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Marijuana Use by Breastfeeding Mothers and Cannabinoid Concentrations in Breast Milk

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Short Title: Cannabinoid Concentration in Breast Milk

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Abbreviations:
Δ9-THC: Delta-9-Tetrahydrocannabinol
CBD: Cannabidiol
11-OH-THC: 11-Hydroxy-Delta-9-Tetrahydrocannabinol
Δ9-carboxy-THC: Delta-9-Carboxy-Tetrahydrocannabinol
CBN: Cannabinol
LC-MS ESI: Liquid chromatography mass spectrometry electrospray ionization

Table of Contents Summary: This study quantified levels of cannabinoids Δ9-THC, 11-OH-THC, CBD, and CBN in 54 milk samples provided by breastfeeding mothers who reported recent marijuana use.

What's Known on This Subject: Previous data quantifying the transfer of Δ9-THC and other cannabinoids into human breast milk following maternal marijuana use are limited to several case reports.

What This Study Adds: In 50 women reporting marijuana use while breastfeeding, Δ9-THC was measurable in 63% of milk samples, up to 6 days after last use; 11-OH-THC and CBD were measurable in 9% of milk samples, and CBN was undetectable in all samples.

Contributors' Statement Page

Mrs. Bertrand designed the data collection instruments, coordinated and supervised data collection and drafted the initial manuscript, and reviewed and revised the manuscript.

Dr. Chambers conceptualized and designed the study and critically reviewed and revised the manuscript.

Drs. Best and Hanan carried out the assay development, sample analysis, and reviewed and revised the manuscript.

Mr. Honerkamp-Smith carried out the study analyses and reviewed and revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Abstract

Background and Objectives

Marijuana is the most commonly used recreational drug among breastfeeding women. With legalization of marijuana in several U.S. states and a 1990 study that documented psychomotor deficits in infants breastfed by mothers using marijuana, there is a need for information on potential exposure to the breastfed infant. The objective of this study was to quantify cannabinoids in human milk following maternal marijuana use.

Methods

Between 2014 - 2017, 50 breastfeeding women who reported marijuana use provided 54 breastmilk samples to a research repository, Mommy's Milk. Concentrations of Δ9-THC, 11-OH-THC, CBD, and CBN were measured using LC-MS ESI.

Results

Δ9-THC was detectable in 34 (63%) of the 54 samples up to approximately six days after last reported use; the median concentration of Δ9-THC was 9.47 ng/mL (range 1.01, 323.00). Five samples had detectable levels of 11-OH-THC (range 1.33, 12.80 ng/mL) or CBD (range 1.32, 8.56 ng/mL). The sample with the highest concentration of CBD (8.56 ng/mL) did not have measurable Δ9-THC. CBN was not detected in any samples. Number of hours since last use was a significant predictor of log Δ9-THC concentrations (-0.03, 95% Confidence Interval [CI] -0.04, -0.01, p=0.005). Adjusted for time since last use, the number of daily uses and time from sample collection to analysis were also significant predictors of log Δ9-THC concentrations (0.51 95% CI 0.03, 0.99, p=0.039; 0.08, 95% CI 0.00, 0.15, p=0.038, respectively).

Conclusions

Δ9-THC was measurable in a majority of breastmilk samples up to approximately six days after maternal marijuana use.

Introduction

Marijuana is the most commonly used recreational drug among breastfeeding women.[1,2] However, potential infant exposure to marijuana through breastfeeding is poorly understood. This question is of critical importance as human milk is the normative standard for infant feeding and nutrition.[3] The World Health Organization recommends exclusive breastfeeding up to six months of age.[4] Being breastfed early in life has been associated with a reduction in subsequent obesity and improved performance on intelligence tests. In mothers, breastfeeding has been associated with lower risks for subsequent breast and uterine cancer and Type II diabetes.[5,6]

However, there is a paucity of data on the effects of maternal marijuana use among infants potentially exposed through breastmilk. Case reports have documented the presence of the primary psychoactive ingredient in marijuana, delta-9-tetrahydrocannabinol (Δ9-THC), in human milk.[7] In one report, the level of Δ9-THC measured in a milk sample provided by a mother who smoked marijuana once daily was 105 ng/mL. The concentration in a sample from a second mother who smoked marijuana seven times per day was 340 ng/mL. In an additional set of paired milk and maternal plasma samples obtained from one of these mothers, the Δ9-THC concentration measured in the milk sample (60.3 ng/mL) was eight times higher than the maternal plasma concentration of 7.2 ng/mL, suggesting that Δ9-THC accumulated in breastmilk.[8] Furthermore, fecal samples from that mother's infant had higher concentrations of other metabolites (11-hydroxy-delta-9-teretrahydrocannabinol [11-OH-THC] and Δ9-carboxy-THC) than the mother's breastmilk, suggesting the infant absorbed and metabolized the Δ9-THC following breastmilk ingestion. In another case report, one human milk sample obtained from a woman with a history of drug abuse was found to have detectable levels of both Δ9-THC (86 ng/mL) and 11-OH-THC (5 ng/mL).[9] A third published study analyzed 109 randomly collected

