Monitoring Perinatal Exposure to Cannabis and Synthetic Cannabinoids

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Purpose: Drug use during pregnancy is a critical global challenge, capable of severe impacts on neonatal development. However, the consumption of cannabis and synthetic cannabinoids is on the rise in pregnant women. Obstetric complications with increased risks of miscarriage, fetal growth restriction, and brain development impairment have been associated with perinatal cannabis exposure, but data on synthetic cannabinoid use during pregnancy are limited.

Methods: We reviewed studies that investigated the risks associated with cannabis and synthetic cannabinoid use and those that reported the concentrations of cannabinoids and synthetic cannabinoids in maternal (breast milk) and neonatal (placenta, umbilical cord, meconium, and hair) matrices during human pregnancy. A MEDLINE and EMBASE literature search to identify all relevant articles published in English from January 1998 to April 2019 was performed.

Results: Cannabis use during pregnancy is associated with increased risks of adverse obstetrical outcomes, although neurobehavioral effects are still unclear. Analyses of cannabinoids in meconium are well documented, but further research on other unconventional matrices is needed. Adverse effects due to perinatal synthetic cannabinoid exposure are still unknown, and analytical data are scarce.

Conclusions: Awareness of the hazards of drug use during pregnancy should be improved to encourage health care providers to urge pregnant women to abstain from cannabis and, if cannabis-dependent, seek treatment. Moreover, substances used throughout pregnancy should be monitored as a deterrent to cannabis use, and potential cannabis-dependent women should be identified, so as to limit cannabis-fetal exposure during gestation, and provided appropriate treatment.

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INTRODUCTION

Cannabis Pharmacology and Metabolism

Cannabis (Cannabis sativa L.) has been consumed for centuries for therapeutic, religious, and recreational purposes, owing to its psychoactive and analgesic effects[1]. D9-tetrahy-drocannabinol (THC) is the primary psychoactive compound in cannabis, along with other minor phytocannabinoids [cannabidiol (CBD), cannabigerol (CBG), tetrahydrocannabivarin (THCV), and cannabinol (CBN), among others][2], flavonoids, terpenes, polyaromatic hydrocarbons, and other chemicals. THC is a partial agonist of the endocannabinoid receptors CB1 and CB2, the activation of which generates central and peripheral effects[2,3]. Consequently, cannabis has relaxing, sedating, exhilarating, orexigenic, and antiemetic properties but is equally associated with acute and chronic cardiovascular and respiratory side effects, impaired cognition, and schizophrenia/psychosis; the risk of long-term cognitive effects increases with an earlier age of onset[3]. Cannabis use can also progress to addiction and dependence and increase vulnerability to abuse and addiction to other substances by altering the brain dopamine reward centers[3]. THC is currently under international control, in accordance with the Convention on Psychotropic Substances of 1971 (schedule I)[4].

Owing to its high lipophilicity, THC is widely distributed in body fat and tissues that represent long-term storage sites. It is predominantly metabolized in the liver, through cytochrome P450 (CYP) enzymes (CYP2C9, CYP2C19, and CYP3A4), leading to the formation of 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH), which undergo further glucuronidation by glucuronosyltransferases (UGT; UGT1A1, UGT1A3, UGT1A9, and UGT1A10). 11-OH-THC is the active metabolite, which presents the psychoactive effects of cannabis. Minor metabolites include 8-hydroxy-THC (8-OH-THC) and 8,11-dihydroxy-THC (8,11-diOH-THC).5 Although CYP3A4 and CYP2C9 are overexpressed during pregnancy, their effects on fetal and maternal THC pharmacokinetics are still unclear[6].

Prevalence of Cannabis Use During Pregnancy

Cannabis is the most widely used illicit drug during pregnancy, and its use has considerably increased in the past decade. According to a national survey conducted in the United States, self-reported past-month cannabis use among 10,587 pregnant women increased from 2.37% in 2002 to 3.85% in 2014, with a higher exposure prevalence during the first trimester[7,8]. Self-report or analytically confirmed cannabis intake during pregnancy among 279,457 women in California increased from 4.2% in 2009 to 7.1% in 2016 (analytically confirmed prevalence was approximately twice that of self-reported use)[9]. A similar trend was observed in Ontario, Canada, where cannabis use increased from 1.2% in 2012 to 1.8% in 2017, based on the medical records and interviews of 732,818 pregnant women.10 Similar patterns were observed in Europe[11–14] and Australia[15]. Maternal depression is a primary risk factor for drug use during pregnancy[16]. More so, pregnant women on cannabis perceive no general or pregnancy-related risks compared with nonusers. They believe cannabis is more natural and safer than other substances, including prescribed medicines[17,18]. Insufficient/ poor communication with health care providers seems to be a key factor in this misconception[18]. Cannabis may even be self-administered to treat nausea and vomiting symptoms during pregnancy[19]. The current depenalization trend of recreational cannabis in Western countries and the increasing interest in cannabis use in medicine may dampen its professed danger and increase its availability, suggesting that exposure prevalence may be more advanced in the future[20,21].

Synthetic Cannabinoids

New psychoactive substances (NPS) are synthetically manufactured molecules capable of inducing the psychoactive effects of legally controlled substances, with often higher potency. In the past decade, the emergence of NPS onto the drug market as “research chemicals” or “legal highs” led to recent important modifications in drug abuse demographics. From 2009 to 2017, 803 NPS in 111 countries and territories have been reported to the United Nations Office on Drugs and Crime Early Warning Advisory, and a dozen “first entries” are detected yearly[22]. NPS are not controlled by the Convention on Psychotropic Substances of 1971; hence, several countries have adopted different strategies to prohibit their use. However, legislations are far outweighed by the constant emergence of new products.

NPS use has been associated with health issues, and they continue to pose a significant risk to public health.

