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Correlation of Breath and Blood Δ^9-Tetrahydrocannabinol Concentrations and Release Kinetics Following Controlled Administration of Smoked Cannabis

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BACKGROUND: Cannabis use results in impaired driving and an increased risk of motor vehicle crashes. Cannabinoid concentrations in blood and other matrices can remain high long after use, prohibiting the differentiation between acute and chronic exposure. Exhaled breath has been proposed as an alternative matrix in which concentrations may more closely correspond to the window of impairment; however, efficient capture and analytically sensitive detection methods are required for measurement.

METHODS: Timed blood and breath samples were collected from 20 volunteers before and after controlled administration of smoked cannabis. Cannabinoid concentrations were measured using LC-MS/MS to determine release kinetics and correlation between the 2 matrices.

RESULTS: Δ9-Tetrahydrocannabinol (THC) was detected in exhaled breath for all individuals at baseline through 3 h after cannabis use. THC concentrations in breath were highest at the 15-min timepoint (median = 17.8 pg/L) and declined to <5% of this concentration in all participants 3 h after smoking. The decay curve kinetics observed for blood and breath were highly correlated within individuals and across the population.

CONCLUSIONS: THC can be reliably detected throughout the presumed 3-h impairment window following controlled administration of smoked cannabis. The findings support breath THC concentrations as representing a physiological process and are correlated to blood concentrations, albeit with a shorter window of detection.

As the legalization of medical and recreational marijuana use expands in the US and globally, the public health concern over marijuana-associated impaired driving is increasing. According to the National Forensic Laboratory Information System's 2017 report, Δ9-tetrahydrocannabinol (THC)[2] was the second most frequently identified drug in drug-related incidents (n = 344 167 reports, 21.7% of cases) *(1)*. National roadside surveys conducted by the US National Highway Traffic Safety Administration have shown THC to be the most prevalent drug detected in a representative sample of drivers, with a 48% increase in prevalence from 2007 to 2014 *(2)*.

Numerous experimental studies indicate that cannabis use results in impaired driving performance and an increased risk of motor vehicle crashes *(3, 4)*. THC, the primary psychoactive component in cannabis, impairs both motor and cognitive functions, including reaction time, tracking, attention, decision-making, impulse control, and memory *(3, 4)*. Performance impairment is associated with time since last use and has been shown to peak within 1 h of smoking marijuana, declining over 2 to 3 h after cannabis use *(4, 5)*. Therefore, reliable testing requires detection throughout the 3-h impairment window. Blood THC concentrations are associated with driving impairment; however, no direct correlation has been observed *(4, 6)*. The practicality of blood testing for THC is also uncertain given substantial delays between arrest and collection *(7, 8)*. Studies on cannabis users' perceptions regarding driving under the influence of cannabis are also concerning. Contrary to scientific evidence, most users wrongly believe that cannabis does not affect their driving performance and that they can compensate for any resulting impairment *(9, 10)*. These findings underscore the need for reliable roadside analytical methods for the detection of driving under the influence of cannabis.

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[2] Nonstandard abbreviations: THC, Δ9-tetrahydrocannabinol; CUDIT-R, Cannabis Use Disorder Test; 11-OH-THC, 11-hydroxy-Δ^9-tetrahydrocannabinol; THC-COOH, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol; CBN, cannabinol; CBD, cannabidiol; LOQ, limit of quantification.

Blood THC concentrations peak during smoking or vaping and decay to a mean of approximately 5 ng/mL at 30 min and 1 ng/mL at 180 min *(11)*. Blood concentrations after consumption are dependent on history of use, with frequent users having higher concentrations than infrequent users *(12)*. Because of its long terminal half-life (20–30 h), THC can remain detectable for many days after last use, prohibiting the differentiation between acute and chronic use *(13, 14)*. Likewise, studies in oral fluid following controlled administration demonstrate long windows of detection. In 1 study, oral fluid THC was still detected in frequent smokers 72 h after use (the final timepoint) *(15)*.

