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Associations of First Trimester Co-Use of Tobacco and Cannabis with Prenatal Immune Response and Psychosocial Well-Being

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Title

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Abstract

Purpose. This study aims to describe the association of first trimester co-use of tobacco and cannabis with maternal immune response and psychosocial well-being, relative to tobacco use only.

Methods. A preliminary midpoint analysis included 138 pregnant women with biologically verified tobacco use, 38 of whom (28%) also tested positive for recent cannabis use. Maternal perceived stress (Perceived Stress Scale), depressive symptoms (Edinburgh Postnatal Depression Scale), and serum immune markers (IL-1β, IL-2, IL-6, IL-8, IL-10, TNFα, CRP, MMP8), were collected, although cytokine data were only available for 122 women.

Results. Participant average age was 29.1 years, approximately half had a high school education or less, and half were unemployed. Compared to tobacco only users, co-users were more likely to be non-White, younger and more economically disadvantaged. In the adjusted linear regression models, TNF-α levels were significantly lower among co-users relative to tobacco only users, after adjusting for age, race/ethnicity, body mass index and tobacco use group (tobacco cigarettes, electronic nicotine delivery devices [ENDS] or both). TNF-α was the only immune marker found to be significant in this analysis. Measured stress levels (M=5.9, SD=3.3; potential range 0-16) and depression scores (M=7.8, SD=5.8; potential range 0-30) were low across all participants and did not differ as a function of co-use.

Conclusion. Preliminary results suggest women co-using during the first trimester exhibit decreased pro-inflammatory immune responsivity on one out of eight markers. Further research is needed to determine the impact of this immune modulation on fetal health outcomes and the unique contribution of cannabis.

Key Words: marijuana; nicotine; cytokines; pregnancy; perceived stress

Introduction

Tobacco and cannabis are the two most common addictive substances used during pregnancy, and are often used concurrently. Nearly 90% of cannabis users are also tobacco smokers (Rabin & George, 2015), and there has been a recent rapid and disproportionate increase in daily cannabis use among female cigarette smokers compared to male smokers (Goodwin et al., 2018). This is of significant concern due to pregnant women being at increased risk of continued use for the duration of their pregnancy (El Marroun et al., 2008; Ko et al., 2015). United States (US) nation-wide survey data reflect that 20% of pregnant women co-use tobacco and cannabis, (Azofeifa, 2016), with those aged 18-25 years old being more likely to co-use tobacco and cannabis than cannabis alone (Coleman-Cowger, Schauer, & Peters, 2017). Yet, other recent studies using large databases from individual prenatal clinics indicate that the number of pregnant concurrent users of cannabis and tobacco is considerably higher (approaching 50%) (Chabarria et al., 2016; Mark, Desai, & Terplan, 2016).

The consequences of tobacco use during pregnancy have been studied extensively. Nicotine, the primary active constituent of tobacco, is a teratogen and classified as a pregnancy class D drug by the US Food and Drug Administration. Tobacco exposure during pregnancy is associated with numerous adverse physical and psychosocial health effects including, but not limited to, spontaneous preterm birth, small for gestational age infant, placenta previa, placenta abruption, impaired fetal lung and brain development and miscarriage (American College of Obstetricians and Gynecologists, 2017; Castles, Adams, Melvin, Kelsch, & Boulton, 1999; Centers for Disease Control and Prevention, 2018; Kharrazi et al., 2004; Warren, Albert, Kraft & Cummins, 2014). Other adverse health effects of prenatal tobacco exposure extend beyond birth and include increased risk for sudden infant death syndrome and numerous respiratory, metabolic, neurobiological and behavioral disorders (e.g. asthma, obesity and attention deficit hyperactivity disorder) (Langely,

Rice & Thapar, 2005; Maritz & Harding, 2011; Oken, Levitan & Gillman, 2008; Weg, Ward, Scarinci, Read, Evans, 2004; Wickstrom, 2007).

Prior work is also suggestive of interactions among prenatal tobacco use, immune dysregulation in the mother, and maternal depression and anxiety (Osborne & Monk, 2013; Coussons-Read, Okun & Nettles, 2007). High levels of maternal depression and anxiety symptoms are associated with shorter gestation, alterations in fetal neurodevelopment (Schetter & Tanner, 2012) and lower visuospatial working memory performance in the offspring (Buss, Davis, Hobel & Sandman, 2011).These maternal psychiatric symptoms are also often associated with immune dysregulation in pregnant women, commonly resulting in high circulating serum C-reactive protein (CRP) and proinflammatory cytokines (i.e., interleukin [IL]-6 and tumor necrosis factor [TNF]-α), and lower levels of the anti-inflammatory cytokine, IL-10 (Christian, Franco, Glaser, & Iams, 2009; Coussons-Read, Okun, Schmitt, & Giese, 2005). Further, nicotine directly affects the immune system. In an animal study by Nouri-Shirazi and Guinet (2013), nicotine significantly depressed antibody responses and T-cell proliferation. A study of microglial activation linked nicotine exposure to significantly decreased levels of pro-inflammatory cytokines including interleukin (IL) -6 and TNF-α (Jia et al., 2016). In pregnancy, first trimester tobacco use has been associated with maternal immune dysregulation. For example, significant anti-inflammatory reductions in cervical IL-10 were observed in women using tobacco early in pregnancy compared to nonsmokers whereas proinflammatory cytokines (IL-1α, 1β, 2, 4, 6, 8, TNFα) did not change. (Ashford, O'Brien, McCubbin, Westneat, & Barnett, 2013; Simhan, Caritis, Hillier, & Krohn, 2005). An examination of serum cytokines in the second and third trimesters of pregnancy revealed significantly higher concentrations of IL-6 and IL-1α among smokers compared to non-smokers (Ashford, Barnett, McCubbin, Kehler, Westneat, 2013). Maternal immune dysregulation is of concern because it has been linked to adverse perinatal outcomes including pre-eclampsia (Ashford et al., 2017) and preterm birth (Goldenberg, Culhane, Iams, & Romero, 2008; Simhan and Krohn, 2009).

Within the past two decades, the perceived risk of cannabis use has decreased (Berg et al., 2015; Sinclair, Foushee, Scarinci, & Carroll, 2013) and public acceptance of cannabis use has increased (Pew Research Center, 2018) in the United States. Perhaps unsurprisingly then, the percentage of national survey respondents reporting past-year cannabis use has also increased (United Nations Office on Drugs and Crime, 2017). Cannabis use in the first trimester of pregnancy has been reported as high as 7.4%, with 16% of users reporting daily cannabis use (Ko, Farr, Tong, Creanga, & Callaghan, 2015). Prenatal cannabis use may occur for various reasons including recreation and self-medication (Ko et al., 2015; Park, McPartland, & Glass, 2004; Wang, Dow-Edwards, Anderson, Minkoff, & Hurd, 2004). Among women who used cannabis during pregnancy, some endorsed its use as a means to treat nausea and vomiting (Westfall, Janssen, Lucas, & Capler, 2006). Although Δ^9- tetrahydrocannabinol (THC; the primary active constituent of cannabis) is FDA-approved as a treatment for nausea and vomiting associated with cancer chemotherapy, it has not been evaluated for hyperemesis gravidarum, a pregnancy complication resulting in severe nausea, vomiting and alteration in serum electrolytes.

