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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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1 **Title**

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

4 **Abstract**

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

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

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

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

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

27 **Introduction**

28

29 Tobacco and cannabis are the two most common addictive substances used during  
30 pregnancy, and are often used concurrently. Nearly 90% of cannabis users are also tobacco  
31 smokers (Rabin & George, 2015), and there has been a recent rapid and disproportionate increase  
32 in daily cannabis use among female cigarette smokers compared to male smokers (Goodwin et al.,  
33 2018). This is of significant concern due to pregnant women being at increased risk of continued  
34 use for the duration of their pregnancy (El Marroun et al., 2008; Ko et al., 2015). United States (US)  
35 nation-wide survey data reflect that 20% of pregnant women co-use tobacco and cannabis,  
36 (Azoifeifa, 2016), with those aged 18-25 years old being more likely to co-use tobacco and cannabis  
37 than cannabis alone (Coleman-Cowger, Schauer, & Peters, 2017). Yet, other recent studies using  
38 large databases from individual prenatal clinics indicate that the number of pregnant concurrent  
39 users of cannabis and tobacco is considerably higher (approaching 50%) (Chabarría et al., 2016;  
40 Mark, Desai, & Terplan, 2016).

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

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

54 Prior work is also suggestive of interactions among prenatal tobacco use, immune  
55 dysregulation in the mother, and maternal depression and anxiety (Osborne & Monk, 2013;  
56 Coussons-Read, Okun & Nettles, 2007). High levels of maternal depression and anxiety symptoms  
57 are associated with shorter gestation, alterations in fetal neurodevelopment (Schetter & Tanner,  
58 2012) and lower visuospatial working memory performance in the offspring (Buss, Davis, Hobel &  
59 Sandman, 2011). These maternal psychiatric symptoms are also often associated with immune  
60 dysregulation in pregnant women, commonly resulting in high circulating serum C-reactive protein  
61 (CRP) and proinflammatory cytokines (i.e., interleukin [IL]-6 and tumor necrosis factor [TNF]- $\alpha$ ),  
62 and lower levels of the anti-inflammatory cytokine, IL-10 (Christian, Franco, Glaser, & Iams, 2009;  
63 Coussons-Read, Okun, Schmitt, & Giese, 2005). Further, nicotine directly affects the immune system.  
64 In an animal study by Nouri-Shirazi and Guinet (2013), nicotine significantly depressed antibody  
65 responses and T-cell proliferation. A study of microglial activation linked nicotine exposure to  
66 significantly decreased levels of pro-inflammatory cytokines including interleukin (IL) -6 and TNF-  
67  $\alpha$  (Jia et al., 2016). In pregnancy, first trimester tobacco use has been associated with maternal  
68 immune dysregulation. For example, significant anti-inflammatory reductions in cervical IL-10  
69 were observed in women using tobacco early in pregnancy compared to nonsmokers whereas  
70 proinflammatory cytokines (IL-1 $\alpha$ , 1 $\beta$ , 2, 4, 6, 8, TNF $\alpha$ ) did not change. (Ashford, O'Brien, McCubbin,  
71 Westneat, & Barnett, 2013; Simhan, Caritis, Hillier, & Krohn, 2005). An examination of serum  
72 cytokines in the second and third trimesters of pregnancy revealed significantly higher  
73 concentrations of IL-6 and IL-1 $\alpha$  among smokers compared to non-smokers (Ashford, Barnett,  
74 McCubbin, Kehler, Westneat, 2013). Maternal immune dysregulation is of concern because it has  
75 been linked to adverse perinatal outcomes including pre-eclampsia (Ashford et al., 2017) and  
76 preterm birth (Goldenberg, Culhane, Iams, & Romero, 2008; Simhan and Krohn, 2009).

77           Within the past two decades, the perceived risk of cannabis use has decreased (Berg et al.,  
78   2015; Sinclair, Foushee, Scarinci, & Carroll, 2013) and public acceptance of cannabis use has  
79   increased (Pew Research Center, 2018) in the United States. Perhaps unsurprisingly then, the  
80   percentage of national survey respondents reporting past-year cannabis use has also increased  
81   (United Nations Office on Drugs and Crime, 2017). Cannabis use in the first trimester of pregnancy  
82   has been reported as high as 7.4%, with 16% of users reporting daily cannabis use (Ko, Farr, Tong,  
83   Creanga, & Callaghan, 2015). Prenatal cannabis use may occur for various reasons including  
84   recreation and self-medication (Ko et al., 2015; Park, McPartland, & Glass, 2004; Wang, Dow-  
85   Edwards, Anderson, Minkoff, & Hurd, 2004). Among women who used cannabis during pregnancy,  
86   some endorsed its use as a means to treat nausea and vomiting (Westfall, Janssen, Lucas, & Capler,  
87   2006). Although  $\Delta^9$ - tetrahydrocannabinol (THC; the primary active constituent of cannabis) is  
88   FDA-approved as a treatment for nausea and vomiting associated with cancer chemotherapy, it has  
89   not been evaluated for hyperemesis gravidarum, a pregnancy complication resulting in severe  
90   nausea, vomiting and alteration in serum electrolytes.

