta-9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCCOOH). J. Anal. Toxicol. 1992; 16:283–290. [PubMed: 1338216]

44. Huestis MA, ElSohly M, Nebro W, Barnes A, Gustafson RA, Smith ML. Estimating time of last oral ingestion of cannabis from plasma THC and THCCOOH concentrations. Ther. Drug Monit. 2006; 28:540–544. [PubMed: 16885722]

45. Huestis MA, Barnes A, Smith ML. Estimating the time of last cannabis use from plasma Δ^9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol concentrations. Clin. Chem. 2005; 51:2289–2295. [PubMed: 16223887]
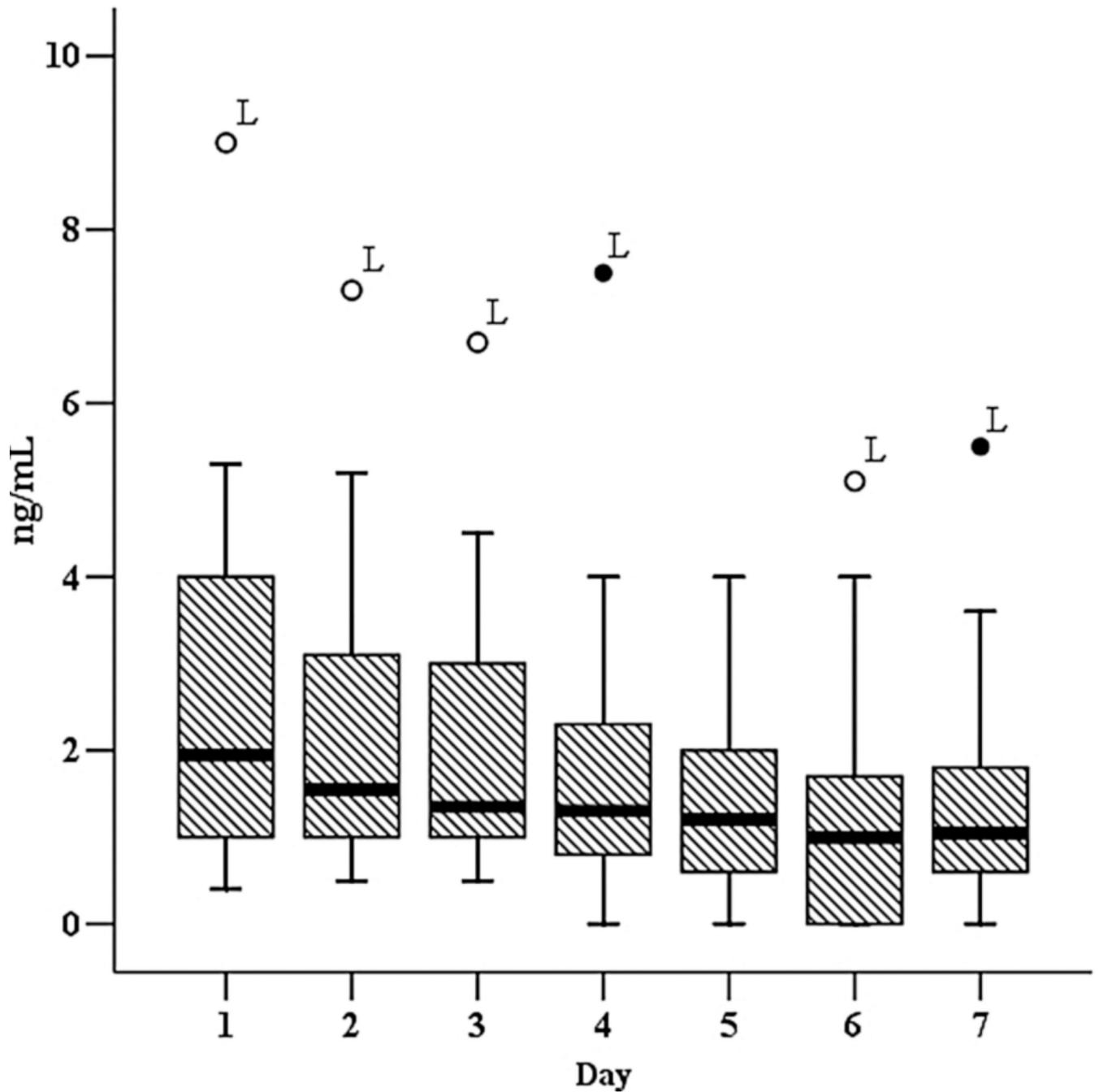
Figure 1.