Synthetic cannabinoids, synthetic cathinones, and new synthetic opioids are currently the most widespread NPS globally[23]. Synthetic cannabinoids are partial or full CB1 and CB2 agonists, eliciting cannabis effects, generally with a higher potency than THC[24,25]. They have been associated with side effects such as tachycardia, agitation, and nausea; can induce acute kidney injuries, chest pain, myocardial infarctions and strokes, seizures, psychosis, panic attacks, hallucinations, and paranoia; and have been involved in several cases of severe intoxication and death by arrhythmia, seizure, or multiorgan failure[26]. Attributable to their relatively recent emergence onto a highly dynamic market, data on synthetic cannabinoid long-term effects and perinatal toxicity are very limited. Moreover, documenting information on synthetic cannabinoid consumption can be challenging because poly-substance use is common among NPS users, new substances are constantly being produced, and detection can be difficult (low active concentrations and no detection with usual toxicological screenings)[27–32].

Objective

The prevalence of cannabis and synthetic cannabinoid use in pregnant and breastfeeding women is on the rise. Data on the risks of in utero cannabis exposure are conflicting and limited, whereas data on synthetic cannabinoids are virtually nonexistent. We aimed to review the most recent studies on the risks associated with perinatal cannabis exposure and synthetic cannabinoids, to encourage health care providers to urge pregnant drug users to seek treatment.

Monitoring cannabis and synthetic cannabis use during pregnancy is an important tool to limit prenatal exposure. Given their short detection windows, drug testing in conventional matrices (maternal oral fluid and urine) only provide information on recent (a few days) intakes. Alternatively, nonconventional matrix (meconium, placenta, umbilical cord, or breast milk) analyses are more comprehensive as they cover longer pregnancy and breastfeeding periods[33]. However, limited data exist on drug concentration in these matrices, making their interpretation challenging. In this article, we reviewed the cannabis and synthetic cannabinoid biomarker concentrations in unconventional matrices, to document their consumption during pregnancy and breastfeeding.

METHODS

A literature search was performed on multidisciplinary research databases, such as PubMed, Scopus, and Web of Science, to identify the literature on perinatal exposure to cannabis and synthetic cannabinoids. The scientific literature from 1998 on cannabis monitoring during pregnancy was reviewed up until April 2019. A combination of the search terms cannabis, cannabinoid, THC, synthetic cannabinoid, pregnancy, in utero, fetal, breastfeeding, neonatal, meconium, umbilical, amniotic, milk, and hair was used. Further studies were retrieved from the reference list of selected articles and reports from international institutions such as the World Health Organization (WHO), US Drug Enforcement Administration, and European Monitoring Centre for Drugs and Drug Addiction. Only articles/reports written in English were selected. All articles were screened independently by 3 authors to determine their relevance in the framework of the current review.

CANNABIS

Risks Associated With Cannabis Use During Pregnancy

THC, CBD, and CBN are lipophilic compounds that readily cross the blood/placenta and blood/breast milk barriers, exposing the fetus or offspring to cannabinoids when cannabis is used during pregnancy or breastfeeding[34–54]. Preclinical and clinical studies have shown that THC concentration in placenta, cord blood, and fetal tissue is lower than that in maternal plasma at the same collection time, suggesting that the fetus has a lower level of exposure than the mother[35,55]. However, cannabinoids can alter endocannabinoid signaling involved in immune regulation, which is important during pregnancy and fundamental gestational events such as decidualization, embryo implantation, and fetal development[56].

Perinatal cannabis exposure has multiple effects that have been addressed in recent review articles[57–62]. Several studies reported an association between in utero cannabis exposure and fetal growth restriction (lower birth weight, height, and head circumference) and a higher risk of perinatal mortality[57–62]. In utero cannabis exposure is reportedly associated with neurodevelopmental impairments, which leads to long-lasting cognitive function effects (deficits in memory, verbal and perceptual skills, reasoning, executive functioning, and reading, and spelling) and behavior (hyperactivity, impulsivity, and aggressiveness). However, because many confounding factors are involved and several studies reached contradictory conclusions, further research on the effects of perinatal cannabis exposure on fetal outcomes and fetal and neonatal neurodevelopment is required[57–61]. The effects of cannabis exposure through breastfeeding are also unclear and have conflicting data[60]. One study observed an association between cannabis exposure during breastfeeding and decreased motor developmental at 1 year, although these results may be confounded by cannabis use during pregnancy. Conversely, another study observed no differences in motor and mental skills at 1 year, after cannabis exposure during breastfeeding[60]. Dong et al[62] reviewed the preclinical and clinical studies on the effects of cannabis exposure on immune regulation. In these studies, perinatal THC exposure was shown to induce long-lasting effects on the immune system of mice pups (atrophy of thymic and splenic tissues and alteration of T-cell populations, through CB1 and CB2 receptor activation). In humans, the effects of perinatal cannabis exposure on the immune system are poorly understood; an increased rate of mutant lymphocytes was observed in cannabis-using mothers and their newborns, and cannabis use during pregnancy may increase the risks of neuroblastoma and acute nonlymphoblastic leukemia in offspring[62]. In the same review, Dong et al[62] reported studies on cannabis exposure-induced epigenetic modifications and long-term effects.

We hereby report the most recent studies on the effects of perinatal cannabis exposure and their mechanisms, to augment available knowledge.

Adverse Obstetrical Outcomes

Recent studies support the theory that perinatal cannabis exposure can induce adverse obstetrical effects. In 2019, Petrangelo et al[63] conducted a retrospective database study on approximately 12.5 million births in the United States, examining the risks of miscarriage and preterm births attributable to in utero cannabis exposure. After adjustment for confounding factors such as maternal age, ethnicity, socioeconomic factors (income, insurance type, and hospital location), preexisting comorbidity (hypertension and diabetes mellitus), tobacco use, alcohol use, and other drug use, women with cannabis use during pregnancy had a 50%, 46%, 40%, 35%, 24%, and 18% increased risk of stillbirth, preterm premature rupture of membranes, preterm birth, growth restriction, placenta previa, and intra-amniotic infection, respectively. However, the risks of hemorrhage, venous thromboembolism, congenital malformation, or death after delivery did not differ significantly[63]. In another retrospective study on 2173 births in the United States, Howard et al[64] found an association (solely based on urine drug tests during prenatal care and delivery) between cannabis use during pregnancy and reduced birth weight (22.6% weight difference after adjustment). The authors also reported a 4.2-fold increase in the risk of perinatal mortality but observed no significant difference (P . 0.05) in gestational age at birth.