The consequences of cannabis use during pregnancy are less clear compared to those of tobacco. Prior research has shown that THC crosses the placenta, although the levels are reduced compared to maternal concentrations (Grant, Petroff, Isoherranen, Stella, & Burbacher, 2017). Some studies have found adverse outcomes such as increased risk of preterm birth (Burns, Mattick, & Cooke, 2006), decreased infant head circumference, growth restriction and decreased birthweight (El Marroun et al., 2009; Fergusson, Horwood, & Northstone, 2002; Metz et al., 2017). However, other studies failed to find negative effects of maternal cannabis use on neonatal outcomes (Conner, Carter, Tuuli, Macones, & Cahill, 2015; Mark et al., 2016; Shiono et al. 1995; van Gelder et al., 2010). Although some studies suggested initial delays in physical development, all milestones are typically reached on time (Grant et al., 2017). Cognitive impairment has most consistently been linked to fetal cannabis exposure (e.g., Fried & Watkinson, 2001; Fried,

Watkinson & Gray, 2003; Huizink & Mulder, 2006; Willford, Chandler, Goldschmidt & Day, 2010). For example, prenatal cannabis exposure has been associated with certain deficits in visual and cognitive function in children (Fried & Watkinson, 2000; Fried, Watkinson & Gray, 2003) and decreased sustained attention in adolescents (Fried & Watkinson, 2001). A review of 36 clinical studies found an association between fetal cannabis exposure and conduct disorder, although causality could not be established (Ruisch, Dietrich, Glennon, Buitelaar, & Hoekstra, 2017). Psychopathological conditions in younger adults, specifically anxiety and depression, are associated with more frequent cannabis use (Hayatbakhsh, Najman, Jamrozik, Mamun, Alati & Bor, 2007) and co-use use (Ramo, Liu & Prochaska, 2012).

Studies have demonstrated that the endogenous cannabinoid system is a key regulator of immune function, with endogenous cannabinoid agonists, as well as exogenous ligands such as THC, having immunosuppressant effects (reviewed in Olah, Szekanecz & Biro, 2017). Surprisingly, however, little information is available regarding the impact of prenatal cannabis use or co-use on immune function. Possible epigenetic mechanisms by which maternal cannabis use might impact transgenerational immune function have been proposed (Dong et al., 2019; Zumbrun, Sido, Nagarkatti & Natarkatti, 2015), but only a single experiment related to maternal cannabis use appears to have been published. In that study, a mouse model was used to demonstrate that prenatal cannabis exposure resulted in T-cell dysfunction in fetal and postnatal animals (Lombard, Hegde, Nagarkatti & Nagarkatti, 2011).

To our knowledge, limited clinical data exist on the consequences of co-use of tobacco and cannabis on maternal or fetal outcomes such as immune function. One recent study reported pre- and postnatal dual exposure to tobacco and cannabis, when compared to tobacco- and cannabis-only groups, increased levels of secretory Immunoglobulin A, an essential antibody for mucosal immunity in early childhood (Molnar et al., 2018). Given that tobacco and cannabis are two of the most widely used substances during pregnancy, and that concurrent cannabis use might confer

additional or synergistic immunity and health risks in pregnant women who use tobacco, this midpoint analysis from an ongoing project sought to describe the effects of first trimester co-use of tobacco and cannabis on serum immune markers (IL-2, IL-6, IL-10, CRP, TNF-α and matrix metalloproteinase [MMP]-8), as well as depression symptoms and perceived stress, compared to tobacco use alone.

2.1. Material and Methods

This report represents a preliminary midpoint analysis of a larger study to determine the impact of prenatal tobacco use, including electronic nicotine delivery systems (ENDS), on immune response and birth outcomes. Therefore, subject groups consisted of tobacco only users compared to tobacco users who also tested positive for recent cannabis use; a cannabis use only group was not included. An institutional review board (IRB) approved, multisite study using quota sampling was used to meet study aims. Participants were recruited from academic and private prenatal clinics in Kentucky via two methods: 1) women were approached at their obstetric screening appointments; and 2) women proactively responded to posted study flyers. A study nurse determined eligibility based on maternal age (18-44 years); first trimester gestation (less than 14 weeks), current tobacco use (within 30 days) and ability to read or write in English. Tobacco use was limited to those who smoked conventional cigarettes and/or any form of ENDS.

A research nurse explained the study to eligible participants and obtained informed consent. At enrollment, participants completed a survey (available via hard copy or iPad) that included demographic, tobacco and psychosocial measures. The survey was written at the 6th grade level and took approximately 20 minutes to complete. Survey responses were stored on REDCap, a secure web-based data management system. Following survey completion, study personnel collected urine and serum samples using previously reported methods (Ashford et al., 2017). These biomarkers were used to determine study groupings (tobacco-only and tobacco plus cannabis). Participants were given a $25 gift card to a local department store at completion of the study visit.

2.1.1. Participants

Demographic information collected via survey included date of birth, race/ethnicity, partner status, education and income. Age was calculated using the participant's date of birth. Race and ethnicity were assessed separately. First, respondents were asked to indicate whether they were 'Hispanic or Latino' or 'Not Hispanic or Latino', and were then asked, 'Which of the following best describes your race?' with response options including 'American Indian/Alaskan Native,' 'Asian,' 'Native Hawaiian or Other Pacific Islander,' 'Black or African American,' 'White' and 'More than 1 race.' Responses from these two questions were combined and a dichotomous variable ('White, non-Hispanic' or 'Non-white or Hispanic') was used in subsequent analyses. Women were asked to select their partner status from response options including 'Single,' 'Married or living with a partner,' 'Divorced or separated,' 'Widowed' or 'Other.' Those who indicated 'Married or living with a partner' were classified as partnered, while all other responses were coded as non-partnered. Employment status was coded as employed ('part-time' or 'full-time') or unemployed ('unemployed,' 'student' or 'homemaker'). For education, women were asked 'What is the highest grade or year of school you have completed?' with response options including 'Less than high school graduate,' 'High school graduate or GED,' 'Some college or vocational/trade school' and 'College graduate or beyond.' For analysis, the latter two categories were collapsed to represent beyond high school education. During the first clinic visit (at enrollment), the research nurse recorded height and weight for each participant, which was used to calculate body mass index.

