Evaluation of Breath and Plasma Tetrahydrocannabinol Concentration Trends Postcannabis Exposure in Medical Cannabis Patients

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Abstract

The legalization of cannabis in Canada brings novel challenges across various fronts, such as policy development, law enforcement, and public health and safety. It is imperative to improve our understanding of the mechanisms and trends surrounding cannabis use to develop efficacious methods of tackling these challenges.

Materials and Methods: Patients' breath collection was achieved using the ExaBreath device from SensAbues. THC measurements in plasma and breath samples were processed and analyzed using LC-MS/MS.

Discussion: We conducted a pragmatic clinical trial on 23 medical cannabis patients, wherein we collected breath and plasma samples intermittently for 4 hours after cannabis consumption. The research participants consumed between 1 and 2 g of cannabis by either vaping, cannabis cigarette, or concentrated wax (dabs) for 10 min. We used standardized laboratory analytical techniques using liquid chromatography–tandem mass spectrometry to analyze both the breath and plasma sample. To analyze the data and find patterns, we developed models using artificial neural network analysis.

Conclusion: Our findings show that tetrahydrocannabinol (THC) breath concentrations peaked in 0.5 hours and reached baseline levels after 2 hours in all the patients. We found an inverse correlation between individuals' body mass index and their peak breath concentrations, and an inverse relationship between age and peak breath concentrations. Male participants had higher peak breath and plasma concentrations than female participants. Our research provides new insight into the correlations between breath and plasma THC concentrations in medical cannabis patients.

Keywords: breath analysis; clinical trial; medical cannabis; *per se* legislation; plasma analysis

Introduction

Medical cannabis use has been on the rise after changes in policies and legislation across many jurisdictions around the world.¹ It is known that cannabis use results in some degree of effect on an individual's physiological condition, and their neurocognitive and psychomotor skills.² However, there is a lack of a consensus on generalizable effects of cannabis use due to the variability across populations, demographics, the type of use, form of consumption, and in terms of frequency, as research is still limited.³

The Canadian legal and health care systems face increasing challenges after the nationwide legalization of

cannabis in 2018; therefore, it is imperative to conduct further scientific and clinical evaluations to increase our understanding.⁴

Although much epidemiological and clinical research has been conducted on cannabis use, there is limited work that has been conducted on the detection of tetrahydrocannabinol (THC) on breath correlated to impairment. This area of research has been receiving growing attention over the past decade. Research studies have detected THC in the breath of research subjects, which increased with consumption and declined over a 3 h period. Some studies have detected THC on the breath up to 4 h after consumption.^{5–7}

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There are also gaps in the research conducted on medical cannabis patients, and in the empirical data and reporting on the correlation between plasma and breath THC concentrations.

The aim of our research was to determine the immediate and short-term impacts of cannabis use on medical cannabis patients. Out of a pool of 300 verified research subjects, we randomly selected 23 medical cannabis patients to participate in our research. We conducted a clinical trial to evaluate the relationship between plasma, breath THC concentrations, and neurocognition in medical cannabis patients.

Materials and Methods

Patients' breath collection was achieved using the ExaBreath device from SensAbues AB, Sweden. The breath collection device is effective for rapid collection according to previous research (Fig. 1).^{8,9} This device is a plastic tube that consists of a filter that traps the aerosols containing the drug compounds. The subjects were asked to breathe into the device and count 25 breaths. The devices were then stored at -25°C until they are processed. Participants were asked to provide a baseline neural assessment and provide plasma and breath samples. They consumed 20% THC through either vaping, dabing, or smoking a cannabis cigarette, the participants were given the choice of consumption method. After 30 min wait period, they provided bio samples and performed cognitive testing. Due to concerns from our Research Ethics Board (REB) regarding the high THC levels, the participants were only allowed 10 min to consume the cannabis. Due to the high daily

dosage of the participants (average 2 ng/day), this restriction was viewed as underdosing by the participants. The bio samples were then provided every hour for the following 3 h. Neurocognition tests were administered at baseline, after consumption, and before leaving. The tests included executive function, language and processing speed, visuomotor processing speed, object naming, and executive function tests.

THC in breath extraction process

THC measurements in breath were performed by the Analytical Facility for Bioactive Molecules, The Hospital for Sick Children, Toronto, Canada. The method is based on the Standard Operating Procedure for Drug Breath Testing V2.0 provided by SensAbues. In brief, 1 ng of internal standard dissolved in methanol, THC-d3 (Sigma-Aldrich), was spotted onto each SensAbues pod. The standard curve was prepared by spotting the appropriate amount of THC onto blank unused SensAbues pods. The range of the standard was 0.001–5000 ng with the lower limit of quantification (LLOQ) being 0.01 ng. Each pod was placed on a conical glass tube and 2 mL of methanol was gently added to each pod and was allowed to saturate for 5 min. After 5 min, 5 mL of methanol was added to each pod and an empty syringe was used to apply pressure to elute residual methanol. Then 20 μL of 1% formic acid was added to the conical glass tubes that were then taken to dryness under a gentle flow of nitrogen at 35°C . The residues were reconstituted in 1:1 water-methanol, yielding a turbid solution, the liquid was then centrifuged at 20,000 g for 30 min at 4°C to reduce turbidity. The supernatant was transferred to an insert in an autosampler vial and analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS).