Other studies sought to understand the obstetrical outcome discrepancy observed in previously published articles. Baer et al[65] examined more closely the risk of premature birth, making the

distinction between preterm birth (before the 36th week of gestation) and early-term birth (between the 37th and 38th week of gestation). In a retrospective database study on approximately 3 million births in California, United States, the authors observed that cannabis use during pregnancy was associated with a 50% increased risk of preterm birth, owing to a 60% increased risk of premature membrane rupture and a 70% increased risk of spontaneous labor, after adjusting for confounders (maternal age, ethnicity, health coverage, education, pregnancy body mass index [BMI], hypertension, diabetes, mental illness, previous preterm births, tobacco use, and alcohol use). Interestingly, there was no significant difference (P . 0.05) in the risk of early birth, although the risk of premature membrane rupture was slightly higher (+10%) with cannabis use during pregnancy, suggesting that previous studies on cannabis and premature births might have been impacted by the gestational age at birth[65]. In 2019, Luka et al[66] examined the records of 243,140 births in Canada and observed a 47% increased risk of growth restriction and 27% increased risk of preterm birth after prenatal cannabis exposure, after adjusting for confounding factors (age, ethnicity, socioeconomic status, pregnancy BMI, tobacco use, alcohol use, and other substance use). More importantly, they found that prenatal cannabis exposure was associated with a 184% increased risk of intrapartum stillbirth (before birth, but after labor onset), although the risks of antepartum stillbirth (before labor onset) and overall stillbirth were not significantly different (P . 0.05), suggesting that the onset of stillbirth could be used for cannabis obstetrical outcome studies. In previous studies, the authors commented that smoking tobacco during pregnancy was also associated with a higher risk of intrapartum stillbirth, which may indicate a similar etiology (fetal distress due to oxygen deprivation or obstructed labor). They hypothesized that growth restriction may be attributable to fetal oxygen deprivation and reduced placenta blood supply, possibly through decreased insulin secretion, which plays a critical role in fetal and placental growth regulation[66]. Maia et al[67] hypothesized that alterations in normal placental development and fetal growth may be attributable to pregnancy-induced dysregulation of endocannabinoid system homeostasis. They studied the effects of 10–40 mM of THC (representing “heavy cannabis consumption”) on the

placental endocannabinoid system in villous explant incubations from human term placenta (n = 12). They observed that endocannabinoid anandamide concentration was significantly increased (P . 0.01) by 40 mM of THC. The expression of N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), involved in anandamide synthesis, increased, whereas that of fatty acid amide hydrolase, involved in anandamide degradation, decreased at all THC concentrations; moreover, CB1 and CB2 receptor expression was unaltered[67]. Low fatty acid amide hydrolase and high anandamide levels were initially associated with lower rates of embryo implantation, impaired decidualization, and higher risks of spontaneous miscarriage[56,68]. Recently, Ashford et al[69] examined the effects of cannabis and tobacco coexposure on the immune response of 138 women in the United States, during their first trimester of pregnancy. They observed decreased proinflammatory immune responses, as reflected by decreased tumor necrosis factor-a (TNF-a) levels, which may affect fetal outcomes. TNF-a levels were 14% lower in cousers, compared with those in pregnant women using tobacco only, after adjusting for confounding variables (age, ethnicity, pregnancy BMI, and other illicit drug use). No significant differences (P , 0.05) were observed in the levels of other inflammatory markers [interleukin-1b (IL-1b), IL-2, IL-6, IL-8, and IL-10, C-reactive proteins, and matrix metalloproteinase-8 (MMP-8)]. In the absence of a cannabis-only group, it was impossible to conclude if the observed inflammatory effects resulted from cannabis only or a synergistic action with tobacco[69].

Neurobehavioral Effects

More data exist on the relationship between perinatal cannabis exposure and long-term cognitive effects. In 2018, Ruisch et al[70] conducted a meta-analysis of the 3 available studies on the behavioral effects of cannabis exposure during pregnancy. The authors found no significant risks of conduct disorder problems after adjusting for confounding factors, although gestational trimester-dependent subeffects were reported. Considering the scarcity and conflicting nature of data, they suggested further research on the bias that may be induced by genetic or epigenetic factors and comorbid externalizing behaviors (attention-deficit/hyperactivity disorder and oppositional-defiant disorder)[70]. In a clinical study involving questionnaires and maternal urine testing on 5903 children aged 7–10 years,

El Marroun et al[71] observed an association between cannabis use during pregnancy and externalized childhood problems (aggressive and rule-breaking behavior) but not with internalized ones (anxiety, depression, withdrawal, and somatic complaints), after adjusting for confounding factors (age, ethnicity, educational level, pregnancy BMI, and alcohol use). However, they observed that externalized problems were equally associated with paternal and maternal cannabis use before pregnancy, which may indicate that behavioral issues are not related to perinatal drug exposure, but other confounding factors such as a genetic/ epigenetic vulnerability, psychiatric parental issues, or parental behavior. Regarding the effects of perinatal cannabis exposure on sociability, Bara et al[72] observed that daily subcutaneous administration of 5-mg/kg THC to pregnant rats (moderate cannabis exposure in humans) reduced social interaction and altered neuronal excitability and synaptic plasticity of prefrontal cortex neurons (a brain region implicated in neuropsychiatric disorders) in male offspring. Interestingly, females were unaffected, suggesting a sex-dependent effect of in utero cannabis exposure. Locomotion, anxiety, and cognition were equally unaffected in both sexes. Neuronal excitability and synaptic plasticity of the nucleus accumbens (another brain region involved in neuropsychiatric diseases) were not affected[72]. In 2018, Fransquet et al73 investigated the addiction vulnerability of 804 newborns with prenatal cannabis exposure but found no evidence of an epigenetic process (gene promoter of dopamine receptor DRD4) that could affect dopamine reward signaling.

Contamination

The contamination of cannabis plants with pesticides raises another concern. Studies showed that postharvest cannabis and manufactured cannabis products could be contaminated by organophosphates, which equally target the endocannabinoid system[74]. Leung et al[74] investigated the theoretical risks of perinatal exposure to chlorpyrifos-contaminated (an organophosphate pesticide) cannabis and proposed an adverse outcome pathway at the molecular, cellular, and tissue levels resulting in developmental neurotoxicity (long-term memory and learning process impairment).