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

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

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

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

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

## 132 **2.1. Material and Methods**

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

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



### 152 2.1.1. Participants

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

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

176

177 **2.1.2. Biological Markers**

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

198 **2.1.3. Psychological Measures**

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

202 2009) and maternal stress was measured using the shortened, 4-item Perceived Stress Scale (PSS)  
203 (Glynn, Schetter, Hobel, & Sandman, 2008; Karam et al., 2012). Both tools have demonstrated  
204 consistent reliability throughout pregnancy (EDPS: Cronbach's  $\alpha = 0.82, 0.83,$  and  $0.84,$   
205 respectively)(Bergink et al., 2011); 4-item PSS with a Cronbach's  $\alpha = 0.79$ ) (Karam et al., 2012).

#### 206 **2.1.4. Statistical Analysis**

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

### 218 **3.1. Results**

#### 219 **3.1.1 Sociodemographic Characteristics**

220 Urine drug tests were performed on 138 tobacco using pregnant women. Overall,  
221 participants were primarily white (82%) and single/not partnered (53%). The average age was  
222 29.1 years; 53% had a high school education or less and approximately half were unemployed.  
223 Approximately one-quarter (24%) of women self-reported using ENDS, either alone or in  
224 combination with cigarettes. Over one-quarter (28%) of women had a positive urine drug screen  
225 for THC-COOH with a median level of 236 ng/ml (IQR=44-401). Pregnant women who reported co-  
226 use of tobacco and cannabis were younger than tobacco only users (27.3 [SD=5.0] vs 29.8 [SD=5.3])

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

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

### 235 3.1.2. Cytokine Levels

236 Cytokine data were available for 122 women in the first trimester. In the bivariate analysis,  
237 there was a significant difference in TNF- $\alpha$  ( $m = 2.0$  pg/mL [IQR=1.7-2.4] vs.  $m = 2.4$  pg/mL  
238 [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  
239 [IQR=3.0-17.1];  $\chi^2_{(df=1)} = 4.6, p = .03$ ; Table 2) levels between the two groups, with tobacco and  
240 cannabis co-users having significantly lower levels compared to tobacco only users for both  
241 inflammatory markers, respectively. In the adjusted linear regression models, there was no  
242 difference in CRP between groups, while TNF- $\alpha$  levels remained lower among co-users ( $b = -0.15$   
243 [SE=0.07],  $p=.03$ ), adjusting for age, body mass index, race/ethnicity, tobacco use group and other  
244 illicit substance use. Because the cytokine values were log-transformed prior to modeling, the  
245 geometric mean was interpreted, which indicated that co-users had approximately 14% lower TNF-  
246  $\alpha$  levels compared to tobacco only users ( $\exp[\beta] = 0.86$ ). There were no differences by use group  
247 for any of the other interleukins or MMP-8 in the unadjusted or adjusted models.

### 248 3.1.3. Psychological Measures

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

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

#### 253 **4.1. Discussion**

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

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

276 addition, a recent study found that pre- and postnatal dual exposure increased secretory  
277 Immunoglobulin-A in early childhood relative to tobacco and cannabis-only exposure (Molnar et al.,  
278 2018). The present preliminary results extend this limited literature by providing initial evidence  
279 that co-use of cannabis and tobacco increases the likelihood of maternal immune system  
280 dysregulation relative to the use of tobacco alone.

