Prenatal Cannabis Exposure and Infant Development
"A Tolerated Matter"

~Hanan El Marroun~

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Prenatal Cannabis Exposure and Infant Development
"A tolerated matter"

Prenatale cannabisblootstelling en de ontwikkeling van het kind
"Een gedoogde materie"

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As long as there are parents and children, the issue of health among life givers and life beginners is timeless.

In dedication to my life givers, mom and dad

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El Marroun H, Tiemeier H, Jaddoe VW, Hofman A, Mackenbach JP, Steegers EA, Verhulst FC, van den Brink W, Huizink AC. Demographic, emotional and social determinants of cannabis use in early pregnancy: The Generation R Study *Drug Alcohol Depend*. 2008;98:218-26.

Chapter 2.2
El Marroun H, Tiemeier H, Jaddoe VW, Hofman A, Verhulst FC, van den Brink W, Huizink AC. Agreement between maternal cannabis use during pregnancy according to self-report and urinalysis in a population based cohort. The Generation R Study *Submitted*

Chapter 3.1
El Marroun H, Tiemeier H, Steegers EA,, Jaddoe VW, Hofman A, Verhulst FC, van den Brink W, Huizink AC. Intrauterine cannabis exposure affects foetal growth trajectories: The Generation R Study *J Am Acad Child Adolesc Psychiatry*. 2009:48:1173-1181

Chapter 3.2
El Marroun H, Tiemeier H, Steegers EA, Roos-Hesselink J, Jaddoe VW, Hofman A, Verhulst FC, van den Brink W, Huizink AC. A prospective study on intrauterine cannabis exposure and foetal blood flow. *Submitted*

Chapter 4.1
El Marroun H, Creemers H, Tiemeier H, Steegers EA, Jaddoe VW, Hofman A, Verhulst FC, van den Brink W, Huizink AC. Parental cannabis use during pregnancy and child behaviour problems at 18 months. *Submitted*

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El Marroun H, Henrichs J, H, Tiemeier H, Steegers EA, Jaddoe VW, Raat H, Hofman A, Verhulst FC, van den Brink W, Huizink AC. Parental cannabis use during pregnancy and cognitive functioning and behavioural problems in early childhood. *Submitted*

Chapter 1

LONG-TERM CONSEQUENCES OF CANNABIS EXPOSURE IN PREGNANCY

Abstract

Since centuries, cannabis is used for recreational, spiritual and medicinal purposes. Today, cannabis is one of the most commonly used illicit substances, also among pregnant women. In the last decades, levels of Δ9-tetrahydrocannabinol in cannabis products have increased, and these higher levels contributed to our interest for investigating the effects of cannabis during pregnancy.

The study described in this thesis was embedded within the Generation R Study, a prospective cohort study from foetal life onwards in a multi-ethnic urban population. In this study, we examined the associations of maternal cannabis use during pregnancy and several offspring outcomes. In order to determine whether cannabis use affects children because of intrauterine exposure, the possible influence of confounding factors should be considered. Moreover, the direct biological effect of intrauterine exposure was addressed by comparing the strength of the associations between maternal and paternal cannabis use during pregnancy and foetal growth using ultrasound measures. Additionally, to determine whether exposure to cannabis has an intrauterine influence or not, the timing of exposure was considered as well, i.e. the comparison between maternal cannabis use only before pregnancy and during pregnancy was made.

This manuscript described the determinants of maternal cannabis use during pregnancy. Additionally, it discussed the agreement between maternal self-report of cannabis use during pregnancy and the presence of cannabis metabolites in urine. We addressed the association between maternal and paternal cannabis use and foetal growth and foetal redistribution observed using ultrasound measurements. Finally, this thesis focuses on the relation between parental cannabis use and child behavioural development and verbal and non-verbal cognitive development.

Background

Evidence of the inhalation of cannabis smoke can be found as far back as the 3rd millennium B.C., as suggested by burnt cannabis seeds found in a ritual brazier at an ancient burial site in current Romania [1]. Among the ancient Hindus of India and Nepal cannabis use was popular; they called the herb *ganjika* in Sanskrit (*ganja* in modern Indic languages) [2]. The ancient Assyrians discovered the psychoactive properties of *qunubu* (i.e. way to produce smoke) and used it in certain religious ceremonies; shamans burned cannabis flowers to induce a state of trance [3]. In the 20th century there was a considerable increase in cannabis use for recreational, spiritual and medicinal purposes. However, the possession, use, or sale of psychoactive cannabis products became illegal in most parts of the world in this time period. For example, in the USA cannabis became illegal in 1937 due to Marihuana Tax Act.

Today cannabis is one of the most commonly used illicit substances in Western countries. It is estimated that approximately 4% of the world's adult population (162 million) use cannabis yearly and 0.6% (22.5 million) on a daily basis [4]. In Europe, the 12-month prevalence of cannabis use among young adults has increased from on average 5% in 1990 to 15% in 2005 [5]. In young Australian women 12-month prevalence of cannabis use in 2000 was 24.4% [6]. In Canadian women, aged between 20-24 years, 12-month prevalence of cannabis use in 2004 was 21.8% [7]. And, among Dutch women 12-month prevalence of cannabis use was 3.1% in 2005 [8]. More specifically, a study conducted in the USA estimated that 2.8% of pregnant women used illicit drugs and that cannabis accounted for three-fourths of this illicit drug use [9]. The Dutch prevalence of cannabis use among pregnant women is unknown. In this thesis we estimated the prevalence of cannabis use in a population-based sample of pregnant women by using self-reported information and urinalysis.

Recently, the media attention for cannabis in Western countries has increased. The higher levels of Δ9-tetrahydrocannabinol (THC) [10], which may yield stronger effects, and a persistent discussion about legalization of cannabis use, in Europe as well as the U.S. and Canada, contributed to this interest. This increased level of THC in cannabis also contributed to our interest for investigating the effects of cannabis use during pregnancy.

First, little is known about specific characteristics of pregnant women that use cannabis. It is important to characterize this group, because both cannabis use and specific demographic and environmental characteristics of pregnant women that may be associated with cannabis use have been shown to affect child development. In 1992, the National Institute on Drug Abuse (NIDA) estimated that, in the United States, 2.9% of all pregnant women used cannabis at some time during pregnancy. Rates of cannabis use were higher for women who were not married, unemployed, and with lower education [11]. Additionally, previous research has shown that substance-abusing pregnant women are characterized by increased exposures to parental and partner violence, sexual abuse, and the presence of psychiatric disorders including depression or antisocial behaviour [12]. Also, recent studies have shown that ethnicity and religion may be associated with cannabis use. For instance, an epidemiological study conducted in 2007 in the US showed that non-Hispanic Whites were more likely to use extra-medical drugs than other race-ethnicity subgroups [13]. This was also found for illicit drug use, including cannabis [14]. A study conducted in the UK, set out to explore ethnic variations in the use of illicit and traditional drugs, showed that lifetime cannabis use was significantly higher amongst black Caribbean and mixed ethnicity young people [15]. The previously mentioned epidemiological study in the U.S. also showed that being non-religious increased the odds of life time cannabis use almost three-fold [13]. In this thesis, we explored how the combination of these demographic, social and emotional determinants contributes to maternal cannabis use during pregnancy in a general population sample in Rotterdam, the Netherlands.

Second, accurate identification of pregnant women who use cannabis during pregnancy could possibly reduce problems associated with cannabis use or abuse during pregnancy [16, 17]. Previous hospital-based research has demonstrated that self-reported use correlates moderately with biochemical measures of exposure to cannabis [18, 19]. Although cannabis use is not prosecuted in the Netherlands, and false negative reporting may therefore occur less frequently than in other countries, denial and thus misclassification cannot be ruled out. It is possible that pregnant women who reported non-use were actually using cannabis before or even during pregnancy and provided false, social desirable answers. Therefore, it is important to verify self-reported information on

maternal cannabis use during pregnancy by means of detecting urinary cannabis metabolites. Thus, the agreement between maternal self-reported cannabis use during pregnancy and the presence of urinary cannabis metabolites was explored in this manuscript.

Third, exposure to high THC-levels in utero may result in a health risk for the developing foetus. This suggestion is based on evidence from several animal studies, showing that administration of high doses of THC in pregnant mice and rats resulted in lower birth weight among offspring [20, 21]. Such effects can be explained by findings from other, biochemical and animal studies, which have shown that THC and its metabolites freely pass the placental barrier [22], and by entering the foetal circulation, may affect the developing foetus. Importantly, molecular research has shown that increased local action of endocannabinoids in the human placenta is present during the first trimester [23]. Although animal studies clearly suggest that intrauterine cannabis exposure is associated with foetal growth retardation [20, 21], human studies are inconclusive. The available literature on human studies is inconsistent with regard to the effects of intrauterine cannabis exposure on foetal growth. Some studies found a positive association between cannabis exposure and growth [24], another study found no association [19], while other studies reported negative associations [16, 25]. To date, results of human studies on foetal consequences of maternal cannabis use during pregnancy remain inconclusive [26, 27]. Since Generation R followed the cohort from pregnancy onwards, foetal growth trajectories could be studied by means of the ultrasound assessments. This also provided the opportunity to investigate whether cannabis use during pregnancy was associated with intrauterine growth retardation.

Furthermore, it has been shown that the endogenous cannabinoid system plays a neuromodulatory role in cardiovascular regulation [28, 29]. Endogenous cannabinoid receptor ligands show cardiovascular effects which are similar to the effects of Δ9-tetrahydrocannabinol (THC); they reduce the blood vessel tone with an associated decrease in blood pressure and increased vascular flow [28]. Prenatal exposure to cannabis in early foetal life could alter the endocannabinoid system and result in adaptations of the vascular system, including a reduction of vascular resistance and an increase in vascular flow. Evidence for effects of prenatal cannabis exposure on vascular development is sparse. However, some information is available on hemodynamic effects of prenatal

tobacco exposure [30-32]. It has been reported that nicotine exposure during pregnancy impairs the uterine vascular function, which may lead to increased vascular resistance and a decrease in uterine blood flow [33], resulting in changes of the uteroplacental circulation. Cannabis has the opposite effect on the vascular system. However, since cannabis and tobacco are used simultaneously, it is unclear which hemodynamic modifications will be present when smoking the combination of cannabis and tobacco during pregnancy. In this study, we described the effect of cannabis over and above that of tobacco on foetal redistribution measured using ultrasound measurements.

Finally, from a psychological and developmental point of view, intrauterine growth retardation may pose a risk for longer-term adverse outcomes as well, since research has demonstrated the importance of indicators of intrauterine growth, such as birth weight, body weight and head size, with regard to subsequent psychological and behavioural development [34-37]. Additionally, in-vitro studies suggest that intrauterine cannabis exposure might harm the development of the child's brain directly as well. First, the endocannabinoid system, present and functional in early prenatal periods, plays an important role in developmental processes of the central nervous system, including cell proliferation, migration and differentiation [38]. Second, intrauterine exposure may alter the expression of key genes for neural development and lead to neurotransmitter and behavioural disturbances [39]. Findings from animal studies show adverse effects of prenatal cannabis exposure on brain development by indicating permanent effects on functional regulation of motor behaviours [40], memory processes [41], and emotional reactivity [42] in offspring. In humans, the available literature reports on associations found between intrauterine cannabis exposure and offspring neurodevelopmental and behavioural problems, such as tremors and startles in newborns [17], developmental retardation according to the Bayley Scales of Infant Development in infants (age of 9 months) [43], lower intelligence measured at the age of three years [44], and more teacher-rated delinquent behaviour at the age of six and ten years [45, 46]. In this thesis, we addressed some important neurodevelopmental outcomes in toddlers, including behavioural developments measured with the Child Behavior Checklist at 18 and 36 months and verbal and non-verbal cognitive development measured with the Language Development Survey

at 30 months and the Parent Report of Children's Abilities at 30 months of age.

Methodological considerations

Until now, only two longitudinal studies addressed the effects of intrauterine cannabis exposure on growth and behavioural outcomes in human offspring [24, 47]. In these studies, rather weak associations were found between intrauterine cannabis exposure and offspring outcomes, including behavioural problems at the ages of six years [45, 48] and ten years [46, 49]. Although these studies are useful in providing more insight in the association between foetal cannabis exposure and offspring long-term outcomes, there are several issues that should be considered.

First, both previous cohort studies started more than 25 years ago. Given the increase in THC-concentrations in cannabis in the last decade, the influence of intrauterine cannabis on offspring is expected to be more pronounced in younger generations.

Second, deviation from normal child development can be best assessed using general population cohorts. Both previous studies, however, examined high-risk cohorts in terms of cannabis use, with 20 and 40% of the pregnant mothers using cannabis, compared to 2.8-4.5% in the general population [9, 50].

Third, in order to determine if cannabis use affects children because of intrauterine influences on foetal development, the influence of confounding factors that could generate non-causal links should be considered. Moreover, by comparing the strength of the associations between maternal and paternal prenatal cannabis use in relation to offspring outcome, one can test for biological effects of intrauterine exposure which should be distinguished from the psychosocial factors associated with cannabis use during pregnancy [51]. That is, if the relation of maternal exposure with offspring outcomes is much stronger than that of paternal exposure, the biological effect of *in utero* exposure is likely to play a more important role in the association on offspring outcome than the psychosocial aspects that are associated with cannabis use in the parents. So far, none of the studies used the approach in which paternal cannabis use during their partners' pregnancy and child outcome was taken into account. In addition, to determine whether exposure to cannabis has a specific intrauterine influence or not, the timing of

exposure should be considered as well, i.e. a comparison between maternal cannabis use only before pregnancy and during pregnancy should be made [51].

The Generation R Study

The study described in this thesis was embedded within the Generation R Study [52,53]. This is a prospective cohort study from foetal life onwards in a general multi-ethnic urban population. The study is designed to identify early environmental and genetic causes of normal and abnormal growth, development and health from foetal life until young adulthood. Eventually, results forthcoming from the Generation R Study may contribute to the development of strategies for optimizing health and healthcare for pregnant women and children. The Generation R Study focuses on four primary areas of research:

1. Growth and physical development
2. Behavioural and cognitive development
3. Diseases in childhood
4. Health and healthcare

The study population consists of children who form a prenatally recruited birth cohort that will be followed until young adulthood. In total, 9778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. Of all eligible children at birth, 61% participate in the study. A large part of this study cohort consists of mothers and children belonging to ethnic minorities. Data collection in the prenatal phase included physical examinations, questionnaires, foetal ultrasound examinations and biological samples. In addition, more detailed assessments are conducted in a subgroup of 1232 pregnant women and their children. At the age of 5 years, all children will be invited to visit the Generation R research centre for detailed assessments.

The aims and outline of this thesis

This thesis aims to extend existing knowledge on intrauterine cannabis exposure and foetal and infant development. The studies were conducted within the Generation R Study, which offers a unique opportunity to investigate the early and late effects of intrauterine environmental factors on growth and development. This is the first birth cohort in the Netherlands, in which children are followed from prenatal life onwards. Moreover, this large cohort provided us more recent data on maternal cannabis use, its determinants and the long-term effects on intrauterine exposure to cannabis, compared to the previous cohorts that started more than 25 years ago. The present thesis addressed the following aims (see also Figure 1.1):

1. To explore which psychosocial characteristics are associated with maternal cannabis use before and during pregnancy;
2. To assess the agreement between maternal self-report on cannabis use during pregnancy and the presence of cannabis metabolites in maternal urine;
3. To test the hypothesis that intrauterine cannabis exposure in humans has adverse effects on foetal growth trajectories and foetal circulatory redistribution;
4. To investigate the association between parental cannabis use and behavioural problems at 18 and 36 months of age;
5. To look into the relation between prenatal parental cannabis use and verbal and non-verbal cognitive development at the age of 30 months;

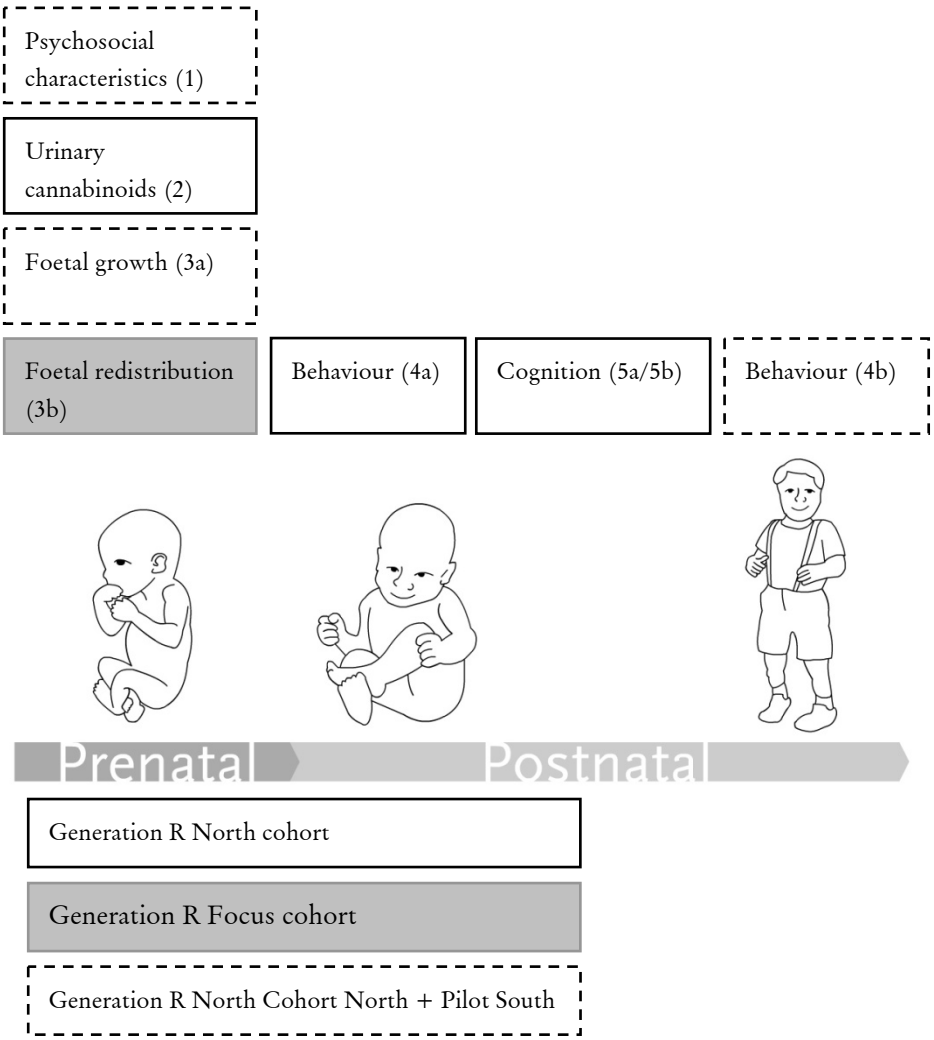

Figure 1.1 A schematic presentation of the tested relationships in this thesis

Table 1.1 shows the number of participants in the study for each specific research question. For the present thesis, data from three Generation R subsamples were used; the Generation R pilot Cohort South, the Generation R (total) cohort North and the Generation R Focus cohort. The studies described in aim 1, 3a, and 4b were conducted in the Generation R total cohort plus pilot Cohort; the studies described in aim 2, 4a, 5a and 5b were conducted in the Generation R total cohort. The study described in aim 3b was conducted within the Generation R Focus Study. In this latter subgroup of 1,232 Dutch pregnant women and their children, detailed assessments, such as Doppler ultrasound measurements in prenatal life were conducted. This subgroup was homogenous in terms of national origin to exclude confounding or effect modification by national origin.