milk samples with no accompanying information on maternal marijuana use; Δ9-THC was detected in two samples and cannabidiol (CBD) in one.[10]

There are very limited data on the potential neurobehavioral effects of infant exposure to cannabis through breastmilk. Astley et al (1990) reported psychomotor deficits in 55 12-month old infants breastfed by mothers using cannabis compared to 81 unexposed.[11] In contrast, Tennes et al (1985) reported no differences in motor and mental development in 27 12-month old infants whose mothers used marijuana while breastfeeding compared to 35 unexposed.[12]

Lacking definitive data on the risk or safety of infant exposure to cannabis through breastmilk, the American Academy of Pediatrics and the American Congress of Obstetricians and Gynecologists advise that marijuana use should be discouraged while breastfeeding.[13,14] However, with current legalization of marijuana for recreational use in 9 U.S. states, there is an urgent need for characterization of cannabinoid distribution in human milk and the corresponding potential exposure of breastfeeding infants and toddlers. The purpose of this study was to measure cannabinoid concentrations in breast milk in relation to dose and time since last maternal marijuana use.

Patients and Methods

Study Design

In 2014, the University of California, San Diego, established Mommy's Milk, the Human Milk Biorepository (HMB) for research. Volunteers residing anywhere in the U.S. and Canada have been recruited into HMB through a variety of sources including social media. After providing written informed consent, for future research uses of milk samples and associated data, breastfeeding mothers completed an interview providing demographics, maternal and child

health history, and details regarding exposures to medications, alcohol, tobacco, and other recreational substances. Participants recalled their exposures for the 14 days prior to milk sample collection and provided additional information on exposure to herbal supplements, prescription medications, or recreational substances since giving birth. Women were instructed to pump and collect 50 mL of milk up to a full expression as close to the time of the scheduled interview as possible and up to 24 hours prior. Any quantity of milk ≥1 mL was accepted. The HMB protocol was approved by the institutional review board at the University of California San Diego, and a National Institutes of Health (NIH) Certificate of Confidentiality was obtained.

Assessment of Exposure

For HMB participants who reported marijuana use at any time since giving birth, the maternal questionnaire data included route of administration (inhaled, ingested, topical), frequency of use, dose, and time since last use prior to sample collection. If mothers indicated a dose unit of joints, puffs or grams, the route of administration was classified as “inhalation only”; those who indicated a dose unit of drops, milligrams or servings were classified as “other only”. Those who reported more than one route of administration were classified as “both”. The frequency of marijuana use was determined by calculating the average number of uses per day during the most recent exposure window within the 14-day recall. Hours since last use was calculated using the end of the exposure interval for the most recent exposure up to the time of milk sample collection.

Collection and Preparation of Human Milk Samples

Prior to sample collection, participants were instructed to clean the nipple and areola of the breast with an alcohol wipe. Milk sampling occurred using one of two methods: 1) if at the HMB research center, participants pumped milk with a hospital grade industrial breast pump

(either the Medela Symphony® or the Hygeia Enjoye®) using a sterile collection kit provided by study staff; or 2) if a home collection, participants pumped milk into a personal milk collection container specific to their own hand or electric breast pump. For home collections, expressed milk was refrigerated at 0-4°C until either transported on ice to the HMB research center or picked up by courier within 24 hours of collection and shipped overnight on ice to the HMB facility. Upon receipt, samples were aliquoted into 1 – 15 mL cryovials or centrifuge tubes and stored at -80°C.

Analytical Procedure

Reference standards (Δ9-THC, 11-OH-THC, CBD, and cannabinol [CBN]) and isotopically labeled internal standards (THC-d3, 11-OH-THC-d3, CBD-d3, CBN-d3) were purchased from Cerilliant (Round Rock, TX). BondElut® certify (Varian, Lake Forest, CA) C-18 cartridges were used for solid-phase extraction. HPLC grade water, 0.1% formic acid in acetonitrile (ACN), and methanol (MeOH) were purchased from Fisher Scientific. Reagent grade ammonium formate and pure sodium hydroxide pellets were purchased from Sigma-Aldrich.

