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Identifying Prenatal Cannabis Exposure and Effects of Concurrent Tobacco Exposure on Neonatal Growth

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BACKGROUND: Cannabis is the most frequently used illicit drug among pregnant women, but data describing the effects of prenatal cannabis exposure and concurrent nicotine and cannabis exposures on neonatal growth are inconsistent. Testing of meconium, the first neonatal feces, offers objective evidence of prenatal cannabis exposure, but the relative ability of meconium testing and maternal self-report to identify affected neonates remains unclear.

METHODS: Eighty-six pregnant women provided detailed self-reports of daily cannabis and tobacco consumption throughout pregnancy. Cannabinoids and tobacco biomarkers were identified in oral fluid samples collected each trimester and quantified in meconium at birth.

RESULTS: Cannabis-using women were significantly more likely to also consume tobacco, and smoked similar numbers of cigarettes as non–cannabis-using tobacco smokers. As pregnancy progressed, fewer women smoked cannabis and those who continued to use cannabis reported smoking a smaller number of cannabis joints, but positive maternal oral fluid tests cast doubt on the veracity of some maternal self-reports. More neonates were identified as cannabis exposed by maternal self-report than meconium analysis, because many women quit cannabis use after the first or second trimester; meconium was more likely to be positive if cannabis use continued into the third trimester. Cannabis exposure was associated with decreased birth weight, reduced length, and smaller head circumference, even after data were controlled for tobacco coexposure.

CONCLUSIONS: Prenatal cannabis exposure was associated with fetal growth reduction. Meconium testing primarily identifies prenatal cannabis exposure occurring in the third trimester of gestation.

Cannabis (marijuana) is one of the most popular drugs among women of child-bearing age in the US, with approximately 11% reporting recent cannabis use *(1)*. After becoming pregnant, some women continue to smoke cannabis for recreational purposes or, in a minority of women, to combat morning sickness *(2)*. The effects of prenatal cannabis exposure on neonatal outcomes are unclear. Some investigators have reported deficits in birth weight, length, and gestational age *(3, 4)*, whereas others observed no adverse effects *(5–10)*. Long-term consequences of prenatal cannabis exposure have also been reported, including poorer performance on intelligence tests *(11)*, increased depressive symptoms *(12)*, and increased likelihood of cannabis use during adolescence *(13)*, but results of other studies have demonstrated no differences between prenatally exposed and nonexposed children and adolescents *(6)*.

A potential reason for the discrepant findings is the difficulty in accurately differentiating exposed from unaffected children. Most investigations rely on maternal interview to differentiate nonexposed controls from affected infants. Self-reported maternal drug histories may be inaccurate, however, owing to feelings of guilt and fear of prosecution, genuine inability to accurately recall drug-use details, poorly constructed screening/assessment tools, and inadequate training of interview staff. Analyzing biological samples from the mother or neonate offers an objective alternative for determining gestational drug use. Meconium, the first neonatal feces, is considered the gold standard for prenatal drug exposure identification because of easy, noninvasive collection and longer drug detection windows. Despite its advantages, meconium has some lim-

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itations including limited sample amount, nonhomogenous distribution of analytes, and more complex analytical requirements. Also, it has been assumed that meconium reflects second- and third-trimester drug use, but prospective data from our laboratory suggest that the detection window is shorter, at least for opioids, cocaine, and nicotine, spanning approximately the third trimester *(14)*. To date, the detection window for cannabinoids in meconium has not been elucidated.

Previous investigations compared the relative ability of maternal self-report and toxicological testing to identify cannabis exposure, and had starkly different results. In the Meconium Project, meconium analysis results led to the identification of 5.3% of the cohort as cannabis exposed, whereas 1.7% of mothers self-reported cannabis use *(8)*. In contrast, in the Infant Development, Environment and Lifestyle study approximately 70% of cannabis-exposed neonates were identified only by maternal self-report, 16.5% had positive maternal self-report and meconium results, and 13.8% were detected by meconium analysis alone *(15)*. Discrepant data may be due to differences in study design and population, analytical test parameters, and the timing, magnitude, and duration of maternal drug use. Thus, it is not yet clear whether maternal self-report or meconium analysis is the best way to identify prenatal cannabis exposure.