Monitoring Perinatal Cannabis Exposure

CBD, CBN, THC, 8-OH-THC, 11-OH-THC,

8,11-diOH-THC, THCCOOH, and THCCOOH-glucuronide were used as biomarkers for perinatal cannabis exposure (Table 1)[34–54]. Cannabinoids were detected in meconium, umbilical cord, and placenta for the documentation of prenatal cannabis exposure and in breast milk for neonatal exposure; fetal and maternal hair testing allowed for monitoring of both prenatal and neonatal exposure.

Meconium Testing

Meconium is the first stool of a newborn, which starts forming at the 12th week of gestation. Meconium testing potentially provides information on drug exposure over the last trimester of pregnancy and the first few days after delivery. For this reason, the detection of cannabinoids in meconium became the most common method for documenting in utero cannabis exposure[39,41,44–46,48,49,51]. Immunoassay screenings and gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) confirmatory methods were used for meconium cannabinoid analyses. Given the volatile and lipophilic nature of cannabi- noids, GC-MS has been the gold standard for quantification, using phenyl-, methyl-, and dimethylpolysiloxane columns[39,44,46,48,49,51]. However, the emergence of sensitive LC-MS/MS technologies requiring less time-consuming sample preparations (no derivatization) is now common (C18 and pentafluorophenylpropyl columns)[41,45]. This is also true for the analyses of other matrices[34–38,40,42,43,47,50,54]. Early methods for quantifying meconium cannabinoids used enzyme hydrolysis (b-glucuronidase) to cleave cannabinoid-glucuronide conjugates[46,48,49]. However, in 2010, Gray et al[51] showed that this type of hydrolysis is critical for the detection of meconium cannabinoids, as it significantly impacts the detection capability of THC metabolites. In this study, b-glucuronidase failed

to efficiently hydrolyze THCCOOH-glucuronide, and potassium hydroxide base hydrolysis provided better results. Conversely, 11-OH-THC-glucuronide b-glucuronidase hydrolysis was more efficient than base hydrolysis. Overall, tandem base-b- glucuronidase hydrolysis proved to be the most suitable treatment for meconium samples for the extensive cleavage of cannabinoid-glucuronide conjugates. In tandem base-b-glucuronidase hydrolysis conditions, THCCOOH was the major THC metabolite (median 107.3 ng/g, for 13.3–546 ng/g, n = 40), followed by 11-OH-THC (median 26.0 ng/g, for 15.0–106 ng/g, n = 23) and 8,11-diOH-THC (median 13.2 ng/g, for 10.7–45.8 ng/g, n = 13); THC was not detected. In b-glucuronidase hydrolysis conditions, THCCOOH and 11-OH-THC had similar concentrations (THCCOOH: median 28.9 ng/g, for 10.6–223 ng/g, n = 24; 11-OH-THC: median 28.5 ng/g, for 15.1–118 ng/g, n = 24), followed by 8,11-diOH-THC (median 14.9 ng/g, for 11.7–47.8 ng/g, n = 9),51 confirming previously reported ranges and metabolite ratios measured in meconium under similar analytical conditions[46,48,49]. THCCOOH was positively identified in all meconium samples in the study by Gray et al[51], negating the need to analyze other cannabinoid analytes. Prego-Meleiro et al[41] and Kim et al[45] quantified THCCOOH-glucuronide in meconium samples and further confirmed the extensive glucuronidation of THCCOOH. The most recent studies involving meconium cannabinoid detection used hydrolysis under basic conditions to improve THCCOOH detection capability[39,44]. CBD and CBN were also proposed as meconium prenatal cannabis exposure markers, although with generally lower concentrations than THCCOOH[41,45,51].

TABLE 1. Concentrations of Cannabinoids and Metabolites in Nonconventional Biological Matrices to Monitor Cannabis Exposure During Pregnancy