Exhaled breath has been suggested as an alternative matrix in which detection of cannabinoids may more closely correspond to the window of impairment. In the first study published in 1983, THC was detected only in breath at 10 min after smoking and was below the analytical limit of detection at 20 min *(16)*. Three decades later, an alternative sampling procedure followed by quantification using LC-MS/MS was evaluated for the detection of cannabinoids in breath samples *(17, 18)*. In 1 study *(18)*, THC was detected in 13 chronic smokers at 30 min, 1 h, and 2 h following controlled administration. THC was not detectable 3 h after cannabis use in all chronic smokers but became detectable again for 1 individual at 4 h. For occasional smokers, THC in exhaled breath was detected at 30 min and 1 h but was not detectable in subsequent timepoints. These results were the first to suggest that the THC detection window in breath might coincide with the window of impairment following smoked cannabis use.

A subsequent study demonstrated a high interindividual variation in the concentration maximum and area under the concentration curve for THC in breath despite small differences in dose *(19)*. THC was detectable for 3 h after smoking cannabis in 13 smokers; however, all individuals had measurable THC in their breath at baseline and significantly higher breath THC concentrations compared with a prior study *(18)*. Contamination from the environment of the oral cavity during the collection of exhaled breath was proposed to explain the discrepancy. Although studies on the measurement of THC in exhaled breath are promising, it is critical to understand whether the concentration of THC in exhaled breath corresponds to systemic physiological exposure rather than simply representing environmental contamination.

The goals of our current study were 3-fold. First, we aimed to carefully control environmental contamination in the test setting to collect only THC related to physiological consumption. Second, we used an ultrasensitive detection *(20)* to assess whether THC was detectable above baseline throughout the 3-h impairment window for both chronic and occasional smokers. Finally, we sought to correlate the concentration of cannabinoids in breath with the concentration in blood to determine whether breath concentrations were indicative of systemic physiological consumption.

Materials and Methods

HUMAN VOLUNTEERS AND BIOLOGICAL SAMPLE COLLECTION

Twenty research volunteers with a history of cannabis use were recruited for the study. The study was approved by the University of California San Francisco Institutional Review Board and conducted on a closed clinical research unit. For inclusion in the study, it was required that each participant be an experienced marijuana user (self-reported) >21 years of age with a minimum use of 1 time in the 14 days before study participation. Exclusion criteria included history of cardiac problems, seizure disorder, pulmonary condition, and/or asthma; physical dependence on any drug other than cannabis, caffeine, or nicotine; drinking >10 alcoholic beverages per week (on average); a current psychiatric illness, mental health disorder, or major depression; and pregnancy (as determined by a urine pregnancy test upon arrival for study participation).

Participants were instructed to abstain from THC for 24 h before participation. The Cannabis Use Disorder Test (CUDIT-R) was completed by each participant. A saline lock was placed in the arm of each participant for all blood collections. Whole-blood samples were collected in potassium oxalate/sodium fluoride Vacutainer tubes (Becton Dickinson). All breath samples were collected using a handheld device with a baffle-based saliva trap (Alcohol Countermeasures Systems) incorporated in the mouthpiece to remove oral fluid (Hound Labs). An accurately measured volume of exhaled breath (18 L ± 10%) was taken into the breath-capture module consisting of a packed bed of 800-μm silica beads and a Technostat electrostatic filter. Blood and breath samples were collected at baseline, before smoking THC. Each study participant smoked marijuana that they purchased and used what was equivalent to a single-use event for them, mimicking real-world cannabis use scenarios. Smoking sessions, which lasted for 10 min with smoking ad libitum, took place in a separate smoking room (with a specialized ventilation system) from all blood and breath measurements. Timed collection of breath and blood samples was done at the following timepoints (from the end of the smoking session): 15, 30, 45, 60, 90, 120, 150, and 180 min.