Table 1.1 The number of participants in the study for each specific research question

	Aims	North & Pilot South	North	Focus
1.	Determinants	7610		
2.	Urinary cannabinoids		3997	
3a.	Foetal growth	7452		
3b.	Foetal redistribution			285
4a.	Behaviour 18 months		3806	
4b.	Behaviour 36 months	3630		
5a.	Verbal cognition 30 months		3086	
5b.	Non-verbal cognition 30 months		3380	

Table note: Numbers depend on available data and chosen design.

In the following chapter, chapter 2.1, we explore which demographic, emotional and social determinants are associated with maternal cannabis use before and during pregnancy. Next, in chapter 2.2, self-reported information on maternal cannabis use during pregnancy is verified by means of detecting urinary cannabis metabolites. Then, chapter 3.1 evaluates the effects of maternal cannabis use independently of maternal smoking during pregnancy on foetal growth characteristics measured with ultrasound assessments in early, mid and late pregnancy. In addition, based on ultrasound measurements in late pregnancy, foetal circulatory distribution and maternal cannabis use were examined in chapter 3.2.

After describing the effects of cannabis exposure on foetal outcomes, our interest was especially in the field of early behavioural and emotional development in children. Therefore, chapter 4.1 focuses on parental cannabis use as a predictor of infant behavioural problems at 18 months. In addition, chapter 4.2 examines the relation between parental cannabis use and infant cognitive development at 30 months of age and behavioural development at 36 months. In both chapters 3 and 4, the relation of maternal as well as paternal cannabis use on the outcome is explored. Finally, chapter 5 provides a general discussion of main findings and discusses some of the methodological aspects of the study. This thesis concludes with some implications for clinical practice and recommendations for future research.

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Chapter 2

MATERNAL CANNABIS USE DURING PREGNANCY

Contents of this chapter

This chapter describes two studies, the first study examined the determinants for cannabis use during pregnancy, and in other words, what specific group of women is likely to use cannabis while pregnant. In order to address this question, demographic, emotional and social determinants were dealt with. The second part of this chapter addressed the agreement between maternal self-reported cannabis use during pregnancy and the presence of cannabis-metabolites in urine. This is important, because reporting on cannabis use in this specific life period may be sensitive to stigma and might lead to misclassification in our study.

Chapter 2.1

PSYCHOSOCIAL DETERMINANTS OF MATERNAL CANNABIS USE DURING PREGNANCY

Abstract

The aim of this study is to ascertain demographic, emotional and social determinants of cannabis use in early pregnancy.

This study was embedded in the Generation R study, a multiethnic population-based cohort of parents and their children, followed from pregnancy to childhood in Rotterdam, The Netherlands. Mothers enrolled in pregnancy who answered questions about their own and their partners substance use before and during pregnancy (n=7610). Using self-report questionnaires, information was collected on maternal demographics, psychopathology, delinquency, childhood trauma, social stress, family functioning, and parental alcohol, tobacco and substance use. Multinomial logistic regression analysis was used, with non-using women as reference.

246 (3.2%) women used cannabis before pregnancy and 220 (2.9%) women used cannabis both before and during pregnancy. The strongest determinant for maternal cannabis use during pregnancy was cannabis use by the biological father of the child (OR=38.56; 95%CI=26.14-58.88). Maternal cannabis use during pregnancy was also independently associated with being single (OR=4.25; 95%CI=2.33-7.75) or having a partner without being married (OR=2.75; 95%CI=1.56-4.85), childhood trauma (OR=1.39; 95%CI=1.22-1.57) and delinquency (OR=3.37; 95%CI=1.90-5.98), but not with maternal age, ethnicity, psychopathology, family functioning and perceived stress. Being religious was protective (Islam: OR=0.25; 95%CI=0.09-0.65) for maternal cannabis use during pregnancy. Additionally, lower educational level determined continued cannabis use in ever-users (OR=3.22; 95%CI=1.54-6.74).

Our results showed that multiple demographic, emotional and social characteristics were associated with maternal cannabis use. These characteristics should be considered when investigating offspring exposed to cannabis in utero, as they may play an important role in mother-child interaction and child development.

Introduction

Cannabis is one of the most commonly used illicit substances in Western countries. Cannabis use represents the use of marijuana, hashish or sensimilla, which are products of different parts of the Cannabis Sativa plant. In Europe, the 12-month prevalence of cannabis use among young adults has increased from on average 5% in 1990 to 15% in 2005 [1]. In young Australian women 12-month prevalence of cannabis use was 24.4% [2]. In addition, in Canadian women, aged between 20-24 years 12-month prevalence of cannabis use was 21.8% [3]. More specifically, a study conducted in the USA estimated that 2.8% of pregnant women used illicit drugs and that marijuana accounted for three-fourths of this illicit drug use [4].

Little is known about the prevalence of cannabis use in pregnant women and about specific characteristics of pregnant cannabis-using women. It is important to characterize this group, because both cannabis use and specific demographic and environmental characteristics of pregnant women that may be associated with cannabis use have been shown to affect child development. For example, animal and human studies have reported associations between in utero cannabis exposure and reduced birth weight in offspring [5-8]. Moreover, literature suggests poorer neonatal outcome [9,10] and poorer cognitive performance in offspring exposed to cannabis in utero [11]. This may be due to certain underlying demographic characteristics of these women, since for instance low socio-economic status is also related with poor health, and poor cognitive and socio-emotional development [12]. Ignoring such characteristics may lead to spurious associations between prenatal cannabis exposure and child development.

In 1992, the National Institute on Drug Abuse estimated that in 2.9% of all pregnant women marijuana was used at some time during pregnancy. Rates of marijuana use were higher for women who were not married, unemployed, and lower educated [13]. Additionally, previous research has shown that substance-abusing pregnant women are characterized by increased exposures to parental and partner violence, sexual abuse, and the presence of psychiatric disorders including depression or antisocial behaviour [14]. Also, recent studies have shown that ethnicity and religion may be associated with cannabis use. For instance, a recent epidemiological study of Degenhardt et al. (2007)

conducted in the US, showed that non-Hispanic Whites were more likely to use extra-medical drugs than other race-ethnicity subgroups [15]. This was also found for illicit drug use, including cannabis, in a study of Sunder et al. (2007) [16]. A study conducted in the UK, set out to explore ethnic variations in the use of illicit and traditional drugs, showed that lifetime cannabis use was significantly higher amongst black Caribbean and mixed ethnicity young people [17]. Since our study was conducted in a urban population-cohort, including several ethnic subgroups, we therefore explored ethnicity as correlate of cannabis use in pregnant women. The study of Degenhardt et al. furthermore showed that being non-religious increased the odds of life time cannabis use almost three-fold [15].

The Generation R study [18,19] follows a population-based urban cohort of parents and their newborn children from early pregnancy to adolescence. This study allowed us to examine the psychosocial characteristics of pregnant cannabis-using women. Specifically, we focused on demographic and lifestyle characteristics such as religion, ethnicity, socio-economic status, emotional problems, family functioning, childhood memories and social stress. In this study, we were able to compare three groups of pregnant women: (1) those who never used cannabis; (2) those who used cannabis only prior to pregnancy and; (3) those who used cannabis both before and during pregnancy.

Methods

Setting and population

The present study is part of an ongoing population-based cohort; the Generation R Study [18], set up to collect data on a sample of urban parents and their newborn children from early pregnancy to adolescence. The study design has been described in detail previously [18]. Briefly, all pregnant women who were resident in Rotterdam at the time of their delivery and whose delivery data was between April 2002 until January 2006 were invited to participate. In total n=9,778 (response rate 61%) mothers were enrolled in Generation R (n=8,880 during pregnancy and n=898 at birth of their child). 71% of the partners were enrolled during pregnancy (n=6,347). The study has been approved by the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam. Written

informed consent was obtained from all participants. For the present analyses mothers enrolled during pregnancy (n=8,880) were eligible. Mothers without information on first trimester substance use were excluded from the present study (14.3%, n=1,270). Mothers using other substances including amphetamines, cocaine or heroin, without using cannabis were excluded from the analyses (0.9%, n=79). In the current analysis 7,531 mothers were included.

Measures

Tobacco, alcohol and substance use were measured using a self-report questionnaire in the first trimester of pregnancy. Participants reported information on timing and frequency of use. A distinction was made between the use of cannabis (marijuana and hashish) and the use of other illicit drugs (cocaine, amphetamines and heroin). We explicitly asked in two separate questions whether pregnant women used drugs before pregnancy, and whether they had used any of these substances in the last three months. In the latter question the answer options were: 'No', 'Yes, until I knew I was pregnant' and 'Yes, I still use substances'. The period of last three months was chosen, because enrolment of participants was aimed at early pregnancy. Mothers were asked about their own substance use and about substance use of the biological father. In addition, participating partners reported on their own substance use.

Demographic information such as age, ethnicity, education, religion, marital status and paternal cannabis use was assessed using self-report. Income and obstetric information (gravidity, parity and planned pregnancy) were based on self-report. Ethnicity of the parents was defined according to the classification of Statistics Netherlands [20,21]. Educational level was categorized in three levels: primary (no or primary education), secondary (lower and intermediate vocational training), and higher education (higher vocational education, and university). Net income was categorized as having an income less than €800,- , between € 800-2000,- and more than € 2000,- a month. The variable religion consisted five categories; Christian, Hindustan, Islamic, having another religion and no religion. Marital status consisted of three categories; being married, having a partner without marriage, and being single. Finally, paternal cannabis use, i.e. Cannabis use by the biological father was regarded as a demographic determinant in the current study as well.

Psychopathology was assessed using the Brief Symptom Inventory (BSI), a validated 53-item (5-point scale) self-report symptom inventory outlined to ascertain the psychological state of individuals [22]. This inventory has a number of dimensions including somatisation, obsessive-compulsivity, depression, (phobic) anxiety, paranoid ideation and psychoticism. The Global Severity Index (GSI) is obtained by dividing the sum of the total items scores by the number of completed items. The internal consistency of the GSI in this sample was high (α=0.96).

The 34-items short version of the Childhood Trauma Questionnaire (CTQ) is a retrospective self-report measure of the frequency and severity of neglect and abuse in childhood/adolescence. Validation ascertained five clinical scales: 1. Physical neglect; 2. Emotional neglect; 3. Physical abuse; 4. Emotional abuse; 5. Sexual abuse [23]. We used the weighted sum score according to Bernstein [23], which had an internal consistency of α=0.92 in this sample. Perceived parental rearing was assessed by the short form of the 'Own memories on parenting questionnaire' (EMBU) [24,25].

The s-EMBU is a validated 23-item inventory and the items are answered for each parent separately [25,26]. Three scales were obtained: 1. Emotional Warmth; 2. Rejection; 3. Overprotection/control attempts [26]. In this sample the internal consistencies for the maternal scales, were α=0.87, α=0.83, α=0.74, respectively. Questions about history of addiction (addicted to alcohol, sleeping medication, tranquillisers, cannabis, other illicit drugs, and gambling) were included using part of the TRAILS Family History Interview [27]. In this review, the concept of addiction was explained with a description of the main DSM-IV criteria, after which information on lifetime and last-year occurrence, professional treatment and medication use was collected. Information on stress was obtained by a sum score of an adjusted version of the Dutch long-lasting difficulties (LLD) list [28]. This list contains 16 items, which addresses problem situations in the preceding year. Women reported whether they have had difficulties with family members, friends, people from the neighbourhood, difficulties at school/work, and reported whether sexual, financial or housing problems had occurred. The internal consistency of LLD in this sample was α=0.89. Family functioning was measured with General Functioning subscale (GF) of the Family Assessment Device (FAD) [29]. GF is a validated measure of well-being and/or pathology of the family situation. Half of the items described healthy functioning, and

the other half defined unhealthy functioning. The internal consistency of GF in this sample was $\alpha=0.90$. Finally, previous delinquent behaviour was assessed using two items: having ever been arrested, or having a criminal record. If at least one of these items was positive, the woman was classified to have a delinquent past.

Statistical Analyses

First, women were categorized in three groups based on their substance use in early pregnancy: 1. no cannabis use; 2. cannabis use only before pregnancy; 3. cannabis use before and during pregnancy. Second, we examined descriptive data of all groups; chi-square for proportions, and analysis of variance (ANOVA) for continuous data were used to determine differences between groups. Multinomial logistic regression models were used as main method of analysis to compare more than two groups at once. The preliminary multinomial logistic models tested the strength and significance of each potential predictor; crude Odds Ratios (OR) with 95% Confidence Intervals (CI) were computed by exponentiation of the logit coefficients. The full model included social determinants, including long-lasting difficulties, family functioning and delinquency, emotional determinants, including psychopathology, childhood trauma and perceived upbringing, as well as demographic determinants, such as education, ethnicity, religion, marital status, cannabis use of the partner and maternal age. In the backwards stepwise selection approach any non-significant variable was dropped one by one ($p>0.10$). Thus, the final model included only significant predictors.

Not all determinants were available for each participant; 2927 participants missed information on one or more variables. The proportion of missing data for all determinants ranged between 0% and 27.9%. We therefore performed multiple imputation of missing data on the determinants [30-32] and generated 10 imputed datasets to achieve a relative efficiency of at least 0.97 with these proportions of missing values [33]. The Markov Chain Monte Carlo method in Proc MI as implemented in SAS version 9.1 was used [34]. Variables with skewed distributions were transformed to meet the procedure's assumption of a multivariate normal distribution of the data. For categorical variables, dummy variables were made and included in the imputation process. Dichotomous variables were not rounded after imputation to avoid the introduction of bias [35]. For the continuous measures standard deviation

scores were used (with the exception of the variable maternal age). After fitting multinomial logistic regression models, we used the Proc MIANALYZE in SAS version 9.1 to combine the parameter estimates generated from each of the 10 imputed datasets and to incorporate between- and within-imputation variance to obtain adjusted standard errors and 95% confidence intervals. Statistical Package for Social Sciences (SPSS) version 15.0 for Windows and SAS version 9.1 were used for data analysis.

Non-response analyses

Analysis of missing data on maternal substance use showed that women without information were younger (n=1270; 29.1±5.5 yrs) than subjects with information (n=7610; 29.7±5.3 yrs), ($F(1,8878)=13.73$, $p<0.001$), were less educated (15.7% primary education vs. 11.3%; $\chi^2=11.77$, df=2, $p<0.001$), had lower incomes (49.4% higher income vs. 62.1%; $\chi^2=54.06$, df=2, $p<0.01$), were more religious (48.3% religious vs. 39.1%; $\chi^2=22.56$, df=1, $p<0.001$) and less often married (21% married vs. 48%; $\chi^2=4.99$, df=1, $p<0.05$).

Results

In our sample, 246 (3.2%) women used cannabis before pregnancy and 220 (2.9%) women used cannabis both before and during early pregnancy. Of these 220 women, only 43 (0.6%) continued using cannabis throughout pregnancy. Maternal report of cannabis use by the father was highly correlated to partner self-report ($r=.813$, $p<0.005$).

Table 2.1.1 shows that women using cannabis were younger ($F(1,7528)=56.81$, $p<0.001$), less educated ($\chi^2=92.02$, df=4, $p<0.001$) and had a lower income ($\chi^2=111.66$, df=4, $p<0.001$) compared to pregnant women who never used cannabis. It also demonstrates that cannabis-using women were more likely to be Surinamese or from Cape Verde, and less likely to be Moroccan or Turkish ($\chi^2=96.68$, df=14, $p<0.001$). Furthermore, cannabis-using women were more often unmarried or single ($\chi^2=387.92$, df=4, $p<0.001$), and reported more often that the biological father of their child used cannabis too ($\chi^2=915.21$, df=2, $p<0.001$). Women using cannabis during pregnancy were at higher risk for an unplanned pregnancy ($\chi^2=94.19$, df=1, $p<0.001$) and were less likely to be pregnant for the first time ($\chi^2=5.18$,

df=1, p<0.05). Finally, foetuses of women who use cannabis during pregnancy are also exposed more often to alcohol (68%), tobacco (85%) and sometimes other substances (10%) in early pregnancy.

When comparing women using cannabis during pregnancy to women using only before pregnancy, we found that the former group was less educated (χ^2=11.15 df=1, p<0.001), less often married (χ^2=5.87 df=1, p<0.05) and had lower incomes (χ^2=5.39 df=1, p<0.05). In addition, these women used tobacco during pregnancy more often (χ^2=12.49 df=1, p<0. 001).

Additional analysis showed that women with a history of cannabis addiction (n=85) were 2.77 times more likely to continue cannabis use during pregnancy (OR=2.77; 95%CI=1.61-1.77; p<0.001) as compared to women without a history of cannabis addiction (n=5,730).

Table 2.1.2 shows that women using cannabis during pregnancy were frequent users (80% used daily or weekly), whereas women using cannabis only before pregnancy were more likely to use cannabis on a monthly basis (χ^2=38.51, df=2, p<0.001). It also displays that during pregnancy hashish is used less often (χ^2=155.88, df=2, p<0.001), while before pregnancy marijuana is consumed just as often as hashish. Unfortunately, we could not further divide the group of cannabis users into smaller subgroups reflecting the intensity of cannabis use, due to too small numbers.

Table 2.1.1 Maternal demographic information

	No exposure (n=7065)		Before pregnancy (n=246)		During pregnancy (n=220)	
Gestational duration at enrolment						
Mean ± sd	15.5	± 4.2	15.8	± 4.4	14.9	± 4.0
Age						
Mean ± sd	29.9	± 5.2	27.6	± 5.9***	26.8	± 5.8***
Gravidity, parity and planning %						
Nulliparous	55.3		74.8	***	72.9	***
Primigravidas	42.9		51.6	***	50.9	*
Unplanned pregnancy	26.8		44.3	***	55.5	***
Education %						
Primary education	11.0		10.2		21.7	***
Secondary education	45.9		58.6	***	59.9	***
Higher education	43.1		31.1	***	18.4	***
Household income %						
< € 800, -	7.8		16.1	***	26.3	***
€ 800 – 2000 ,-	28.8		34.4		44.2	***
> € 2000,-	63.5		49.5	***	29.5	***
Ethnicity %						
Dutch	49.1		55.3	*	50.7	
Cape Verdean	3.7		4.9		10.0	***
Moroccan	6.9		1.6	***	0.9	***
Dutch Antillean	3.5		4.9		4.1	
Surinamese	8.8		12.2		17.4	***
Turkish	9.6		2.0	***	1.8	***
Other Western	8.8		10.2		8.7	
Other Non-western	9.6		8.9		6.4	
Religion %						
Not religious	59.3		82.6	***	81.9	***
Christian	19.6		11.6	**	10.3	**
Hinduism	2.4		1.1		0.6	
Islam	15.5		3.2	***	4.5	***
Other religion	3.2		1.6		2.6	
Marital status %						
Married	51.3		14.6	***	7.8	***
Having a partner	35.6		54.9	***	44.2	**
Being single	13.1		30.5	***	47.9	***

Table 2.1.1 Continued

	No exposure (n=7065)	Before pregnancy (n=246)		During pregnancy (n=220)	
Paternal cannabis use					
Maternal report	6.5	63.0	***	78.8	***
Alcohol use %					
None	34.5	9.1	***	12.4	***
Before	23.9	35.1	***	18.9	
During stopped	25.7	34.6	***	48.8	***
During continued	15.8	21.8		19.8	
Tobacco use %					
None	62.8	13.7	***	4.1	***
Before	16.3	32.4	***	10.1	*
During stopped	10.8	22.8	***	31.3	***
During continued	10.1	31.1	***	54.4	***
Other substances use %					
None	100.0	75.2	***	75.9	***
Before	-	22.8		14.1	
During stopped	-	2.0		9.5	
During continued	-	-		0.4	

Table note: Values are means ± SD for continuous variables and percentages for categorical variables. ANOVA for continuous variables and χ^2 tests for categorical variables was used with 'no cannabis exposure' as reference group
**** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$*

Table 2.1.2 Maternal self-report of frequency of marijuana and hashish use before and during pregnancy

	Cannabis use before pregnancy (n=246)	Cannabis use during pregnancy (n=220)
Frequency of use before pregnancy %		
Daily	30.9	49.1 [a] ***
Weekly	26.0	30.9 [a]
Monthly	39.8	14.1 [a] ***
Unknown	3.3	5.9 [a]
Frequency of use during pregnancy %		
Daily	n.a.	31.8 [b] ***
Weekly	n.a.	33.6 [b]
Monthly	n.a.	16.4 [b] ***
Unknown	n.a.	18.2 [b]
What was used before pregnancy %		
Marijuana	54.1	49.5 [a]
Hash	21.1	19.1 [a]
Both	24.8	26.8 [a]
Unknown	0.0	4.5 [a]
What was used during pregnancy %		
Marijuana	n.a.	60.5 [b] ***
Hash	n.a.	39.5 [b] ***
Both	n.a.	0.0 [b]
Unknown	n.a.	0.0 [b]

*Table note: Values are percentages; for categorical variables χ^2 tests were used. [a]Comparison of use in women using cannabis before pregnancy with women using during pregnancy. [b]Comparison of use before and during pregnancy in women using cannabis during pregnancy*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ n.a. not applicable*

Crude bivariate analysis shows that among pregnant women age, ethnicity, education, religion, marital status, cannabis use of the biological father, psychopathology, childhood trauma, childhood upbringing, long lasting difficulties, family functioning and delinquency were all significantly associated with cannabis use before or during pregnancy (Table 2.1.3). The unadjusted associations of the determinants with cannabis use before or during pregnancy of the imputed and non-imputed dataset were compared, and these associations were similar. We, therefore, only report results based on the imputed data. In the final model CTQ was used at the expense of the EMBU subscales, because the CTQ and the EMBU subscales were highly correlated ($r \approx 0.65$) and the CTQ showed a stronger association with the outcome (Table 2.1.3).