Use of conventional and electronic cigarettes was assessed separately. For each product, the research nurse asked 'Have you *used e-cigarettes/smoked cigarettes* within the last 30 days?' Women who responded 'yes' were coded as current users of the respective product. Those who responded 'yes' to electronic cigarettes were coded as dual or ENDS only users, while those who responded 'no' were coded as conventional cigarette only users.

2.1.2. Biological Markers

Urine and serum samples were collected in the first trimester (8-14 weeks gestation). Urine samples were assayed for the presence of nicotine and cannabis metabolites. Cotinine, a metabolite of nicotine, has a half-life of approximately 9 hours in pregnant women (Bernert et al., 1997; NicAlert, 2007) and was used to confirm tobacco status using a validated commercial assay (NicAlert®). Cotinine levels greater than or equal to 100 ng/mL validated current tobacco use (Ashford et al., 2010; Bernert, Harmon, Sosnoff, & McGuffey, 2005). 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), a major metabolite of THC, was measured using a validated analytical method for measurement of THC-COOH in urine using solid phase extraction and high performance liquid chromatography coupled with negative mode electrospray ionization tandem mass spectrometry. Similar methods have been used previously to assess cannabis use in pregnant women (El Marroun et al., 2010; Westin, Huestis, Aarstad, & Spigset, 2008). Maternal serum cytokines IL-1β, IL-2, IL-6, IL-8, IL-10, TNFα, CRP and MMP8 were determined from plasma samples using methods previously reported (Ashford et al., 2017). The iCup Drug Screen (BioScan Screening Systems, Inc., Smyrna, TN) was used to validate illicit drug use (McCarberg, 2011. The iCup employs enzyme-linked immune assays (ELIZA) to detect the presence or absence of the following drugs/drug classes: buprenorphine, morphine/opiates, methadone, oxycodone, benzodiazepines, amphetamines, methamphetamine, cocaine, and THC. An indicator variable for other illicit drug use was created to represent a positive test for any illicit substance use other than cannabis. Only one participant tested positive for alcohol and this participant also tested positive for illicit drug use other than cannabis.

2.1.3. Psychological Measures

Maternal depressive symptoms and perceived stress were measured using tools validated both during and after pregnancy. The 10-item Edinburgh Postnatal Depression Scale was used to measure prenatal depressive symptoms (Gibson, McKenzie-McHarg, Shakespeare, Price, & Gray,

2009) and maternal stress was measured using the shortened, 4-item Perceived Stress Scale (PSS) (Glynn, Schetter, Hobel, & Sandman, 2008; Karam et al., 2012). Both tools have demonstrated consistent reliability throughout pregnancy (EDPS: Cronbach's α = 0.82, 0.83, and 0.84, respectively)(Bergink et al., 2011); 4-item PSS with a Cronbach's α =0.79) (Karam et al., 2012).

2.1.4. Statistical Analysis

Descriptive statistics summarized study variables. The two-sample t-test or chi-square test of association, as appropriate, examined associations among sociodemographic variables and subject group (i.e., co-use of tobacco and cannabis or tobacco use only). Multiple linear regression models were used to determine differences in stress and depression by group, controlling for age, race/ethnicity, partner status, education, income, tobacco use group (conventional cigarette only versus dual or ENDS only user) and other illicit drug use. For the cytokine analysis, the Mann-Whitney U test compared users of both tobacco and cannabis to tobacco only users. Cytokine values were log-transformed as an adjustment for lack of normality in the raw values and multiple linear regression models tested for differences by subject group, adjusting for age, race/ethnicity, body mass index, tobacco use group and other illicit drug use. All analysis was conducted using SAS, version 9.4, with an alpha level of .05 throughout.

3.1. Results

3.1.1 Sociodemographic Characteristics

Urine drug tests were performed on 138 tobacco using pregnant women. Overall, participants were primarily white (82%) and single/not partnered (53%). The average age was 29.1 years; 53% had a high school education or less and approximately half were unemployed. Approximately one-quarter (24%) of women self-reported using ENDS, either alone or in combination with cigarettes. Over one-quarter (28%) of women had a positive urine drug screen for THC-COOH with a median level of 236 ng/ml (IQR=44-401). Pregnant women who reported co-use of tobacco and cannabis were younger than tobacco only users (27.3 [SD=5.0] vs 29.8 [SD=5.3]

years old; $t_{(df=136)}$ = 2.5, p=.02; Table 1). A higher proportion of co-users defined their race/ethnicity to be other than White (41% vs 10%; $\chi^2_{(df=1)}$ = 16.5, p<.01) compared to tobacco only users. In addition, compared to tobacco only users, a greater percentage of co-users listed their job status as unemployed (68.7% vs 43.3%; $\chi^2_{(df=1)}$ = 6.2, p=.01).

Thirty percent of the participants (n=42) were positive for recent use of an illicit substance other than cannabis in the first trimester. The most common substances were methamphetamine (n = 36), prescription opioids (n = 34) and cocaine (n =7). There was no difference in the rate of urine drug screens positive for any illicit drug between the groups (p=.86).

3.1.2. Cytokine Levels

Cytokine data were available for 122 women in the first trimester. In the bivariate analysis, there was a significant difference in TNF-α (m = 2.0 pg/mL [IQR=1.7-2.4] vs. m = 2.4 pg/mL [IQR=2.0-2.8]; $\chi^2_{(df=1)}$ = 8.0, p = .01) and CRP (m = 5.3 mg/L [IQR=1.3-12.9] vs. m = 8.2 mg/L [IQR=3.0-17.1]; $\chi^2_{(df=1)}$ = 4.6, p = .03; Table 2) levels between the two groups, with tobacco and cannabis co-users having significantly lower levels compared to tobacco only users for both inflammatory markers, respectively. In the adjusted linear regression models, there was no difference in CRP between groups, while TNF-α levels remained lower among co-users (b =-0.15 [SE=0.07], p=.03), adjusting for age, body mass index, race/ethnicity, tobacco use group and other illicit substance use. Because the cytokine values were log-transformed prior to modeling, the geometric mean was interpreted, which indicated that co-users had approximately 14% lower TNF-α levels compared to tobacco only users (exp[*beta]* = 0.86). There were no differences by use group for any of the other interleukins or MMP-8 in the unadjusted or adjusted models.

3.1.3. Psychological Measures

On average, all participants had low stress levels (M=5.9, SD=3.3; potential range 0-16) and depression scores (M=7.8, SD=5.8; potential range 0-30). There was no significant difference in

perceived stress or depressive symptoms as a function of use group in the unadjusted or adjusted analysis.

4.1. Discussion

There are well characterized adverse maternal, prenatal and child health effects of tobacco cigarette use during pregnancy. Of concern is the recent escalation in daily cannabis use that has been observed among female cigarette smokers (Goodwin et al., 2018) because concurrent cannabis use might confer additional or synergistic maternal and/or fetal immunity and health risks above those of tobacco. The present midpoint analysis from an ongoing project therefore sought to describe the effects of first trimester co-use of tobacco and cannabis on serum immune markers, as well as depression symptoms and perceived stress, compared to prenatal tobacco use alone. Preliminary results from this analysis suggest that pregnant women co-using tobacco and cannabis during the first trimester have decreased pro-inflammatory immune responsivity as reflected by reduced TNF-α levels. There were no differences in the other seven markers.