CHEMICALS AND REAGENTS

All cannabinoid standards were purchased from Cerilliant: THC, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), cannabinol (CBN), cannabidiol

(CBD), THC-D_3, 11-OH-THC-D_3, THCCOOH-D_3, CBN-D_3, and CBD-D_3. LC-MS/MS-grade methanol, acetonitrile, and water were purchased from Honeywell Burdick & Jackson. Diazonium salt Fast Red RC was purchased from Sigma-Aldrich. WAX-S tips (1-mL tip containing 20 mg of resin and 40 mg of salt) were purchased from DPX Technologies.

SAMPLE PREPARATION FOR BREATH AND WHOLE-BLOOD SAMPLES

All breath and whole-blood cannabinoid measurements (THC, 11-OH-THC, THC-COOH, CBN, CBD) were done using previously published and analytically validated LC-MS/MS methods *(20)*. Samples captured from exhaled breath (18 L) were eluted from the breath-capture module with 1.0 mL of methanol. For sample preparation, 5.0 μL of internal standard mix (1.0 ng/mL) was added to 200 μL of eluted breath sample. The fortified sample was then derivatized for 30 min at room temperature with 45 μL of a diazonium solution (1.5 mmol/L Fast Red RC in 5.0 mmol/L ammonium acetate buffer).

For whole-blood samples, 200 μL was fortified with 10 μL of internal standard mix (200 ng/mL), and 500 μL of acetonitrile was added for protein precipitation. Following vortex-mixing and centrifugation at 1250*g* for 20 min, 500 μL of supernatant was transferred to a 96-well plate and 200 μL of 5.0% formic acid was added. The plate was loaded to a VIAFLO ASSIST pipetting robot (Integra Biosciences) equipped with WAX-S tips, and 4 aspiration/dispense cycles were implemented. Then 50 μL of the upper layer was mixed with 50 μL of mobile phase A. To derivatize the analytes, 350 μL of methanol and 50 μL of diazonium solution (1.5 mmol/L Fast Red RC in 5.0 mmol/L ammonium acetate buffer) were added, and the sample was incubated at room temperature for 0.5 h.

LC-MS/MS METHOD FOR BREATH AND WHOLE-BLOOD SAMPLES

LC-MS/MS analysis was performed using a Shimadzu Prominence LC-20ADXR and QTRAP® 4500 triple-quadrupole mass spectrometer (Sciex). Mobile phases consisted of 5.0 mmol/L ammonium formate with 0.05% formic acid and acetonitrile with 0.05% formic acid. A Kinetex C18 column (3.0 mm × 50 mm, 2.6-μm particle, temperature 30 °C) (Phenomenex) was used with gradient elution for HPLC separation (flow rate, 0.5 mL/min): equilibration at 80% B for 1 min, increased to 100% B over 1.9 min and held for 4 min, returning to 80% B over 0.1 min and held for 0.9 min. Sample injection volume was 50 μL (breath) or 20 μL (whole blood). Mass spectrometry conditions included ESI positive-ion mode; curtain gas, 20 ψ; collision gas, 10 ψ; ion source gas 1, 55 ψ; ion source gas 2, 35 ψ; ion spray voltage, 4000 V; and temperature, 650 °C. The THC derivative was monitored using 2 transitions (*m*/*z* 483[rarr]142, 361). The defined limits of quantification (LOQ) for THC using the LC-MS/MS derivatization methods were 0.5 pg/mL, for breath eluted in methanol (equivalent to 0.03 pg of THC/L of breath for this study), and 100 pg/mL for whole blood. The analyte peak identification criteria were retention time within ±0.1 min of the mean calibrator RT and qualifier/quantifier transition peak area ratios ±20% of the mean calibrator transition ratio. For a full description of the analytical method and complete validation data, please refer to Luo et al. *(20)*.