The aims of this research were to (*a*) investigate self-reported cannabis use patterns, (*b*) compare self-reported cannabis use history and meconium cannabinoid results, (*c*) determine if the presence or concentration of cannabinoids in meconium predict growth deficits, and (*d*) evaluate whether there is an association between cannabis exposure and neonatal outcomes among tobacco-exposed neonates.

METHODS

Pregnant women between 12 and 20 weeks' gestation were invited to participate in this study. The research protocol was approved by the institutional review board of the University at Buffalo. The primary objective of this protocol was to evaluate the effects of prenatal and environmental tobacco exposure on child self-regulation. For this report, however, we evaluated the effects of cannabis use on fetal growth and considered tobacco coexposure as a potential confounder. Exclusion criteria included maternal age <18 years, multiple-birth pregnancy, illicit drug use (other than cannabis), heavy alcohol or cannabis consumption (>1 drink or >5 joints per day or >4 drinks or >4 joints on a single occasion after pregnancy recognition). After providing written informed consent, participants completed prenatal assessments near the end of each trimester and 1 postpartum assessment at approximately 2 months of infant age corrected for prematurity. At each appointment, a time-line follow-back interview *(16, 17)* was used to gather retrospective data on daily tobacco, alcohol, and cannabis use for the previous 3 months, except for the first assessment that covered 3 months before conception through the appointment; thus, self-reported data spanned the time period from 3 months before conception through delivery. If a woman reported cannabis amounts in grams, blunts, or bowls, she was asked how many joints could have been rolled from an equivalent amount of cannabis. If she did not know, bowls and blunts were converted to 3 and 4 joints, respectively.

At each assessment, an oral fluid sample was collected for toxicological evaluation, providing objective evidence of recent exposure to cannabis and tobacco *(18–20)*. Oral fluid samples were analyzed by the US Drug Testing Laboratory (Des Plaines, IL) for Δ^9-tetrahydrocannabinol (THC),[3] the psychoactive component of cannabis, by immunoassay screening (4.0 μg/L cutoff) and GC-MS confirmation (4.0 μg/L cutoff). Cotinine, the primary nicotine biomarker, was assayed with ELISA or liquid chromatography–tandem mass spectrometry; a 10-μg/L cutoff was employed for both methods.

After birth, meconium samples were collected from soiled diapers twice daily until the appearance of milk stool. The meconium samples were transferred to storage containers and frozen until transport to the National Institute on Drug Abuse, where analysis was performed. Meconium samples were assayed with a validated 2-dimensional GC-MS analytical method for THC; 11-hydroxy-THC; 8β,11-dihydroxy-THC; 11-nor-9-carboxy-THC (THCCOOH); and cannabinol *(21)*. Meconium samples were well mixed and 0.25 g was analyzed. Samples were homogenized in methanol before tandem β-glucuronidase (Type IX-A from *Escherichia coli*) and alkaline hydrolyses to liberate glucuronide conjugates. Free cannabinoids were isolated from matrix components by mixed-mode anion-exchange solid-phase extraction and derivatized with N,O-bis(trimethylsilyl) trifluoroacetamine with 1% trimethylchlorosilane. Limits of quantification were 10 ng/g for all analytes, except 11-hydroxy-THC at 15 ng/g. Coexposure to tobacco was identified by the presence of nicotine (limit of quantification 2.5 ng/g), cotinine (1 ng/g), and/or *trans*-3′-hydroxycotinine (5 ng/g) with a validated liquid chromatography–tandem mass spectrometry method for meconium *(22)*.

[3] Nonstandard abbreviations: THC, Δ^9-tetrahydrocannabinol, THCCOOH, 11-nor-9-carboxy-THC.

Table 1. Maternal demographics for 86 pregnant women classified by self-reported cannabis-smoking status.