Backward selection in the multivariate model retained several significant independent determinants of cannabis use during pregnancy. Cannabis use of the biological father was the strongest predictor (OR=38.56; 95%CI=26.14-58.88; $p<0.001$). Significant demographic, social and emotional predictors were: being single (OR=4.25; 95%CI=2.33-7.75; $p<0.001$) or having a partner without marriage (OR=2.75; 95%CI=1.56-4.85; $p<0.001$), childhood trauma (OR=1.39; 95%CI=1.22-1.57; $p<0.001$), and delinquency (OR=3.37; 95%CI=1.90-5.98; $p<0.001$). Being Islamic (OR=0.25; 95%CI=0.09-0.65; $p<0.05$) was found to be protective against cannabis use. For cannabis use before pregnancy, the same independent predictors were found with somewhat lower Odds Ratios. However, being Christian (OR=0.56; 95%CI=0.35-0.91; $p<0.05$), Hindu (OR=0.22; 95%CI=0.05-0.96; $p<0.05$) or having another religion (OR=0.25; 95%CI=0.08-0.73; $p<0.05$) and maternal age (OR=0.97; 95%CI=0.94-0.99; $p<0.05$) were protective determinants for cannabis use before pregnancy (Table 2.1.3).

Finally, we examined which determinants predicted whether ever-users continued using cannabis during pregnancy. Interestingly, this analysis showed that determinants for continued use of cannabis in pregnancy were lower educational level (OR=3.22; 95%CI=1.54-6.74; $p<0.01$), being single (OR=2.55 95%CI=1.25-5.22; $p<0.01$), and cannabis use of the biological father (OR=2.28; 95%CI=1.65-4.07; $p<0.001$).

Table 2.1.3 Associations between maternal cannabis use habits in early pregnancy and demographic, social and emotional determinants

	Before pregnancy (n=246)		During pregnancy (n=220)	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI) a	Unadjusted OR (95% CI)	Adjusted OR (95% CI) a
Demographic predictors				
Maternal age	0.92 (0.90 – 0.94) ***	0.97 (0.94 – 0.99) *	0.90 (0.88 - 0.92) ***	0.98 (0.95 – 1.01)
Ethnicity				
Dutch	1.0	1.0	1.0	1.0
Cape Verde	1.15 (0.63 – 2.10)	n.s.	2.58 (1.60 – 4.15) ***	n.s.
Moroccan	0.21 (0.08 – 0.57) **	n.s.	0.13 (0.03 – 0.51) **	n.s.
Antillean	1.21 (0.66 – 2.22)	n.s.	1.09 (0.54 – 2.18)	n.s.
Surinamese	1.22 (0.82 – 1.83)	n.s.	1.88 (1.28 – 2.74) ***	n.s.
Turkish	0.19 (0.08 – 0.46) ***	n.s.	0.19 (0.07 – 0.50) ***	n.s.
Other Western	1.03 (0.92 – 1.59)	n.s.	0.95 (0.58 – 1.57)	n.s.
Other Non-Western	0.82 (0.52 – 1.30)	n.s.	0.63 (0.36 –1.10)	n.s.
Education				
Primary education	1.21 (0.76-1.92)	n.s.	4.35 (2.85-6.64) ***	n.s.
Secondary education	1.71 (1.29-2.27) ***	n.s.	2.84 (2.00-4.03) ***	n.s.
Higher education	1.0	1.0	1.0	1.0
Religion				
Not religious	1.0	1.0	1.0	1.0
Christian	0.47 (0.29 – 0.78) **	0.56 (0.35 – 0.91) *	0.49 (0.28 – 0.85) **	0.53 (0.27 – 1.03)
Hinduism	0.21 (0.05 –0.94) *	0.22 (0.05 – 0.96) *	0.21 (0.05 –0.99) *	0.21 (0.04 – 1.08)
Islam	0.13 (0.06 – 0.28) ***	0.23 (0.10 – 0.53) ***	0.13 (0.05 – 0.27) ***	0.25 (0.09 – 0.65) ***
Other religion	0.24 (0.06 – 0.90) *	0.25 (0.08 – 0.73) *	0.43 (0.12 – 1.49)	0.33 (0.09 – 1.16)

Table 2.1.3 Continued

	Before pregnancy (n=246)				During pregnancy (n=220)			
	Unadjusted OR (95% CI)		Adjusted OR (95% CI) a		Unadjusted OR (95% CI)		Adjusted OR (95% CI) a	
Marital status								
Married	1.0		1.0		1.0		1.0	
Having a partner	5.39 (3.73 – 7.81)	***	2.19 (1.45 – 3.32)	***	7.90 (4.74 –13.18)	***	2.75 (1.56 – 4.85)	***
Being single	8.11 (5.42 – 12.13)	***	1.79 (1.09 – 2.92)	*	23.50 (14.12 – 39.06)	***	4.25 (2.33 – 7.75)	***
Cannabis use of biological father								
	25.70 (19.30 – 34.23)	***	16.37 (11.92 – 22.47)	***	66.51 (46.28 – 95.57)	***	38.56 (11.92 – 58.88)	***
Emotional predictors								
General symptoms, sds	1.24 (1.10 – 1.38)	***	n.s.		1.41 (1.27 – 1.55)	***	n.s.	
Childhood trauma, sds	1.54 (1.38 – 1.73)	***	1.40 (1.22 – 1.61)	***	1.61 (1.46 – 1.77)	***	1.39 (1.22 – 1.57)	***
Childhood upbringing [b]								
Emotional warmth, sds	0.85 (0.71 – 1.03)		-		0.81 (0.70 – 1.93)	**	-	
Overprotection, sds	1.04 (0.87 – 1.25)		-		0.96 (0.82 – 1.13)		-	
Rejection, sds	1.41 (1.26 – 1.57)	***	-		1.41 (1.24 – 1.58)	***	-	
Social predictors								
Lasting difficulties, sds	1.36 (1.21 – 1.53)	***	n.s.		1.52 (1.30 – 1.77)	***	n.s.	
Family functioning, sds	1.25 (1.09 – 1.44)	***	n.s.		1.41 (1.18 –1.67)	***	n.s.	
Delinquency	7.00 (4.72 – 10.37)	***	2.95 (1.85 – 4.68)	***	9.43 (6.13 – 14.49)	***	3.37 (1.90 – 5.98)	***

*Table note: Models were constructed using multinomial logistic regression with backward selection; non significant (n.s.) predictors were dropped out the model; Values are regression coefficients (OR) and 95% confidence intervals (95% CI) relative to the non-using group. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; [a]Adjusted=final model; [b]not included in full mode; [c] category is used as reference; sds: standard deviation score*

Discussion

The aim of the present study was to determine demographic, emotional and social determinants associated with cannabis use in early pregnancy. To our knowledge, this is the first study that examined these characteristics in a urban population-based sample of pregnant women, including a group that used cannabis. The results demonstrate that cannabis use during pregnancy is associated with several characteristics that may by themselves also influence foetal growth and offspring outcomes.

The strongest predictor for cannabis use before and during pregnancy was cannabis use by the biological father. Positive correlations between spouses for cannabis use, abuse and dependence are well known [36]. We further found that childhood maltreatment and delinquent behaviour of pregnant women to be significant independent predictors for using cannabis before and during pregnancy as well. Previous research reported that childhood maltreatment is related to cannabis use [37] and it is known that substance use increases the risk of committing antisocial, aggressive and delinquent acts [38]. Additionally, lower educational level is an important determinant for continued cannabis use in ever-users. Finally, women using cannabis were more likely to be single. This is in line with the MHPCD marijuana cohort, in which 71% of the cannabis-using women were single at the beginning of pregnancy and only 4% were married [39]. In addition, it has previously been reported that being married is a protective factor for cannabis use or substance use in young women [2]. Such a protective factor was found in the current study as well. Being religious is a significant protective factor for cannabis use before or during pregnancy. This may result from most religions' disapproval of psychotropic substances use [40,41]. Finally, based on the existing literature we expected that long-lasting difficulties (social stress), psychological difficulties and unhealthy family functioning would be significant independent predictors of cannabis use in pregnancy [42-44]. In the final model, however, these predictors no longer reached significance. A plausible explanation for the non-significance of psychopathology in the full model could be that "childhood trauma" accounted for the effect of psychopathology. Importantly, it seems that cannabis use during pregnancy might reflect addictive behaviour. This idea is supported by

two findings. First, women with a history of cannabis dependence continued using cannabis during pregnancy more often and second, women using cannabis during pregnancy were more likely to be frequent users.

Our results are important when investigating the relationship between prenatal cannabis exposure and offspring outcomes. It is plausible that the previously reported associations between cannabis exposure in utero and offspring outcomes are at least partially explained by the cumulative effect of pre-existing prenatal adverse family environment that is likely to persist after childbirth. However, strong correlations between cannabis use and other prenatal adverse situations will make it difficult to disentangle the effect of these determinants and to establish the independent contribution of prenatal cannabis exposure on the child's development. Likewise, the vast majority of cannabis-using pregnant women also used tobacco, which complicates the study of cannabis exposure effects on offspring even more. A possible way to solve this issue is to compare children of pregnant cannabis-using women with children exposed to prenatal tobacco use.

Our findings have important implications for clinicians and public health workers. First, they facilitate the recognition of women at risk for using cannabis in pregnancy. This could improve education and prevention of cannabis use in pregnant women, which could start even before pregnancy, because often, pregnancy was unplanned in cannabis users. Preconception and prenatal care directed at young women with a partner that uses cannabis and at women with a history of emotional and social difficulties might be an efficient approach to reduce the exposure in utero to cannabis, as well as to tobacco and alcohol. An important strategy for preconception and prenatal care should be to provide comprehensible education about cannabis and its effects on the unborn child, because future mothers might not be aware of the fact that cannabis can affect their child by passing the placental barrier and by breast-feeding milk. Yet, it should be noted that women in our study who used cannabis in early pregnancy already tried to reduce the risk of exposing their child to cannabis by changing their use of hashish (more potent) to marijuana (less potent) and by using cannabis less often than before pregnancy. Nonetheless, current THC-levels in marijuana are rather high and can pass through the placenta.

The strengths of this study are the large population-based sample of pregnant women, the multiethnic composition of the cohort, and the information on numerous potential confounding variables. However, the study also has some limitations. First, we used only self-reported data on substance use. Although cannabis use is not prosecuted in the Netherlands and false-negative reporting may therefore occur less frequently than in other countries, some residual denial cannot be ruled out. Second, the proportion of missing data on substance use (14.3%) is relatively high. Particularly, since women without information about substance use are younger, lower educated and have less income than women with information, they may have been at higher risk for using cannabis. However, in large populations it is sensible to use self-reported information on prenatal drug exposure, because urine toxicology is expensive and timeframe-specific; similarly hair examination is limited, because cannabis incorporation depends on hair growth rate, anatomical region, age, gender, ethnicity, hair colour and individual variability [45]. Third, in total 61% of all eligible women participated in the Generation R study [18] and they may not be completely representative of the general Rotterdam population. This may have led to an unfavourable selection of non-using women, as our study population is somewhat higher educated as compared to the general Rotterdam population [46], which perhaps has provided us a lower prevalence of cannabis use. However, this selection bias does not necessarily mean that the relationship between the predictors and cannabis use in pregnancy presented in this study is distorted. A final limitation is that not all participants had complete information on every determinant and therefore we had to impute the missing information. The multiple imputation method resulted in similar estimates in the univariate regression analyses compared to the estimates in the non-imputed dataset; therefore, we assume that the imputation process estimated the data correctly.

In conclusion, our findings confirm the numerous demographic, social and emotional determinants associated with cannabis use among young pregnant women in urban environments. These characteristics should be considered when examining the effects of prenatal cannabis exposure on offspring outcomes.

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Chapter 2.2

AGREEMENT BETWEEN MATERNAL SELF - REPORT AND URINARY SCREENS

Abstract

It is important to verify self-reported information on drug use during pregnancy, because reporting in this specific period is sensitive to stigma and might lead to misclassification.

Using semi-quantitative immunochemical analysis, the presence of the main urinary cannabinoid metabolite (11-nor-Δ9-THC-9-COOH) was compared to self-reported cannabis use during pregnancy. Sensitivity and specificity for self-report and urinalysis outcomes were calculated and Yule's Y was used as an agreement measure.

Urine samples were available for 3997 pregnant women. Of these 3997 women, 92 reported having used cannabis during pregnancy (2.3%) and 71 had positive urine screens (1.8 %). In total 33 (35%) of the 92 women with self-reported cannabis use also had a positive urine screen. Positive urines were relatively frequent in women reporting cannabis use before pregnancy only (7.6%) and in women with missing self-reported information (2.6%). Sensitivity and specificity of urinalysis compared to self-report were 0.46 and 0.98. Sensitivity and specificity of self-report compared to urinalysis were 0.36 and 0.99. Yule's Y amounted to 0.77, indicating substantial agreement between self-report and urinalysis.

Our findings illustrate the difficulties in obtaining valid information on prenatal cannabis use. Nonetheless, self-report seems an acceptable single method to determine cannabis use during pregnancy in epidemiological studies. In order to improve the quality of cannabis use data, we suggest a two-step approach starting with self-report, followed by urinalysis in women reporting cannabis use before pregnancy and in women who refuse to answer questions about substance use during pregnancy.

Introduction

Cannabis is the most commonly used illicit substance in Western countries. In Europe, the 12-month prevalence of cannabis use among young adults increased from an average of 5% in 1990 to 15% in 2005 [1]. In young Australian women 12-month prevalence of cannabis use was 24.4% [2], while in Canadian women, aged 20-24 years, 12-month prevalence of cannabis use was 21.8% [3]. In pregnant women, prevalences of cannabis use were much lower, i.e. in 2007 2.9% of Dutch pregnant women and 1.8% of American pregnant women reported cannabis use during pregnancy [4,5].

In epidemiological studies, researchers often use different methods to assess substance use, such as biochemical measures, self-reports and/or reports from other informants, like family members. Each of the different methods has its advantages and disadvantages. Biochemical measures provide clear information on recent substance use, yet are restricted by error rates, brief detection time periods and high costs. Self-reports are less invasive and permit the evaluation of substance use over longer periods in time, though are influenced by possible reporter social desirability and forgetfulness. Methods that are frequently used to assess self-reported information on substance use are quantity-frequency measures (participants report the frequency and/or quantity of substance use over a specific time period) and calendar methods (participants also report substance over a specific time while specific cues and reminders are used to enhance recall) [6]. An example of a frequently used calendar method is the Timeline Follow Back Interview (TLFB), which was developed in order to gather information in a more detailed and precise manner than is done using the conventional quantity/frequency index [7]. This method was initially designed to collect information on drinking behaviour in alcoholics, and has been modified for gathering information on substance use as well [8-10]. While employing the TLFB method in a clinical population of adolescents, Godley and colleagues reported self-report (last-month) to be in high agreement with on-site urine testing ($\kappa > 0.75$) [11].

Although multiple studies on consistency and validity of multiple assessment methods among adults and adolescents have been reported, [12], little information is available on the agreement between self-reported cannabis use and urinalysis in pregnancy. Accurate identification of

pregnant women using cannabis during pregnancy, followed by medical and or behavioural interventions to improve obstetrical and neonatal outcomes, could possibly reduce problems associated with prenatal cannabis use or abuse [13,14]. Previous hospital-based research has demonstrated that self-reported use correlates moderately with biochemical measures of exposure to cannabis. Among pregnant women participating in a large multi-centre study (n=585) in whom cannabis metabolites in their blood serum was found, 69.2% (n=405) denied smoking cannabis, and only 43.2% (n=180) of the women reporting cannabis use also had positive serum assay results [15]. Another study among women who were in the early stages of pregnancy (weeks of gestation <22 weeks) showed that, of those who indicated using of cannabis in the past, 23.9% had positive urine assays. Among those reporting never having used cannabis, 5.7% had positive urine screens and among those reporting current use, 86.6% had positive urine findings [16].

In a previous study of a population-based cohort in the Netherlands, we based maternal cannabis use during pregnancy on self-reported information collected using a questionnaire in the first trimester of pregnancy [4]. Although self-report is a commonly used measure to assess substance use, it is acknowledged that individuals may underreport or deny substance use, especially during pregnancy [17]. Cannabis use is not prosecuted in the Netherlands, and false negative reporting may therefore occur less frequently than in other countries, however, denial and thus misclassification cannot be ruled out. Factors that may lead pregnant women to underreport substance use could possibly be social desirability, forgetfulness, perceived norms about acceptability of substance use and fear of consequences such as intervention of child services. Therefore, it is important to verify self-reported information on maternal cannabis use during pregnancy by means of detecting urinary cannabis metabolites. Moreover, we were particularly interested in exploring whether or not missing information on self-reported cannabis use was related to an increased risk of positive urine screens. In this study, the focus is on cannabis use exclusively, as in a general population-based cohort the prevalence of other illicit drug use during pregnancy (e.g. cocaine, amphetamines) is expected to be very low. In addition, urinary detection time of other substances (e.g. cocaine assesses 1-3

days) is much shorter as compared to detection time of cannabis biomarkers (10-14 days at a level of 50 μg/l).