STATISTICAL ANALYSIS

Statistical analyses were performed using R (version 3.4.0). Linear regression with ordinary least squares was used to calculate the fit coefficients. For each data set, corresponding to a single individual and sample matrix (breath or blood), timepoints after smoking were considered for the analysis. For a selected data set, linear regression was performed for log[C], where C is the THC concentration as a function of time, t. This resulted in a linear regression model:

$$\ln(C) = \text{intercept} + \text{slope} \times t$$

Breath and blood THC concentrations were first transformed to their natural logs, i.e., *ln*[Breath] and *ln*-[Blood], and the Pearson correlation coefficient and the corresponding *P* values were calculated.

Results

The demographics and self-reported cannabis use histories for the 20 volunteers are shown in Table 1. The 15 male and 5 female participants were racially diverse and ranged from 22 to 48 years of age (median age, 29 years). Based on self-reported cannabis use histories, 9 participants would be classified as chronic cannabis users (≥4 times/week) and 11 as occasional cannabis users (<twice/week). Four participants had CUDIT-R scores indicating hazardous cannabis use (CUDIT-R, ≥8), and 5 participants' scores identified a possible cannabis use disorder (CUDIT-R, ≥12). THC was the major cannabinoid detected in all breath samples. No samples tested positive for THC-COOH or 11-OH-THC. CBD was quantified in 50% of samples, corresponding to whether the product used by the participant contained CBD. CBN was above the LOQ in all breath samples and showed a linear correlation with THC ($R^2 = 0.918$).

Fig. 1 shows the concentration–time curves of THC in breath and blood for each participant. Intercept and slope values and the coefficient of determination (R^2) for all fits are summarized in Table 2. Table 2 also summarizes the blood vs breath correlation coefficients and *P* values for each individual. All measurements in both

Table 1. Demographics and self-reported cannabis use histories for study participants.

Participant	Sex	Race	Age, years	Body mass index, kg/m^2	Form of use for study	Product label[a]	Days since last use	Age first used, years	Days used in past 14	Average use per day	CUDIT-R	Primary route of use	Other routes of use	Medications/ supplements	Tobacco use
A01	M	White	31	25.4	Joint	NA[b]	3	18	1	0–1	6	Vaping	Smoking, edibles	None	No
A02	M	Mixed	39	27.1	Joint	15% THC, 0% CBD	2	18	14	5	15	Vaping	Smoking, edibles, topical	HIV meds, turmeric	Yes
A03	M	Black	26	24.6	Joint	16%THC, 0% CBD	2	21	10	3	12	Smoking	Vaping	None	No
A04	M	White	26	27.6	Joint	NA	3	25	3	0–1	4	Edibles	Smoking	Loratadine	No
A05	F	White	26	21.0	Joint	18% THC, 0% CBD	12	21	2	0–1	4	Edibles	Vaping	None	No
A06	M	White	48	24.1	Pipe	NA	7	16	13	1	7	Smoking	Edibles	Methotrexate, folic acid, aspirin	Yes
A07	F	White	27	23.5	Joint	NA	2	16	3	0–1	4	Vaping	Smoking, edibles	OCP	No
A08	M	Asian	26	28.0	Joint	20% THC, 0% CBD	2	17	14	2	10	Smoking	Vaping, edibles	None	No
A09	M	Mixed	28	23.5	Joint	15%THC, 0% CBD	3	17	3	0–1	10	Edibles	Smoking	None	No
A10	M	White	24	26.5	Pipe	NA	2	18	14	1	9	Smoking	Vaping, edibles	Emtricitabine, tenofovir	No
A11	M	Asian	22	27.2	Pipe	28% THC, 0% CBD	12	20	2	0–1	7	Smoking	Vaping	none	No
A12	F	Mixed	24	22.7	Joint	28% THC, 0% CBD	2	20	1	0–1	9	Smoking	Vaping	Oral contraceptive	No
A13	M	Asian	27	31.5	Joint	18% THC, 0% CBD	13	24	1	0–1	3	Vaping	Edibles	None	No
A14	M	White	29	19.3	Joint	21%THC, 0% CBD	14	18	1	0–1	2	Edibles	Smoking, vaping	None	No
A15	M	Hispanic	24	21.4	Joint	NA	2	16	14	2	6	Vaping	Smoking	None	No
A16	M	Hispanic	43	23.7	Joint	NA	1	13	14	2	12	Smoking	Edibles	Hydrocodone, acetaminophen	No
A17	M	White	28	24.8	Joint	NA	2	17	10	2	6	Smoking	Edibles	None	No
A18	F	Hispanic	26	24.9	Joint	19% THC, 0.6% CBD	1	19	8	2	12	Smoking	Edibles	Escitalopram	No
A19	M	Hispanic	26	33.1	Joint	21% THC, 0% CBD	5	22	5	1	13	Smoking	Vaping, edibles	None	No
A20	F	White	26	26.7	Joint	22% THC, 0.05% CBD	6	18	2	0–1	5	Smoking	Edibles	Loratadine, spironolactone	No