	Cannabis nonusers (n = 48)	Cannabis users (n = 38)	Total (N = 86)	*P*
Age, mean (SD) (range), y	24.3 (5.2) (19–39)	24.4 (5.1) (19–38)	24.3 (5.1) (18–39)	0.909
Para, mean (SD) (range)	2.3 (2.4) (1–9)	2.2 (2.5) (1–10)	2.2 (2.4) (0–10)	0.800
Gravida, mean (SD) (range)	1.9 (1.5) (0–5)	1.4 (1.8) (0–8)	1.7 (1.6) (0–8)	0.318
Race, n (%)				0.031
White	13 (27.1)	9 (23.7)	22 (25.6)	
African American	22 (45.8)	16 (42.1)	38 (44.2)	
Hispanic	11 (22.9)	3 (7.9)	14 (16.3)	
Multiracial	2 (4.2)	10 (26.3)	12 (14.0)	
Tobacco use				<0.001
Active tobacco smoker in third trimester, n (%)r	22 (45.8)	33 (86.8)	55 (64.0)	
Quit tobacco use during pregnancy, n (%)	4 (8.3)	3 (7.9)	7 (8.1)	
Non–tobacco smoker, n (%)	22 (45.8)	2 (5.3)	24 (27.9)	
Cigarettes per day in third trimester, median (interquartile range)	3.5 (1.7–6.1)	5.2 (3.1–7.6)	4.3 (2.6–7.1)	0.142

Neonatal birth parameters, including sex, gestational age, birth weight, length, head circumference, and 1- and 5-min Apgar scores were extracted from medical records.

Statistical evaluations were completed with SPSS for Windows. We used Kolmorgov-Smirnov tests to assess the normality of data distribution. For normally distributed variables, *t*-tests were employed, whereas variables with nonnormal distributions were evaluated with Mann–Whitney *U*- and Spearman ρ tests. We used χ^2 tests to examine variable independence for race, marital status, and tobacco use. A *P* value <0.05 was considered statistically significant.

Results

Initially, 120 women met eligibility criteria. Of these, 16 did not return for second or third prenatal interviews or elected to drop out, 1 suffered a miscarriage, and 1 relocated. Thus, 102 women were enrolled and remained in the study through the delivery period. Meconium was not collected from 14 neonates, 3 for whom only milk stool samples were available, 6 for whom meconium samples were discarded by mistake, and 5 who were not delivered at the recruitment hospital. Another meconium sample had insufficient amounts for analysis. An additional woman moved soon after delivery, before the final interview. In total, 86 woman-infant dyads had complete self-report data and meconium analyzed for cannabinoids and tobacco biomarkers.

SOCIODEMOGRAPHIC CHARACTERISTICS AND SELF-REPORTED CANNABIS AND TOBACCO USE

Thirty-eight women (44.2%) self-reported cannabis use during pregnancy. The sociodemographic characteristics from cannabis users and nonusers are presented in Table 1. No differences in maternal age, obstetric history, employment status, or martial status were noted. Significant differences in race and concurrent tobacco consumption were observed between cannabis users and nonusers. Hispanic women were less likely to consume cannabis, whereas multiracial women were more frequently cannabis users. Cannabis-using women were also more likely to be tobacco smokers and smoked a similar number of cigarettes per day as tobacco smokers who did not use cannabis.

According to self-report, women tended to stop using cannabis as pregnancy progressed. Thirty-seven women reported that they had smoked cannabis in the first trimester, 22 that they had smoked cannabis in the second trimester, and 14 that they had smoked cannabis in the third trimester. Stated differently, the self-reported final trimester during which cannabis was smoked was the first trimester for 15 women and the second trimester for 9 women, and 14 women smoked cannabis throughout pregnancy. For 4 women, however, positive results of oral fluid tests conflicted with self-reports; in 2 cases, women denied cannabis use throughout pregnancy, yet oral fluid tests were positive for 1 woman in the first trimester and for the other woman in the second trimester. The remaining 2 women reportedly ceased cannabis use in the second

Table 2. Cannabinoid biomarker prevalence and concentrations in 20 neonates with positive meconium results following enzyme-alkaline tandem hydrolysis.