We followed the Wei *et al* saponification-solid-phase extraction method for sample clean up and extraction with minor modifications.[15]

Chromatographic separation was achieved on reverse-phase C-18 column (MAC-MOD Ace 5 C-18, 15cm • 2.1mm). The mobile phase consisted of 5 mM ammonium formate in 0.05% formic acid (Solvent A) and ACN in 0.1% formic acid (Solvent B). A gradient elution was performed over 15 minutes at a flow rate of 200 µL/min. Retention times for 11-OH-THC, CBD, THC were 9.35, 10.62, and 11.70 minutes in positive ion mode, respectively. Quantitation of THC, 11-OH-THC, and CBD and isotopically labeled internal standards was achieved on a triple

quadrupole mass spectrometer (API-4000, AB Sciex) in positive ion mode. CBN and CBN-d3 were detected in negative ion mode.

Statistical Analysis

Demographic and clinical variables for mothers and infants were summarized using frequencies and percentages. For each cannabis metabolite, concentrations were categorized as either above or below the quantification limit which was defined as 1 ng/mL. Summary statistics for those samples with concentrations above the limits of quantification for each metabolite were computed. Concentrations of Δ9-THC were log transformed. Linear regression models were used to estimate associations between the log concentrations of Δ9-THC and time since last cannabis exposure, frequency of use, dose, and route of administration. Other covariates that were considered included maternal body mass index, age and sex of the infant, time from sample collection to analysis (sample age), time of day of sample collection, time since last feed or pump, and feeding frequency. Effect estimates and 95% confidence intervals (CIs) as well as p-values were reported.

To estimate potential infant exposure and infant plasma concentrations, we used the Δ9-THC concentrations from the linear regression models to compute the mean cumulative dose of Δ9-THC hypothetically ingested over a 24-hour period by a nursing 3 month old infant that weighs 6.1 kg, assuming 3.1875 ounces of milk was consumed at each feeding over eight equally-spaced feedings with 6% oral bioavailability.[16] All statistical analyses were performed using R version 3.4.1 with a two-sided p-value below 0.05 judged as significant.

Results

Between 2014 and 2017, 50 mothers who participated in the HMB research repository reported recent marijuana use and provided a breast milk sample. Four mothers provided two samples at different time points. Characteristics of the participants are shown in Table 1. Two-thirds of the women were breastfeeding a child <1 year of age. The most common route of administration was by inhalation only (64%) and most women in the sample (88%) reported at least daily marijuana use.

As shown in Table 2, Δ9-THC was detectable in 34 (63%) of 54 samples; among these, the median concentration of Δ9-THC was 9.47 ng/mL (range 1.01, 323.00). Only 5 (9%) of the 54 samples had measurable concentrations of 11-OH-THC. Similarly, 5 (9%) of the 54 samples had measurable concentrations of CBD. Only one sample had measurable levels of all three cannabinoids (Δ9-THC, 11-OH-THC, CBD); in this sample, the concentration of Δ9-THC was the highest measured in the series (323 ng/mL). Similarly, only one other sample had measurable levels of two of the cannabinoids (11-OH-THC and CBD but not Δ9-THC); in that sample, the concentration of CBD was the highest measured in the series (8.56 ng/mL). There were no samples with detectable levels of CBN.

As shown in Table 3 and in Figure 1 a there was a significant association between log Δ9-THC concentrations and hours since last marijuana use in the fitted regression model. On average, for each additional hour between last exposure to marijuana and the milk sample collection, there was a reduction of 0.03 in the log concentration of Δ9-THC (95% CIs -0.04, -0.01, p=0.005). On the unlogged scale, this corresponds to a reduction in Δ9-THC concentration of 3% per hour after last exposure, which can be used to estimate a half-life of approximately 27 hours for Δ9-THC in human milk. The longest duration between last use of marijuana and measurable Δ9-THC was approximately 140 hours or six days (Figure 1).

As shown in Table 3, in each of the separate regression models, hours since last use was a significant negative predictor of log Δ9-THC concentration. Number of uses per day was also significantly associated with increasing concentrations of log Δ9-THC (0.51 95% CI 0.03, 0.99, p=0.039), as was sample age in months (0.08, 95% CI 0.00, 0.15, p=0.038). No other covariates among those considered were significant predictors of log Δ9-THC concentration.