[a] Information on label for product used in the study.
[b] NA, information not available.

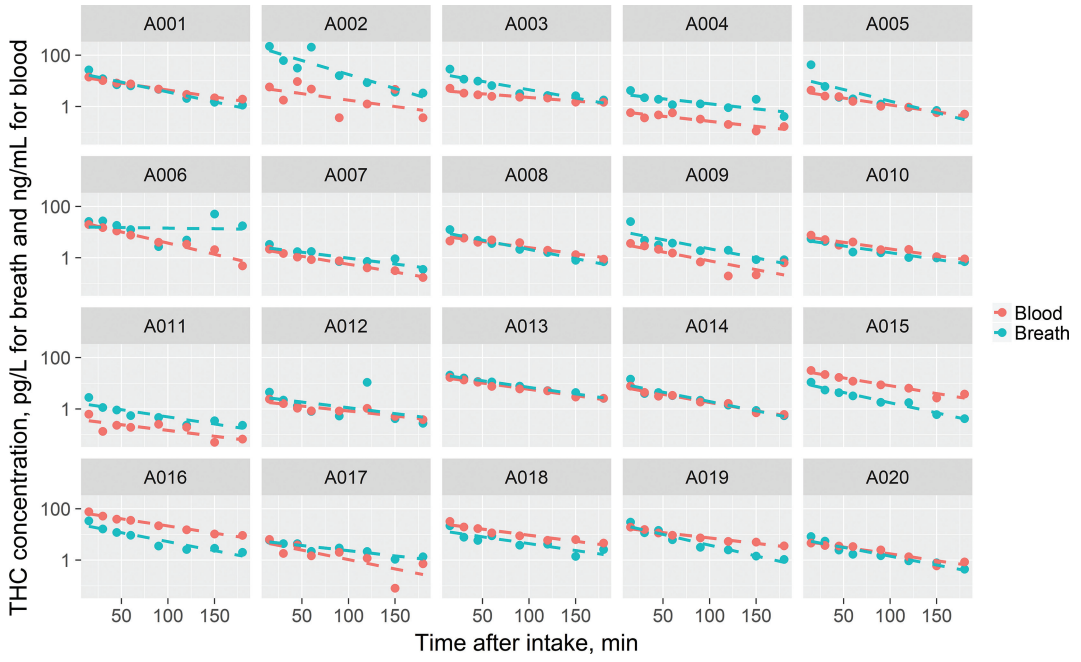

Fig. 1. Individual THC concentration–time curves in breath and blood.
Dashed lines correspond to fits of the measured kinetic data using linear regression with ordinary least squares.