In the current study, self-reported information on cannabis use during pregnancy was compared to the presence of the cannabis metabolite 11-nor-Δ9-THC-9-COOH using semi-quantitative immunochemical urinalysis. This metabolite is not psychoactive itself, but has a long half-life in the body of up to several days or even weeks, depending on the frequency of use, making it a suitable marker in urine for recent cannabis use [18]. Although available data suggest that self-reported cannabis use correlates only moderately with biochemical measures, we hypothesize substantial agreement between these measures as cannabis use is not prosecuted in the Netherlands [19]. Our hypotheses were as follows:

1. When women reported having used cannabis during pregnancy, we assume that this information is valid and therefore that we will find a high proportion of positive urine screens;
2. When women reported having used cannabis prior to pregnancy only, we will find a substantial proportion with positive urine screens for cannabis during pregnancy;
3. When women reported never having used cannabis, we assume that the possibility of finding a relatively small percentage of positive urine screens exists; and
4. When women specifically did not answer the question about substance use, we expect to find an intermediate proportion of positive urine screens.

Methods

Setting and population

This study was conducted within the Generation R study, a population-based birth cohort in Rotterdam, the Netherlands [20,21] set up to collect data on a sample of urban parents and their children from early pregnancy onwards. All children were born between April 2002 and January 2006 and constitute a prenatally enrolled birth cohort that is currently followed until young adulthood. In total n=9,778 (response

rate 61%) pregnant women were enrolled in Generation R (n=8,880 during pregnancy and n=898 at child birth). The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki, and has been approved by the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all participants.

Measures

Alcohol, tobacco and cannabis use were measured using a self-report questionnaire at enrolment (usually in the first trimester of pregnancy; response rate for report on cannabis use was 85.7%). These questionnaires were handled anonymously by using barcodes instead of names and birthdates. Participants reported information on timing and frequency of use. We explicitly asked them to retrospectively reply to two separate questions of whether they had used these substances before pregnancy, and whether they had used them in the last three months. In the latter question the response options were: 'No', 'Yes, until I knew I was pregnant' and 'Yes, I still use substances'.

This information was therefore not specific for the entire gestational period. Mothers also provided information on the substance use of the biological father of the child. Based on these questions, we grouped the total population (n=8880) for this study in 4 non-overlapping categories:

1. Cannabis use before and during pregnancy (n=220)
2 Cannabis use before pregnancy only (n=246)
3. Missing information on cannabis use (n=1270)
4. No cannabis use before nor during pregnancy (n=7144)

We assume that the self-reported data reflect rather reliable report on cannabis use; self-reported numbers of cannabis before pregnancy (2.8%) an during pregnancy (2.5%) are in agreement with national numbers of cannabis use among Dutch women aged between 15 and 64 years (recent use: 3.1% and current use: 1.5%) in the same period [22].

Substance use was unknown for 1270 women of the total population (n=8880). Causes for missing information were failure to return the questionnaire (n=764) or failure to fill out the specific question on substance use (n=506).

Details of biological specimen collection have been described previously [21]. Women enrolled during pregnancy were asked to provide a urine sample at their first visit to the research centre. Maternal urine samples were collected in early, mid- and late pregnancy between February 2004 and November 2005. Depending on the gestational duration in this period, the mother provided one or multiple urine samples. For example, if a mother enrolled in December 2003, this mother would only provide two samples (mid and late pregnancy during urine sample collection period), and if a mother enrolled after February 2004 she would provide three urine samples. All samples were collected in 100-mL polypropylene collection containers and were kept in a cold room (4 °C) for maximally 20 hours before being frozen in 20-mL portions in 25-mL polypropylene vials at -20 °C. After collection, the urine samples were transported to the STAR-MDC laboratory (Stichting Trombosedienst & Artsenlaboratorium Rijnmond) for further processing and storage. From April to July 2008, about 14 batches (n=300 per batch) of 3-mL of urine were sent to the Delta laboratory in Poortugaal, the Netherlands and were tested on the presence of 11-nor-Δ9-THC-9-COOH with a cut-off value of 50 μg/l as recommended by the Substance Abuse and Mental Health Security Agency (SAMSHA). The Delta Laboratory used a semi-quantitative immunochemical technique EMIT II ®, i.e. Enzyme Multiplied ImmunoTechnique (Dade-Behring). For each urine sample, creatinine concentrations were determined, as this provides information on possible urinary dilution. Creatinine concentrations in normal human urine should be greater than 1.8 mmol/l. Urinary creatinine concentrations less than 1.8 mmol/l are considered dilute, whereas concentrations of less than 0.4 mmol/l are inconsistent with human urine [23,24]. Of all samples, 5.6% (n=222) were diluted; we took the dilution into account by calculating a THC/Creatinine Ratio and depending on this ratio, we (re)classified the urine sample as being positive or negative. Based on the creatinine levels only one sample was deemed as inconsistent with human urine. Deleting or including the diluted urine samples did not change any of the results. For women who reported having used cannabis before or during pregnancy, all available samples were analysed to detect the presence of 11-nor-Δ9-THC-9-COOH; if multiple urine samples were positive, they were counted as positive only once. For the women who reported not having used cannabis and for women who had missing information on

cannabis use, the first available urine sample was analysed. Urine samples were missing in almost half of the cohort because urine sample collection was performed during a limited period in the prenatal phase of the study [21]. For the current study, we could therefore use urine samples of 3997 pregnant women. Due to this limited period of urine collection, 78.9% (n=2,375) of the pregnant women filled out the questionnaire after urine collection, and the remaining 21.1% filled out the questionnaire before urine sample collection. Mothers were not aware of this specific validation study at the time of completing the questionnaire.

Statistical Analysis

First, we compared women with urine samples (n=3997) and women without urine samples (n=4883) on several characteristics such as maternal age, ethnicity [25], educational level [26], maternal psychopathology [27], and maternal report on alcohol, tobacco and cannabis use during pregnancy using independent t-tests for continuous variables and χ^2-test for categorical variables. The average creatinine levels of the urine samples were compared using ANOVAs among the following groups: 1. Women reporting cannabis during pregnancy; 2. Women reporting cannabis before pregnancy; 3. Women reporting no cannabis use; and 4. Women without information on cannabis use.

Self-reported data on cannabis use and the presence of 11-nor-Δ9-THC-9-COOH in maternal urine were compared using a 2x2 contingency table. Using this table, sensitivity and specificity were calculated for urinalysis compared to self-report, and vice versa. We calculated the sensitivity and specificity in both directions, because we did not consider either of the two measurements as the golden standard. Significant positive cannabinoid metabolite levels in urine may be indicative of cannabis use and may be regarded as an indicator of exposure. In addition to sensitivity and specificity, Yule's Y [28] was calculated as a measure of overall agreement between self reported cannabis use and urinalysis findings. Yule's Y, also called the coefficient of colligation for dichotomous variables, is exactly equivalent to Hoehler's adjusted κ of agreement [29]. Yule's' Y is based on the odds ratio and a symmetric measure taking on values between -1 and +1; -1 or +1 imply a perfect negative or positive association, and zero implies no association. Yule's' Y is calculated using the 2x2 table with the following formula: $Y = (\sqrt{ad} - \sqrt{bc}) / (\sqrt{ad} + \sqrt{bc})$.

Results were considered significant at $p<0.01$. We chose to use this conservative significance level because it is known that, given a sufficiently large sample, as we have in this study, extremely small and negligible differences can be found to be statistically significant. Statistical analyses were performed using the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc. Chicago, IL).

Non-response analyses

Of the 8880 pregnant mothers, 3997 urine samples were available (Table 2.2.1). Non-response analyses showed some small differences between women with and without urine samples (Table 2.2.1). Women without urine samples were significantly younger (0.5 years), somewhat lower educated (39.4% higher educated vs. 45.5%) and smoked slightly more often during pregnancy (25.7% smoked during pregnancy vs. 22.2%) as compared to women with urine samples. No statistically significant differences in maternal and paternal cannabis use, maternal alcohol use and ethnicity were found.

Results

Of 3997 pregnant women, 92 reported having used cannabis during pregnancy (2.3%), while we found 71 positive urine screens (1.8%). The numbers were not completely overlapping (Table 2.2.2). Of the 92 mothers who reported having used cannabis during pregnancy, 33 mothers had a positive urine screen. These data indicate that 92 of the 130 women with probable cannabis use during pregnancy (70.7%) reported their cannabis use. Based on these data, the total number of women with at least some evidence of cannabis use during pregnancy amounts to 92 (self-reported) + 15 + 14 + 9 (positive urine tests without self-reported cannabis use) = 130, i.e. 3.3% of the group with urinalysis data (Table 2.2.2, lower part). In addition, based on a combination of positive self-report and positive urinalysis data, at least 130 of the 3997 pregnant women used cannabis during pregnancy (3.3%), i.e. 1.43 times the prevalence of cannabis use during pregnancy based on self-reported data only and 1.83 times the prevalence based on urinalysis data only.

Table 2.2.1 Characteristics of mothers with and without urine samples

	With urine samples (n=3997)	Without urine samples (n=4883)	
Maternal age			
Mean ± s.d.	29.9 ± 5.2	29.4 ± 5.4	*
Maternal education (%)			
Primary education	12.3	13.3	*
Secondary education	42.3	47.3	
Higher education	45.5	39.4	
Marital status (%)			
Non-married	49.4	51.9	
Maternal psychopathology (mean ± s.d.)			
General Symptom Index	0.29 ± 0.38	0.31 ± 0.39	
Maternal Ethnicity (%)			
Dutch	48.2	50.3	
Non-Dutch	51.8	49.4	
Maternal Tobacco use (%)			
No	61.3	56.9	*
Before pregnancy	16.5	17.4	
During pregnancy	22.2	25.7	
Maternal Alcohol use (%)			
No	33.1	32.5	
Before pregnancy	24.8	24.5	
During pregnancy	42.1	43.1	
Maternal Cannabis use (%)			
No	80.1	80.7	
Before pregnancy	3.0	2.6	
During pregnancy	2.3	2.6	
Unknown	14.7	14.0	
Cannabis use of the biological father			
No	88.7	87.7	
Yes	10.2	10.7	
Unknown	1.2	1.6	

Table note: Values are means ± standard deviations (s.d.) for continuous variables and percentages for categorical variables. Statistical significance was derived from independent t-tests for continuous variables and χ^2-test for categorical variables ($p<0.01$)*

Table 2.2.2 shows that all hypotheses were at least partially confirmed: a sizeable proportion of positive urine screens was found in women who reported cannabis use during pregnancy (35.9%), a substantial proportion of positive screens was found in women who reported cannabis use only before pregnancy (7.6%), a very small proportion of positive screens was found in women who reported no cannabis use (0.4%), and an intermediate proportion of positive screens was found in women without self-report data on cannabis use (2.6%).

Average creatinine levels were not statistically different across groups ($F=2.23$, $p=0.082$) (Table 2.2.2). Moreover, no significant difference between mean maternal urinary creatinine level in women who reported having used cannabis during pregnancy (9.4 ± 5.5 mmol/litre) and of mothers who reported not having used cannabis during pregnancy (8.4 ± 5.4 mmol/litre) was found ($t=1.78$; $p=.074$). Mean creatinine levels for cannabinoid-positive ($n=71$, $\mu=12.5 \pm 7.0$ mmol/litre) and cannabinoid-negative urine samples ($n=3926$, $\mu=8.4 \pm 5.3$ mmol/litre) were significantly different ($t=6.41$; $p<.001$), indicating that on average, negative screens were reached in samples that were slightly diluted.

Sensitivity and specificity of the urinalysis as compared to self-report were $33/71=0.46$ and $3867/3926=0.98$, respectively. In addition, sensitivity and specificity of self-report as compared to urinalysis were $33/92=0.36$ and $3867/3905=0.99$. These findings (based on Table 2.2.2) indicate that both approaches perform very well in the identification of non-cannabis users, but that both measures seem to identify partially different subpopulations of cannabis users during pregnancy. Based on Table 2.2.2, it was possible to calculate overall Yule's' Y: $(\sqrt{33*3867} - \sqrt{59*38}) / (\sqrt{33*3867} + \sqrt{59*38}) = 0.77$, which implies that the geometric mean of the surplus of consistent over inconsistent data pairs as a percentage of all non-tied pairs is .77, indicating substantial agreement between self-reported cannabis use and urinalysis. When calculated by comparing pregnant cannabis users and women who reported non-use (Group B_3 in Table 2.2.2), Yule's Y was somewhat higher: $(\sqrt{33*3187} - \sqrt{59*14}) / (\sqrt{33*3187} + \sqrt{59*14}) = 0.84$.

Table 2.2.2 2x2 contingency table for calculating sensitivity, specificity and Yule's' Y with absolute and relative numbers of available urine samples, positive detected urine screens and mean creatinine level.

	Urinalysis							
	Positive	(%) [a]	Negative	(%) [a]	Total	(%) b	Creatinine in mmol/l mean	± s.d.
Maternal self-report								
Cannabis use during pregnancy (Group A) (n=220)	33	(35.9)	59	(64.1)	92	(41.8)	9.4	± 5.5
All other women (Group B) (n=8660)	38	(1.0)	3867	(99.0)	3905	(45.1)	8.4	± 5.4
Total (n=8880)	71	(1.8)	3926	(98.2)	3997	(45.0)	8.4	± 5.4
Maternal self-report								
Cannabis use during pregnancy (Group A) (n=220)	33	(35.9)	59	(64.1)	92	(41.8)	9.4	± 5.5
Cannabis before pregnancy (Group B_1) (n=246)	9	(7.6)	109	(92.4)	118	(47.9)	8.6	± 4.2
Missing information (Group B_2) (n=1270)	15	(2.6)	571	(97.6)	586	(46.1)	8.8	± 5.0
No cannabis use during pregnancy (Group B_3) (n=7144)	14	(0.4)	3187	(99.6)	3201	(44.8)	8.4	± 5.5
Total (n=8880)	71	(1.8)	3926	(98.2)	3997	(45.0)	8.4	± 5.4

Table note: Group B consists of three subgroups: B_1 Women reporting cannabis before pregnancy; B_2 Women reporting no cannabis use; and B_3 Women without information on cannabis use; [a] Proportion of positive and negative urine screens based on the number of urine samples; [b] Proportion of urine samples based on the total number of participants

Discussion

In this study, we compared self-reported information on maternal cannabis use during pregnancy with the presence of the main urinary cannabis metabolite, 11-nor-Δ9-THC-9-COOH, measured by semi-quantitative immunochemical urinalysis. Our data demonstrate that both self-report and urinalyses provide important though different information on cannabis use during pregnancy: self-report provides a higher estimate of cannabis use during pregnancy (2.3%) than urinalysis (1.8%) and urinalysis provides additional cases of women using cannabis during pregnancy. Based on a combination of positive self-report and positive urinalysis data, at least 130 of the 3997 pregnant women used cannabis during pregnancy (3.3%), i.e. 1.43 times the prevalence of cannabis use during pregnancy based on self-reported data only and 1.83 times the prevalence based on urinalysis data only. These findings are consistent with Shiono's previously published report describing the numbers of self reported cannabis use and positive biochemical assays [15]. Thus, this study demonstrates that reliance on self reported cannabis alone underestimates the prevalence of cannabis use during pregnancy even in a country where neither cannabis possession nor cannabis use is prosecuted. However, reliance on urinalysis alone underestimates the prevalence of cannabis use during pregnancy more profoundly and may be biased toward long-term or heavy users, as they are more likely than occasional users to be detected through urinalysis.

The non-response analysis showed small differences between women with and without urine samples. Women without urine samples were somewhat younger, somewhat lower educated, and smoked more often during pregnancy. These small differences between the groups may increase the likelihood for cannabis use in women without urine samples. However, as we reported previously, cannabis use of the biological father is one of the main determinants for cannabis use of pregnant women before and during pregnancy [4], and it is therefore important to consider paternal cannabis use. These data showed that women with and without urine samples did not differ in this respect.

Additionally, a total of 61% of all eligible women participated in the Generation R study [30] and they may not be completely representative of the general Rotterdam population. This may have led to an unfavourable

selection of non-using women, as our study population is somewhat higher educated compared to the general Rotterdam population [31], which perhaps has led to a lower prevalence estimate of cannabis use. However, this selection bias does not necessarily mean that the relationship between self-reported cannabis use and urinalysis presented in this study was distorted.

Compared to previous studies, we report a higher agreement between self-report measures of cannabis use during pregnancy and urinalysis (Yule's' Y varied from 0.77 to 0.84, indicating substantial to good agreement). Previous studies with different populations have reported moderate agreement between self-reports on cannabis use and urinalysis. For example, Perrone and colleagues reported a moderate agreement (Cohen's κ=0.41) between self-reported cannabis use and positive urine screens in psychiatric patients at an hospital emergency department [32]. Buchan and colleagues also reported moderate agreement between self-report of cannabis use and urinalysis in adolescents entering substance abuse treatment for cannabis use disorders [12]. Consistent with our data, the discrepancy between self-report and urinalysis was bidirectional; two-thirds of frequent cannabis users had a positive urine sample and one-third tested positive even though they reported non-use [12].

However, our findings were not fully consistent with the data of Markovic et al., who reported much higher proportions of positive urine screens (86.6%) in women reporting current cannabis use in early pregnancy [16]. A logical explanation for this finding is that the urine-samples were collected at the same time as the self-reported data on recent substance use. In addition, this sample was not representative of the general population, but was a selected group of 570 (out of 1347) pregnant women with a high prevalence of cannabis use during pregnancy measured by self-report (16.8%) and urinalysis (21.5%) [33].

Sensitivity, of both self-report and urinalysis, was moderate, whereas specificity of both assessment procedures was high, indicating that the number of false negatives was low. Positive urine screens were mainly in the group that reported cannabis use before pregnancy (7.6%) and in the group that refused to report on their cannabis use (2.6%). In addition, a small percentage of positive urine screens was present in the non-users group (0.4%). Therefore, it is important to consider women who reported using cannabis before pregnancy (i.e. past users) as a separate

group, and not simply as non-users during pregnancy. However, the size of the group reporting non-use should also be taken into account. Although the percentage of self-reported non-users with positive urine screens is small, the total group size is relatively large and could contribute significantly to the absolute number of cannabis users during pregnancy.

If it can be assumed that false positive self-reports for cannabis use are unlikely, the negative urine screens of pregnant women reporting cannabis use during pregnancy are of interest. The main metabolite excreted in the urine (11-nor-Δ9-THC-9-COOH) is found within hours of exposure and remains detectable in the urine for 3-10 days after smoking a single dose. However, the length of time following cannabis use for a positive urine screen is dependent upon multiple factors, including the frequency and amount of cannabis, metabolic rate, excretion rate, half-life time, storage of the urine samples, and the cannabis-user's age, body fat content, activity, and diet [34,35]. Therefore, possible explanations for "false positive self-reports" include infrequent cannabis use, time-frame specificity, and individual variety in metabolism rate. These difficulties may explain the finding of negative urine screens in 59 of the 92 (64%) women who reported cannabis use during pregnancy. This means that immunochemical urinalysis cannot be regarded as the golden standard in assessing maternal cannabis use during pregnancy in large epidemiological studies. This finding should be taken into account, as it means that in the group of mothers who did not answer the question about substance use or reported non-use, the true prevalence of cannabis use may be higher. In fact, if the prevalence of cannabis use during pregnancy were estimated based on the urinalysis findings, the fact that at least two-thirds of cannabis using women was not detected in our study with urinalysis should be taken into account.