Based on our assumptions regarding breastfeeding frequency, quantity of milk ingested, and 6% oral bioavailability, the estimated plasma concentration of Δ9-THC in a hypothetical 3 month old infant weighing 6.1 kg was 0.040 ng/ mL. Compared to the plasma concentration of an adult who consumed 10 mg of Δ9-THC, the estimated infant dose ingested via breastmilk would be approximately 1000 times lower.[24]

Δ9-THC was not detectable in 20 (37%) of 54 samples. Fewer women with undetectable levels of Δ9-THC reported more than daily use compared to women with measurable quantities (41.2% vs. 21.1%). In addition, the mean number of hours since last use was higher among those women with levels below limits of detection compared to those above, with wide variability across samples (53.06 hours [SD 76.54] vs. 24.48 hours [SD 32.87]). However, the only statistically significant factor differentiating the two groups was route of administration. The majority of women with quantifiable levels of Δ9-THC used marijuana exclusively via inhalation (76.5%) compared to only 36.8% in the group with levels of Δ9-THC below limits of detection (p=0.010) (data not shown).

Discussion

This is the first study to quantify levels of specific cannabinoids detectable in human milk in a relatively large sample of mothers with detailed and varied histories of recent

marijuana use of currently available products. Δ9-THC, 11-OH-THC, and CBD were all detectable at 1 ng/mL or above in at least one human milk sample. Δ9-THC was detected in 34 (63%) of the 54 samples analyzed. This finding is consistent with the few previously published case reports in which Δ9-THC was measurable in human milk.

Cannabinoids are highly lipophilic compounds.[16,17] Therefore, it is not surprising that metabolites would be detected in human milk which is comprised of 3-5% fat.[18] However, the fat content of human milk is the most variable of its macronutrient components; hindmilk may contain two to three times the concentration of milk fat found in foremilk.[19,20] As a result, we might have expected that women in this study who did not provide a full expression of the breast would have lower concentrations of cannabinoids; however, 80% of the samples were collected from a full expression (Table 1), and sample collection type as a covariate was not significant in any of our models (Table 3). Further, 11-OH-THC is less lipophilic than Δ9-THC, which might explain why only five of the milk samples had measurable concentrations for this cannabinoid.[16,17]

The number of times a woman used marijuana per day was a positive predictor of log Δ9-THC concentrations in milk. This finding was expected and consistent with the few previous case reports in which higher concentrations were found in one mother who smoked marijuana seven times per day compared to another who smoked one time per day.[8]

In our study, the sample age was also a significant predictor of log Δ9-THC concentrations in milk, which was unexpected. Cannabinoids are stable with minimal degradation long-term when frozen, and there is no evidence that more metabolites are formed during storage.[15] However, sample age was also positively correlated with the number of times a

woman used marijuana per day, i.e., dose (eFigure1). Thus, the association between sample age and log Δ9-THC may be explained by this artifact in the data.

In this study, we did not measure infant plasma concentrations. However, based on our assumptions, we estimated the mean infant plasma concentration of Δ9-THC obtained from breastfeeding to be about 1000 times lower than the concentration in an adult after a single dose of 10 mg of Δ9-THC[25]. A previous case report showed that a nursing infant exposed to marijuana via breast milk had high concentrations of Δ9-THC, 11-OH-THC and Δ9-carboxy-THC in feces indicating that the child absorbed, metabolized and excreted marijuana metabolites despite the lower Δ9-THC levels present in the mother's milk[8]. If a child is exposed to low levels of Δ9-THC in milk daily, there is a concern for accumulation of the various cannabinoids in the nursing infant due to slow elimination from body fat stores and continuous daily exposure.[16]

Since the brain rapidly develops during the time period when, ideally, a child's main source of nutrition is human milk, brain development may be altered by Δ9-THC exposure. Previous studies have suggested that *prenatal* exposure to cannabis may interfere with brain development resulting in deficits in cognitive and behavioral function.[21-23] It is reasonable to speculate that Δ9-THC, 11-OH-THC or CBD exposure during breastfeeding, depending on the dose and timing, could influence normal brain development of a child.

This study had several limitations as well as strengths. Samples were collected under different conditions, not all breast milk collections were directly observed, and we relied on maternal report of marijuana exposure. However, all participants completed a 14-day recall guided by trained study staff who prompted for specific daily use with the aid of a calendar. We had detailed information on timing and dose as well as information on a wide variety of other relevant covariates. Furthermore, the samples were collected from mothers who were using

currently available cannabis products. In contrast, previously published data were collected at a time when Δ9-THC concentrations in marijuana were far less than is typical today.[24]

We did not have infant plasma samples; instead we estimated potential infant Δ9-THC exposure based on several assumptions. Our infant exposure estimates may be an under- or overestimation depending on infant age and other factors. Future studies are required to better characterize the distribution of cannabinoids in human milk through more intensive and paired (milk/plasma) sampling and with more detailed data about the cannabinoid content of the product and exact route used by the mothers. In addition, longitudinal sampling of plasma of breastfeeding infants with either single-dose exposure (mothers who are occasional users) or steady-state (mothers who are frequent users) is needed to determine the extent to which cannabinoids accumulate in the breastfeeding infant.