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

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

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

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

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

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

### 345 **5.1. Summary and Conclusion**

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



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

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374 **References**

- 375 Aghaeepour, N., Ganio, E. A., Mcilwain, D., Tsai, A. S., Tingle, M., Van Gassen, S., ... & Ghaemi, M. S.  
376 (2017). An immune clock of human pregnancy. *Science immunology*, 2(15), ean2946.
- 377 American College of Obstetricians and Gynecologists (ACOG). (2017). Smoking cessation during  
378 pregnancy. Committee Opinion No. 721. *Obstetrics & Gynecology* 130:e200-4.
- 379 Ashford, K., Barnett, J., McCubbin, A., Kehler, S., & Westneat, S. (2013). Prenatal Systemic Immune  
380 Response in Smoking and Nonsmoking Women. *Journal of Obstetric, Gynecologic, & Neonatal*  
381 *Nursing*, 42(s1), S82-S83.
- 382 Ashford, K., Chavan, N., Ebersole, J., Wiggins, A., Sharma, S., McCubbin, A., Barnett, J., O'Brien, J.  
383 (2017). Patterns of Systemic and Cervicovaginal Fluid Inflammatory Cytokines throughout  
384 Pregnancy. *American journal of perinatology*, 35(05), 455-462.
- 385 Ashford, K. B., Hahn, E., Hall, L., Rayens, M. K., Noland, M., & Ferguson, J. E. (2010). The Effects of  
386 Prenatal Secondhand Smoke Exposure on Preterm Birth and Neonatal Outcomes. *Journal of*  
387 *Obstetric, Gynecologic, & Neonatal Nursing*, 39(5), 525-535. doi: 10.1111/j.1552-  
388 6909.2010.01169.x
- 389 Ashford, K., O'Brien, J., McCubbin, A., Westneat, S., & Barnett, J. (2013). 15: The influence of prenatal  
390 smoking status on cervical cytokine distribution. *American Journal of Obstetrics &*  
391 *Gynecology*, 208(1), S10.
- 392 Azofeifa, A. (2016). National estimates of marijuana use and related indicators—National Survey on  
393 Drug Use and Health, United States, 2002–2014. *MMWR. Surveillance Summaries*, 65.
- 394 Berg, C. J., Stratton, E., Schauer, G. L., Lewis, M., Wang, Y., Windle, M., & Kegler, M. (2015). Perceived  
395 harm, addictiveness, and social acceptability of tobacco products and marijuana among  
396 young adults: marijuana, hookah, and electronic cigarettes win. *Substance use & misuse*,  
397 50(1), 79-89.

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

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

404 Bernert, J. T., Jr., Turner, W. E., Pirkle, J. L., Sosnoff, C. S., Akins, J. R., Waldrep, M. K., . . . Sampson, E. J.  
405 (1997). Development and validation of sensitive method for determination of serum  
406 cotinine in smokers and nonsmokers by liquid chromatography/atmospheric pressure  
407 ionization tandem mass spectrometry. *Clin Chem*, 43(12), 2281-2291.

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

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

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

414 Centers for Disease Control and Prevention CDC. February 6, 2018. Smoking During Pregnancy.  
415 Retrieved on November 21, 2018 from  
416 [http://www.cdc.gov/tobacco/basic\\_information/health\\_effects/pregnancy/index.htm](http://www.cdc.gov/tobacco/basic_information/health_effects/pregnancy/index.htm)

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

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

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

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

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

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

435 Coussons-Read, M. E., Okun, M. L., Schmitt, M. P., & Giese, S. (2005). Prenatal stress alters cytokine  
436 levels in a manner that may endanger human pregnancy. *Psychosomatic Medicine*, 67(4),  
437 625-631.

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

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

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

448 El Marroun, H., Tiemeier, H., Jaddoe, V., Hofman, A., Verhulst, F., van den Brink, W., & Huizink, A.  
449 (2010). Agreement between maternal cannabis use during pregnancy according to self-  
450 report and urinalysis in a population-based cohort: the Generation R Study. *European*  
451 *addiction research*, 17(1), 37-43.

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

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

457 Fried, P. A., & Watkinson, B. (2000). Visuo-perceptual functioning differs in 9-to 12-year olds  
458 prenatally exposed to cigarettes and marihuana. *Neurotoxicology and Teratology*, 22(1), 11-  
459 20.

460 Fried, P. A., & Watkinson, B. (2001). Differential effects on facets of attention in adolescents  
461 prenatally exposed to cigarettes and marihuana. *Neurotoxicology and teratology*, 23(5), 421-  
462 430.

463 Fried, P. A., Watkinson, B., & Gray, R. (2003). Differential effects on cognitive functioning in 13-to  
464 16-year-olds prenatally exposed to cigarettes and marihuana. *Neurotoxicology and*  
465 *teratology*, 25(4), 427-436.

466 Gibson, J., McKenzie-McHarg, K., Shakespeare, J., Price, J., & Gray, R. (2009). A systematic review of  
467 studies validating the Edinburgh Postnatal Depression Scale in antepartum and postpartum  
468 women. *Acta Psychiatrica Scandinavica*, 119(5), 350-364. doi: 10.1111/j.1600-  
469 0447.2009.01363.x

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

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

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

477 Goodwin, R. D., Pacek, L. R., Copeland, J., Moeller, S. J., Dierker, L., Weinberger, A., . . . Hasin, D. S.  
478 (2018). Trends in Daily Cannabis Use Among Cigarette Smokers: United States, 2002–2014.  
479 *American journal of public health, 108*(1), 137-142.