Box plot of Δ^9-tetrahydrocannabinol (THC) plasma elimination during continuous monitored abstinence in 18 frequent cannabis users. Thin horizontal lines specify concentration range, boxes the 25th and 75th percentiles (interquartile range, IQR), and the median concentration is the darker line within the box. Outliers (greater than 1.5 times IQR) are marked with an open circle and extreme cases (greater than 3 times IQR) by a closed circle.
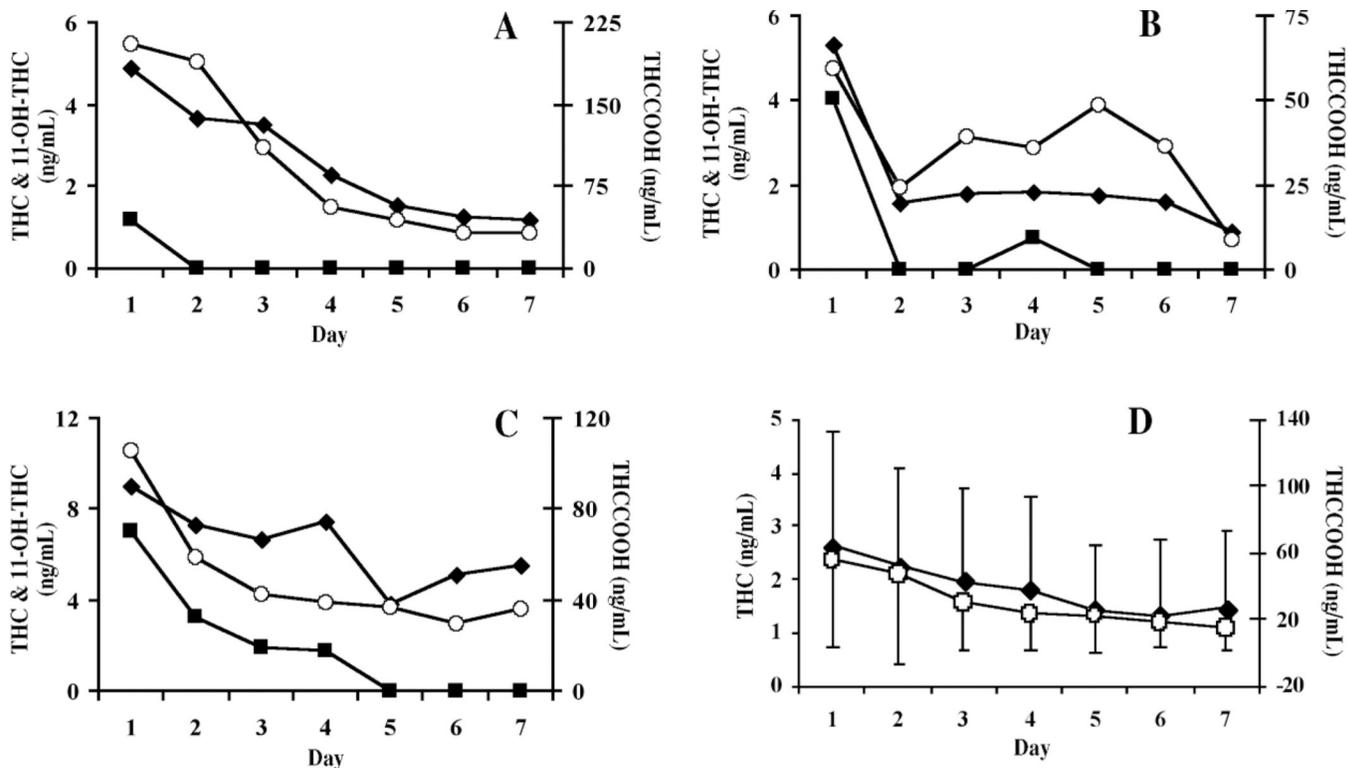
Figure 2.
Δ^9-Tetrahydrocannabinol (THC, ♦), 11-hydroxy-THC (11-OH-THC, ■), and 11-nor-9-carboxy-THC (THCCOOH, ○) concentrations in participant A (A), who smoked cannabis for 22 years (longest use), participant K (B), who smoked cannabis for the last 2 years (shortest use), and participant L (C), who had the highest THC concentrations for 6 of 7 days of continuous monitored abstinence. THC and THCCOOH mean ± SD concentrations, including 0 for specimens with no drug detected ($n = 18$) (D).
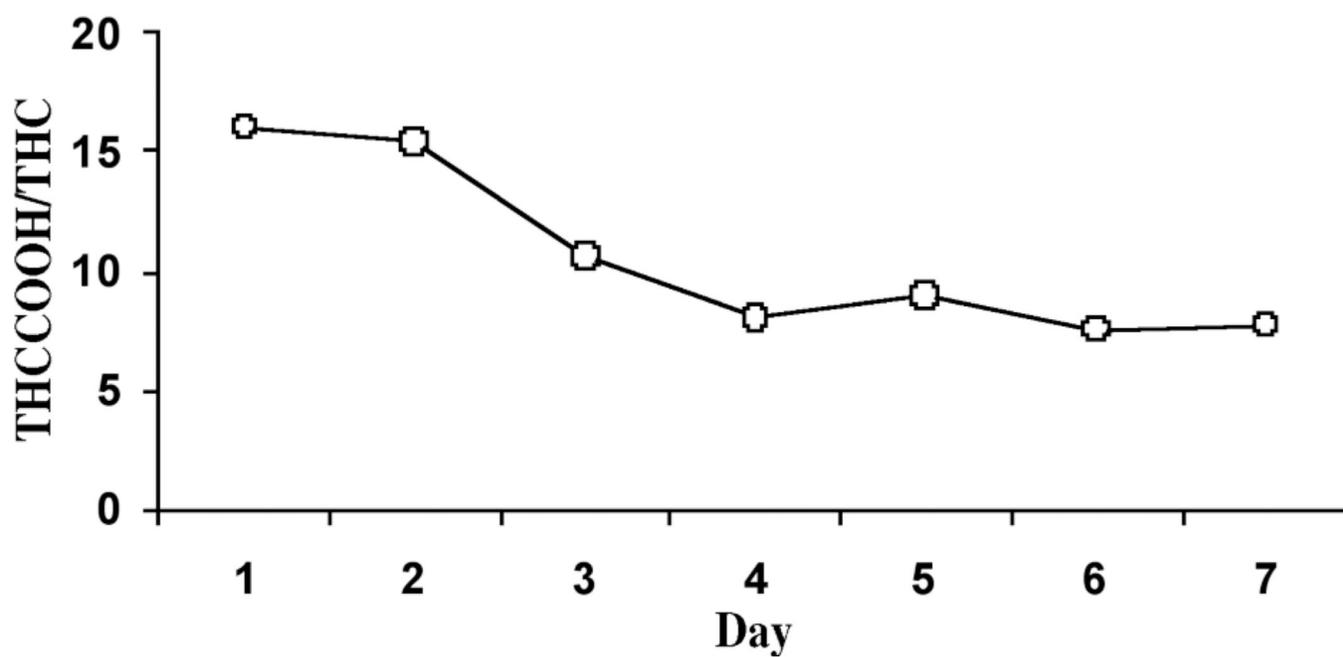
Figure 3.
11-nor-9-carboxy-Δ^9-Tetrahydrocannabinol (THCCOOH, ○) to Δ^9-tetrahydrocannabinol (THC, ♦) median ratios in 11 participants with THC and THCCOOH concentrations greater than the 0.25 ng/mL limits of quantification for each of seven days of continuous monitored abstinence.