breath and blood are presented in Table 1 of the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol65/issue9. All THC breath measurements were above the defined LOQ of the assay at all timepoints. The median baseline THC concentration in breath was 0.25 pg/L (range, 0.06–3.2 pg/L). Baseline measurements were not subtracted from subsequent measurements. THC breath concentrations were highest at the 15-min timepoint for 1 of 20 participants. The median breath THC concentration at 15 min was 17.8 pg/L and ranged from 2.8 to 222.6 pg/L. The baseline and maximum breath THC concentrations did not correlate with any demographic or cannabis use variables, including days since last use and CUDIT-R score. At 3 h after smoking, the median THC breath concentration was 0.78 pg/L (range, 0.22–3.4 pg/L, excluding 1 outlier at 17.6 pg/L). Similarly, THC blood concentrations were all <1 ng/mL at baseline with maximum concentrations observed at the 15-min timepoint for 18 of 20 participants (median, 6.13 ng/mL; range, 0.58–77.45 ng/mL). The THC concentrations decreased significantly with time after smoking in both matrices. THC concentrations in blood and breath were significantly correlated in 17 of 20 participants, following outlier analysis, with correlation coefficients ranging from 0.76 to 0.99 (Table 2).

The THC breath concentrations in 4 participants indicated potential outliers that were verified using an unsupervised outlier analysis as described in the online Data Supplement file. For participant A02, 2 peaks were observed in both matrices, indicating a possible second exposure to THC. The participants were on a closed research unit but were not directly observed between timepoints. This participant also had significantly higher THC concentrations in breath measuring 222.62 pg/L at 15 min after smoking and then 206.33 pg/L again at 60 min. The next highest concentration of THC in breath at 15 min was 42.78 pg/L. For participant A04, there was no correlation between blood and breath THC concentrations. Second peaks were observed in both matrices, however, at different timepoints. This could also indicate a second exposure. For participants A06 and A12, there were isolated outlier breath measurements observed that were not correlated with blood THC concentrations; however, for both participants the breath and blood THC were still significantly correlated after removal of the outlier (see Table 2 here and also the online Data Supplement file). Contamination of breath samples with oral fluid using the collection device was ruled out by measurement of salivary amylase and determination of the ratio between phosphatidylcholines 16:0/16:0 and phosphatidylcholines 16:0/18:1 (data not shown).

The median THC concentration over time in breath and blood for all participants is shown in Fig. 2 with no exclusion of outliers. Plotting the median concentrations demonstrated the strong association of the THC-release

Table 2. Blood and breath THC release kinetics (intercept, slope, and the coefficient of determination of the fit), correlation coefficients (blood vs breath), and *P* values for each participant.

	Breath			Blood			Breath and blood correlation		Breath and blood correlation (outliers removed)	
Participant	Intercept	Slope	R^2	Intercept	Slope	R^2	Pearson *r*	*P* value	Pearson *r*	*P* value
A01	3.08	−0.018	0.94	2.72	−0.012	0.98	0.99	<0.001	0.99	<0.001
A02	5.39	−0.025	0.81	1.73	−0.012	0.30	0.51	0.193	0.46	0.305
A03	3.00	−0.015	0.87	1.47	−0.007	0.88	0.95	<0.001	0.95	<0.001
A04	1.15	−0.009	0.60	−0.42	−0.009	0.79	0.48	0.225	0.48	0.225
A05	2.57	−0.021	0.74	1.39	−0.012	0.95	0.92	0.001	0.94	0.001
A06	2.77	−0.001	0.00	3.32	−0.020	0.95	0.10	0.805	0.94	0.005
A07	1.02	−0.011	0.80	0.83	−0.014	0.98	0.90	0.003	0.90	0.003
A08	2.41	−0.017	0.96	2.01	−0.011	0.90	0.88	0.004	0.88	0.004
A09	2.44	−0.016	0.78	1.31	−0.016	0.70	0.78	0.023	0.76	0.049
A10	1.66	−0.012	0.91	2.00	−0.012	0.93	0.91	0.002	0.91	0.002
A11	0.58	−0.013	0.80	−0.90	−0.010	0.60	0.70	0.055	0.70	0.055
A12	1.14	−0.010	0.24	0.71	−0.009	0.81	0.76	0.028	0.98	<0.001
A13	3.13	−0.012	0.99	2.90	−0.011	0.97	0.97	<0.001	0.97	<0.001
A14	2.34	−0.017	0.92	2.03	−0.015	0.96	0.97	<0.001	0.97	<0.001
A15	2.40	−0.018	0.96	3.48	−0.014	0.93	0.97	<0.001	0.97	<0.001
A16	3.28	−0.016	0.88	4.35	−0.013	0.97	0.97	<0.001	0.97	<0.001
A17	1.78	−0.009	0.84	1.74	−0.017	0.57	0.90	0.003	0.90	0.003
A18	2.71	−0.012	0.76	3.32	−0.011	0.91	0.86	0.006	0.86	0.006
A19	3.28	−0.019	0.94	2.96	−0.010	0.96	0.97	<0.001	0.97	<0.001
A20	1.94	−0.016	0.91	1.79	−0.012	0.91	0.86	0.006	0.86	0.006