Little empirical information is available regarding the consequences of co-use of tobacco and cannabis during pregnancy. Analyses of secondary data from a larger study on illicit and prescription drug use during pregnancy indicated that relative to the use of only tobacco or cannabis, co-use was significantly and positively correlated with smaller infant head circumference and birth defects (Coleman-Cowger, Oga, Peters & Mark, 2018). Similarly, another study found that smaller head size, an increased risk of preterm birth and decreased birth weight in the neonates was associated with prenatal co-use of tobacco and cannabis compared to use of cannabis alone (Chabarria et al., 2016). With respect to childhood effects of co-use of tobacco and cannabis, offspring born to women who reported "decreasing co-use" (i.e., primarily during prenatal and preschool periods) were more likely to be co-users themselves, and children of chronic co-users were more likely to have a substance use disorder, relative to those whose mothers reported no co-use or only postnatal co-use (De Genna, Goldschmidt, Richardson, Cornelius & Day, 2018). In

addition, a recent study found that pre- and postnatal dual exposure increased secretory Immunoglobulin-A in early childhood relative to tobacco and cannabis-only exposure (Molnar et al., 2018). The present preliminary results extend this limited literature by providing initial evidence that co-use of cannabis and tobacco increases the likelihood of maternal immune system dysregulation relative to the use of tobacco alone.

Lower socio-economic status, unemployment, and belonging to a racial minority group are common in women who use cannabis during pregnancy (Chabarria et al., 2016; Conner et al., 2015; Metz et al., 2017; van Gelder et al., 2010). Among tobacco users, co-users of cannabis are more likely to be younger and non-Hispanic Black or Hispanic relative to tobacco only users (Coleman-Cowger et al., 2017), consistent with the current findings. Although demographic characteristics differ between groups, a comprehensive and inclusive approach for identifying and providing cessation interventions should be provided to all co-users of tobacco and cannabis. Future research may also explore the efficacy of interventions tailored to meet the unique needs of distinct demographic groups.

Pregnancy is characterized by a physiologic systemic inflammatory response that fluctuates over the course of the pregnancy (Romero, Gotsch, Pineles & Kusanovic, 2007). Tobacco use during pregnancy is associated with a maternal shift in anti-inflammatory and pro-inflammatory cytokines that can negatively affect fetal outcomes (Ashford et al., 2013; Simhan et al., 2005). To our knowledge, no clinical research has been conducted on the effects of prenatal cannabis use, or co-use of tobacco and cannabis, on maternal, fetal or child cytokine composition. The present study is the first to report that women who co-use tobacco and cannabis exhibit a depressed pro-inflammatory response, as evidenced by significantly lower TNF-α levels, relative to tobacco-only users. TNF-α is a byproduct of macrophages that are responsible for apoptosis, and during pregnancy, are lowest in the first trimester compared to the third trimester (Ashford et al., 2017).

Current research reporting the effects of tobacco or co-use of tobacco and cannabis on cytokine levels is mixed (Klein, T., Lane, B., Newton, C. & Friedman, H., 2000), yet largely examines the effects of medical marijuana in patients with chronic inflammatory conditions (e.g. rheumatoid arthritis) (Nagarkatti, P., Pandey, R., Rieder, S., Hegde, V., & Nagarkatti, M., 2009). In other in-vivo and murine work independently examining cannabis and tobacco, potential effects contributing to TNF-α suppression included the use of unheated THC (Verhoeckx, K. et al., 2006) and higher doses of nicotine (Li-Sha, G. et al., 2015). Further reductions in first trimester TNF-α by the co-use of tobacco and cannabis could compromise the immune system balance between maintaining maternal health and tolerating the semiallogeneic fetus, thereby negatively affecting birth outcomes (Dong et al., 2019). These group differences in TNF-α could be due to the use of cannabis or additive/synergistic effects of tobacco and cannabis in the co-use group, and/or the differing demographic characteristics of the two groups. Further research is needed to uncover the factors driving these group differences.

Maternal psychosocial factors such as stress, depression, and anxiety have been linked with tobacco use (Goodwin, Keyes, Simuro, 2007; Hauge, Torgersone, Vollrath, 2012; Zhu & Valbo, 2002) and cannabis use during pregnancy (Conner et al., 2015; Hayatbakhsh et al., 2007; Mark et al., 2016; Oh, Salas-Wright, Vaughn, & DiNitto, 2017; Ramo, Liu, Prochaska, 2012), although we are not aware of any studies that have specifically examined the presence of psychiatric disorders in pregnant co-users. The present midpoint analysis suggests that maternal stress and depressive symptoms do not differ between these groups. Prior studies have compared tobacco-using women and non-tobacco-using women and found an association between tobacco use and psychological stress and depression (Husky, Mazure, Paliwal, & McKee, 2008). The present study exclusively recruited tobacco-users, so it is plausible that differences in perceived stress or depressive symptoms were not detected due to the homogeneity of having a sample consisting of all prenatal tobacco users.

Another possibility is that stress and depressive symptom scores were relatively low in both groups making group differences difficult to detect (i.e., a floor effect).

Some study limitations warrant mentioning. Weaknesses of this midpoint analysis include the small sample size, the lack of additional comparison groups (i.e., women without exposure to tobacco, and those with cannabis only), and the inability to control for exposures to various medications that might impact study outcomes, such as antibiotics or anxiolytics. Further, there was only one participant with a multiple pregnancy, therefore we were unable to address the potential impact this may have on both psychosocial and immune function. Another limitation is that self-reported cannabis data were not collected. Instead, the presence of urinary THC and level of cotinine (> 100 ng/mL) were used for co-use group assignment. Given the varied detection window for urinary THC, it is possible that one or more co-users were misclassified as a tobacco only user, which might have impacted our ability to detect relationships between co-use and maternal outcomes. In addition, the lack of quantitative self-reported use data precluded determination of dose-response relationships. Although standards for the expected concentrations of immune markers at different timepoints during pregnancy have not been established, one possibility is that the varied collection times within the first trimester (weeks 8-13) might have yielded variability in immune markers (Aghaeepour, N. et al., 2017), which also might have impacted our ability to detect relationships between co-use and maternal outcomes. Despite these limitations, the preliminary findings from this midpoint analysis provide the premise for future studies to examine changes in cytokines over the course of pregnancy in women who use tobacco and cannabis.