Table I

Participant Demographics and Self-Reported Cannabis Smoking Histories

Participant	Sex*	Age Years	Ethnicity†	BMI	Mean Cannabis per Day	# Days Used Last 14	Days Since Last Use‡	Years of Use	Age at 1st Use
A	M	36	AA	21.2	0.25 ounce	14	0	22	14
B	M	24	AA	32.1	1 dime	10	1	10	14
C	M	33	AA	21.6	1 blunt	8	1	5	17
D	M	25	AA	23.5	6 blunts	14	0	9	14
E	M	22	AA	25.7	2 blunts	14	1	4	18
F	M	28	AA	23.0	2 blunts	14	0	13	14
G	F	21	AA	31.3	0.5 ounce	14	0	12	9
H	F	29	AA	28.2	2 blunts	14	1	10	12
I	F	22	AA	24.4	5 blunts	14	0	8	14
J	F	30	AA	23.0	2 blunts	10	1	12	18
K	F	26	C	26.6	7 joints	11	0	2	21
L	F	21	AA	22.8	4 blunts	3	0	7	14
M	F	22	AA	28.3	4–5 blunts	14	1	8	14
N	F	28	AA	27.2	3 blunts	14	0	11	16
O	F	26	AA	27.8	4 blunts	14	1	9	17
P	F	23	AA	39.0	8 blunts	14	0	8	15
Q	F	23	AA	32.0	4 blunts	14	0	3	20
R	F	23	AA	42.5	2 blunts	7	1	5	18
Mean		25.7		27.8	0.25 ounce	12.1	0.4	9.0	15.5
SD		4.2		5.8	1 dime	3.2	0.5	4.7	2.9
Median		24.5		26.9	1 blunt	14.0	0.0	8.5	14.5

*
 M, male and F, female.

†
AA, African American and C, Caucasian.

‡
Days since last use = 0 (used day of admission); 1 (used day prior to admission).

Table II

Median (*n* = 18) and Range of Plasma Cannabinoid Concentrations During Seven Days of Continuous Monitored Abstinence, Number of Participants With Concentrations Above Common Analytical Cutoffs (1, 2, and 5 ng/mL) and LOQ of 0.25 ng/mL for THC and THCCOOH and 0.5 ng/mL for 11-OH-THC

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
THC (ng/mL)							
Median	1.9	1.6	1.4	1.3	1.2	1.0	1.1
Range	0.5–9.0	0.5–7.3	ND–6.7	ND–7.5	ND–4.0	ND–5.1	ND–5.5
n ≥ LOQ	18	18	17	17	14	13	16
n ≥ 1.0 ng/mL	15	14	13	11	12	9	9
n ≥ 2.0 ng/mL	9	6	5	6	5	4	4
11-OH-THC (ng/mL)							
Median	0.0	0.0	0.0	0.0	0.0	–	–
Range	ND–7.0	ND–3.3	ND–2.0	ND–1.7	ND–1.2	–	–
n ≥ LOQ	8	3	3	2	1	–	–
n ≥ 1.0 ng/mL	8	3	3	1	1	–	–
n ≥ 2.0 ng/mL	4	3	1	0	0	–	–
THCCOOH (ng/mL)							
Median	35.7	24.2	18.1	14.1	11.9	11.9	9.3
Range	12.1–205.4	7.1–189.4	6.3–111.1	4.3–88.3	3.8–89.7	2.9–53.8	2.8–45.6
n ≥ LOQ	18	18	18	18	18	18	18
n ≥ 5.0 ng/mL	18	18	18	17	16	16	15

Table III

Median, Minimum, and Maximum Percent Increases and Decreases in THC ($n = 11$) and THCCOOH ($n = 18$) Concentrations Per Day During Seven Days of Continuous Monitored Abstinence

	Days 1 to 2	Days 2 to 3	Days 3 to 4	Days 4 to 5	Days 5 to 6	Days 6 to 7
THC						
n with decrease	7	6	6	6	7	6
Median % THC decrease	24.6	6.7	26.2	31.7	15.6	18.3
Minimum decrease (%)	6.6	1.0	5.3	3.4	6.4	4.5
Maximum decrease (%)	70.3	14.3	42.3	59.0	51.9	44.4
n with increase	4	5	5	5	4	5
Median % THC increase	13.1	13.9	3.7	17.9	27.5	16.8
Minimum increase (%)	5.8	3.2	1.5	0.5	0.5	5.1
Maximum increase (%)	31.3	63.3	11.8	108.0	90.9	35.9
THCCOOH						
n with decrease	15	17	15	13	14	14
Median % THCCOOH decrease	23.6	32.0	27.6	20.1	15.5	26.1
Minimum decrease (%)	0.1	7.6	3.4	3.7	1.5	2.7
Maximum decrease (%)	59.1	53.7	49.1	51.6	40.0	75.5
n with increase	3	1	3	5	4	4
Median % THCCOOH increase	15.9	60.9	4.5	23.9	9.2	28.7
Minimum increase (%)	3.5	–	4.4	1.6	0.3	1.2
Maximum increase (%)	39.3	60.9	27.5	35.4	33.9	40.8