Study	Drugs and Metabolites	Matrix (n = nb. Samples)	Concentration	Analytical Procedure	Reference
Cannabis					
Case report nursing woman with daily cannabis use	THC	Breast milk	105 ng/mL	Gas–liquid chromatography-MS (no detail)	Perez-Reyes and Wall[54]
Case report nursing woman with daily cannabis use, 7X/d; samples collected 8 mo after delivery X2 visits (2nd 1 h after last use)	THC	Breast milk (1st visit)	340 ng/mL	Gas–liquid chromatography-MS (no detail)	Perez-Reyes and Wall[54]
		Breast milk (2nd)	60.3 ng/mL		
		Maternal plasma (2nd)	7.2 ng/mL		
		Neonatal feces (2nd)	347 ng		
	11-OH-THC	Breast milk (1st)	4.0 ng/mL		
		Breast milk (2nd)	1.1 ng/mL		
		Maternal plasma (2nd)	2.5 ng/mL		
		Neonatal feces (2nd)	67 ng		
	THCCOOH	Breast milk (2nd)	1.6 ng/mL		
		Maternal plasma (2nd)	19.0 ng/mL		
		Neonatal feces (2nd)	611 ng		
Survey 10 self-reported pregnant "heavy" cannabis users during 3rd trimester from 2 Denver public hospitals, United States	THC	Maternal blood (n = 5)	Median 1.0 ng/mL (0.4–6.0)	GC-MS analysis (no detail)	Blackard and Tennes[55]
		Cord blood (n = 3)	Median 0.3 ng/mL (0.3–1.0)		
	THCCOOH	Maternal blood (n = 10)	Median 16.0 ng/mL (2.3–125)		
		Cord blood (n = 10)	Median 2.9 ng/mL (0.4–18.0)		
Newborn meconium after prenatal cannabis exposure (n = 16)	THC	Meconium (n = 3)	Median 3.0 ng/g (2.4–4.0)	MeOH homogenization; LLE with potassium phosphate monobasic and chloroform; b-glucuronidase hydrolysis; LLE with HCl and Hex:EtOAc and further LLE of both phases; BSTFA derivatization of acidic fraction; SIM GC-MS (5%-phenyl-methylpolysiloxane column)	ElSohly and Feng[46]
	8-OH-THC	Meconium (n = 1)	16.7 ng/g		
	11-OH-THC	Meconium (n = 16)	Median 57.0 ng/g (7.0–29.0)		
	8,11-diOH-THC	Meconium (n = 13)	Median 14.9 ng/g (2.8–68.6)		
	THCCOOH	Meconium (n = 14)	Median 9.5 ng/g (2.3–259)		
Newborn meconium after prenatal cannabis exposure (n = 24)	11-OH-THC	Meconium (n = 18)	Median 6.4 ng/g (1.2–48.1)	MeOH homogenization; protein precipitation with IPA; b-glucuronidase hydrolysis; immunoaffinity chromatographic extraction; BSTFA derivatization of acidic fraction; SIM GC-MS (5%-phenyl-methylpolysiloxane column)	Feng et al[48]
	8,11-diOH-THC	Meconium (n = 3)	Median 3.6 ng/g (2.4–8.9)		
	THCCOOH	Meconium (n = 15)	Median 3.8 ng/g (1.0–30.2)		
Premature dizygotic twins with prenatal cannabis exposure (delivery after 28 wk)	Cannabinoids	Meconium (1st twin)	1.2 ng/mg	Immunoassay (no detail)	Boskovic et al[52,53]
		Meconium (2nd twin)	0.05 ng/mg		
Study on a pair of premature dizygotic twins with prenatal exposure to cannabis in the United States (delivery after 34 weeks)	Cannabinoids	Fetal hair (1st twin)	1.9 ng/mg	Immunoassay (no detail)	Boskovic et al[52,53]
		Fetal hair (2nd twin)	0.2 ng/mg		
Clinical survey 1151 mother–newborn dyads (n = 300 samples)	THC	Meconium (n = 5)	Median 41.8 ng/g (25.6–81.2)	b-Glucuronidase hydrolysis; protein precipitation with MeOH, LLE with KOH and Hex:EtOAc and further LLE of both phases; BSTFA derivatization; SIM GC-MS (5%-phenyl-methylpolysiloxane column)	Marchei et al[49]
	11-OH-THC	Meconium (n = 23)	Median 67.6 ng/g (20.7–493)		
	THCCOOH	Meconium (n = 24)	Median 65.7 ng/g (33.2–182)		
Placenta (n = 64) from women after induced abortion in 1st trimester	THCCOOH	Placenta (n = 1)	123 ng/g	PCA homogenization; SPE on reverse phase, strong cation exchange; TMS derivatization; SIM GC-MS (5%-phenyl-methylpolysiloxane column)	Jova et al[50]

TABLE 1. (*Continued*) Concentrations of Cannabinoids and Metabolites in Nonconventional Biological Matrices to Monitor Cannabis Exposure During Pregnancy

Study	Drugs and Metabolites	Matrix (n = nb. Samples)	Concentration	Analytical Procedure	Reference
Meconium (n = 19)	THC	Meconium (n = 4)	Median 6.5 ng/g (4.2–7.7)	MeOH homogenization; SPE on nonpolar, cation exchange; LC gradient on C_{18} (0.1% FA in water, ACN); MS/MS analysis in MRM in POS mode	Prego-Meleiro et al[41]
	11-OH-THC	Meconium (n = 1)	11.9 ng/g		
	8,11-diOH-THC	Meconium (n = 4)	Median 145 ng/g (53.2–332)		
	THCCOOH	Meconium (n = 4)	Median 49.4 ng/g (24.1–289)		
	CBD	Meconium (n = 4)	Median 94.3 ng/g (7.1–252)		
	CBN	Meconium (n = 4)	Median 81.4 ng/g (30.7–93.3)		
	THCCOOH-gluc	Meconium (n = 3)	Median 291 ng/g (190–307)		
Meconium and maternal hair 513 mother–newborn dyads	THC	Maternal hair (n = 5) (12 hair segments)	Median 0.41 ng/mg (0.17–1.2)	DCM and MeOH wash; acidic buffer digestion; dilute in water; LC-MS/MS (no detail)	Cortès et al[42]
	CBD	Maternal hair (n = 4) (8 hair segments)	Median 0.98 ng/mg (0.46–1.8)		
	CBN	Maternal hair (n = 5) (8 hair segments)	Median 0.16 ng/mg (0.05–0.33)		
Umbilical cord (n = 44) after prenatal cannabis exposure	THC	Umbilical cord (n = 11)	0.2–1.3 ng/g	MeOH homogenization; base hydrolysis; SPE on nonpolar, strong anion exchange; LC gradient on C_{18} (5-mM ammonium bicarbonate, MeOH); MS/MS analysis in MRM in NEG mode (dual ionization source)	Wu et al[43]
	11-OH-THC	Umbilical cord (n = 10)	0.2–3.1 ng/g		
	THCCOOH	Umbilical cord (n = 30)	0.2–20.9 ng/g		
Meconium (n = 6) after prenatal cannabis exposure	THCCOOH	Meconium (n = 4)	11.7–64.5 ng/g	Accelerated solvent extraction with base hydrolysis and SPE on C8, strong anion exchange; MTBSTFA derivatization; SIM GC-MS ((5%-phenyl)-methylpolysiloxane)	Mantovani, et al[44]
Meconium and umbilical cord (n = 13) after prenatal cannabis exposure	THC	Meconium (n = 3)	Median 5.6 ng/g (3–15.6)	Meconium: MeOH homogenization; SPE on cation exchange; LC gradient on C_{18} (0.1% FA in water, ACN); MS/MS analysis in MRM in POS mode	Kim et al[45]
	11-OH-THC	Meconium (n = 2)	3.7 and 164 ng/g	Umbilical cord: wash with water; MeOH homogenization; SPE on strong cation exchange; LC gradient on pentafluorophenylpropyl (0.1% FA in water, 0.1% FA in ACN); MS/MS analysis in MRM in POS mode (dual ionization source)	
	8,11-diOH-THC	Meconium (n = 11)	Median 47.6 ng/g (5.4–887)		
	THCCOOH	Meconium (n = 12)	Median 17.8 ng/g (3.9–118)		
	THCCOOH-gluc	Meconium (n = 4)	Median 89.6 ng/g (19.4–190)		
		Umbilical cord (n = 12)	Median 4.7 ng/g (1.6–19.1)		
	CBD	Meconium (n = 9)	Median 31.4 ng/g (9.5–335)		
Survey n = 50 nursing women, ≤ 6 days after last cannabis use (n = 54 samples)	THC	Breast milk (n = 34)	Median 9.5 ng/mL (1.0–323)	Saponification and SPE on C_{18}; LC gradient on C_{18} (0.05% FA in 5-mM ammonium formate, 0.1% FA in ACN); MS/MS analysis in MRM in POS and NEG mode	Bertrand et al[47]
	11-OH-THC	Breast milk (n = 5)	Median 2.4 ng/mL (1.3–12.8)		
	CBD	Breast milk (n = 5)	Median 5.0 ng/mL (1.3–8.6)		