In addition, some other limitations in this study should be considered. First, extended frozen storage may have possibly led to degradation of cannabinoids over time, and may have led to an underestimation of the prevalence of cannabinoids in urine. However, several studies have shown that average change in concentrations of the cannabis biomarker (11-nor-Δ9-THC-9-COOH) in urine after long-term storage was not extensive (<15%) [36,37]. Second, although we cannot rule out the possibility that women may wonder about specific aims for urine collection, they were blind to all study questions that could be

answered using urinalysis. Therefore, we expected that knowing urine samples were collected (without a specific reason) did not influence cannabis use among these women. Finally, exact information on the amount of time that had elapsed between filling out the questionnaire and urine collection was not available, however, in general women who visited the research centre for anthropometrics, ultrasound measurements and blood and urine collection, filled out the questionnaire at approximately the same time. Alternative detection methods such as hair examination is limited, as cannabis incorporation depends on growth rate, anatomical region, age, gender, ethnicity, hair colour and individual variability [38]. More recently, a novel detection technique of cannabinoid metabolites in fingerprints (i.e. sweat) using nanoparticle-enhanced fluorescence imaging has been developed, which could be a first relevant step for routine detection of cannabis consumption through sweat testing [39]. However, detection of THC in sweat is subjected to the same pitfalls as urinalysis, because the elimination period for THC in sweat appears to be similar to that of THCCOOH in urine [40]. Therefore, the most sensible solution is to use self-reported information on prenatal drug exposure in large populations, as this method is likely to provide the best estimate for the lowest price (highest cost effectiveness).

The observed underreporting of cannabis use among this population of pregnant women is a significant finding that has both research and clinical implications. Researchers and clinicians, such as obstetricians, should acknowledge that pregnant women may underreport current cannabis use, a situation that seems most prevalent in women admitting past cannabis use (i.e. cannabis use before the pregnancy) and in women refusing to provide information on cannabis use during pregnancy.

In conclusion, our findings illustrate the difficulties in obtaining valid information on prenatal cannabis use. Nonetheless, self-report seems to be an acceptable single method to determine cannabis use during pregnancy in epidemiological studies. Importantly, in order to improve the quality of cannabis use data, we suggest a two-step approach, starting with self-report information and followed by urinalysis in women who reported cannabis use before pregnancy and in women who refused to answer questions about substance use during pregnancy.

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Chapter 3

INTRAUTERINE CANNABIS EXPOSURE AND FOETAL CONSEQUENCES

Contents of this chapter

In the previous chapter we showed that multiple demographic, emotional and social characteristics were associated with maternal cannabis use during pregnancy. We concluded that these characteristics should be considered when investigating offspring exposed to cannabis in utero, as they may play an important role in mother-child interaction and child development. In this chapter, we investigated the associations between maternal cannabis use during pregnancy and foetal outcomes such as growth until birth and foetal redistribution. When investigating these associations, we took into account several maternal demographic, emotional and social factors that may be underlying to these associations.

Chapter 3.1

INTRAUTERINE CANNABIS EXPOSURE AND FOETAL GROWTH UNTIL BIRTH

Abstract

Among illicit drug use of pregnant women, cannabis is most often consumed and intrauterine exposure may result in a risk for the developing fetus. The importance of intrauterine growth regarding subsequent psychological and behavioral child development has been demonstrated. This study examines the relation between maternal cannabis use and fetal growth until birth in a population sample.

In total, 7452 mothers enrolled during pregnancy with information on substance use and fetal growth. Fetal growth was determined using ultrasound measures in early, mid- and late pregnancy. Additionally, birth weight was assessed.

Maternal cannabis use during pregnancy was associated with growth restriction in mid- and late pregnancy, and with lower birth weight. For fetuses exposed to continued maternal cannabis use in pregnancy this growth reduction was most pronounced. Fetal weight in cannabis-exposed fetuses showed a growth reduction of -14.44 grams/week (95% Confidence Interval (CI): -22.94,-5.94) and head circumference (-0.21 mm/week, 95%CI: -0.42, 0.02), compared to non-exposed fetuses. Maternal cannabis use during pregnancy resulted in more pronounced growth restriction than maternal tobacco use. Paternal cannabis use was not associated with fetal growth restriction.

Maternal cannabis use, even for a short period, may be associated with several adverse fetal growth trajectories.

Introduction

Among illicit drug use of pregnant women, cannabis is most often consumed [1]. In the last decade Δ9-tetrahydrocannabinol (THC) concentration in marijuana and hashish has increased strongly, particularly in Dutch cannabis due to improved breeding and greenhouse technology [2, 3]. Exposure to these high THC-levels in utero may result in a risk for the developing fetus. This suggestion is based on evidence from several animal studies, showing that administration of high doses of THC in pregnant rodents resulted in lower birth weight among offspring [4, 5]. Such effects can be explained by findings from studies showing that THC and its metabolites freely pass the placental barrier [6], and by entering the fetal circulation, may affect the developing fetus [7]. Importantly, molecular research has shown that local actions of endocannabinoids in the human placenta are present in early pregnancy [8]. This may pose a risk for longer-term adverse outcomes as well, since research has demonstrated the importance of indicators of intrauterine growth, such as birth weight, body weight and head size, regarding subsequent child development [9-13].

Maternal cannabis use could affect fetal growth by several underlying mechanisms. First, tobacco use leads to attenuated intrauterine growth due to fetal oxygen deprivation caused by a combination of increased carboxyhemoglobin levels in the blood and a decreased maternal blood supply to the placenta [14, 15]. Second, the cannabinoid receptor system is present and functional in early pregnancy [16] and cannabis (metabolites) could directly affect the brain and body by altering cannabinioid and related neurotransmitter or neuroendocrine systems. Recent studies showed that endocannabinoids bind to cannabinoid receptors in pancreatic β-cells and regulate the intracellular calcium concentration and consequently decrease glucose-dependent insulin secretion [17-20]. The major fetal growth factors are IGF-2 (insulin-like growth factor) and IGF-1, which are regulated by insulin itself [21]. Therefore, it may be that fetuses prenatally exposed to cannabis have lower insulin levels compared to controls, which then induces impaired growth. Finally, epiphenomena of maternal cannabis use including, maternal stress, co-use of other substances, or poor nutritional status during pregnancy may have contributed to the differences in fetal growth between cannabis users, tobacco users and non-users [22].

Until now, two longitudinal studies addressed the influence of maternal cannabis use during pregnancy on behavioral outcomes in human offspring [23, 24]. These studies found associations between intrauterine cannabis exposure and offspring neurodevelopmental and behavioral problems, such as tremors and startles in newborns [25], decreased mental scores measured with the Bayley Scales of Infant Development in infants (age of 9 months) [26], lower intelligence scores measured with the Stanford-Binet Intelligence Scale at the age of three years [27], and more teacher-rated delinquent behavior at the age of six and ten years [28, 29]. Part of these findings may originate in adverse fetal growth trajectories. To date, results of human studies on fetal consequences of maternal cannabis use in pregnancy remain inconclusive [30-37]. Several reasons could account for the inconsistencies across studies. First, birth weight was a main focus of many studies, while birth weight is just a proxy for fetal growth that can be reached through different individual growth patterns. Second, most studies lacked sufficient power, and inferred their conclusion from observations in small non-representative samples. Finally, differences in potency of cannabis used in these studies may have accounted for the inconsistencies.

The current investigation takes these methodological pitfalls into account and is the first to focus on the relation between potent maternal and paternal cannabis use during pregnancy and fetal growth using ultrasound measurements in early, mid- and late pregnancy in a large population-based cohort. Because available data suggest that birth weight is negatively associated with intrauterine cannabis exposure, we hypothesized to find a negative association between fetal growth trajectories and cannabis exposure as well.

Method

Setting and population

This study was embedded in the Generation R Study, a multiethnic population-based prospective cohort study from fetal life onwards, designed to identify early environmental and genetic determinants of growth, development and health [38, 39]. The cohort included 9778 mothers and their children living in Rotterdam, the Netherlands. Enrolment was aimed at early pregnancy (gestational age <18 weeks), but was possible

until birth of the child. Measurements were planned in early (<18 weeks), mid- (18-25 weeks) and late pregnancy (≥25 weeks). Seventy-one per cent of the partners were enrolled during pregnancy (n = 6347). The Medical Ethics Committee of Erasmus MC in Rotterdam, the Netherlands, has approved the study in accordance with the Declaration of Helsinki of the World Medical Association. Written informed consent was obtained from all participants.

All pregnant women resident in the study area at their delivery date from April 2002 until January 2006 were invited to participate. In total 8880 mothers were enrolled during pregnancy and were eligible for the present analyses [38]. Mothers without information about substance use (14.3%, n=1270) and women who used other drugs but not cannabis (n=79) were excluded. Mothers with twin pregnancies (n=78) were excluded. One mother did not have any ultrasound examination and was also excluded. Of the remaining mothers n=7452 who were included in the analyses, 71.4% (n=5324) had three ultrasound assessments, 23.7% (n=1763) had two and 4.9% (n=365) had only one ultrasound assessment. In this study, 74.0% of the fathers participated and 81.1% of them (n=4475) provided substance use information.

Measures

Timing and frequency (daily, weekly, monthly) substance use were measured using a self-report questionnaire at enrolment. A distinction was made between the use of cannabis (marijuana and hashish) and other illicit drugs (cocaine, amphetamines and heroin). At enrolment, we explicitly asked with two questions whether pregnant women used substances (tobacco, alcohol and illicit substances) before pregnancy and whether they had used any of the substances in the last three months. The period of the last three months was chosen in this question, because enrolment was aimed at early pregnancy. In the second question the answer options were: 'No', 'Yes, until I knew I was pregnant' and 'Yes, I still use substances'. Mothers were asked about their own substance use and about substance use of the biological father. In addition, participating partners reported on their own substance use. Identical questions were answered for tobacco use. Maternal cannabis and tobacco use were combined in one variable with five categories of non-overlapping groups: 1. continued cannabis use; 2. cannabis use in early pregnancy; 3. cannabis

use only before pregnancy; 4. tobacco use during pregnancy; 5. non-use (no cannabis or tobacco during pregnancy).

Fetal ultrasound assessments were performed at research centers and were carried out in early-, mid- and late pregnancy. Femur length, abdominal and head circumference, and transcerebellar diameter, were measured using standardized techniques [40]. Fetal weight was estimated using femur length, head and abdominal circumference in the formula of Hadlock. [41] The intra- and inter-observer reliability of fetal biometry in early pregnancy within Generation R were good; all intraclass correlation coefficients were >0.98 [42]. The ultrasound measurements were performed using an Aloka® Model SSD-1700 (Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Seattle, Washington, USA) equipped with a 5.0 MHz, high frequency curved array transducer.

Maternal age, educational level, national origin, alcohol use, parity (0 or ≥1) and gravidity (1 or ≥2) were assessed with questionnaires and considered as possible confounders. Maternal national origin was defined according to the classification of Statistics Netherlands [43]: 1. Dutch, 2. Cape Verdean, 3. Moroccan, 4. Turkish, 5. Surinamese 6. Antillean and 7. Other national origin. Educational level was categorized in primary, secondary, and higher education [44]. Timing and frequency of alcohol use were measured with similar questions as for tobacco and substance use during pregnancy. Information on maternal anthropometrics was collected in the research centers. The Brief Symptom Inventory (BSI), a validated 53-item self-report symptom inventory was used to ascertain the psychological state [45]. The standard deviation score of the Global Severity Index (GSI) scale of the BSI was used to determine general psychopathology. Fetal gender was obtained from midwives and hospital registries.

Statistical Analysis

To examine the associations between maternal cannabis and tobacco use with fetal parameters in mid-, and late pregnancy and at birth, multiple linear regression models were used. These models were also used to examine a potential dose-response association of cannabis use with birth weight. First, the effects of cannabis use during pregnancy were investigated using non-users as reference group. Second, the same analyses were performed with tobacco-users as reference group.

The association between maternal cannabis and tobacco use and repeatedly measured fetal growth parameters were analyzed using longitudinal multilevel analysis to account for the dependency between measurements in the same subject [46]. First, the best fitting model with the outcome as a function of gestational age was constructed using fractional polynomials [47]. Since fetal size and transcerebellar diameter were not measured reliably in early pregnancy, the analyses were conducted with mid- and late pregnancy measures and birth measures for estimated fetal weight and with mid- and late pregnancy measures for transcerebellar diameter. The measurements from early until late pregnancy were used for head circumference. Then, maternal cannabis and tobacco use were entered into the model as the main determinant. The final curve was fitted with random effects for both intercept and gestational age, because it takes within and between-individual variation into account. All other covariates were fitted as fixed effects, because there was no a priori reason to assume that these covariates have varying effects for each individual. The interaction term of maternal cannabis/tobacco use with gestational age was included in the model to compare the slope of the curves of different categories of maternal cannabis/tobacco use with the reference group (non-use). Additional analyses were performed with the tobacco-users as the reference group to determine whether effects of cannabis during pregnancy significantly differed from the effects of tobacco. Moreover, we used an alternative approach in which maternal cannabis and tobacco use were put in the model as separate variables. By doing so, we could assess the effect of cannabis adjusted for the effect of tobacco. Furthermore, to account for residual confounding supplementary analyses were performed which calculated the association of paternal cannabis without maternal cannabis use and fetal growth.

All models were adjusted for gestational age, maternal age, body mass index, height, education, national origin, maternal alcohol use, parity, gravidity, fetal gender and maternal psychopathology [48]. SPSS for Windows (version 11.0) and SAS v.8.2 (SAS Institute Inc. Cary, NC, USA) including the Proc Mixed module for longitudinal multilevel analysis were used for data analysis.

Non-response Analysis

Women without information on substance use (n=1270) were excluded. Non-response analyses showed that these women were somewhat younger (29.1±5.5 yrs) than women with information on substance use (29.7±5.3 yrs) (P<0.01), less educated (15.7% primary educated vs. 11.3%; P<0.01) and less likely to be married (21.3% married vs. 48.1%; P<0.05). No significant differences in national origin were present.

Results

Table 3.1.1 shows the characteristics of pregnant women per cannabis use category (n=7452). In this study sample, 245 (3.3%) women used cannabis only before pregnancy; 214 (2.9%) women used cannabis before and during early pregnancy. Of these 214 women, 173 (81%) quit using cannabis in early pregnancy, while 41 (19%) continued using cannabis throughout pregnancy. In total, 1453 mothers (19.5%) smoked tobacco during pregnancy (but used no cannabis), All other women (n=5540) did not use cannabis or tobacco during pregnancy (non-users). Of the women using cannabis during pregnancy, 85% also smoked tobacco during pregnancy. The number of daily smoked cigarettes did not significantly differ between women who smoked tobacco and used cannabis versus women smoking tobacco only; 50% smoked more than five cigarettes per day (χ^2=15.35, df=10, P=0.120). Table 3.1.1 shows that cannabis-users were lower educated and more likely to drink alcohol than non-users. No difference in gestational duration among the groups was present (Table 3.1.1).

Table 3.1.1 Maternal and child characteristics in cannabis-using and non-using subgroups

First trimester cannabis use	Continued use (n=41)		In early pregnancy (n=173)		Before pregnancy (n=245)		Non-use (n=5540)
Maternal Characteristics							
Age (yrs)	28.3 (5.4)		26.4 (5.8)	**	27.6 (5.9)	**	30.1 (5.1)
Height (cm)	166.5 (7.5)		168.4 (6.7)		168.3 (6.7)		167.3 (7.5)
Body mass index (kg/m^2)	24.7 (4.4)		23.6 (4.4)	**	23.8 (4.0)	**	24.8 (4.5)
Parity (% primiparous)	58.5		73.4	**	71.4	**	53.4
Gravidity (% primigravidus)	39.0		53.2	**	52.3	*	43.9
Educational level (%)							
Primary education	29.3	**	20.2	**	10.2	**	10.1
Secondary education	56.1		58.4		58.0		41.9
Higher education	14.6		19.7		31.0		45.7
First trimester alcohol use (%)							
None	19.5	*	11.2	**	8.7	**	36.4
Before	29.3		15.9		34.7		24.6
During, stopped	22.0		56.5		34.7		23.7
During, continued	29.3		16.5		21.9		15.3
First trimester tobacco use [A] (%)							
None	0.0		5.3		13.8		79.4
Before	12.5		9.4		32.1		20.6
During, stopped	12.2		35.7		22.9		0.0
During, continued	73.2		49.7		31.3		0.0
Maternal psychopathology							
General symptom index	0.97 (0.83)		1.22 (1.15)	**	0.87 (1.02)	**	0.64 (0.83)
Child anthropometrics							
Birth weight	3122 (544)	**	3206 (535)	**	3372 (549)		3450 (554)
Gestational age at birth	39.5 (1.7)		39.6 (1.7)		39.8 (1.5)		39.8 (1.7)
Gender (% boys)	65.9	*	56.1		51.4		48.7

*Table note: Values are means (SD) for continuous variables and percentages for categorical variables. ** $P < 0.01$; * $P < 0.05$. ANOVA with post-hoc comparison for continuous variables and χ^2 tests for categorical variables vs. non-users was used. [A] Statistical analysis on tobacco use was not performed, because the groups were selected on the basis of this variable.*

Table 3.1.2 and 3.1.3 represent the associations between maternal cannabis use and different fetal growth parameters. Using cannabis before pregnancy does not affect fetal growth in mid- and late pregnancy or at birth (Table 3.1.2). However, when mothers used cannabis in early pregnancy, fetuses showed a reduced growth. It affected fetal weight from late pregnancy onwards and resulted in a decrease of 156 grams in birth weight. It also affected growth of head circumference from mid-pregnancy onwards (Table 3.1.3). Continued cannabis use during pregnancy showed the largest growth reduction, which was already present in mid-pregnancy (-13.58 grams) and resulted in a growth reduction of 277 grams at birth. Fetal weight was also negatively affected by tobacco use (-23.21, 95%CI:-34.41,-12.00, P<0.001) from late pregnancy onwards, and resulted in approximately 85 grams less birth weight (-87.41, 95%CI:-113.14,-61.67, P<0.001) as compared to non-exposed fetuses. Similarly, maternal tobacco use in pregnancy attenuated growth of the head in mid- (-0.41, 95%CI:-0.80,-0.02, P<0.038) and late pregnancy (-1.05, 95%CI:-1.60,-0.50, P<0.001). No statistically significant associations were found between maternal cannabis and tobacco use and transcerebellar diameter (data not shown).

Furthermore, we examined the effects of intrauterine cannabis exposure using the smokers as reference. At birth, neonates exposed to cannabis use in early pregnancy were 95 grams lighter (-95.40, 95%CI:-168.27,-22.54, P=0.010) and continued cannabis-exposed neonates were 172 grams lighter (-171.68, 95%CI:-308.29,-35.07, P=0.014) as compared to neonates exposed to only tobacco. In late pregnancy, fetuses exposed to cannabis in early pregnancy weighed 40 grams less (-40.56, 95%CI:-71.53,-9.60, P=0.010), and fetuses exposed to persistent cannabis use weighed 67 grams less (-67.12, 95%CI:-124.32,-9.92, P=0.021) than fetuses exposed to only tobacco. In mid-pregnancy, the effects of cannabis exposure on the growth parameters were not significantly different from tobacco effects.