5.1. Summary and Conclusion

This analysis appears to be the first to compare markers of immune and psychosocial function in first trimester pregnant women who co-use tobacco and cannabis to those in tobacco-only users. These preliminary results suggest that pregnant women co-using tobacco and cannabis

are more likely to be non-White, younger and more economically disadvantaged compared to tobacco-only users. These preliminary results also suggest that co-use in the first trimester is associated with a depressed proinflammatory immune response, as reflected by one immune marker, TNF-α. Additional research to measure the range of maternal and fetal immune responsiveness during gestation, as well as long-term follow-up of offspring of women who co-use tobacco and cannabis, is warranted. These findings also support research that includes appropriate comparison groups to disentangle the unique contribution of cannabis use relative to tobacco and cannabis co-use on maternal immune function.

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References

Aghaeepour, N., Ganio, E. A., Mcilwain, D., Tsai, A. S., Tingle, M., Van Gassen, S., ... & Ghaemi, M. S. (2017). An immune clock of human pregnancy. *Science immunology*, *2*(15), eaan2946.

American College of Obstetricians and Gynecologists (ACOG). (2017). Smoking cessation during pregnancy. Committee Opinion No. 721. *Obstetrics & Gynecology 130:e200-4*.

Ashford, K., Barnett, J., Mccubbin, A., Kehler, S., & Westneat, S. (2013). Prenatal Systemic Immune Response in Smoking and Nonsmoking Women. *Journal of Obstetric, Gynecologic, & Neonatal Nursing*, *42*(s1), S82-S83.

Ashford, K., Chavan, N., Ebersole, J., Wiggins, A., Sharma, S., McCubbin, A., Barnett, J., O'Brien, J. (2017). Patterns of Systemic and Cervicovaginal Fluid Inflammatory Cytokines throughout Pregnancy. *American journal of perinatology, 35(05), 455-462*.

Ashford, K. B., Hahn, E., Hall, L., Rayens, M. K., Noland, M., & Ferguson, J. E. (2010). The Effects of Prenatal Secondhand Smoke Exposure on Preterm Birth and Neonatal Outcomes. *Journal of Obstetric, Gynecologic, & Neonatal Nursing, 39*(5), 525-535. doi: 10.1111/j.1552-6909.2010.01169.x

Ashford, K., O'Brien, J., McCubbin, A., Westneat, S., & Barnett, J. (2013). 15: The influence of prenatal smoking status on cervical cytokine distribution. *American Journal of Obstetrics & Gynecology, 208*(1), S10.

Azofeifa, A. (2016). National estimates of marijuana use and related indicators—National Survey on Drug Use and Health, United States, 2002–2014. *MMWR. Surveillance Summaries, 65*.

Berg, C. J., Stratton, E., Schauer, G. L., Lewis, M., Wang, Y., Windle, M., & Kegler, M. (2015). Perceived harm, addictiveness, and social acceptability of tobacco products and marijuana among young adults: marijuana, hookah, and electronic cigarettes win. *Substance use & misuse, 50*(1), 79-89.

Bergink, V., Kooistra, L., Lambregtse-van den Berg, M. P., Wijnen, H., Bunevicius, R., van Baar, A., & Pop, V. (2011). Validation of the Edinburgh Depression Scale during pregnancy. *Journal Of Psychosomatic Research, 70*(4), 385-389.

Bernert, J. T., Harmon, T. L., Sosnoff, C. S., & McGuffey, J. E. (2005). TECHNICAL NOTE: Use of Cotinine Immunoassay Test Strips for Preclassifying Urine Samples from Smokers and Nonsmokers Prior to Analysis by LCMSMS. *Journal of Analytical Toxicology, 29*, 814-818.

Bernert, J. T., Jr., Turner, W. E., Pirkle, J. L., Sosnoff, C. S., Akins, J. R., Waldrep, M. K., . . . Sampson, E. J. (1997). Development and validation of sensitive method for determination of serum cotinine in smokers and nonsmokers by liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. *Clin Chem, 43*(12), 2281-2291.

Burns, L., Mattick, R. P., & Cooke, M. (2006). The use of record linkage to examine illicit drug use in pregnancy. *Addiction, 101*(6), 873-882.

Buss, C., Davis, E. P., Hobel, C. J., & Sandman, C. A. (2011). Maternal pregnancy-specific anxiety is associated with child executive function at 6–9 years age. *Stress*, *14*(6), 665-676.

Castles A, Adams EK, Melvin CL et al (1999) Effects of smoking during pregnancy. Five meta-analyses. Am J Prev Med 16:208–215

Centers for Disease Control and Prevention CDC. February 6, 2018. Smoking During Pregnancy. Retrieved on November 21, 2018 from http://www.cdc.gov/tobacco/basic_information/health_effects/pregnancy/index.htm

Chabarria, K. C., Racusin, D. A., Antony, K. M., Kahr, M., Suter, M. A., Mastrobattista, J. M., & Aagaard, K. M. (2016). Marijuana use and its effects in pregnancy. *American Journal of Obstetrics & Gynecology, 215*(4), 506. e501-506. e507.

Christian, L. M., Franco, A., Glaser, R., & Iams, J. D. (2009). Depressive symptoms are associated with elevated serum proinflammatory cytokines among pregnant women. *Brain, behavior, and immunity, 23*(6), 750-754.

Coleman-Cowger, V. H., Oga, E. A., Peters, E. N., & Mark, K. (2018). Prevalence and associated birth outcomes of co-use of Cannabis and tobacco cigarettes during pregnancy. *Neurotoxicology and teratology*, *68*, 84-90.

Coleman-Cowger, V. H., Schauer, G. L., & Peters, E. N. (2017). Marijuana and tobacco co-use among a nationally representative sample of US pregnant and non-pregnant women: 2005–2014 National Survey on Drug Use and Health findings. *Drug & Alcohol Dependence, 177*, 130-135.

Conner, S. N., Carter, E. B., Tuuli, M. G., Macones, G. A., & Cahill, A. G. (2015). Maternal marijuana use and neonatal morbidity. *American Journal of Obstetrics & Gynecology, 213*(3), 422. e421-422. e424.

Coussons-Read, M. E., Okun, M. L., & Nettles, C. D. (2007). Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. *Brain, behavior, and immunity*, *21*(3), 343-350.

Coussons-Read, M. E., Okun, M. L., Schmitt, M. P., & Giese, S. (2005). Prenatal stress alters cytokine levels in a manner that may endanger human pregnancy. *Psychosomatic Medicine, 67*(4), 625-631.

De Genna, N. M., Goldschmidt, L., Richardson, G. A., Cornelius, M. D., & Day, N. L. (2018). Trajectories of pre-and postnatal co-use of cannabis and tobacco predict co-use and drug use disorders in adult offspring. *Neurotoxicology and teratology*, *70*, 10-17.