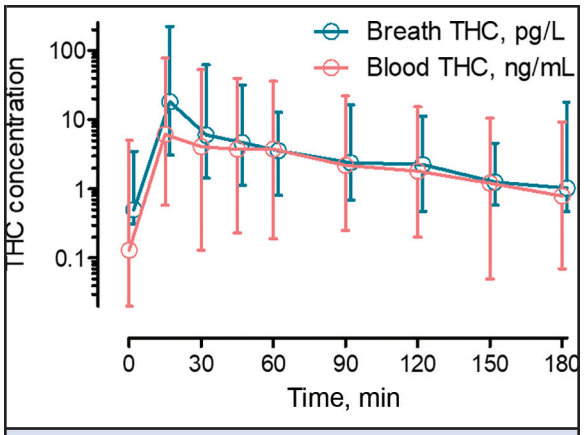

Fig. 2. Median THC concentration over time in breath and blood for all participants.
Points are the median values, and bars represent the range.

kinetics in breath and blood. Fig. 3 shows the scatter plot of all corresponding breath and blood THC measurements (n = 180) summarizing the correlation between THC in blood and THC in breath across all volunteers and timepoints after smoking.

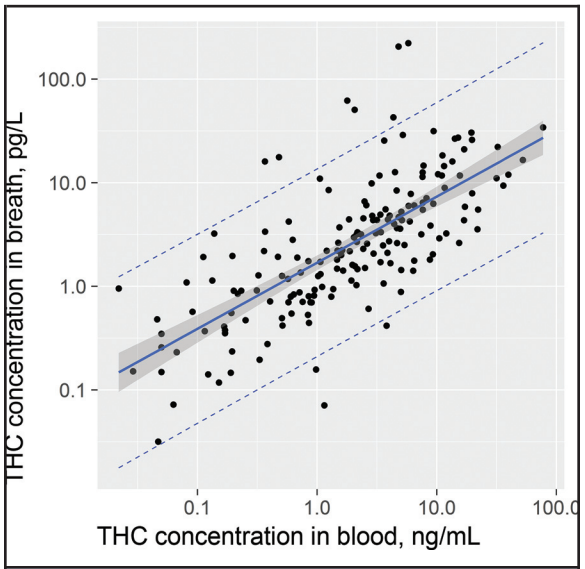

Fig. 3. Correlation between THC in breath and blood across all individuals and timepoints after smoking.
The solid line is calculated using linear regression of *ln*[Breath] and *ln*[Blood] across all individuals. The shaded region around the line represents the CI for the fit. The dashed lines represent the prediction interval for the calculated fit.