11-OH-THC, 11-hydroxy-THC; 8,11-diOH-THC, 8,11-dihydroxy-THC; 8-OH-THC, 8-hydroxy-THC; ACN, acetonitrile; BSTFA, N,O-bis (trimethylsilyl) trifluoroacetamide; CBD, cannabidiol; CBN, cannabinol; DCM, dichloromethane; Et2O, diethyl ether; EtOAc, ethyl acetate; FA, formic acid; Hex, hexane; HRMS, high-resolution MS; HS, head space; IPA, isopropanol; LLE, liquid–liquid extraction; MeOH, methanol; MRM, multiple reaction monitoring; MTBSTFA, N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide; NEG, negative; PBS, phosphate buffer; PCA, perchloric acid; POS, positive; SIM, single ion monitoring; SPE, solid phase extraction; SPME, solid phase microextraction; THC, D9-tetrahydrocannabinol; THCCOOH, 11-nor-9-carboxy-THC; THCCOOH-gluc, 11-nor-9-carboxy-THC-glucuronide; TMCS, trimethylchlorosilane; TMS, trimethylsilyl.

TABLE 1. (*Continued*) Concentrations of Cannabinoids and Metabolites in Nonconventional Biological Matrices to Monitor Cannabis Exposure During Pregnancy

Study	Drugs and Metabolites	Matrix (n = nb. Samples)	Concentration	Analytical Procedure	Reference
Meconium (n = 19)	THC	Meconium (n = 4)	Median 6.5 ng/g (4.2–7.7)	MeOH homogenization; SPE on nonpolar, cation exchange; LC gradient on C_{18} (0.1% FA in water, ACN); MS/MS analysis in MRM in POS mode	Prego-Meleiro et al[41]
	11-OH-THC	Meconium (n = 1)	11.9 ng/g		
	8,11-diOH-THC	Meconium (n = 4)	Median 145 ng/g (53.2–332)		
	THCCOOH	Meconium (n = 4)	Median 49.4 ng/g (24.1–289)		
	CBD	Meconium (n = 4)	Median 94.3 ng/g (7.1–252)		
	CBN	Meconium (n = 4)	Median 81.4 ng/g (30.7–93.3)		
	THCCOOH-gluc	Meconium (n = 3)	Median 291 ng/g (190–307)		
Meconium and maternal hair 513 mother–newborn dyads	THC	Maternal hair (n = 5) (12 hair segments)	Median 0.41 ng/mg (0.17–1.2)	DCM and MeOH wash; acidic buffer digestion; dilute in water; LC-MS/MS (no detail)	Cortés et al[42]
	CBD	Maternal hair (n = 4) (8 hair segments)	Median 0.98 ng/mg (0.46–1.8)		
	CBN	Maternal hair (n = 5) (8 hair segments)	Median 0.16 ng/mg (0.05–0.33)		
Umbilical cord (n = 44) after prenatal cannabis exposure	THC	Umbilical cord (n = 11)	0.2–1.3 ng/g	MeOH homogenization; base hydrolysis; SPE on nonpolar, strong anion exchange; LC gradient on C_{18} (5-mM ammonium bicarbonate, MeOH); MS/MS analysis in MRM in NEG mode (dual ionization source)	Wu et al[43]
	11-OH-THC	Umbilical cord (n = 10)	0.2–3.1 ng/g		
	THCCOOH	Umbilical cord (n = 30)	0.2–20.9 ng/g		
Meconium (n = 6) after prenatal cannabis exposure	THCCOOH	Meconium (n = 4)	11.7–64.5 ng/g	Accelerated solvent extraction with base hydrolysis and SPE on C8, strong anion exchange; MTBSTFA derivatization; SIM GC-MS ((5%-phenyl)-methylpolysiloxane)	Mantovani, et al[44]
Meconium and umbilical cord (n = 13) after prenatal cannabis exposure	THC	Meconium (n = 3)	Median 5.6 ng/g (3–15.6)	Meconium: MeOH homogenization; SPE on cation exchange; LC gradient on C_{18} (0.1% FA in water, ACN); MS/MS analysis in MRM in POS mode	Kim et al[45]
	11-OH-THC	Meconium (n = 2)	3.7 and 164 ng/g	Umbilical cord: wash with water; MeOH homogenization; SPE on strong cation exchange; LC gradient on pentafluorophenylpropyl (0.1% FA in water, 0.1% FA in ACN); MS/MS analysis in MRM in POS mode (dual ionization source)	
	8,11-diOH-THC	Meconium (n = 11)	Median 47.6 ng/g (5.4–887)		
	THCCOOH	Meconium (n = 12)	Median 17.8 ng/g (3.9–118)		
	THCCOOH-gluc	Meconium (n = 4)	Median 89.6 ng/g (19.4–190)		
		Umbilical cord (n = 12)	Median 4.7 ng/g (1.6–19.1)		
	CBD	Meconium (n = 9)	Median 31.4 ng/g (9.5–335)		
Survey n = 50 nursing women, ≤ 6 days after last cannabis use (n = 54 samples)	THC	Breast milk (n = 34)	Median 9.5 ng/mL (1.0–323)	Saponification and SPE on C_{18}; LC gradient on C_{18} (0.05% FA in 5-mM ammonium formate, 0.1% FA in ACN); MS/MS analysis in MRM in POS and NEG mode	Bertrand et al[47]
	11-OH-THC	Breast milk (n = 5)	Median 2.4 ng/mL (1.3–12.8)		
	CBD	Breast milk (n = 5)	Median 5.0 ng/mL (1.3–8.6)		