Dong, C., Chen, J., Harrington, A., Vinod, K., Hedge, M., & Hegde, V., (2019). Cannabinoid exposure during pregnancy and its impact on immune function. *Cellular and Molecular Life Sciences.* February 2019, Volume 76, Issue 4, pp 729–743. https://doi-org.ezproxy.uky.edu/10.1007/s00018-018-2955-0

El Marroun, H., Tiemeier, H., Jaddoe, V. W., Hofman, A., Mackenbach, J. P., Steegers, E. A., . . . Huizink, A. C. (2008). Demographic, emotional and social determinants of cannabis use in early pregnancy: the Generation R study. *Drug & Alcohol Dependence, 98*(3), 218-226.

El Marroun, H., Tiemeier, H., Jaddoe, V., Hofman, A., Verhulst, F., van den Brink, W., & Huizink, A. (2010). Agreement between maternal cannabis use during pregnancy according to self-report and urinalysis in a population-based cohort: the Generation R Study. *European addiction research, 17*(1), 37-43.

El Marroun, H., Tiemeier, H., Steegers, E. A., Jaddoe, V. W., Hofman, A., Verhulst, F. C., . . . Huizink, A. C. (2009). Intrauterine cannabis exposure affects fetal growth trajectories: the Generation R Study. *Journal of the American Academy of Child & Adolescent Psychiatry, 48*(12), 1173-1181.

Fergusson, D. M., Horwood, L. J., & Northstone, K. (2002). Maternal use of cannabis and pregnancy outcome. *BJOG: An International Journal of Obstetrics & Gynaecology, 109*(1), 21-27.

Fried, P. A., & Watkinson, B. (2000). Visuoperceptual functioning differs in 9-to 12-year olds prenatally exposed to cigarettes and marihuana. *Neurotoxicology and Teratology*, *22*(1), 11-20.

Fried, P. A., & Watkinson, B. (2001). Differential effects on facets of attention in adolescents prenatally exposed to cigarettes and marihuana. *Neurotoxicology and teratology*, *23*(5), 421-430.

Fried, P. A., Watkinson, B., & Gray, R. (2003). Differential effects on cognitive functioning in 13-to 16-year-olds prenatally exposed to cigarettes and marihuana. *Neurotoxicology and teratology*, *25*(4), 427-436.

Gibson, J., McKenzie-McHarg, K., Shakespeare, J., Price, J., & Gray, R. (2009). A systematic review of studies validating the Edinburgh Postnatal Depression Scale in antepartum and postpartum women. *Acta Psychiatrica Scandinavica, 119*(5), 350-364. doi: 10.1111/j.1600-0447.2009.01363.x

Glynn, L. M., Schetter, C. D., Hobel, C. J., & Sandman, C. A. (2008). Pattern of perceived stress and anxiety in pregnancy predicts preterm birth. *Health Psychology: Official Journal Of The Division Of Health Psychology, American Psychological Association, 27*(1), 43-51.

Goldenberg, R. L., Culhane, J. F., Iams, J. D., & Romero, R. (2008). Epidemiology and causes of preterm birth. *The lancet, 371*(9606), 75-84.

Goodwin R.D., Keyes K., Simuro N. (2007) Mental disorders and nicotine dependence among pregnant women in the United States. Obstet Gynecol. 2007;109(4):875–83.

Goodwin, R. D., Pacek, L. R., Copeland, J., Moeller, S. J., Dierker, L., Weinberger, A., . . . Hasin, D. S. (2018). Trends in Daily Cannabis Use Among Cigarette Smokers: United States, 2002–2014. *American journal of public health, 108*(1), 137-142.

Grant, K. S., Petroff, R., Isoherranen, N., Stella, N., & Burbacher, T. M. (2017). Cannabis use during pregnancy: pharmacokinetics and effects on child development. *Pharmacology & therapeutics*.

Hauge, L. J., Torgersen, L., & Vollrath, M. (2012). Associations between maternal stress and smoking: findings from a population-based prospective cohort study. *Addiction (Abingdon, England)*, *107*(6), 1168-73.

Hayatbakhsh, M. R., Najman, J. M., Jamrozik, K., Mamun, A. A., Alati, R., & Bor, W. (2007). Cannabis and anxiety and depression in young adults: a large prospective study. *Journal of the American Academy of Child & Adolescent Psychiatry*, *46*(3), 408-417.

Huizink, A. C., & Mulder, E. J. (2006). Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring. *Neuroscience & Biobehavioral Reviews*, *30*(1), 24-41.

Husky, M. M., Mazure, C. M., Paliwal, P., & McKee, S. A. (2008). Gender differences in the comorbidity of smoking behavior and major depression. *Drug and Alcohol Dependence, 93*(1), 176-179. doi: https://doi.org/10.1016/j.drugalcdep.2007.07.015

Jia, J., Peng, J., Li, Z., Wu, Y., Wu, Q., Tu, W., & Wu, M. (2016). Cannabinoid CB2 receptor mediates nicotine-induced anti-inflammation in n9 microglial cells exposed to β amyloid via protein kinase C. *Mediators of inflammation*, *2016*.

Karam, F., Bérard, A., Sheehy, O., Huneau, M. C., Briggs, G., Chambers, C., . . . Koren, G. (2012). Reliability and validity of the 4-item Perceived Stress Scale among pregnant women: Results from the OTIS antidepressants study. *Research in nursing & health, 35*(4), 363-375.

Kharrazi, M., DeLorenze, G. N., Kaufman, F. L., Eskenazi, B., Bernert Jr, J. T., Graham, S., ... & Pirkle, J. (2004). Environmental tobacco smoke and pregnancy outcome. *Epidemiology*, 660-670.

Klein, T. W., Lane, B., Newton, C. A., & Friedman, H. (2000). The Cannabinoid System and Cytokine Network (44546). *Proceedings of the Society for Experimental Biology and Medicine*, *225*(1), 1-8.

Ko, J. Y., Farr, S. L., Tong, V. T., Creanga, A. A., & Callaghan, W. M. (2015). Prevalence and patterns of marijuana use among pregnant and nonpregnant women of reproductive age. *American Journal of Obstetrics & Gynecology, 213*(2), 201. e201-201. e210.

Langley, K., Rice, F., & Thapar, A. (2005). Maternal smoking during pregnancy as an environmental risk factor for attention deficit hyperactivity disorder behaviour. A review. *Minerva pediatrica*, *57*(6), 359-371.

Li-Sha, G., Jing-Lin, Z., Guang-Yi, C., Li, L., De-Pu, Z., & Yue-Chun, L. (2015). Dose-dependent protective effect of nicotine in a murine model of viral myocarditis induced by coxsackievirus B3. *Scientific reports*, *5*, 15895.

Lombard, C., Hegde, V. L., Nagarkatti, M., & Nagarkatti, P. S. (2011). Perinatal exposure to Δ9-tetrahydrocannabinol triggers profound defects in T cell differentiation and function in fetal and postnatal stages of life, including decreased responsiveness to HIV antigens. *The Journal of pharmacology and experimental therapeutics*, *339*(2), 607-17.