Discussion

Here we show that THC can be reliably detected at concentrations above baseline throughout the suggested 3-h impairment window following controlled administration of smoked cannabis. Baseline measurements after self-reported THC abstinence for 24 h ranged from 0.06 to 1.26 pg/L, excluding 1 outlier participant. The median THC breath concentrations were highest at the 15-min timepoint (median, 17.8 pg/L) and declined to <5% of this concentration (median, 0.78 pg/L) within 3 h after use. Although the THC release kinetics in breath were similar for all participants, there was a high degree of interindividual variation in maximum THC concentrations observed (range, 2.83–222.6 pg/L). Maximum THC breath concentrations did not correlate with any demographic or cannabis use variables (days since last use, mean use per day, and CUDIT-R score). The median concentration at the 15-min timepoint was not significantly different between chronic and occasional cannabis users.

Breath THC concentrations in this study are difficult to compare with previous studies, which have all reported concentrations in picograms per breath-collection pad or filter. The concentrations ranged from 180 to 773 pg/sample, 85.3 to 209 pg/sample, and 136 to 20 948 pg/sample in 3 different studies *(17–19)*. The latter 2 studies used the same collection device and measured THC following smoked cannabis. The concentrations measured were significantly different, bringing into question the sampling device and the potential for external contamination from the environment or oral fluid or variations in the method for sample elution. Assuming the collection of approximately 30 L of breath/pad or filter, as referenced in the most recent study, the breath THC concentrations in these 2 studies would range from approximately 2.8 to 7.0 pg/L and 4.5 to 698 pg/L, respectively. In our study presented here, the THC breath concentrations ranged from 0.06 to 42.8 pg/L (excluding outlier participant A02). Himes et al. reported measurement of breath THC in 13 chronic smokers at 2 h postsmoking; however, THC was not detectable at the 3-h timepoint. One sample became positive again at 4 h. In 13 occasional smokers, breath THC was detected in only 7 participants 1 h after smoking cannabis. Coucke et al. reported THC breath concentrations at 3 h postsmoking in all 13 participants; however, the values are significantly higher than those reported by Himes et al. We believe that the lower THC concentrations recorded in our study, which are in line with the Himes data, reflect the careful control of environmental and oral fluid contamination.

In our study, we evaluated corresponding breath and blood THC concentrations following controlled administration of smoked cannabis. The findings support

breath THC concentrations as representing a physiological process that correlates to blood concentrations albeit with a shorter window of detection. The decay curve kinetics observed for blood and breath were highly correlated within individuals and across the population. Thus, THC concentrations in breath may reflect recent use. It is important to note that THC elimination from blood is best shown by a multicompartment model. The window of measurement for the current study is much shorter, enabling a linear relationship for log-transformed data between blood and breath.

This study showed significant interindividual variation. This may be because, in part, participants smoked cannabis ad libitum, resulting in variable cannabinoid doses that were likely reflective of typical variability in standard conditions of use. Despite the different doses, blood and breath concentrations were still correlated, and THC breath concentrations dropped to <5% of maximum concentrations by 3 h in all participants, both occasional and chronic cannabis users. This standard kinetic profile suggests that measurement of THC above a threshold concentration can be used to indicate probable use within the proposed 3-h window of impairment. No timepoint data were collected beyond 3 h. It remains unknown how long THC is detectable at low concentrations beyond the 3-h timepoint using our LC-MS/MS assay. The cause of the high variability in maximum concentrations between individuals remains to be determined. Normalizing concentrations to breath volume may not be enough. Individuals may exhale variable amounts of breath particles in the same volume of breath, which could account for some of this variation.

A considerable breadth of research has been devoted to the evaluation of biomarkers in exhaled breath for clinical and forensic investigations *(21)*. Breath is an ideal matrix as a measure of impairment because the collection is noninvasive and can be done with a portable collection device. Although connection to level of impairment has not yet been investigated, our results verify that THC is detectable above baseline throughout the presumed 3-h impairment window following smoking in both chronic and occasional cannabis users. Moreover, correlation with blood measurements shows that breath THC concentrations correspond to systemic physiological consumption, suggesting that breath testing may also be applicable to different routes of use such as vaping and consumption of edibles.

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