11-OH-THC, 11-hydroxy-THC; 8,11-diOH-THC, 8,11-dihydroxy-THC; 8-OH-THC, 8-hydroxy-THC; ACN, acetonitrile; BSTFA, N,O-bis (trimethylsilyl) trifluoroacetamide; CBD, cannabidiol; CBN, cannabinol; DCM, dichloromethane; Et2O, diethyl ether; EtOAc, ethyl acetate; FA, formic acid; Hex, hexane; HRMS, high-resolution MS; HS, head space; IPA, isopropanol; LLE, liquid–liquid extraction; MeOH, methanol; MRM, multiple reaction monitoring; MTBSTFA, N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide; NEG, negative; PBS, phosphate buffer; PCA, perchloric acid; POS, positive; SIM, single ion monitoring; SPE, solid phase extraction; SPME, solid phase microextraction; THC, D9-tetrahydrocannabinol; THCCOOH, 11-nor-9-carboxy-THC; THCCOOH-gluc, 11-nor-9-carboxy-THC-glucuronide; TMCS, trimethylchlorosilane; TMS, trimethylsilyl.

Umbilical Cord and Placenta Testing

The umbilical cord and placenta also provide information on recent prenatal cannabis exposure. Similar to meconium, their sampling is noninvasive, and the samples are large and can be used for multiple drug tests. Moreover, potential interferences with medications given to the newborn before meconium collection are avoided[75]. However, information on umbilical cord and placenta cannabinoid concentration is limited.

In 1984, Blackard and Tennes reported the postdelivery THC and THCCOOH umbilical cord blood concentrations of women with "heavy" cannabis use during the third trimester of pregnancy. THCCOOH median concentration (2.9 ng/mL, for 0.4–18.0 ng/mL, n = 10) was higher than that of THC (0.3 ng/mL, for 0.3–1.0 ng/mL, n = 3), and maternal blood concentrations were higher than that of cord blood (THCCOOH: median 16.0 ng/mL, for 2.3–125 ng/mL, n = 10; THC: median 1.0 ng/mL, for 0.4–6.0 ng/mL, n = 5), suggesting that fetal exposure may be limited to some extent. THC transfer was higher in early pregnancy[35]. More recently, cannabinoids were quantified in umbilical cord samples.38,43,45 In 2018, Wu et al quantified THC, 11-OH-THC, and THCCOOH in

44 samples from perinatal cannabis exposure cases. After base hydrolysis, THCCOOH was the major THC metabolite (0.2–20.9 ng/g, n = 30), followed by 11-OH-THC (0.2–3.1 ng/g, n = 10) and THC (0.2–1.3 ng/g, n = 11).43 In the same year, Kim et al quantified THCCOOH-glucuronide in umbilical cord samples from 13 newborns exposed to cannabis during pregnancy. THCCOOH-glucuronide was detected in 12 cord samples (median 4.7 ng/g, for 1.6–19.1 ng/g), and only 4 meconium samples (median 89.6 ng/g, for 19.4–190 ng/g), probably attributable to the different sensitivities between the 2 analytical methods (limits of quantification were 1 and 10 ng/g, respectively); THC, 11-OH-THC, 8,11-diOH-THC, THCCOOH, THC-glucuronide, and CBD were only detected in meconium and not cord samples. There was a good agreement between the umbilical cord and meconium (92.3% match), suggesting that the umbilical cord may be a good matrix to evaluate in utero cannabis exposure. However, analytical methods with higher sensitivity may be required[45].

Investigations on placenta cannabinoid disposition started in the late 1960s, after acute intravenous, intraperitoneal, or subcutaneous administration of THC to animal models (rodents and dogs). Studies showed that THC concentration was higher in the placenta than in fetal tissue but lower than that in maternal plasma, suggesting that the fetus is less exposed to cannabinoids compared with the mother[55]. In 2010, Joya et al[50] reported the first human placenta cannabinoid concentration after induced abortion in the first trimester of pregnancy. THCCOOH concentration was 123 ng/g; THC was not quantified. Two years later, Falcon et al[36] quantified THC in the placenta of 10 of 280 fetuses after induced abortion in the first trimester of pregnancy, although cannabis consumption was documented in 60 cases through maternal hair testing. THC mean concentration (6SD) was 197 6 110 ng/g; metabolites were not quantified. As observed in the animal models, THC concentration was lower in fetal tissue (119 6 110 ng/g, n = 9) than in the placenta. No correlation was found between placental and fetal concentrations.36 Thus, the umbilical cord and placenta are promising matrices to evaluate perinatal cannabis exposure, but more data comparing umbilical cord, placenta, and meconium cannabinoid concentrations, with and without hydrolysis, are required.

Breast Milk Testing

Breast milk was investigated to document neonatal cannabinoid exposure in nursing mother[34,40,47,54]. In 1982, Perez-Reyes and Wall34 reported the first cannabinoid concentrations in human breast milk in 2 frequent cannabis users, 7 and 8 months after delivery. The breast milk THC concentration in the first patient was 105 ng/mL, whereas THC and 11- OH-THC concentrations were 340 and 4.0 ng/mL, respectively, in the second patient. The second patient underwent a second visit 1 hour after her last cannabis use, where her breast milk THC, 11-OH-THC, and THCCOOH concentrations were 60.2, 1.1, and 1.6 ng/mL, respectively, whereas simultaneously collected maternal plasma concentrations were 7.2, 2.5, and 19.0 ng/mL, respectively, confirming recent use. Interestingly, breast milk cannabinoid concentrations were 8 times higher than that of maternal plasma, suggesting that breast milk may be a suitable matrix for monitoring neonatal cannabis exposure. However, 11-OH-THC and THCCOOH

concentrations were lower in breast milk, probably attributable to their lower lipophilicity[34]. It is important to note that the presumption that THC concentrates in breast milk is based solely on 1 maternal plasma sample: breast milk pair. Additional data on the relationship of THC in breast milk and maternal plasma are urgently needed in the new world of medicinal and recreational cannabis. Recently, Bertrand et al measured cannabinoids in the breast milk of 50 nursing mothers 6 days after their last reported cannabis use. THC and 11- OH-THC were detected in 34 (median: 9.5 ng/mL, for 1.0–323 ng/mL) and 5 (median: 2.4 ng/mL, for 1.3–12.8 ng/mL) of 54 samples, respectively; 20 and 49 samples were negative for THC and 11-OH-THC, respectively (,1 ng/mL), which may indicate that both cannabinoids are rapidly eliminated from breast milk and more sensitive detection methods are required.47 CBD was equally used as a cannabis neonatal exposure breast milk biomarker, although the THC:CBD ratio is cannabis source-dependent[40,47].