Our findings also highlight a critical need for further research on neurodevelopmental outcomes in infants breastfed by mothers using marijuana. The one previous study that found psychomotor deficits in 12-month old infants breastfed by mothers using cannabis was conducted in an era when Δ9-THC concentrations in marijuana were estimated to be one third of what they are today.[11,24]

In summary, Δ9-THC was measurable in highly variable concentrations in the breast milk of approximately two-thirds of samples from women who reported marijuana use during breastfeeding and up to approximately six days since last reported dose. While the estimated median daily dose of Δ9-THC ingested by the infant is very low compared to adult doses, the high variability in breastmilk concentrations means that some infants may be exposed to daily amounts of cannabinoids closer to (but still lower than) typical adult amounts. Furthermore, the extent of oral absorption in breastfeeding infants, metabolism and accumulation patterns, and

pharmacologic effects of even low levels of cannabinoids on neurodevelopment in infants are unknown and require further study. As marijuana is the most commonly used recreational drug among breastfeeding women, information regarding risks to breastfeeding infants is urgently needed.

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Table 1. Selected characteristics of maternal and infant participants, n = 54, 2014-2017

Characteristic	N= 50 mothers, No. (%)	N=4 mothers who gave a repeat sample, No. (%)
Maternal Age (yrs)		
22-25	7 (14)	
25-30	17 (34)	
30-35	18 (36)	
35-41	8 (16)	
Maternal Ethnicity		
Hispanic	9 (18)	
Non-Hispanic	41 (82)	
Maternal Race		
Caucasian	44 (88)	
Black	1 (2)	
Asian	2 (4)	
Native American	3 (6)	
Maternal Education (yrs)		
Partial High School	1 (2)	
High School Graduate/GED	4 (8)	
Some College/ Specialization	27 (54)	
College Graduate	14 (28)	
Post-Graduate	4 (8)	
Maternal Body Mass Index (BMI)[a]		
<18.5	0 (0)	
18.5-24.99	17 (34)	
25-29.99	17 (34)	
>30	9 (18)	
Parity		
1	21 (42)	
>1	29 (58)	
Route of Marijuana Exposure[b]		
Inhalation Only	32 (64)	2 (50)
Other Only	7 (14)	0 (0)
Both	11 (22)	2 (50)
Frequency of Marijuana Use		
<1 use/day	6 (12)	1 (25)
1 use/day	23 (46)	3 (75)
>1 use/day	21 (42)	0 (0)
Full Expression of the Breast[c]		
No	9 (18)	0 (0)
Yes	41 (82)	4 (100)
Infant Age (months)[d]		
0-3	3 (6)	0 (0)
3-6	20 (40)	0 (0)

6-12	11 (22)	0 (0)
>12	16 (32)	4 (100)
Infant Sex		
Female	22 (44)	
Male	28 (56)	

[a] Body Mass Index (BMI)= bodyweight in kilograms divided by height in meters squared

[b] Inhalation only was defined as a dose unit of joints, puffs, or grams. Other only was defined as a dose unit of drops, milligrams or servings. Both was defined as a dose unit from the both the inhalation only and the other only groups

[c] A full expression of milk was defined as emptying the breast of milk entirely during one pump session

[d] If the mother of the baby was breastfeeding >1 child, only data from child 1 was included

Table 2: Concentrations of cannabinoids in human milk samples, n= 54

	Min.	1st Quartile	Median	3rd Quartile	Max.	AQL	BQL
Delta-9-Tetrahydrocannabinol (ng/mL)	1.01	2.29	9.47	46.78	323.00	34	20
11-hydroxy-tetrahydrocannabinol (ng/mL)	1.33	1.35	2.38	5.45	12.80	5	49
Cannabidiol (ng/mL)	1.32	2.92	4.99	5.97	8.56	5	49

*AQL, above quantification limits, was defined as ≥1ng/ mL; BQL, below quantification limits, was defined as <1 ng/mL

** The concentration of CBN was BQL in all 54 samples

Table 3. Log Δ-9-THC concentration by characteristics of maternal cannabis use and sample collection, n=34 samples