519 Maritz, G. S., & Harding, R. (2011). Life-long programming implications of exposure to tobacco
520 smoking and nicotine before and soon after birth: evidence for altered lung
521 development. *International journal of environmental research and public health*, *8*(3), 875-
522 898.
523 Mark, K., Desai, A., & Terplan, M. (2016). Marijuana use and pregnancy: prevalence, associated
524 characteristics, and birth outcomes. *Archives of women's mental health, 19*(1), 105-111.
525 McCarberg, B. H. (2011). A critical assessment of opioid treatment adherence using urine drug
526 testing in chronic pain management. *Postgraduate medicine*, *123*(6), 124-131.
527 Metz, T. D., Allshouse, A. A., Hogue, C. J., Goldenberg, R. L., Dudley, D. J., Varner, M. W., . . . Silver, R. M.
528 (2017). Maternal marijuana use, adverse pregnancy outcomes, and neonatal morbidity.
529 *American Journal of Obstetrics & Gynecology, 217*(4), 478. e471-478. e478.
530 Molnar, D. S., Granger, D. A., Shisler, S., & Eiden, R. D. (2018). Prenatal and postnatal cigarette and
531 cannabis exposure: Effects on Secretory Immunoglobulin A in early
532 childhood. *Neurotoxicology and teratology*, *67*, 31-36.
533 Nagarkatti, P., Pandey, R., Rieder, S. A., Hegde, V. L., & Nagarkatti, M. (2009). Cannabinoids as novel
534 anti-inflammatory drugs. *Future medicinal chemistry*, *1*(7), 1333-1349.
535 NicAlert. (2007). Expressing the results as cotinine concentration ranges. Retrieved May 2, 2007,
536 from http://www.nicalert.net/nicalert/er.html
537 Nouri-Shirazi M, Guinet E. Evidence for the immunosuppressive role of nicotine on human dendritic
538 cell functions. Immunology. 2013 [accessed 2017 October 18]; 109(3): 365-373.
539 http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2567.2003.01655.x/abstract. doi:
540 10.1046/j.1365-2567.2003.01655.x.
541 Oh, S., Salas-Wright, C. P., Vaughn, M. G., & DiNitto, D. M. (2017). Marijuana use during pregnancy: A
542 comparison of trends and correlates among married and unmarried pregnant women. *Drug*
543 *& Alcohol Dependence, 181*, 229-233.

544 Oken, E., Levitan, E. B., & Gillman, M. W. (2008). Maternal smoking during pregnancy and child
545 overweight: systematic review and meta-analysis. *International journal of obesity*, *32*(2),
546 201.
547 Oláh, A., Szekanecz, Z., & Bíró, T. (2017). Targeting Cannabinoid Signaling in the Immune System:
548 "High"-ly Exciting Questions, Possibilities, and Challenges. *Frontiers in immunology*, *8*, 1487.
549 doi:10.3389/fimmu.2017.01487
550 Osborne, L. M., & Monk, C. (2013). Perinatal depression—the fourth inflammatory morbidity of
551 pregnancy?: theory and literature review. *Psychoneuroendocrinology*, *38*(10), 1929-1952.
552 Park, B., McPartland, J. M., & Glass, M. (2004). Cannabis, cannabinoids and reproduction.
553 *Prostaglandins, leukotrienes and essential fatty acids, 70*(2), 189-197.
554 Pew Research Center. (2018). About six-in-ten Americans support marijuana legalization.
555 Retrieved 03/19/18, from http://www.pewresearch.org/fact-
556 tank/2018/01/05/americans-support-marijuana-legalization/
557 Rabin, R. A., & George, T. P. (2015). A review of co-morbid tobacco and cannabis use disorders:
558 Possible mechanisms to explain high rates of co-use. *The American journal on*
559 *addictions*, *24*(2), 105-116.
560 Ramo, D. E., Liu, H., & Prochaska, J. J. (2012). Tobacco and marijuana use among adolescents and
561 young adults: a systematic review of their co-use. *Clinical psychology review*, *32*(2), 105-121.
562 Romero, R., Gotsch, F., Pineles, B., & Kusanovic, J. P. (2007). Inflammation in pregnancy: its roles in
563 reproductive physiology, obstetrical complications, and fetal injury. *Nutrition*
564 *reviews*, *65*(suppl_3), S194-S202.
565 Ruisch, I. H., Dietrich, A., Glennon, J. C., Buitelaar, J. K., & Hoekstra, P. J. (2017). Maternal substance
566 use during pregnancy and offspring conduct problems: A meta-analysis. *Neuroscience &*
567 *Biobehavioral Reviews*.

Schetter, C. D., & Tanner, L. (2012). Anxiety, depression and stress in pregnancy: implications for mothers, children, research, and practice. *Current opinion in psychiatry, 25*(2), 141.

Shiono, P. H., Klebanoff, M. A., Nugent, R. P., Cotch, M. F., Wilkins, D. G., Rollins, D. E., . . . Behrman, R. E. The impact of cocaine and marijuana use on low birth weight and preterm birth: A multicenter study. *American Journal of Obstetrics & Gynecology, 172*(1), 19-27. doi: 10.1016/0002-9378(95)90078-0

Simhan, H. N., Caritis, S. N., Hillier, S. L., & Krohn, M. A. (2005). Cervical anti-inflammatory cytokine concentrations among first-trimester pregnant smokers. *American Journal Of Obstetrics And Gynecology, 193*(6), 1999-2003.

Simhan, H. N., & Krohn, M. A. (2009). First-trimester cervical inflammatory milieu and subsequent early preterm birth. *American Journal Of Obstetrics And Gynecology, 200*(4), 377.e371-374. doi: 10.1016/j.ajog.2008.10.038

Sinclair, C. F., Foushee, H. R., Scarinci, I., & Carroll, W. R. (2013). Perceptions of harm to health from cigarettes, blunts, and marijuana among young adult African American men. *Journal of health care for the poor and underserved, 24*(3), 1266-1275.

United Nations Office on Drugs and Crime. (2017). *World Drug Report 2017*. (ISBN: 978-92-1-148291-1, eISBN: 978-92-1-060623-3). United Nations publication Retrieved from http://www.unodc.org/wdr2017/field/Booklet_5_NEXUS.pdf.

van Gelder, M. M., Reefhuis, J., Caton, A. R., Werler, M. M., Druschel, C. M., & Roeleveld, N. (2010). Characteristics of pregnant illicit drug users and associations between cannabis use and perinatal outcome in a population-based study. *Drug & Alcohol Dependence, 109*(1), 243-247.

Verhoeckx, K. C., Korthout, H. A., van Meeteren-Kreikamp, A. P., Ehlert, K. A., Wang, M., van der Greef, J., ... & Witkamp, R. F. (2006). Unheated Cannabis sativa extracts and its major

compound THC-acid have potential immuno-modulating properties not mediated by CB1 and CB2 receptor coupled pathways. *International immunopharmacology*, *6*(4), 656-665.