Participant number	Trimester of last use[a]	THC, ng/g	11-hydroxy-THC, ng/g	8β,11-dihydroxy-THC, ng/g	THCCOOH, ng/g	Cannabinol, ng/g
12	3		46.6	13.2	459.6	46.6
20	3		18.1	12.0	164.3	40.8
23	3		105.5	27.9	443.5	186.0
29	3		44.3		245.2	79.5
34	3				22.7	
37	1				13.7	
38	3		20.6		136.3	19.3
39	3		19.0		29.7	15.9
41	3		16.0		54.8	
45	2				17.0	12.2
52	3				13.3	
60	3		39.8	13.2	270.5	23.9
61	[b]				17.2	
62	3				39.5	
66	2				107.3	
72	3		26.9	45.8	43.9	12.1
73	3				21.9	
74	1				18.7	
76	3				15.4	12.5
78	2				48.1	10.6
No., positive		0	9	5	20	11
%		0	45	20	100	55
Median			26.9	13.2	41.7	19.3
Interquartile range			18.6–45.5	12.6–36.9	17.6–157	12.2–46.6

[a] As determined by maternal self-report and/or oral fluid testing.
[b] No self-reported cannabis use or positive oral fluid results throughout pregnancy.

trimester, but oral fluid samples collected in the third trimester were positive. Therefore, based on the combination of self-report and results of oral fluid testing, 46 women (53.5%) had no cannabis exposure, 16 (18.6%) smoked cannabis only in the first trimester, 8 (9.3%) stopped use in the second trimester, and 16 (18.6%) smoked throughout pregnancy.

The number of cannabis joints smoked per trimester decreased from a median (interquartile range) of 70.8 (11.1–308) cannabis joints in the first trimester to 30.2 (1.9–170) in the second and 5.8 (1.8–7.4) in the third.

PREVALENCE OF BIOMARKERS IN MECONIUM BASED ON SELF-REPORTED CANNABIS USE

Twenty meconium samples contained 1 or more cannabinoids, documenting prenatal cannabis exposure (Table 2). The presence of cannabinoids in meconium was influenced by the timing of maternal cannabis use. One neonate had positive meconium results despite maternal denial of cannabis use and negative results for oral fluid tests throughout pregnancy. Based on the combination of self-report and/or oral fluid testing, 2 meconium samples were positive among 16 women who reported using cannabis only in the first trimester, and 3 meconium samples were positive among women who reported ceasing cannabis use during the second trimester (n = 8). For women who reported that their cannabis use continued into the third trimester (n = 16), 14 meconium samples were positive. Of the 2 women whose infants had negative meconium samples, 1 woman reported smoking a total of 1 and the other a total of 3 joints during the third trimester. The number of self-reported joints consumed in the third trimester was not correlated to individual or total cannabinoid biomarker concentrations found in meconium.

Table 3. Neonatal growth comparisons based on the presence of cannabis biomarkers in meconium or positive self-report, oral fluid tests, or meconium results (N = 86).

	Independent of tobacco results			Among tobacco-positive mothers[b]		
	Cannabinoid-negative meconium (n = 66)	Cannabinoid-positive meconium (n = 20)	*P*	Cannabinoid-negative meconium (n = 33)	Cannabinoid-positive meconium (n = 19)	*P*
Estimated gestational age at delivery, median (interquartile range), weeks[a]	39 (38.7–40.2)	39 (37.2–39.4)	0.012	39 (38–40)	39 (37–39.5)	0.166
Birth weight, mean (SD), g	3429 (544)	2853 (618)	<0.001	3386 (622)	2831 (627)	0.003
Head circumference, mean (SD), cm	34.4 (1.7)	33.0 (2.4)	0.003	34.1 (1.7)	32.8 (2.3)	0.025
Length, mean (SD), cm	50.8 (2.4)	48.8 (4.4)	0.010	50.9 (2.8)	48.7 (4.5)	0.031
Apgar score, median[a]						
1 min	9	9	0.231	9 (8–9)	9	0.128
5 min	9	9	0.653	9	9	0.188
	No cannabis exposure[b] (n = 45)	**Cannabis exposure[b] (n = 41)**	***P***	**No cannabis exposure[b] (n = 23)**	**Cannabis exposure[b] (n = 39)**	***P***
Estimated gestational age at delivery, median (interquartile range)[a]	39 (38.0–40.1)	39 (38.0–40.0)	0.685	38.4 (37–40)	39 (38–40)	0.464
Birth weight, mean (SD), g	3417 (504)	3161 (689)	0.051	3376 (550)	3152 (690)	0.190
Head circumference, mean (SD), cm	34.6 (1.7)	33.5 (2.2)	0.011	34.4 (1.5)	33.5 (2.2)	0.081
Length (cm, mean (SD)	50.8 (2.2)	49.8 (3.8)	0.156	50.6 (2.3)	49.8 (3.9)	0.360
Apgar score, median (interquartile range)						
1 min[a]	9 (8–9)	9	0.709	9 (8–9)	9	0.392
5 min[a]	9	9	0.496	9	9	0.443