	Estimate	95% CI	p-value
Hours Since Last Use			
Hours[a]	-0.02	(-0.04, 0.00)	0.005
Uses per Day			
Hours	-0.02	(-0.04, 0.00)	0.032
Uses/Day[b]	0.51	(0.03, 0.99)	0.039
Route[c]			
Hours	-0.02	(-0.04, -0.01)	0.008
Route: Other Only	-0.69	(-3.06, 1.67)	0.553
Route: Both	-1.11	(-2.64, 0.41)	0.146
Puffs[d]			
Hours	-0.02	(-0.04, 0.00)	0.031
Puffs	-0.04	(-0.34, 0.26)	0.785
Heavy Use[e]			
Hours	-0.02	(-0.04, 0.00)	0.062
Heavy Use	1.13	(-0.28, 2.53)	0.110
Sample Age[f]			
Hours	-0.02	(-0.04, 0.00)	0.044
Sample Age (months)	0.08	(0.00, 0.15)	0.038
Time of Sample Collection [g]			
Hours	-0.03	(-0.04, -0.01)	0.008
Time of Collection (PM)	0.27	(-0.97, 1.51)	0.662
Time Since Last Feed/ Pump[h]			
Hours	-0.03	(-0.04, -0.01)	0.005
Time Since Last Feed./Pump (Hours)	-0.05	(-0.18, 0.09)	0.470
Feeding Frequency[i]			
Hours	-0.02	(-0.04, 0.00)	0.019
Feeding Frequency (Feeds/Day)	0.15	(-0.04, 0.34)	0.117

Separate multivariable linear regression models for each covariate with log-transformed Δ9-THC as the outcome

[a] Hours was defined as the number of hours between the most recent exposure end date and the date of sample collection

[b] Uses per day was defined as the average number of uses of marijuana during the most recent exposure period

[c] Route was defined as categorical variable with three levels: Inhalation, Other, and Both, with Inhalation as the reference category. Inhalation only was defined as a dose unit of joints, puffs, or grams. Other only was defined as a dose unit of drops, milligrams or servings. Both was defined as a dose unit from both the inhalation only and the other only groups

[d] For the covariate puffs, the dataset was restricted to samples where the dose unit was indicated as "puffs" (n=27). The covariate "puffs" was reported as the number of puffs taken per use of marijuana

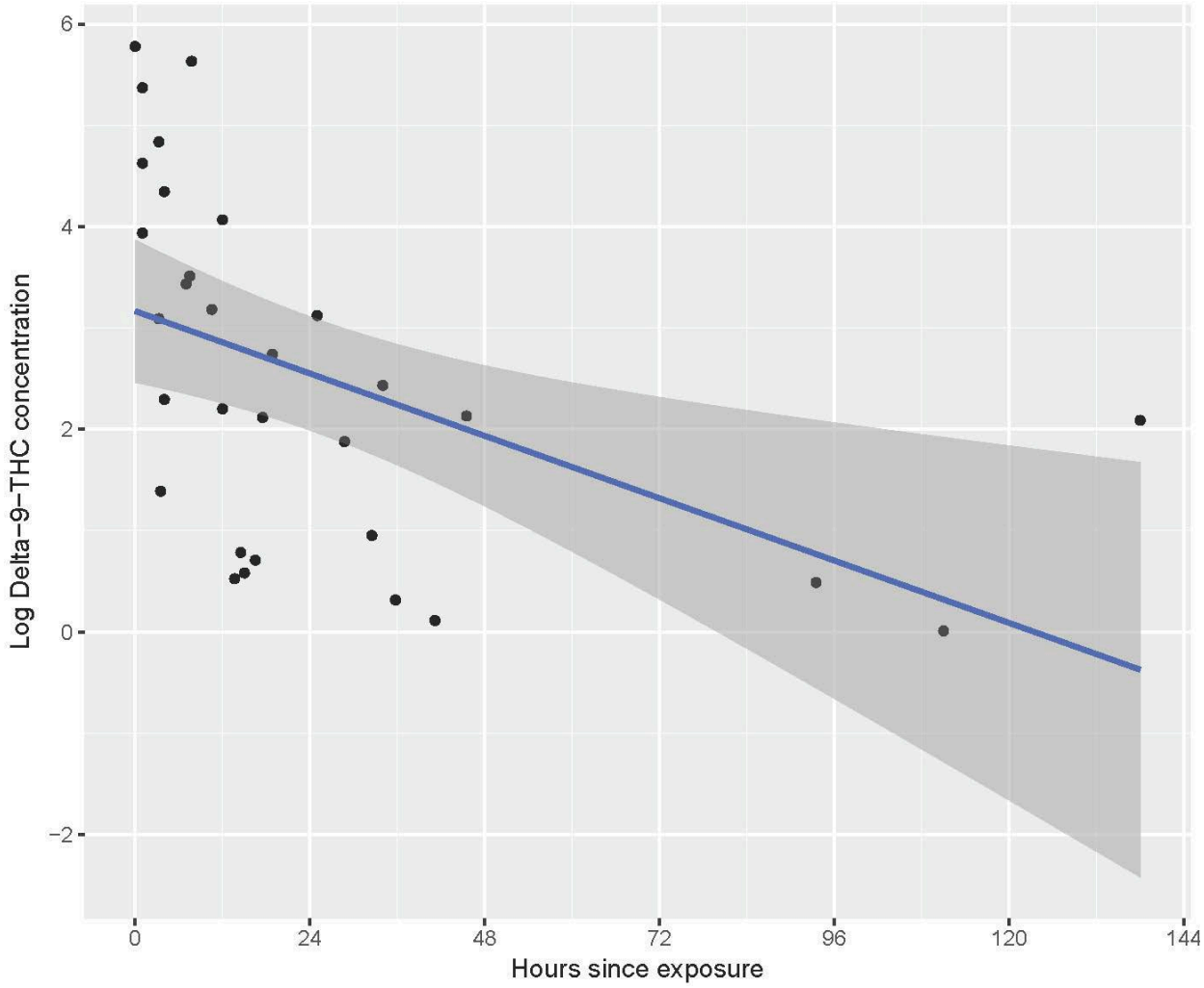
[e] For the covariate Heavy Use, the dataset was restricted to samples where the dose unit was indicated as "Puffs" (n=27). The covariate "puffs" was reported as the number of puffs taken per use of marijuana. Heavy use was defined as women who reported uses per day and puffs greater than the median values in the sample (1 use/ day and 3 puffs per use, respectively)