Wang, X., Dow-Edwards, D., Anderson, V., Minkoff, H., & Hurd, Y. L. (2004). In utero marijuana exposure associated with abnormal amygdala dopamine D2 gene expression in the human fetus. *Biological psychiatry, 56*(12), 909-915.

Warren, G. W., Alberg, A. J., Kraft, A. S., & Cummings, K. M. (2014). The 2014 Surgeon General's report:"The Health Consequences of Smoking–50 Years of Progress": a paradigm shift in cancer care. *Cancer*, *120*(13), 1914-1916.

Weg, M. W. V., Ward, K. D., Scarinci, I. C., Read, M. C., & Evans, C. B. (2004). Smoking-related correlates of depressive symptoms in low-income pregnant women. *American Journal of Health Behavior*, *28*(6), 510-521.

Westfall, R. E., Janssen, P. A., Lucas, P., & Capler, R. (2006). Survey of medicinal cannabis use among childbearing women: patterns of its use in pregnancy and retroactive self-assessment of its efficacy against 'morning sickness'. *Complementary Therapies in Clinical Practice, 12*(1), 27-33.

Westin, A. A., Huestis, M. A., Aarstad, K., & Spigset, O. (2008). Short communication: Urinary excretion of 11-nor-9-carboxy-Delta (9)-tetrahydrocannabinol in a pregnant woman following heavy, chronic cannabis use. *Journal of analytical toxicology, 33*(9), 610-614.

Wickström R. Effects of nicotine during pregnancy: human and experimental evidence. *Current Neuropharmacology.* 2007;5(3):213-222.

Willford, J. A., Chandler, L. S., Goldschmidt, L., & Day, N. L. (2010). Effects of prenatal tobacco, alcohol and marijuana exposure on processing speed, visual–motor coordination, and interhemispheric transfer. *Neurotoxicology and teratology*, *32*(6), 580-588.

Zhu SH, Valbo A. Depression and smoking during pregnancy. Addict Behav. 2002;27(4):649–58.

Zumbrun, E. E., Sido, J. M., Nagarkatti, P. S., & Nagarkatti, M. (2015). Epigenetic Regulation of

Immunological Alterations Following Prenatal Exposure to Marijuana Cannabinoids and its Long Term Consequences in Offspring. *Journal of neuroimmune pharmacology: the official journal of the Society on NeuroImmune Pharmacology*, *10*(2), 245-54.

Table 1. Sociodemographic characteristics of the study sample

Variable	Total sample (*N* = 138) *Mean* (SD) or *n* (%)	Prenatal exposures: **Tobacco and cannabis** (*n* = 38) *Mean* (SD) or *n* (%)	Prenatal exposures: **Tobacco only** (*n* = 100) *Mean* (SD) or *n* (%)	test statistic	*p*
Age	29.1 (5.4)	27.3 (5.0)	29.8 (5.3)	$t_{(df=136)} = 2.5$	.02
Body mass index (kg/m^2)	29.4 (8.3)	28.1 (8.1)	29.8 (8.4)		.29
Race/ethnicity				$\chi^2_{(df=1)} = 16.5$	<.01
Non-White or Hispanic	24 (18.1%)	14 (41.2%)	10 (10.1%)		
White	109 (81.9%)	20 (58.8%)	89 (89.9%)		
Partnered				$\chi^2_{(df=1)} = 1.6$	.20
Yes	61 (47.3%)	12 (37.5%)	49 (50.5%)		
No	68 (52.7%)	20 (62.5%)	48 (49.5%)		
Education				$\chi^2_{(df=2)} = 0.9$	.63
Less than high school	18 (13.7%)	5 (15.1%)	13 (13.2%)		
High school graduate	52 (39.7%)	15 (45.5%)	37 (37.8%)		
Beyond high school	61 (46.6%)	13 (39.4%)	48 (49.0%)		
Employment status				$\chi^2_{(df=1)} = 6.2$	.01
Employed	65 (50.4%)	10 (31.3%)	55 (56.7%)		
Unemployed	64 (49.6%)	22 (68.7%)	42 (43.3%)		
Tobacco use group				$\chi^2_{(df=1)} = 0.3$	.60
Conventional cigarettes only	99 (76.2%)	27(79.4%)	72 (75.0%)		
Dual or ENDS only	31 (23.8%)	7 (20.6%)	24 (25.0%)		
Other illicit drug use				$\chi^2_{(df=1)} < 0.1$	.86
Yes	38 (27.5%)	12 (31.6%)	30 (30.0%)		
No	100 (72.5%)	26 (68.4%)	70 (70.0%)		
Stress[a]	5.9 (3.3)	5.8 (3.4)	6.0 (3.2)	$t_{(df=128)} = 0.4$	.72
Depression[b]	7.8 (5.8)	7.5 (6.0)	7.9 (5.8)	$t_{(df=136)} = 0.1$	.75

Note: Numbers vary due to missing data. Abbreviation: ENDS, electronic nicotine delivery system

[a] Stress measured by the 4-item Perceived Stress Scale; potential scores range from 0-16, with higher scores reflecting more perceived stress

[b] Depressive symptoms measured using the 10-item Edinburgh Postnatal Depression Scale; potential scores range from 0-30

Table 2. Unadjusted and adjusted associations among cytokines and cannabis use

	Prenatal exposures			
	Tobacco and Cannabis (*n* = 34)	Tobacco only (*n* = 87)	Unadjusted	Adjusted
Cytokine	*Median* (IQR)	*Median* (IQR)	p^a	p^b
IL 1β (pg/mL)	0.09 (0.05 – 0.13)	0.08 (0.06 – 0.11)	.36	.43
IL 2 (pg/mL)	0.13 (0.07 – 0.26)	0.11 (0.07 – 0.22)	.59	.28
IL 6 (pg/mL)	0.74 (0.49 – 1.04)	0.69 (0.50 – 1.12)	.95	.18
IL 8 (pg/mL)	2.52 (2.06 – 3.38)	2.91 (2.28 – 4.02)	.06	.11
IL 10 (pg/mL)	0.30 (0.21 – 0.42)	0.27 (0.22 – 0.46)	.81	.58
TNF-α (pg/mL)	2.03 (1.69 – 2.36)	2.35 (1.98 – 2.75)	<.01	.03
CRP (mg/L)	5.34 (1.26 – 12.94)	8.18 (3.03 – 17.05)	.03	.26
MMP 8 (ng/mL)	27.70 (16.01 – 38.33)	36.33 (19.25 – 60.73)	.10	.76

[a] p-value from Mann-Whitney U test

[b] p-value from multiple linear regression model adjusting for age, body mass index, race/ethnicity, tobacco use group and other illicit substance use.