[a] Data were not normally distributed.
[b] Positive self-report, or oral fluid or meconium test result.

Meconium testing had a positive predictive value of 95.0% and a negative predictive value of 68.2% if cannabis exposure was self-reported or oral fluid test results were positive at any time during pregnancy. If use was self-reported or oral fluid tests were positive in the second and/or third trimester, the positive predictive value decreased to 85% and the negative predictive value increased to 89.3%. If self-reports or oral fluid tests indicated cannabis use in the third trimester, the positive predictive value of meconium testing was 70% and the negative predictive value was 97.0%. The increase in negative predictive value reflected a decrease in false-negative test results, which was attributable to the improved ability of meconium testing to detect third-trimester drug exposure. The decreases in positive predictive values most likely reflect increased false-negative self-reports later in gestation, when mothers are hesitant to report continued drug use owing to guilt or fear of negative consequences such as loss of custody.

All but 1 cannabinoid-positive meconium sample also contained 1 or more tobacco biomarkers. Total tobacco-biomarker concentrations were higher in samples that contained cannabinoids (median 268 ng/g, interquartile range 204–334 ng/g) than in samples that did not contain a cannabinoid (median 137.6 ng/g, interquartile range 89.6–266 ng/g; $P = 0.001$).

MECONIUM CONCENTRATION AS A PREDICTOR OF NEONATAL GROWTH DEFICITS

The presence and concentration of cannabis biomarkers were evaluated as predictors of neonatal growth deficits. If meconium test results were positive for 1 or more cannabis biomarkers, we observed lower gestational age, birth weight, head circumference, and birth length than those observed in nonexposed children (Table 3), whereas we found no difference in 1- and 5-min Apgar scores. Also, 3 cannabis-exposed and 1 nonexposed infants were premature (<37 weeks' gestation). If the criteria for cannabis exposure status were expanded to include any positive self-report, oral fluid test, or meconium result, only differences in head circumference remained significant (Table 3).

The high prevalence of tobacco coexposure in cannabis-exposed neonates potentially confounds neonatal growth deficits. Therefore, we evaluated whether neonates with cannabinoid- and tobacco-positive meconium were more affected than those with only tobacco-positive meconium (Table 3). In children exposed to cannabis, birth weight, length, and head circumference were significantly lower than in children who were not exposed to cannabis, but gestational age and Apgar scores were not affected. Again, if we expanded cannabis and tobacco-exposure status to include any positive self-report, oral fluid test, or meconium result, we found that growth parameters were no longer significantly affected by cannabis exposure (Table 3).

Although the presence of cannabinoid biomarkers in meconium was associated with growth deficits, there were no correlations between any cannabinoid biomarker concentration or total cannabinoid biomarker concentration and birth weight, length, head circumference, gestational age, or Apgar scores. The number of joints consumed by mothers during the third trimester did not correlate with growth deficits in children.

Multiple regression analysis of the effects of several variables, including race, gestational age, cannabis-positive meconium, and tobacco-positive meconium on birth weight ($R^2 = 0.532$) showed that only gestational age ($\beta = .568$), being white ($\beta = .272$), and the presence of cannabinoids in meconium ($\beta = -.229$) were found to impact the model's ability to predict birth weight.