[f] Sample age was defined as the length of time between the date of milk collection and the date the sample was analyzed, measured in months.

[g] Time of collection was defined as the time of day at which the sample was expressed, measured in number of hours after midnight and dichotomized into before noon (AM) and after noon (PM).

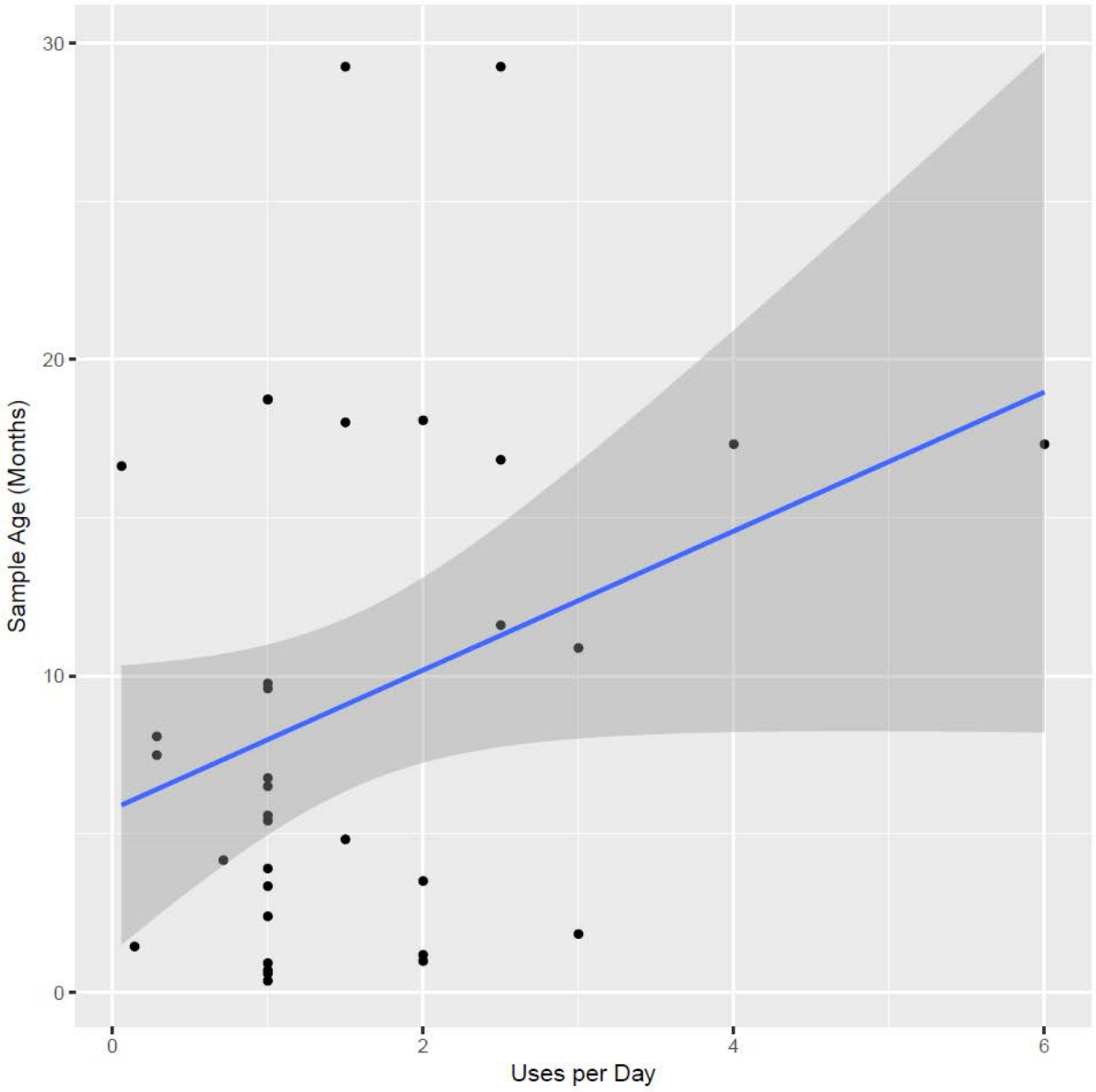
[h] Time since last feed/pump was defined as the difference between the time of the sample collection and the time of the most recent feed or pump, measured in hours.