THC in plasma extraction

THC measurements in plasma were also performed by the Analytical Facility for Bioactive Molecules, The Hospital for Sick Children, Toronto, Canada. In brief, 1 ng of internal standard dissolved in methanol, THC-d3 (Sigma-Aldrich), was added to a series of 2 mL Eppendorf-style tubes. The standard curve was prepared by adding the appropriate amount of THC into the appropriate tube. The range of the standard was 0.001–5000 ng with the LLOQ being 0.01 ng. To each tube, 100 μL of matrix (SigMatrix Serum Diluent; Sigma-Aldrich) or sample was added followed by 0.1% formic acid in methanol. Tubes were vortexed and centrifuged at 20,000 g for 10 min at 4°C . The supernatant

FIG. 1. SenseAbues breath collection device.

was removed into a conical glass tube and evaporated to dryness under a gentle flow of nitrogen at 35°C. The residues were reconstituted in 1:1 water–methanol, yielding a turbid solution, the liquid was then centrifuged at 20,000 *g* for 30 min at 4°C to reduce turbidity. The supernatant was transferred to an insert in an autosampler vial and analyzed by LC-MS/MS.

LC-MS/MS details for breath and plasma processing
THC was measured by LC-MS/MS at the Analytical Facility for Bioactive Molecules, The Hospital for Sick Children, Toronto, Canada, using a QTRAP 5500 triple-quadrupole mass spectrometer (SCIEX, Framingham, MA) in positive electrospray ionization mode by multiple reaction monitoring data acquisition with an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA). Chromatography was performed by automated injection on a Kinetex Biphenyl column, 50 × 2.1 mm, 2.6 μ m particle size (Phenomenex, Torrance, CA). HPLC flow was maintained at 400 μ L/min with mobile phases consisting of A = 0.1% formic acid in water and B = 0.1% formic acid in acetonitrile. Initial conditions were 50% A and the gradient was ramped to 5% A by 2.5 min and then immediately returned to 50% A. Total run time was 7 min. Data acquisition and quantification were performed with Analyst 1.6.2 software (Sciex).

Analyses

For data analysis, we employed an artificial intelligence technique called deep neural network analysis to develop models for identifying patterns between the breath and plasma THC concentrations. The clinical trial data variables used as input parameters include the following:

- Time elapsed since cannabis consumption
- Breath THC concentration
- Gender of the participant
- Age of the participant
- Dosage consumed
- Body mass index (BMI) of the participant

The output from the models was used to draw the correlations between the parameters (Table 1).

Results

Our analysis revealed that THC plasma concentrations increased less rapidly relative to breath concentrations in the medical cannabis research subjects. All of the

research participants, plasma concentration levels exceeded the legal limits of 5 ng/mL when converted to blood values. Figure 2 shows that the breath THC concentrations reached a peak value after 0.5 h of consumption on average. Breath concentration levels reached baseline levels after 2 h. It was also observed that the peak breath concentrations showed a decreasing trend as the participants' BMI increased, with the peak breath concentrations exceeding 100 ng for the participants with a BMI <30 kg/m².

When comparing both the breath and the plasma THC concentration levels between our participants, we found that males generally had higher concentrations relative to females. The plasma and breath concentration peak levels exhibited a decreasing trend as the age of the participants increased.

When conducting the baseline THC concentration tests at the beginning of the study, Figure 3 reveals that <20% of the participants had equivalent blood concentration levels below the lower legal limit of 2 ng/mL at baseline, which is the minimum concentration required to be considered impaired according to the Canadian Federal Impaired Driving Act legislation. We used a plasma–blood conversion model used in the literature^{10,11} to estimate blood levels for *per se* limits comparison. A total of 29% of participants exceeded the estimated blood concentration level of 5 ng/mL legal limit at baseline, which is the higher *per se* limit under the Canadian legislation, while 38% exceeded 10 ng/mL.

When tested 30 min after consumption, 100% of the study participants had estimated blood THC concentrations more than the 5 ng/mL maximum Canadian legal *per se* limit. At 150 min after consumption, 100% of the participants were still more than the 2 ng level, with 85% more than the 5 ng limit.

The neurocognitive test results were contrary to our expectations; the difference in performance on cognitive testing between baseline (Time 1) and post-THC administration (Time 2) was either nonsignificant (i.e., no change) or revealed an improvement at Time 2. Although at face value these findings suggest that THC either enhances or does not affect cognitive functioning, the current design failed to account for learning effects, as it did not include a control group in which participants were tested repeatedly without being exposed to THC.