Discussion

The primary aim of the Growing Up Healthy study was to determine how prenatal and environmental tobacco exposure affected child self-regulation; cannabis coexposure was included as a potential confounder. Because of the high prevalence of cannabinoid exposure in this cohort, we also sought to evaluate maternal self-reported cannabis use patterns. We compared these patterns to the presence of cannabinoids in meconium to determine whether meconium cannabinoid results predicted growth deficits, and whether the effects of cannabis and nicotine coexposure on neonatal growth are interactive.

Many cannabis-using women in our study population self-reported stopping or decreasing their cannabis consumption as pregnancy continued. Self-reported reduction or cessation of cannabis use in pregnant women as pregnancy progressed has been observed in other investigations, including the National Survey on Drug Use and Health, in which fewer pregnant respondents admitted cannabis use in the second (2.9%) and third trimesters (1.4%) than in the first trimester (4.6%). Similarly, in a study based in the United Kingdom, during the course of pregnancy progressively fewer women smoked and those who did smoked a progressively smaller number of joints, but the rate of decline for both the number of cannabis users and the amount used was much less than that observed for ecstasy and cocaine *(23)*. Although some women in the present study claimed cannabis cessation, objective evidence provided by oral fluid testing suggested otherwise. A strength of this study was the inclusion of maternal biological monitoring during pregnancy, which helped us to properly classify children as "cannabis exposed" or "nonexposed." Of 48 women, 2 (4.2%) who denied cannabis use throughout pregnancy had positive oral fluid samples, and 2 of the 23 women who reported that they quit cannabis during the first or second trimester had a THC-positive oral fluid sample in the third trimester. The presence of THC in oral fluid, a matrix with a short detection time window *(19)*, indicated recent, active cannabis use by the mother. After an individual has smoked cannabis, THC remains detectable in the oral fluid of the smoker for approximately 24 h *(18)*. At the cutoff concentration used in this study, passive exposure to cannabis smoke would not be expected to generate a positive result *(24)*. A limitation of this research was the infrequent collection of oral fluid samples. Because oral fluid samples were collected only once each trimester, at the time of maternal interview, additional cannabis use could have been missed in women who self-reported no cannabis smoking or abstinence from cannabis beginning early in pregnancy. More frequent oral fluid collection might have enabled us to identify additional erroneous maternal self-reports.

Similarly to other studies performed to evaluate the disposition of cannabinoids in meconium *(25–27)*, our study revealed that THCCOOH was the most prevalent and abundant analyte, but results of previous research by our laboratory demonstrated that the hydrolysis procedure affects detection and prevalence of specific cannabinoids in meconium *(21)*. We observed that analysis of THCCOOH performed after a basic hydrolysis procedure maximized identification of cannabis-exposed neonates, but if a tandem enzymatic and alkaline hydrolysis procedure was used, greater concentrations of THCCOOH and greater prevalence of the minor metabolites 11-hydroxy-THC and 8β,11-dihydroxy-THC were obtained. For the current investigation, we chose to analyze samples with tandem hydrolysis because this method yielded the broadest spectrum of cannabinoids. It was important to have as many analytes as possible because it was not clear whether the presence or concentration of a particular cannabinoid was associated with adverse neonatal outcomes.

Because of the uncertainty of self-reported abstinence, particularly among pregnant women who supposedly ceased cannabis use in the second trimester, too few data were available to determine if the detection window for cannabis in meconium extended into the second trimester. However, third-trimester cannabis exposure was readily identified by meconium analysis; 14 of 16 women with positive self-report or oral fluid test results in the third trimester had neonates with cannabinoid-positive meconium. We previously documented a short detection window of only the last 3 months of gestation for opiates and cocaine, a finding based on prospective testing of 3 maternal urine samples each week of pregnancy *(14)*. More research is necessary to determine how well second-trimester cannabis exposure can be documented in meconium samples. Improved estimates for the detection window of cannabinoids in meconium could be made if biological monitoring of pregnant women's drug use during gestation was employed.

Whereas timing of maternal cannabis use was demonstrated to influence cannabinoid disposition in meconium, the magnitude of maternal consumption was not reflected in higher cannabinoid concentrations in meconium or more severe growth deficits in children. The elucidation of a clear dose effect on meconium concentration and neonatal outcome was potentially complicated by several factors. First, we relied on self-report to determine the number of joints smoked per day. Self-reports cannot be assumed to be accurate, and it is possible that women underreported their actual cannabis use. Second, the THC content of the cannabis smoked likely differed between participants. Third, differences in smoking topography (e.g., depth of breath, length of hold) probably affected THC bioavailability and dose *(28)*.