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

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

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

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

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

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

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

501 Kharrazi, M., DeLorenze, G. N., Kaufman, F. L., Eskenazi, B., Bernert Jr, J. T., Graham, S., ... & Pirkle, J.  
502 (2004). Environmental tobacco smoke and pregnancy outcome. *Epidemiology*, 660-670.

503 Klein, T. W., Lane, B., Newton, C. A., & Friedman, H. (2000). The Cannabinoid System and Cytokine  
504 Network (44546). *Proceedings of the Society for Experimental Biology and Medicine*, 225(1),  
505 1-8.

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

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

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

515 Lombard, C., Hegde, V. L., Nagarkatti, M., & Nagarkatti, P. S. (2011). Perinatal exposure to  $\Delta^9$ -  
516 tetrahydrocannabinol triggers profound defects in T cell differentiation and function in fetal  
517 and postnatal stages of life, including decreased responsiveness to HIV antigens. *The Journal*  
518 *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., Szekanez, 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-](http://www.pewresearch.org/fact-tank/2018/01/05/americans-support-marijuana-legalization/)  
556 [tank/2018/01/05/americans-support-marijuana-legalization/](http://www.pewresearch.org/fact-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*.

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

570 Shiono, P. H., Klebanoff, M. A., Nugent, R. P., Cotch, M. F., Wilkins, D. G., Rollins, D. E., . . . Behrman, R.  
571 E. The impact of cocaine and marijuana use on low birth weight and preterm birth: A  
572 multicenter study. *American Journal of Obstetrics & Gynecology*, 172(1), 19-27. doi:  
573 10.1016/0002-9378(95)90078-0

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

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

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

583 United Nations Office on Drugs and Crime. (2017). *World Drug Report 2017*. (ISBN: 978-92-1-  
584 148291-1, eISBN: 978-92-1-060623-3). United Nations publication Retrieved from  
585 [http://www.unodc.org/wdr2017/field/Booklet\\_5\\_NEXUS.pdf](http://www.unodc.org/wdr2017/field/Booklet_5_NEXUS.pdf).

586 van Gelder, M. M., Reefhuis, J., Caton, A. R., Werler, M. M., Druschel, C. M., & Roeleveld, N. (2010).  
587 Characteristics of pregnant illicit drug users and associations between cannabis use and  
588 perinatal outcome in a population-based study. *Drug & Alcohol Dependence*, 109(1), 243-  
589 247.

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

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

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

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

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

603 Westfall, R. E., Janssen, P. A., Lucas, P., & Capler, R. (2006). Survey of medicinal cannabis use among  
604 childbearing women: patterns of its use in pregnancy and retroactive self-assessment of its  
605 efficacy against 'morning sickness'. *Complementary Therapies in Clinical Practice*, 12(1), 27-  
606 33.

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

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

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

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

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

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

FINAL PEER REVIEWED

620 **Table 1. Sociodemographic characteristics of the study sample**

Variable	Total sample (N = 138) Mean (SD) or n (%)	Prenatal exposures		test statistic	p
		<b>Tobacco and cannabis</b> (n = 38) Mean (SD) or n (%)	<b>Tobacco only</b> (n = 100) Mean (SD) or n (%)		
Age	29.1 (5.4)	27.3 (5.0)	29.8 (5.3)	$t_{(df=136)} = 2.5$	.02
Body mass index (kg/m <sup>2</sup> )	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 <sup>a</sup>	5.9 (3.3)	5.8 (3.4)	6.0 (3.2)	$t_{(df=128)} = 0.4$	.72
Depression <sup>b</sup>	7.8 (5.8)	7.5 (6.0)	7.9 (5.8)	$t_{(df=136)} = 0.1$	.75

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

622 <sup>a</sup> Stress measured by the 4-item Perceived Stress Scale; potential scores range from 0-16, with

623 higher scores reflecting more perceived stress

624 <sup>b</sup> Depressive symptoms measured using the 10-item Edinburgh Postnatal Depression Scale;

625 potential scores range from 0-30

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

Cytokine	Prenatal exposures		Unadjusted <i>p</i> <sup>a</sup>	Adjusted <i>p</i> <sup>b</sup>
	Tobacco and Cannabis ( <i>n</i> = 34) <i>Median</i> (IQR)	Tobacco only ( <i>n</i> = 87) <i>Median</i> (IQR)		
IL 1 $\beta$ (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- $\alpha$ (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

<sup>a</sup> p-value from Mann-Whitney U test

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