Hair Testing

Xenobiotics are incorporated from the bloodstream into hair through their roots and carried out of the skin through hair growth. Hair testing can therefore theoretically provide an overview of the history of maternal cannabis exposure during pregnancy and after delivery. However, THC and, in particular, THCCOOH (the main metabolite) are not properly incorporated into hair, resulting in low concentrations. Moreover, external contamination, through cannabis smoke, cosmetic treatments, and ethnicity, greatly affects hair cannabinoid concentrations, making the interpretation of result challenging. THCCOOH detection in hair clearly confirms cannabis consumption and, therefore, is the analyte of choice for identifying cannabinoids in hair[76]. However, highly sensitive methods are required for this identification. Although controversial, the Society of Hair Testing recommends a 50-pg/mg THC cutoff for hair screening methods used to identify cannabis consumption and a cutoff of 50 and 0.2 pg/mg THC and THCCOOH, respectively, for confirmation screens, with THCCOOH detection only used when proving active use[77]. The interpretation for maternal hair cannabinoid concentration during pregnancy is similar to that of nonpregnant individuals. Falcon et al36 quantified cannabinoids in 60 women with induced abortion in the first trimester of pregnancy. Mean (6SD) THC and THCCOOH hair concentrations were 2.7 6 3.4 ng/mg (0.1–14.1 ng/mg) and

0.09 6 0.12 ng/mg (0.001–0.700 ng/mg), respectively. Cannabis consumption was confirmed in 10 cases by analyzing placenta and fetal remains.36 Lendoiro et al37 measured THC in 6 women who acknowledged cannabis consumption during pregnancy. The median concentration was 129 pg/mg (42.6– 197 pg/mg, n = 16 segments).

Although limited in quantity/concentration, fetal hair cannabinoids can equally be measured to document maternal cannabis use. In 2001, Boskovic et al measured cannabinoids in fetal hair of dizygotic twins who were exposed to cannabis during pregnancy, in the United States.52,53 Although the twins were theoretically exposed to the same quantity of cannabis, the cannabinoid concentration was 1917 pg/mg in

1 twin, but only traces (,200 pg/mg) were detectable in the other twin. The authors hypothesized a difference in metabolism between the twins, but the varying cannabinoid concentration can equally be attributed to vasculature and placenta differences.52,53

SYNTHETIC CANNABINOIDS

Animal Studies

Gilbert et al[78] determined the toxicity of CP-55,940, a synthetic cannabinoid, in mice and their offspring. Acute intraperitoneal administration of 0.0625–2.0 mg/kg CP- 55,940 on the 8th day of pregnancy induced dose-dependent teratogenicity, involving craniofacial, ocular, and brain abnormalities.

Human Studies

Few reports on synthetic cannabinoid exposure during pregnancy were reported in humans. In 2013, Berry-Cabán et al[79] reported the case of a pregnant woman with a history of synthetic cannabinoid and synthetic cathinone abuse. The patient had terminated her drug use at approximately the 10th week of gestation, and the baby was born without complications or health issues. In 2015, Oztürk80 reported another case of synthetic cannabinoid exposure during pregnancy. The pregnant woman was on escitalopram, quetiapine, venlafaxine, lamotrigine, and tobacco and regularly smoked synthetic cannabinoids for the 6 months preceding gestation. Her combination antidepressant therapy was replaced with quetiapine monotherapy after her pregnancy was discovered (5th week), but the patient was unable to terminate other drug use. Eventually, a healthy baby was born, void of obstetrical complications[80]. Despite the favorable outcome of these cases, perinatal morbidity and mortality related to synthetic cannabinoid use are highly expected considering their mechanism of action (disruption of endocannabinoid system homeostasis), potency, and toxicity in healthy individuals[81].

CONCLUSIONS

Drug use during pregnancy poses an important global health issue affecting maternal and neonatal outcomes. Cannabis is the most widely spread illicit drug consumed during gestation and is associated with the risk of adverse obstetrical outcomes and long-term behavioral effects. However, the symptomology of cannabis perinatal exposure is still unclear. The profile of cannabis users is often associated with additional adverse obstetrical complications and pediatric neuropsychiatric disorders such as a low socioeconomic status, psychiatric disorders, abnormal BMI, tobacco use, alcohol use, and other harmful substance abuse. Although most studies adjust for confounding factors in their models, these effects are difficult to isolate and may create an interpretation bias. More so, several studies are based on self-reports or administrative database exploration, which may result in further bias. Finally, further research is needed on genetic and epigenetic influences.

Although women generally tend to limit drug use during pregnancy, education efforts on cannabis adverse effects on pregnancy should be improved and pharmaceutical, psychological, and neurological support provided. Another way to limit perinatal cannabis exposure is the monitoring of maternal drug use during gestation. Drug testing in unconventional matrices (meconium, placenta, umbilical cord, and breast milk) is a good alternative to the conventional matrices (oral fluid and urine). However, considering the paucity of information available on drug concentration in nonconventional matrices, data interpretation may be challenging. For this reason, we reviewed the concentrations of cannabis biomarkers in unconventional matrices, to document consumption during pregnancy and breastfeeding.

The relatively recent emergence of NPS onto the drug market and the dynamic nature of this market limit our current knowledge of their toxicities. Data on perinatal toxicity due to chronic prenatal exposure to synthetic cannabinoids are virtually nonexistent in humans, although there is evidence (preclinical studies and toxicity in healthy individuals), suggesting that they may seriously affect pregnancy and neonatal outcomes. Considering the growing trend, synthetic cannabinoids may be increasingly used by pregnant women in the next few years, and research efforts should focus on their harmful effects on embryos/fetuses.

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