Table 3.1.2 The association between maternal cannabis use and estimated weight in mid- and late pregnancy and birth weight

	Estimated fetal weight in in mid-pregnancy		**Estimated fetal weight in late pregnancy**		**Weight at Birth**	
	Beta (95%CI) [#]	*P* -value	Beta (95%CI) [#]	*P* -value	Beta (95%CI) [#]	*P* -value
Maternal cannabis use						
Non-use	Reference		Reference		Reference	
Continued use	-13.58 (-27.73, 0.12)	0.052	-96.44 (-152.45, -40.43)	<0.001	-277.27 (-409.15, -145.39)	<0.001
In early pregnancy	-3.50 (-10.64, 3.65)	0.34	-57.66 (-86.68, -28.65)	<0.001	-156.61 (-224.00, -89.23)	<0.001
Before pregnancy	-2.26 (-8.28, 3.76)	0.46	-20.42 (-44.59, 3.76)	0.098	-24.91 (-80.60, 30.78)	0.38

Table note: Models were constructed using multiple linear regressions. All values were adjusted for maternal age, body mass index, height, educational level, national origin, maternal alcohol use, gestational age, parity, gravidity, fetal gender and maternal psychopathology. [#] Beta represents the increase or decrease of fetal size characteristics in millimeters in the cannabis subgroups using the non-users as reference

Table 3.1.3 The association between maternal cannabis use and fetal growth parameters in mid- and late pregnancy

	Head circumference in mid-pregnancy		Head circumference in late pregnancy	
	Beta (95%CI) #	*P* -value	Beta (95%CI) #	*P* -value
Maternal cannabis use				
Non-use	Reference		Reference	
Continued use	-0.80 (-2.75, 1.15)	0.42	-2.45 (-5.20, 0.30)	0.081
In early pregnancy	-1.01 (-2.02, -0.01)	0.048	-1.78 (-3.21, -0.34)	0.015
Before pregnancy	-0.64 (-1.49, 0.20)	0.14	-1.29 (-2.48, -0.09)	0.035

Table note: Models were constructed using multiple linear regressions. All values were adjusted for maternal age, body mass index, height, educational level, national origin, maternal alcohol use, gestational age, parity, gravidity, fetal gender and maternal psychopathology.# Beta represents the increase or decrease of fetal size characteristics in millimeters in the cannabis subgroups using the non-users as reference

Table 3.1.2 demonstrates that any intrauterine cannabis exposure cannabis was associated with reduced birth weight. Therefore, all pregnancy cannabis-users were pooled for examining potential dose-response associations. Occasional cannabis use (monthly, n=36) was not significantly associated with a lower birth weight as compared to non-users (-123.0, 95%CI: -263.4,17.4, P=0.086), and as compared to tobacco users (-83.2, 95%CI:-230.3,63.9, P=0.268). Moderate cannabis use (weekly=72) was associated with lower birth weight (-149.7, 95%CI:-249.7,-49.7, P=0.003) compared to non-users, but not when compared to tobacco-users (-85.8, 95%CI:-191.3, 19.7, P =0.111). Finally, heavy cannabis use (daily=69) is associated with the lowest birth weight (-225.7, 95%CI -330.7,-120.8, P<0.001) also when compared to tobacco-users (-149.4; 95%CI -260.0, -38.8, P =0.008).

Table 3.1.4 shows the associations between maternal cannabis and tobacco use and prospectively measured growth parameters. Maternal cannabis use was negatively related to head growth and fetal weight. Using cannabis in early pregnancy or throughout pregnancy results in a reduced fetal growth; fetuses exposed to cannabis in early pregnancy or to continued use grew respectively 11.18 grams/week and 14.44

grams/week less than fetuses of non-users. Tobacco-exposed fetuses grew 4.07 grams/week (95%CI:-5.60,-2.54, P<0.001) less than non-exposed fetuses. This stronger effect of intrauterine cannabis exposure on growth was also found for head circumference. No statistically significant associations were found between maternal cannabis and tobacco use and transcerebellar diameter (data not shown). Further, we examined the effects of cannabis use compared to the tobacco users as reference. These analyses showed that fetuses exposed to cannabis in early pregnancy grew 7.08 grams/week less (95%CI:-11.40,-2.77, P=0.001) and continued cannabis-exposed fetuses grew 10.29 grams/week less (95%CI:-19.01,-1.57, P=0.021) than tobacco-exposed fetuses. No significant differences for growth of head circumference between cannabis- or tobacco-exposed fetuses.

The alternative approach, using cannabis and tobacco as two separate but overlapping variables, showed an association of intrauterine cannabis exposure corrected for smoking and fetal growth reduction. According to this approach fetuses exposed to cannabis in early pregnancy grew 7.45 grams/week less (95%CI:-11.65,-3.25, P<0.001) and continued cannabis-exposed fetuses grew -8.90 (95%CI:-17.50,-0.30, P=0.043) less as compared to non-exposed fetuses. For the growth of head circumference no statistically significant association for cannabis exposure corrected for tobacco use was found (continued exposure -0.10 mm/week with 95%CI:-0.33, 0.12, P=.363; early pregnancy exposure -0.06 mm/week with 95%CI:-0.17,-0.05, P=0.281). No interaction effects were found of cannabis and tobacco exposure on fetal growth characteristics.

Table 3.1.4 The association of maternal cannabis use and fetal growth characteristics throughout pregnancy compared to maternal non-use

	Regression coefficients (and 95% confidence intervals)			
	Fetal weight	*P*-value	Head circumference	*P*-value
Intercept of the model in cannabis use categories				
Non-use	*Reference*		*Reference*	
Continued use	294.23 (101.45, 487.01)	0.003	2.66 (-1.61, 6.93)	0.22
In early pregnancy	243.18 (151.17, 335.20)	<0.001	1.80 (-0.25, 3.86)	0.090
Before pregnancy	39.72 (-38.76, 118.16)	0.32	1.69 (-0.07, 3.46)	0.060
Fractional polynomial model as a function of gestational age				
Gestational age (GA)	-792.60 (-804.93, -780.26)	<0.001	4.57 (2.97, 6.17)	<0.001
GA^2	-	-	1.25 (1.07, 1.43)	<0.001
GA^2 * ln(GA)	-	-	-0.30 (-0.34, -0.26)	<0.001
GA * ln(GA)	216.95 (214.14, 219.76)	<0.001	-	-
Slope of the model in cannabis use categories				
Non-use	*Reference*		*Reference*	
Continued use	-14.44 (-22.94, -5.94)	0.001	-0.21 (-0.42, 0.02)	0.070
In early pregnancy	-11.18 (-15.26, -7.10)	<0.001	-0.13 (-0.24, -0.02)	0.016
Before pregnancy	-1.98 (-5.44, 1.49)	0.26	-0.11 (-0.20, -0.02)	0.020

Table note: Longitudinal multilevel models were constructed using fractional polynomials for gestational age. All values were adjusted for maternal age, body mass index, height, educational level, national origin, maternal alcohol use, parity, gravidity, fetal gender and maternal psychopathology. The slope represents the decrease of fetal size characteristics in grams (fetal weight) and millimeters (head circumference) per week in the cannabis subgroups using the non-users as reference.

Additional analyses on paternal cannabis use and estimated fetal weight showed that, when fathers use cannabis during pregnancy (without maternal cannabis use), fetal growth was not affected. The intercept of growth curve was not different between paternal users (n=115) and non-users (n=2527) (40.68; 95%CI:-56.71, 138.06, P=0.41) and the growth rate did not differ either (-1.40 grams/week; 95%CI:-5.71, 2.92, P=0.53). Similar results were found when mothers reported on the cannabis use of the biological father of their child. The growth rate was not different (-3.08 grams/week; 95%CI:-6.49, 0.34, P=0.077) between fetuses of fathers who used cannabis (n=192) and of fathers who did not use cannabis (n=4098). However, the intercept of the curve was different (81.19 grams/week; 95%CI: 3.90, 158.47, P=0.040).

Figure 3.1.1 visualizes the differences between the growth curves of estimated fetal weight (with non-users as reference group) and maternal cannabis use obtained from fitting the fractional polynomial model. This figure shows that using cannabis before pregnancy did not result in a significantly different fetal weight. Further, fetuses exposed to cannabis in early pregnancy had a significantly lower growth rate, compared to the reference group; continued cannabis use was associated with the strongest fetal growth reduction.

Discussion

This population-based study shows that exposure to potent cannabis in utero may be related to a reduced fetal growth and fetal head size, which are risk factors for neurodevelopmental and behavioral problems, while it is not related to gestational duration. Importantly, we found that cannabis use during pregnancy, often combined with tobacco, has an additive effect in late pregnancy and at birth over and above tobacco use. In contrast, in mid pregnancy, fetal growth deficits seem to be due to tobacco use only. Our findings further suggest that this may be particularly true for those women who continued their cannabis use throughout pregnancy. Even short-term intrauterine cannabis exposure seems to be associated with impaired fetal growth. These associations between maternal cannabis use and fetal growth were independent of lifestyle and socio-economic factors and are in line with some previous studies reporting lower birth weight in cannabis exposed babies [33-35].

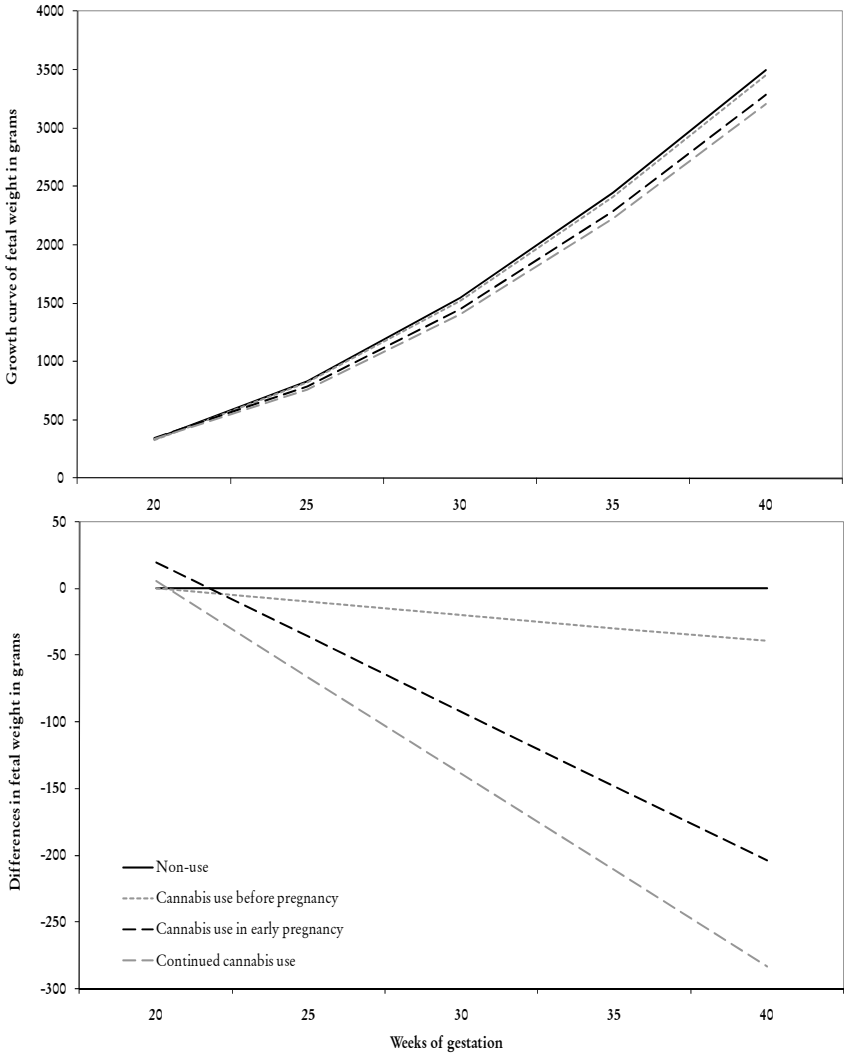

Figure 3.1.1 The estimated growth curve and difference in fetal weight due to maternal cannabis use in pregnancy compared to fetuses of mothers who did not use cannabis or tobacco.

Figure note: Estimates of differences were obtained from fitting the fractional polynomial model, adjusted for maternal age, body mass index, height, educational level, national origin, first trimester alcohol use, parity, gravidity, foetal gender and maternal psychopathology.

Furthermore, this study provided information on a tendency towards a dose-response association; it showed that particularly heavier cannabis use during pregnancy is associated with lower birth weight, independently of other related lifestyle and socioeconomic factors. Interestingly, paternal cannabis use during pregnancy was not associated with fetal growth restriction, which supports the idea that the negative association between maternal cannabis use and fetal growth could be due to intrauterine exposure [49]. Additionally, this idea is supported by the fundamental role of the cannabinoid system in prenatal development [7]. Finally, no effects of cannabis or tobacco use in pregnancy were found on cerebellar size, which was previously shown to apply to tobacco exposed fetuses in the same study population as well [50, 51].

To our knowledge, this is the first cohort examining the associations of maternal cannabis use in pregnancy with fetal growth characteristics. Strengths of this investigation include the large population-based prospective cohort we used to examine these associations, the use of ultrasound measurements in combination with information collected at birth, which enabled us to determine growth trajectories throughout gestation until birth, and the possibility to control for many important confounding factors, including lifestyle factors, socio-economic factors, and known determinants of fetal growth. Moreover, we were able to compare pregnant cannabis users, who often use cannabis in combination with tobacco, with a group of pregnant women who only used tobacco. Since cannabis and tobacco use often co-occur, this is an important addition to the existing literature. In addition, we were able to compare the strength of the associations between maternal and paternal cannabis use and fetal growth.

Nevertheless, our findings may be viewed with several limitations in mind and therefore should be cautiously interpreted. First, we used self-reported data on substance use. Both potential misclassification and selection bias may have led to an underestimation of the prevalence of cannabis use and an underestimation of the effects of cannabis exposure on the fetal growth and brain parameters. Second, the effects of maternal cannabis use on fetal characteristics may be underestimated, because the early pregnancy measurements were used for pregnancy dating, assuming that the variation in growth before the first measurement is zero. Third, because in Western-Europe cannabis use is often combined with tobacco, we compared our cannabis users with tobacco-only users, or controlled

for tobacco use. Our findings thus provided information on the effects of cannabis over and above that of tobacco. Information on the effects of cannabis use only (without tobacco), could not be retrieved from our non-clinical population-based sample. Thus, it remains desirable to study the effects of cannabis in samples of pregnant women, who use cannabis only with similar THC-levels and to replicate these findings in other population-based cohorts. Finally, we were not able to determine which teratogenic compounds of cannabis in combination with tobacco bring about the alteration in bodily proportions.

Of interest is our finding that cerebellar size was not affected by maternal cannabis use. This is consistent with the idea that the cerebellum, which is evolutionarily conserved, may be spared when intrauterine growth is impaired [52]. Although intrauterine cannabis exposure did not affect cerebellar size, it may be that cannabis acted upon the cerebellum on a molecular level. Therefore, adaptation of the cannabinoid system and related neurotransmitter systems due to stimulation of the cannabinoid receptors at critical stages cannot be excluded. Such changes in the ontogeny of neurotransmitter systems might lead to substantial and long-lasting effects in different behavioral patterns [53, 54]. However, more research is needed to elucidate the underlying mechanisms associated with the potential harmful long-term effects of intrauterine cannabis exposure.

Our findings suggest the importance to educate future mothers about the consequences of prenatal maternal cannabis use. Our findings may imply that different messages could be transmitted to tobacco and cannabis users. Our findings likely reflect that the effects of cannabis exposure, even restricted to early pregnancy, may not be reversible, while quitting smoking tobacco in early pregnancy is known to be beneficial. Thus, to prevent the potential harmful effects of intrauterine cannabis exposure, women should quit using cannabis before conception.

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Chapter 3.2

INTRAUTERINE CANNABIS EXPOSURE AND FOETAL CIRCULATORY REDISTRIBUTION

Abstract

Cannabis is a commonly used illicit drug among pregnant women. In the last decade, the potency of cannabis products has increased enormously. It is unclear whether maternal cannabis use causes hemodynamic modifications in the fetus, like tobacco does. The aim of this study is to ascertain fetal blood redistribution due to maternal cannabis use during pregnancy.

This study was embedded in the Generation R Focus Study, a population-based cohort of parents and their children followed from pregnancy to childhood. In late pregnancy fetal hemodynamics were assessed with ultrasound measurements in cannabis-exposed and non-exposed fetuses. Pregnant women reported about substance use before and during pregnancy. A distinction was made between mothers with continued cannabis use (n=9), mothers with cannabis use only in early pregnancy (n=14), mothers with continued tobacco use (n=85), mothers with tobacco use only in early pregnancy (n=92), and mothers with no tobacco or cannabis use during pregnancy (n=85).

Cannabis use during pregnancy affected the placental and cardiac fetal blood flow in late pregnancy. Continued cannabis use was associated with an increased pulsatility and resistance index of the uterine artery, while discontinued cannabis use was associated with a decreased pulsatility, and resistance index, as compared to controls. Additionally, continued cannabis exposure resulted in a significantly higher uterine pulsatility index and uterine resistance index compared to intrauterine tobacco exposure. Continued cannabis use was found to be associated with a smaller aortic diameter, as well. No association between intrauterine cannabis exposure and the fetal cerebral vascular system was found.

Our findings suggest that intrauterine cannabis exposure was associated with changes in hemodynamic programming of the vascular system of the fetus in late pregnancy mainly due to tobacco exposure, but intrauterine cannabis exposure did demonstrate a specific effect on the uterine blood flow.

Introduction

Cannabis is one of the commonly used illicit substances in Western countries. In Europe, the last-year prevalence of cannabis use among young adults has increased from on average 5% in 1990 to 15% in 2005 [1]. In pregnancy much lower prevalence rates for cannabis use are found, i.e. in 2007 2.9% of Dutch women and 1.8% of American women reported cannabis use [2, 3]. In the Netherlands, potency of cannabis products has increased considerably in the last years [4]. This study aims to study whether exposure in utero to such potent cannabis products is related to early changes in the fetal blood flow characteristics.

We focus on fetal hemodynamics because it has been shown that the endogenous cannabinoid system plays an important neuromodulatory role in cardiovascular regulation [5, 6]. Two cannabinoid (CB) receptors have been identified: the CB_1-receptor, highly expressed in the brain, but also present in peripheral tissues including the heart and vascular tissues, and the CB_2-receptor, predominantly expressed in immune and hematopoietic cells, but also present in the heart and endothelial cells of various origins [7]. Endogenous cannabinoid receptor ligands show cardiovascular effects which are similar to the effects of Δ9-tetrahydrocannabinol (THC); they reduce the blood vessel tone with an associated decrease in blood pressure and increased vascular flow [5]. Biochemical and animal studies have shown that THC and its metabolites freely pass the placental barrier [8, 9], and molecular research has shown that local actions of endocannabinoids in the human placenta are already present in early pregnancy [10]. Thus, prenatal exposure to cannabis in early fetal life could alter the endocannabinoid system and result in adaptations of the vascular system, including a reduction of vascular resistance and an increase in vascular flow.

Evidence for effects of prenatal cannabis exposure on vascular development is sparse. However, somewhat more information is available on hemodynamic effects of prenatal tobacco exposure. Epidemiological research suggests that intrauterine exposure to maternal smoking is associated with high blood pressure later in life [11, 12]. In addition, animal studies have demonstrated that prenatal nicotine exposure is associated with epigenetic modification of vascular contractility in adult offspring [13]. It has also been reported that nicotine exposure during pregnancy impairs the uterine vascular function, which may lead to an increased

vascular resistance and a decrease in uterine blood flow [14], resulting in changes of the uteroplacental circulation. As compared to tobacco, cannabis has an opposite effect on the vascular system. However, since cannabis and tobacco are often used simultaneously, it is unclear which hemodynamic modifications will be present when smoking cannabis and tobacco during pregnancy.