Contradictory results have been reported by previous investigators who compared maternal self-report and meconium testing for identification of prenatal cannabis exposure *(8, 15)*. In our cohort, more neonates were identified by maternal self-report ($n = 38$) than meconium testing ($n = 20$), results similar to our findings in the Infant Development, Environment and Lifestyle study *(15)*. Because meconium is not yet formed during the first trimester, it does not reflect first-trimester maternal drug use. In addition, data from our previous research indicate that second-trimester drug use is poorly reflected in meconium *(14)*. Another biological matrix or improved self-report tool is necessary to identify cannabis exposure in early pregnancy. Maternal hair is a potential matrix for objectively detecting gestational cannabis exposure, but consent to collect hair is often difficult to obtain for cosmetic reasons, and the hair quantities obtained may be insufficient for testing. Moreover, cannabinoids are difficult to detect in the hair of nondaily users; in previously reported research approximately half of men smoking 1–5 joints per week had cannabinoid-negative hair *(29)*.

In our study, we compared neonatal growth outcomes in cannabis-exposed and nonexposed neonates (as determined by meconium analysis), and found reductions in gestational age, birth weight, head circumference, and length, yet most neonates were within the expected range for weight, head circumference, and length. Gestational age, birth weight, head circumference, and length are highly interdependent; therefore, any potential effects of cannabis exposure on these parameters should not be interpreted as independent effects. Race was investigated as a potential influencer of fetal growth, and being white was the only racial variable that significantly influenced the regression model.

For most sociodemographic parameters, women who smoked cannabis were no different from women who were abstainers. However, cannabis-smoking pregnant women were far more likely also to be tobacco smokers, and those who were consumed similar numbers of cigarettes as non–cannabis-using tobacco smokers. The high prevalence of tobacco coconsumption also was observed in previous prenatal cannabis exposure investigations *(4, 23, 30)*. Tobacco use in pregnancy is widely associated with impaired fetal growth and obstetrical complications *(31)*, thus research elucidating the effects of cannabis exposure should consider nicotine coexposure as a potential confounder. After we controlled data for nicotine exposure by comparing infants with cannabis- and tobacco-positive meconium to those with only tobacco-positive meconium, cannabis exposure was still associated with decreased birth weight, length, and head circumference, but again, results for most children were within reference intervals. Our results are consistent with those of El Marroun et al. *(4)*, who found an additive effect of cannabis and tobacco coexposure on birth weight, length, and head circumference deficits. In our study, we observed no linear concentration-outcome effects for any growth parameter. Because of our small sample size, however, our study may have lacked the power to detect correlations. If meconium concentrations are not indicative of more severe deficits, meconium analyses could be limited to qualitative (positive/negative) assessments.

We observed that fetal growth restriction was associated with presence of cannabinoids in meconium (indicative of third-trimester exposure) but not cannabis exposure at any point in pregnancy. This finding suggests that cannabis may have a more profound effect on growth if exposure occurs later in pregnancy, or that exposure into the third trimester may also reflect a

higher cumulative dose than if drug use stopped earlier in gestation. The largest increases in fetal growth occur in the last trimester, because fetal weight nearly triples between gestational weeks 26 and 40. *(32)* However, other evidence indicates that cannabis-related growth inhibition may occur during midgestation as well. Hurd et al. *(33)* found that 17- to 22-week-old terminated fetuses exposed to cannabis were lighter in weight and had shorter foot length than nonexposed fetuses. Similarly, El Marroun et al. *(4)* showed that midgestational growth deficits associated with cannabis use in early pregnancy persisted to birth; moreover, continued cannabis use in late pregnancy resulted in more severe growth inhibition.

In summary, our results showed that prenatal cannabis exposure results in fetal growth reduction, even after data were controlled for tobacco coexposure. Meconium testing primarily identified prenatal cannabis exposure occurring in the third trimester of gestation.

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