[i] Feeding frequency was defined as the number of feedings per 24 hours.

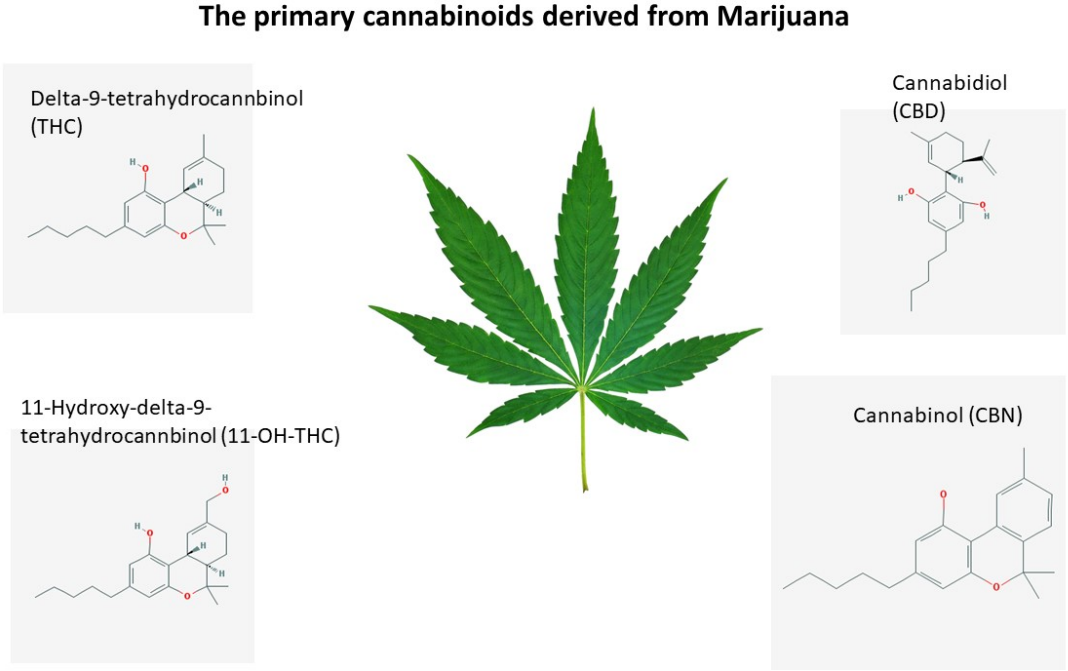


*The fitted regression line is shaded with 95% confidence limits around the regression line.

Figure 1. Scatterplot and fitted regression line of log concentration of Δ-9-THC by hours since last use of marijuana, N=34



eFigure 1. Distribution of sample age in months by number of times used per day, n=34 human milk samples



eFigure 2. The four primary cannabinoids, Delta-9-THC, 11-OH-THC, CBD, and CBN, derived from Cannabis