This study investigates the effects of cannabis and tobacco exposure in early pregnancy on placental, cardiac and cerebral blood flow in late pregnancy. We hypothesize that intrauterine cannabis and tobacco exposure will have effects on fetal circulation. Additionally, our expectation is that the effect of tobacco and cannabis together will be different than the effects of tobacco alone; i.e. we hypothesize to detect vasoconstrictive effects of tobacco exposure as compared to the non-exposed group, while we expect that the combination of smoking tobacco and cannabis will lead to a less vasoconstrictive effect since cannabis itself has a vasodilatative effect. Vasoconstriction in the placenta may cause a decreased blood flow in the fetus, which will indirectly result into decreased blood supply to the brain.

Methods

Setting and population

The present study is part of an ongoing population-based cohort; the Generation R Study [15] , set up to follow a sample of urban parents and their newborn children from early pregnancy to adolescence. The study design has been described in detail previously [15]. Detailed assessments of fetal growth and development were conducted in a subgroup of 1,232 Dutch mothers and children, referred to as the Generation R Focus Study. Of all approached women, 80% were enrolled in this subgroup in late pregnancy. This subgroup has a homogeneous ethnic background in order to exclude possible confounding or effect modification by ethnicity. For the present study, fetal circulation variables were assessed in this subset between 28 to 34 weeks of gestation.

The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki, and has been approved by the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all participating parents.

For the present analyses mothers enrolled in the Generation R Focus cohort (n=1,232) were eligible. Mothers without information on first trimester substance use were excluded from the present study (12.4%, n=153). Of the remaining mothers, all women who used cannabis during pregnancy (n=23) and all women who used tobacco during pregnancy (n=177) were included in our sample of analyses. Additionally, a random selection of women who did not use tobacco or cannabis was included as the reference group (n=85). The present analyses were performed in a total of n=285 subjects.

Measures

Routine ultrasound measurements were carried out in the whole Generation R cohort in a research setting at a regional health facility in the centre of Rotterdam in early, mid and late pregnancy. These fetal ultrasound procedures were used to establish gestational age and to assess fetal growth characteristics [16]. Estimated fetal weight was calculated with the formula by Hadlock using femur length, head and abdominal circumference [17]. Fetal circulation variables were assessed by pulsed-wave Doppler between 28 and 34 weeks' gestation in the Generation R Focus Group, obtained once in each participant. Additional information about the Doppler measurements has been described elsewhere [18]. For each measurement, three consecutive uniform waveforms were recorded by pulsed Doppler ultrasound, during fetal apnea and without fetal movement. The mean of three measurements was used for further analyses. All measurements were performed by three experienced sonographers. Doppler measurements in different vascular beds were used to determine various aspects of the fetal circulation, including the placental, cardiac and cerebral blood flow.

Placental vascular resistance was evaluated with recorded flow-velocity waveforms from the umbilical and uterine arteries. A raised umbilical artery pulsatility index (PI) and uterine artery resistance index (RI) indicate an increased placental resistance [19]. Uterine artery PI and RI were measured in the uterine arteries near the crossover with the external iliac artery. Umbilical artery PI was measured in a free-floating loop of the umbilical cord. Umbilical vein volume flow (in milliliters per minute) was determined online with the inner diameter and by placing the sample volume over the entire venous vessel, parallel to the ultrasound beam with maximal time-averaged velocity [20].

Cardiac outflow flow-velocity waveforms from the aorta and pulmonary artery were recorded from the four-chamber view and the short-axis view of the fetal heart just above the semilunar valves, respectively. Peak systolic velocity (PSV), fetal heart rate (FHR), and the inner diameter during systole of both the aorta and the pulmonary artery were recorded.

The redistribution of blood flow in favor of the fetal brain was quantified by the middle and anterior cerebral artery pulsatility indices using color Doppler visualization of the circle of Willis in the fetal brain. Reductions in middle and anterior cerebral artery PI are indicators for the brain-sparing effect and fetal redistribution [21, 22]. Flow-velocity waveforms were obtained in the proximal part of the cerebral arteries. High intraclass correlation coefficient values (>0.80) with corresponding low coefficient of variation values (<10%), which indicate adequate reproducibility for all Doppler measurements, have been reported [18]. All ultrasound examinations were performed with an ATL-Philips model HDI 5000 (Seattle, Wash) equipped with a 5.0-MHz high-frequency, curved-array transducer.

Illicit substance use was measured using a self-report questionnaire in the first trimester of pregnancy. Tobacco and alcohol use were also measured by self-report in mid- and late pregnancy. Participants reported information on timing and frequency of use. A distinction was made between the use of cannabis (marijuana and hashish) and the use of other illicit drugs (cocaine, amphetamines and heroin). We explicitly asked in two separate questions whether pregnant women used drugs before pregnancy and whether they had used any of these substances in the last three months.

In the second question the answer options were: 'No', 'Yes, until I knew I was pregnant' and 'Yes, I still use substances'. The period of last three months was chosen in this question, because enrolment was aimed at early pregnancy. Information on maternal age and education was assessed using self-report. Educational level was categorized in three levels: primary (no or primary education), secondary (lower and intermediate vocational training), and higher education (higher vocational education, and university). Information on child characteristics, such as birth weight and fetal gender were obtained from midwife and hospital registries at birth.

Statistical Analysis

Mothers were categorized in five groups based on their cannabis and tobacco use in early pregnancy: 1. continued cannabis use during pregnancy (n=9); 2. cannabis use in early pregnancy (n=14); 3. continued tobacco use during pregnancy (n=85); 4. tobacco use in early pregnancy (n=92); 5. non-users, i.e. no cannabis or tobacco use during pregnancy (n=85).

First, descriptive statistics such as maternal age and education were examined, chi-square for proportions and analysis of variance (ANOVA) for continuous data were used to determine differences between the cannabis and tobacco groups. Then, for the fetal blood flow measures, analysis of variance (ANOVA) was used to determine differences between the groups with non-use as the reference group. When a difference was present, we used linear regression analysis to determine whether cannabis and tobacco use were independently associated with these blood flow parameters. We adjusted for estimated fetal weight in late pregnancy, fetal gender and maternal education. Additionally, we also compared the effect of cannabis exposure to the effect of tobacco exposure. Statistical Package for Social Sciences (SPSS) version 15.0 was utilized for calculations. A p-value of <0.05 was considered as statistically significant.

Results

Table 3.2.1 shows the maternal characteristics of each group. Cannabis-using women are somewhat younger, and cannabis and tobacco-using women had a lower level of education than non-users. No significant differences between the groups were observed in the percentage of

mothers using alcohol during pregnancy. Importantly, this table shows that women, who continued using cannabis during pregnancy, also continued using tobacco during pregnancy. In addition, Table 3.2.2 demonstrates that pregnant women who use cannabis during pregnancy are in general frequent users; they use marijuana and/or hashish on a daily to weekly basis.

Table 3.2.3 shows that the pulsatility index of the umbilical artery late in pregnancy is significantly higher in the fetuses of tobacco using compared to non-using mothers. This trend is also seen in the fetuses of cannabis-using mothers, but the difference is not significant. The table also demonstrates that in the fetuses of continued cannabis-users the pulsatility index of the uterine artery is significantly higher than in the fetuses of non-using mothers, while in fetuses of cannabis-using mothers that stopped in early pregnancy this index is lower than in fetuses of the non-using mothers. This same pattern is seen in the resistance index of the uterine artery. For continued tobacco use and tobacco use only in early pregnancy similar, but non-significant trends are present. Table 3.2.3 further shows that the inner diameter of the aorta in fetuses of continued cannabis using mothers and continued tobacco using mothers is smaller than in fetuses of non-using mothers. When cannabis or tobacco is used only in early pregnancy, no difference in aortic diameter is observed. The diameter of the pulmonary artery was not affected by cannabis or tobacco use. However, pulmonary peak systolic velocity was lower when cannabis was used throughout pregnancy. No differences were found on fetal heart rate measured at the aorta and pulmonary artery. No association is found between maternal cannabis use and the cerebral blood flow parameters (Table 3.2.3). Tobacco use throughout pregnancy does result in a somewhat higher (non-significantly) pulsatility index in the middle cerebral artery, but tobacco use does not result in higher resistance indices (pulsatility index and peak systolic velocity) in the anterior cerebral artery. Finally, when cannabis or tobacco was used throughout pregnancy, the offspring has a significantly lower birth weight, although no differences in gestational age were observed. Cannabis use only in early pregnancy also resulted in a significantly lower birth weight, but tobacco use in early pregnancy did not (Table 3.2.3).

Table 3.2.1 Demographic sample characteristics

First trimester cannabis/ tobacco use	Continued cannabis use (n=9)	Cannabis in early pregnancy (n=14)	Continued tobacco use (n=85)	Tobacco in early pregnancy (n=92)	Non-use (n=85) random sample
Maternal age (years; SD)					
	30.2 ± 4.8	28.8 ± 3.2 *	30.8 ± 5.4	31.1 ± 4.1	31.8 ± 3.7
Maternal educational level (%)					
Low	33.3 **	21.4 **	6.0 **	5.4	1.2
Intermediate	55.6	35.7	59.5	34.8	37.6
High	11.1	42.9	34.5	59.8	61.2
Maternal tobacco use (%)					
Not in pregnancy	-	7.1	-	-	100.0
During , stopped	-	21.4	-	100.0	-
During , continued	100.0	71.4	100.0	-	-
Maternal alcohol use (%)					
Not in pregnancy	22.2	28.6	38.8	16.3 *	35.3
During , stopped	0.0	28.6	13.0	25.0	16.5
During , continued	77.8	42.9	48.2	58.7	47.1

*Table note: Values are means ± SD for continuous variables and percentages for categorical variables. ** $p \leq 0.01$; * $p \leq 0.05$; ANOVA for continuous variables and χ^2 tests for categorical variables was used with 'non-use' as reference group.; Statistical analysis on maternal tobacco use was not performed, because the groups were selected on the basis of this variable*

Table 3.2.2 Maternal cannabis use before and during pregnancy

Maternal cannabis use	Continued cannabis use (n=9)	Cannabis use in early pregnancy (n=14)
Frequency of use before pregnancy		
Daily	66.7	35.7
Weekly	33.3	57.1
Monthly	n.a.	7.1
Unknown	n.a.	n.a.
Frequency of use during pregnancy		
Daily	33.3	35.7
Weekly	66.7	28.6
Monthly	n.a.	14.3
Unknown	n.a.	21.4
What was used before pregnancy		
Marijuana	44.4	64.3
Hashish	n.a.	28.6
Both	55.6	7.1
What was used during pregnancy		
Marijuana	55.6	64.3
Hashish	44.4	35.7
Both	n.a.	n.a.

Table note: Values are percentages for categorical variables

Based on the significant results of the ANOVA's displayed in Table 3.2.3, linear regression analyses were performed, and in the adjusted models fetal gender, estimated weight at time of the measurement as well as maternal educational level were taken into account. The results of these regression analyses in Table 3.2.4 show that continued cannabis use after adjustment for fetal weight, gender and maternal education, showed no significant effects. In contrast, there is a negative relationship between cannabis use in early pregnancy and pulsatility and resistance index in the uterine artery, which remained statistically significant after adjustment for the covariates. Similar findings are found for tobacco use in early pregnancy with differences remaining significant after controlling for possible confounding factors. Finally, both continued cannabis use and continued tobacco use are related to a smaller inner diameter of the aorta. These associations remain significant after adjustment for gender and fetal weight, and become borderline non-significant when including maternal education in the model.

Additionally, we compared the effects on the fetal parameters of cannabis-using groups to the tobacco-using groups. These additional analyses showed that nearly all effects found in the cannabis-using groups were not statistically different as compared to the effects in the tobacco-using groups. However, only the effect of continued cannabis exposure on the resistance indices in uterine artery remained significantly different from the flow observed in continued tobacco exposure. The uterine pulsatility index was higher in continued cannabis exposed fetuses compared to continued tobacco exposure, even after controlling for the covariates ($\beta=0.19$; 95%CI 0.01-0.36; $p< 0.05$). The same holds for the resistance index of the uterine artery ($\beta=0.07$; 95%CI 0.01-0.13; $p<0.05$).

Table 3.2.3 Fetal blood flow characteristics

First trimester cannabis and tobacco use	Continued cannabis use (n=9)	Cannabis in early pregnancy (n=14)	Continued tobacco use (n=85)	Tobacco in early pregnancy (n=92)	Non-use (n=85 random sample)
Fetal blood flow in late pregnancy					
Placental blood flow					
Umbilical artery (PI)	0.99 ± 0.26	1.01 ± 0.18	1.03 ± 0.18 **	0.99 ± 0.17	0.95 ± 0.15
Umbilical vein flow	203.7 ± 76.5	239.6 ± 68.4	234.2 ± 55.7	255.2 ± 62.2	236.1 ± 61.6
Uterine artery (PI)	0.58 ± 0.14 *	0.44 ± 0.07 *	0.51 ± 0.09	0.47 ± 0.09 *	0.50 ± 0.11
Uterine artery (RI)	1.00 ± 0.44 *	0.63 ± 0.17 *	0.80 ± 0.26	0.70 ± 0.22 *	0.79 ± 0.30
Cardiac blood flow					
FHR Aorta	140.38 ± 8.80	139.80 ± 10.93	139.4 ± 8.0	138.1 ± 9.8	138.8 ± 9.8
Inner Diameter Aorta	0.59 ± 0.10 **	0.62 ± 0.07	0.62 ± 0.07 **	0.65 ± 0.07	0.66 ± 0.06
Aortic PSV (cm/s)	85.8 ± 9.6	88.8 ± 11.4	98.8 ± 11.6	92.1 ± 13.1	89.9 ± 13.3
FHR Pulmonary artery	139.38 ± 14.70	141.08 ± 8.91	136.7 ± 10.3	138.7 ± 8.7	139.3 ± 9.4
Diameter of pulmonary art.	0.77 ± 0.10	0.79 ± 0.08	0.78 ± 0.08	0.80 ± 0.09	0.80 ± 0.09
Pulmonary PSV (cm/s)	66.8 ± 7.8 *	72.6 ± 8.2	72.5 ± 8.2	74.3 ± 9.3	73.3 ± 9.3
Cerebral blood flow					
Middle cerebral artery PI	1.87 ± 0.29	1.93 ± 0.31	2.03 ± 0.34	1.98 ± 0.29	1.92 ± 0.33
Middle cerebral artery PSV	40.4 ± 12.0	44.7 ± 5.6	42.8 ± 8.9	42.7 ± 9.0	43.9 ± 9.3
Anterior cerebral artery PI	1.75 ± 0.45	1.64 ± 0.34	1.74 ± 0.25	1.70 ± 0.27	1.8 ± 0.4
Anterior cerebral artery PSV	40.5 ± 9.7	39.1 ± 12.9	38.6 ± 13.1	40.9 ± 13.5	41.2 ± 14.5

Table 3.2.3 Continued

First trimester cannabis and tobacco use	Continued cannabis use (n=9)	Cannabis in early pregnancy (n=14)	Continued tobacco use (n=85)	Tobacco in early pregnancy (n=92)	Non-use (n=85 random sample)
Child characteristics at measurement and at birth					
Estimated weight (30wks)	1589 ± 423	1410 ± 225 **	1566 ± 266 **	1622 ± 278	1694 ± 268
Birth weight	3158 ± 628 *	3191 ± 640 *	3334 ± 575 *	3482 ± 610	3553 ± 532
Gender (% boys)	77.8	35.7	64.7	42.4 *	61.2
Gestational age at birth	39.9 ± 2.4	39.3 ± 1.8	39.9 ± 1.8	39.9 ± 1.6	39.9 ± 1.8

*Table note: Values are means ± SD for continuous variables and percentages for categorical variables; PI = Pulsatility Index; RI = Resistance Index; FHR = Fetal Heart rate; PSV = Peak Systolic Velocity; ANOVA for continuous variables and χ^2 tests for categorical variables was used with 'non-use' as reference group [a]; ** $p \leq 0.01$; * $p \leq 0.05$*

Table 3.2.4 The association of maternal cannabis and tobacco use and fetal blood flow characteristics

First trimester cannabis and tobacco use	Continued cannabis use (n=9)	Cannabis use in early pregnancy (n=14)	Continued tobacco use (n=85)	Tobacco use in early pregnancy (n=92)	Non-use (n=85 random sample)
	Regression coefficients with 95% confidence intervals				
Placental blood flow in late pregnancy					
Model 1					
Umbilical artery (PI)	.05 (-.07; .17)	.07 (-.03; .16)	.08 (.03; .13) **	.04 (-.01; .09)	1.0 $
Umbilical vein flow	-32.5 (-77.1; 12.1)	3.4 (-32.7; 39.5)	-3.0 (-23.2; 17.3)	19.5 (-.2; 39.1)	1.0 $
Uterine artery (PI)	.21 (.01; .41) *	-.16 (-.32; .00) *	.02 (-.07; .10)	-.09 (-.16; -.01) *	1.0 $
Uterine artery (RI)	.08 (.01; .15) *	-.06 (-.12; -.00) *	.01 (-.03; .04)	-.03 (-.06; -.01) *	1.0 $
Model 2					
Umbilical artery (PI)	-.02 (-.13; .09)	-.03 (-.12; .07)	.04 (-.01; .09)	.02 (-.03; .07)	1.0 $
Umbilical vein flow	-5.9 (-50.5; 38.6)	31.0 (-5.3; 67.2)	9.5 (-10.6; 29.5)	21.6 (2.9; 40.4) *	1.0 $
Uterine artery (PI)	.19 (-.02; .39)	-.22 (-.39; -.05) *	-.01 (-.10; .08)	.10 (-.18; -.02) *	1.0 $
Uterine artery (RI)	.07 (-.00; .14)	-.08 (-.14; -.02) **	-.00 (-.04; .03)	-.04 (-.07; -.01) *	1.0 $
Cardiac blood flow in late pregnancy					
Model 1					
FHR Aorta	1.55 (-5.1; 8.2)	0.95 (-4.5; 6.4)	0.62 (-2.5; 3.7)	-1.44 (-4.4; 1.5)	1.0 $
Diameter Aorta	-.07 (-.12; -.02) **	-.03 (-.07; .01)	-.04 (-.06; -.01) **	-.01 (-.03; 0.2)	1.0 $
Pulmonary PSV (cm/s)	-6.4 (-12.9; .06) *	-.70 (-6.1; 4.7)	-.60 (-3.6; 2.4)	1.06 (-1.9; 4.0)	1.0 $
Model 2					
FHR Aorta	0.78 (-6.3; 7.9)	0.92 (-4.9; 6.7)	0.41 (-2.9; 3.7)	-1.54 (-4.6; 1.5)	1.0 $
Diameter Aorta	-.04 (-.09; .01)	-.01 (-.05; .03)	-.02 (-.04; .00)	-.00 (-.02; .02)	1.0 $
Pulmonary PSV (cm/s)	-4.4 (-11.2; 2.4)	-.03 (-5.6; 5.8)	.16 (-3.0; 3.3)	1.20 (-1.7; 4.1)	1.0 $

Table note: PI = Pulsatility Index; RI = Resistance Index; FHR = Fetal Heart rate; PSV = Peak Systolic Velocity; Model 1: Unadjusted analyses; Model 2: Model 1 + adjustment for fetal weight, gender and maternal education; $ is Reference category
*** $p \leq .01$ * $p \leq .05$*

Discussion

The aim of the present study was to determine the effect of maternal cannabis use during pregnancy on fetal hemodynamic adaptations. To our knowledge, this is the first study that examined these characteristics in fetuses exposed to cannabis in a population-based sample. Investigating these hemodynamic adaptations is important, as several studies have shown that intrauterine cannabis exposure is negatively related to birth outcomes [23-25]. Importantly, in a previous study we showed that in utero cannabis exposure at any time during pregnancy is negatively associated with fetal growth. We found reduced growth on several growth parameters, including abdominal and head circumference, femur length, and birth weight [26]. Moreover, several studies have shown that maternal cannabis use during pregnancy is associated with behavioral and cognitive development later in life [27-30]. The long-term effects of cannabis exposure during pregnancy could be caused by environmental factors, since maternal cannabis use is associated with multiple unfavorable characteristics [2], but it could also have a physiological foundation, such as an altered vascular system. Our results demonstrate that cannabis use during pregnancy is indeed associated with adaptations in fetal placental and cardiac blood flow, but not with cerebral blood flow. However, this association could be explained by the co-occurrence of tobacco use during pregnancy in this group as we have found that the blood flow parameters did not significantly differ between cannabis-exposed fetuses and tobacco-exposed fetuses. Importantly, we found statistically significant specific associations between maternal cannabis use and the uterine resistance indices, which remained present after taking into account maternal tobacco use.