Discussion

Our research provides new insights into some physiological trends that impact the time to return to baseline

Table 1. Plasma—Breath Comparison Data Table

Participant	BMI kg/m ²	Age	Usage/day (g)	Gender	Breath 1 baseline	Baseline ng THC/mL plasma	Breath 2 consumption +30 min	ng THC/mL plasma consumption +30 min	Breath 3 consumption +90 min	ng THC/mL plasma consumption +90 min	Breath 4 consumption +150 min	ng THC/mL plasma consumption +150 min	Breath 5 consumption +210 min	ng THC/mL plasma consumption +210 min
1	28.14	32	3	Male	34.7	22.8	148	43.8	34.2	48.8	7.36	29.7	Cnt	23.9
2	29.18	42	2	Female	6.55	1.98	95.9	22.7	26.4	8.11	3.91	6.82	Cnt	0
3	31.31	30	3.5	Male	10.4	1.65	74.6	63.6	7.03	21.7	4.42	14.7	Cnt	8.02
4	19.67	44	1	Female	80.3	10	183	17.7	23.9	5.13	5.20	3.04	Cnt	0
5	26.57	33	2	Female	50.1	7.95	108	49.8	7.95	22.8	8.98	12.1	Cnt	15.8
6	22.36	37	2.5	Female	11.3	10	112	n/a	35.9	n/a	1.75	n/a	Cnt	n/a
7	44.26	36	5	Male	2.49	34.7	147	135	7.80	84	na	32.2	Cnt	39.8
8	na	25	1	Male	19.2	19.5	165	59.6	8.47	31.7	7.15	25.9	Cnt	23.2
9	46.68	26	2	Female	67.9	7.32	102	29.7	6.72	20.3	0	11.7	Cnt	9.87
10	34.26	35	2	Female	4.34	10.9	167	74.4	12.0	36.5	6.06	21.4	Cnt	15.4
11	22.83	37	3	Male	36.8	39.7	4.36	72.8	2.33	66.3	na	33.2	Cnt	0
12	17.77	27	2	Male	22.2	0.717	206	59.4	150	18.2	49.2	7.78	Cnt	4.89
13	19.59	33	2	Male	13.9	45.2	213	100	31.4	53.4	1.32	28.9	Cnt	26.4
14	20.90	34	2	Male	11.7	10.7	36.2	28.9	44.8	13.5	na	9.08	Cnt	8.23
15	34.08	39	1.25	Male	18.3	n/a	na	n/a	na	n/a	na	n/a	Cnt	n/a
16	22.54	31	3	Female	36.8	7.65	230	72.9	65.9	30.2	1.51	17.5	Cnt	21.3
17	35.62	58	1.5	Female	13.9	2.72	59.4	20.4	10.1	6.93	2.86	4.74	Cnt	3.52
18	30.64	59	14	Male	17.1	32.9	195	59.5	17.0	18.6	na	16.7	Cnt	11.9
19	29.10	32	9	Male	96.9	19.7	181	55.7	19.6	35.7	9.02	31.1	Cnt	23.8
20	27.70	39	3.5	Female	8.26	7.02	104	89.8	29.0	26.1	0.711	13.4	Cnt	0
21	18.73	24	3	Male	2.73	28.8	182	118	10.4	47.7	5.59	33.7	Cnt	27.8
22	51.62	30	1.5	Male	9.37	n/a	65.1	n/a	23.1	n/a	na	n/a	Cnt	n/a
23	41.53	41	3	Male	7.27	4.03	120	32.4	20.8	11.7	8.67	8.56	Cnt	6.85

Breath, ng THC/25 breaths; Cnt, contaminated breath sample due to ambient cannabis smoke; na, not available. Some participants opted out of blood draws. Plasma, ng THC mL.
BMI, body mass index; THC, tetrahydrocannabinol.

FIG. 2. Average plasma–breath THC levels over the study period. THC, tetrahydrocannabinol.

FIG. 3. Blood levels (estimated from plasma) related to the Canadian impairment *per se* limits over the study time period.

after consumption of cannabis. These factors are consumed dosage, an individual's BMI, age, and gender. We observed that certain patients had a persistent estimated blood THC concentration level far exceeding the legal limit even at baseline, and others had an estimated blood concentration level well below legal limits, THC was also detectable on the breath at this lower *per se* limit. This has implications for law enforcement, with regard to generating false positive and false negative impairment tests, as tolerance is more likely to develop in chronic users.¹²

When considering the implications, the limitations of our research need to be considered. Some major limitations of our study are the sample size and the scope, which is a challenge present in many clinical trials due to resource constraints. For future studies, it will be necessary to increase the sample of the study and increase the number of variables to account for additional factors.

In summary, our findings validate existing research and provide additional evidence for using exhaled breath testing as an effective means of THC impairment and field sobriety detection. Our research provides further insight and considerations with regard to recommendations for policy making, benchmarks for law enforcement, and public health and safety guidelines.

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Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

BMI = body mass index
LLOQ = lower limit of quantification
REB = Research Ethics Board
THC = tetrahydrocannabinol