Continued cannabis use was associated with an increased fetal pulsatility index and resistance index of the uterine artery, which may indicate an increased placental resistance during pregnancy [19]. Insufficient placental circulation is an important cause of fetal growth restriction [19]. Previous work has shown that increased placental impedance is also associated with a reduced umbilical vein volume flow [18]. Although we visually observed a lower umbilical vein volume flow in continued cannabis users, this was not a statistically significant difference. Unexpectedly, in women who used cannabis only in early pregnancy we found the opposite effect; uterine pulsatility index and resistance index

were lower than in the non-using mothers. This latter finding may reflect the differential result of occasional cannabis and chronic cannabis use during pregnancy. Animal studies have shown that cannabis receptors are expressed in placental tissues in early pregnancy [10], and that endocannabinoid levels decrease gradually during pregnancy [31]. Moreover, high endocannabinoid levels are associated with failure of developing a normal pregnancy [32]. Thus, exposure to cannabinoids during pregnancy could lead to inappropriate activation of the CB-mediated pathways in the placental cells. Moreover, we found a similar trend in fetuses exposed to tobacco only; tobacco use in early pregnancy only also resulted in lower uterine resistance indices, while continued tobacco did not.

In terms of cardiac blood flow, we found effects on the aortic diameter in the continued cannabis users and the continued tobacco users. Possibly, this might be a lasting effect due to non-optimal development of the blood vessels and therefore these vessels were smaller. Moreover, the effect of cannabis exposure was not different from the effect of tobacco exposure. Thus, even though THC-administration in in-vitro experiments leads to vasorelaxation of the aorta [33], we did not observe this in our study. THC-induced vasorelaxation does not neutralize the tobacco-induced vasoconstriction; despite frequent cannabis use in this sample is frequent (daily/weekly). Moreover, it could be possible that THC does cause acute vasorelaxation, but does not cause long-term vasodilatation in prenatal exposed fetuses. In continued cannabis-users it seems that a lower peak systolic velocity in the pulmonary artery is present, though not statistically significant after controlling for confounding. A progressive decrease of PSV in the cardiac outflow has been observed in growth restricted fetuses [34] and may indicate a diminished cardiac function.

We did not find any differences in blood flow of the cerebral arteries in the cannabis exposed fetuses. It may be possible that this difference was not observed due to preferential perfusion of the fetal central nervous system, i.e. brain sparing, caused by placental insufficiency in cannabis and tobacco exposed fetuses.

The strength of this study is that we were able to focus in-depth on blood flow distribution in cannabis-exposed fetuses using ultrasound measurements in a prospective design. However, the main limitation in this study is the small sample size. First, cannabis use in pregnancy is

rare. Second, underestimation due to misclassification is possible, since we used self-reported data on substance use. Third, due to the small sample size, this study may not have enough power for adjustment with multiple confounding factors in the analysis, which leads to non-significance of the observed effects.

In conclusion, our findings suggest that intrauterine cannabis exposure is associated with changes in the hemodynamic programming of vascular system in late pregnancy. However, the association found was rather weak and was probably to be induced by tobacco exposure instead of cannabis exposure during pregnancy. However, an effect induced by cannabis itself cannot be ruled out. Further research in larger samples is important to evaluate the long-lasting effects of prenatal cannabis use on fetal blood flow parameters.

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Chapter 4

INTRAUTERINE CANNABIS EXPOSURE AND INFANT CONSEQUENCES

Contents of this chapter

In the previous chapter, we described the association between maternal cannabis use and foetal outcomes, such as foetal growth reduction in cannabis-exposed children. This chapter focuses on the long-lasting effects of intrauterine cannabis exposure in children and describes the associations between maternal cannabis use during pregnancy and child behavioural problems at 18 and 36 months of life. This chapter also depicts the association between maternal cannabis use and verbal and non-verbal cognitive functioning at the age of 30 months. Again, like in the previous chapters, we took into account several maternal demographic, emotional and social factors that may be underlying to these associations.

Chapter 4.1

PARENTAL CANNABIS USE AND CHILD BEHAVIOUR AT 18 MONTHS

Abstract

This study compared the strength of associations between intrauterine cannabis exposure and infant behavioral problems at 18 months of age, with the association between maternal cannabis use only prior to pregnancy, or paternal cannabis use, and these offspring outcomes.

Within a population-based birth cohort, the Generation R Study, parents reported on their cannabis use habits. Behavioral problems were assessed with the Child Behavior Checklist. In total, information was available in n=3,806 children.

After adjustment for confounders (age and gender of the child, parental education, national origin and alcohol use), maternal cannabis use during pregnancy was associated with more Externalizing Problems in exposed children compared to non-exposed children (odds ratio (OR) 1.86, 95% confidence interval (CI):1.06-3.27). Paternal cannabis use was not associated with a higher risk of offspring behavioral problems when considering confounding factors. Maternal cannabis use during pregnancy was specifically related to Externalizing Problems in girls (OR=3.57, 95%CI: 1.64-7.77), but not in boys. Moreover, after adjustment for maternal psychopathology, the association in girls remained statistically significant (OR=3.07, 95%CI: 1.40-6.79).

Parental cannabis use during pregnancy is associated with problem behavior in children at 18 months of age. Importantly, a gender-specific association was found for maternal cannabis use during pregnancy and child behavior, which may be partially explained by a biological mechanism due to intrauterine cannabis exposure, and partially explained by an unfavorable environment in which these cannabis-exposed children grow up.

Introduction

Lately, the media attention for cannabis in Western countries has increased. The higher levels of Δ9-tetrahydrocannabinol (THC) [1], which may yield stronger effects, and a persistent discussion of legalization of cannabis use, in Europe as well as the U.S. and Canada, contributed to this interest.

Consuming cannabis with high THC-levels during pregnancy may be harmful for the child, since cannabinoids pass the placental barrier [2]. In-vitro studies suggest that intrauterine cannabis exposure might particularly harm the development of the child's brain. First, the endocannabinoid system, present and functional in early prenatal periods, plays an important role in developmental processes of the central nervous system, including cell proliferation, migration and differentiation [3]. Second, intrauterine exposure may alter the expression of key genes for neural development and lead to neurotransmitter and behavioral disturbances [4]. Findings from animal studies support the adverse influence of prenatal cannabis exposure on brain development by indicating permanent effects on functional regulation of motor behaviors [5], memory processes [6], and emotional reactivity [7].

Whereas the influence of prenatal cannabis exposure on brain developmental processes has been the focus of several studies, only two longitudinal studies addressed the influence on behavioral outcomes in human offspring [8,9]. In these studies, rather weak associations were found between intrauterine cannabis exposure and offspring behavioral problems at the ages of six years [10,11] and ten years. [12,13]. Although these studies are useful in providing more insight in the association between fetal cannabis exposure and offspring behavioral problems, there are several issues that should be considered. First, both previous studies used cohorts that started more than 25 years ago. Given the increase in THC-concentrations in cannabis in the last decade(s), the influence of intrauterine cannabis on behavioral problems is expected to be more pronounced in younger generations. Second, deviation from normal child development can be best assessed using general population cohorts. The previous studies, however, examined high-risk cohorts in terms of cannabis use, with 20 and 40% of the pregnant mothers using cannabis, compared to 2.8-4.5% in the general population [14,15]. Third, in order to determine if cannabis use affects child behavior because of intrauterine

influences on fetal development, the influence of confounding factors that could generate non-causal links should be considered. Moreover, comparing the associations between maternal and paternal prenatal cannabis use in relation to offspring outcome, may provide information about the potential effect of intrauterine exposure [16]. So far, none of the studies used the approach in which paternal cannabis use during their partners' pregnancy and child outcome was taken into account. Finally, literature has suggested that gender may be an important modulating factor in a variety of cannabinoid effects and that when gender-specific effects are found, females are usually more sensitive than males to cannabinoids [17]. However, it is unknown whether prenatal cannabis exposure also results in gender-specific effects in offspring.

In the current study, we examined the hypothesis that prenatal parental cannabis use is negatively associated with child behavioral problems at the age of 18 months in a low-risk general population sample. Moreover, we were able to determine whether parental cannabis use during pregnancy was differentially associated in boys and girls.

Method

Setting and population

This study was conducted within the Generation R study, a population based birth cohort in Rotterdam, the Netherlands, [18] set up to collect data on a sample of urban parents and their newborn children from early pregnancy onwards. All children were born between April 2002 and January 2006. The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki, and has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participating parents. For the current study, the parents of 7,893 children were approached for postnatal participation. Mothers of 598 children did not give full consent for post-natal participation. Children without information on maternal substance use habits in pregnancy were excluded (n = 1,490, 20.5%). Women who used other illicit substances during pregnancy without using cannabis were also excluded (n=27). Women who used tobacco only in early pregnancy were not suitable for the reference group and were also excluded (n=647). The remaining 5,131 children were eligible for the present study. We received in total

3,858 18-months questionnaires. Information on child behavioral problems at 18 months was available in 3,806 children (74.2% of 5,131), and these children were included in the analyses.

Measures

Tobacco, alcohol and substance use were measured using a self-report questionnaire in the first trimester of pregnancy. Participants reported information on timing and frequency of use. A distinction was made between the use of cannabis (marijuana and hashish) and the use of other illicit drugs (cocaine, amphetamines and heroin). We explicitly asked in two separate questions whether pregnant women used drugs before pregnancy and whether they had used any of these substances in the last three months before completing this questionnaire. Because enrollment was aimed at early pregnancy, the period of last three months was chosen in this question. In the second question the answer options were: 'No', 'Yes, until I knew I was pregnant' and 'Yes, I still use substances' (timing). Also mothers indicated whether they had used cannabis before and/or during pregnancy on a daily, weekly or monthly basis (frequency). Women that reported the use of cannabis during pregnancy often continued cigarette smoking as well (85%). In order to assess the influence of cannabis above and beyond the influence of tobacco, we categorized intrauterine exposure in 4 groups, according to cannabis and tobacco use before or during pregnancy. Thus, our population was divided in:

1. Non- use (N=3,272)
2. Cannabis use during pregnancy (N=95)
3. Cannabis use before pregnancy (N=163)
4. Tobacco use only throughout pregnancy (N=276)

Both mothers and fathers were questioned about paternal cannabis use habits during pregnancy of their partner (cannabis use versus non-use). We used maternal information on paternal cannabis use when fathers did not complete this questionnaire. Maternal report on paternal cannabis use was highly correlated to partner's self-reported cannabis use ($r=0.83$, $p<0.001$).

The Child Behavior Checklist for toddlers (CBCL 1½–5 yrs) was used to acquire a standardized maternal report of children's problem

behaviors. The Total Problems score consists of a sum score of the 99 problem items. The internalizing scale is the sum score of items in four syndrome scales: Emotionally Reactive, Anxious/Depressed, Somatic Complaints and Withdrawn. The externalizing scale is the sum score of Attention Problems and Aggressive Behavior. Each item is scored 0=not true, 1=somewhat or sometimes true and 2=very true or often true, based on the preceding two months. Good reliability and validity have been reported for the CBCL [19]. We used the borderline cut-off score (83rd percentile of a Dutch norm group [20]) to classify children as having behavioral problems in the borderline/clinical range.

Demographic and obstetric information such as maternal age, ethnicity, education, and parity was assessed using self-report. Parental educational level and national origin was defined according to the classification of Statistics Netherlands. [21,22] Educational level was categorized in three levels: primary (no or primary education), secondary (lower and intermediate vocational education), and higher education (higher vocational education and university). Parental national origin was classified into seven categories: 1. Dutch, 2. Cape Verdean, 3. Moroccan, 4. Turkish, 5. Surinamese 6. Antillean and 7. other national origin. Parental psychopathology was measured using the Brief Symptom Inventory (BSI), a validated 53-item (5-point scale) self-report symptom inventory outlined to ascertain the psychological state of individuals. [23] The Global Severity Index (GSI) subscale of the BSI was used to determine general psychopathology symptoms. Gender of the child was obtained from midwives and hospital registries at birth.

Statistical Analysis

Differences in maternal and paternal characteristics were analyzed using χ^2-tests for categorical variables and ANOVA's for continuous variables with the non-users as the reference group. Successive logistic regression analyses models were performed to determine whether the association between parental cannabis use and behavioral problems remained present when the following covariates were taken into account: age and gender of the child, parental education, national origin and psychopathology. To determine whether gender-specific effects of parental cannabis use on behavioral problems were present, we first introduced an interaction term of parental cannabis use and child gender in the model. When this interaction term was significant, we performed stratified analysis for the

effects of parental cannabis use and behavioral problems for boys and girls. Measures of association (Odds Ratios, ORs) are presented with the 95% Confidence Intervals (CIs). Statistical analyses were performed using the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc. Chicago, IL).

Non-response analysis

Response analyses showed that mothers of children included in the current analyses were older (31.2 ± 4.6 vs. 28.3 ± 5.5, $p<0.001$), higher educated (56.3% vs. 26.2% higher educational level, $\chi^2=762.8$, $p<0.001$) and more often of Dutch national origin (62.5% vs. 32.8%, $p<0.001$) as compared to mothers of children who were not in the analyses. The mothers included in the analyses had lower psychopathology scores (0.26 ± 0.31 vs. 0.36 ± 0.44, $p<0.001$) and these mothers were more likely to be non-users of cannabis and tobacco (85.6% vs. 77.8% non-users, $p<0.001$).

The fathers included in the analyses were older (33.7 ± 5.5 vs. 31.7 ± 6.5, $p<0.001$), higher educated (60.7% vs. 37.1% higher educational level, $p<0.001$) and more often of Dutch national origin (64.5% vs. 38.5%, $p<0.001$) as compared to fathers who were not in the analyses. The fathers included in the analyses had lower psychopathology scores (0.12 ± 0.19 vs. 0.17 ± 0.28, $p<0.001$) and these fathers were more likely to be cannabis non-users (90.0% vs. 86.8% non-users, $p<0.001$).

Results

Mothers who use cannabis during pregnancy are younger, more often pregnant of their first child, and often consumed alcohol during pregnancy as well (Table 4.1.1). Moreover, they were lower educated, and more often of Surinamese or Antillean national origin, and had a higher level of psychopathology. Fathers of children exposed to intrauterine cannabis were younger, lower educated, more often of Cape Verdean and Surinamese ethnicity. When mothers used cannabis during pregnancy, 85.2% of fathers used cannabis as well. Finally, Table 4.1.1 demonstrates that children exposed to intrauterine cannabis or tobacco have an increased level of Externalizing Problems as compared to non-exposed children.

Parental cannabis use and child behavior

The unadjusted analyses (Table 4.1.2) showed that maternal cannabis use during pregnancy was not related to a higher risk of CBCL Internalizing Problems (OR=0.81; 95%CI:0.29-2.25) compared with non-exposed children. However, maternal cannabis use during pregnancy was related to a higher risk of Externalizing Problems in children compared with the risk of Externalizing Problems in children when there was no prenatal cannabis or tobacco exposure (OR=2.16; 95%CI:1.24-3.74). Introduction of the confounders attenuated the risk for Externalizing Problems, but the risk remained statistically significant (OR=1.86; 95%CI: 1.06-3.27). Finally, parental psychopathology was introduced into the multivariate model; findings indicate that the included covariates accounted for the effects of maternal cannabis use during pregnancy on child behavior (OR=1.56; 95%CI:0.87-2.77). Similar findings are displayed in Table 4.1.2 for tobacco use. No associations were found between maternal cannabis use before pregnancy and problem behavior in children.

Similarly, we examined the contribution of these covariates in the association between paternal cannabis use and child behavior. Unadjusted analyses showed that paternal cannabis use was not related to a higher risk of Internalizing Problems (OR=1.33; 95%CI:0.80-2.24) compared with non-exposed, but it was associated to CBCL Total Problems (OR=1.69; 95%CI:1.11-2.57) and Externalizing Problems (OR=1.54; 95%CI:1.09-2.18). However, after controlling for the influences of confounders, the associations were no longer statistically significant.

Table 4.1.1: Maternal cannabis use and parental and child characteristics

	Cannabis use during pregnancy (n=95)		Cannabis use before pregnancy (n=163)		Tobacco use during pregnancy (n=276)		Non-use (n=3,272)
Maternal characteristics							
Age (yrs)	28.5 ± 5.2	**	30.4 ± 4.9	**	30.6 ± 5.5	**	31.3 ± 4.3
Nulliparous (%)	76.8	**	68.7	**	51.1	**	58.0
Alcohol use (%)							
Use during pregnancy	68.4	**	47.8		44.9		49.1
Educational level (%)							
Primary	13.7	**	4.3	*	10.1	**	5.1
Secondary	56.7		46.1		63.7		33.5
Higher	28.3		48.4		23.5		60.3
National origin (%)							
Dutch	63.2	*	62.6	*	60.9	**	62.7
Cape Verdean	2.1		3.0		4.2		2.1
Moroccan	2.1		0.5		1.0		4.1
Turkish	1.0		1.1		14.8		5.9
Surinamese	11.5		5.4		5.7		5.4
Antillean	5.2		3.6		2.1		1.6
Other origin	14.6		23.2		9.4		17.5
Psychopathology							
Global Severity Index	0.42 ± 0.42	**	0.28 ± 0.32		0.33 ± 0.35	**	0.22 ± 0.26
Paternal characteristics							
Paternal age	31.5 ± 6.1	**	31.9 ± 5.6	**	32.3 ± 6.1	**	33.9 ± 5.3
Educational level (%)							
Primary	11.3	**	13.5	**	9.6	**	3.8
Secondary	60.3		39.2		58.7		32.0
Higher	28.2		47.1		31.5		64.1
National origin (%)							
Dutch	55.2	**	52.1	**	59.1	**	65.6
Cape Verdean	6.8		3.3		4.0		1.8
Moroccan	3.3		0.7		1.4		4.8
Turkish	2.2		7.5		3.2		2.1
Surinamese	12.5		10.8		7.0		4.7
Antillean	2.2		1.6		13.1		5.5
Other origin	17.1		23.4		11.8		15.1
Psychopathology							
Global Severity Index	0.18 ± 0.23		0.15 ± 0.17		0.15 ± 0.20		0.12 ± 0.18
Paternal cannabis use	85.2	**	56.2	**	16.9	**	5.1

Table note: Values are means ± SDs for continuous variables and percentages for categorical variables. P-values are derived from ANOVAs for continuous variables and $\chi 2$-tests for categorical variables ($p<0.05$, ** $p<0.01$) with non-use as the reference.*

Table 4.1.2 Logistic regression models of the effects of parental cannabis use and child behavior

	Unadjusted model		Confounder adjusted		Confounder adjusted + psychopathology
	Risk for child problem behavior in children (OR + 95% CI)				
Maternal cannabis use					
	Total problems				
Cannabis during	2.03 (1.06 -3.90)	*	1.83 (0.93-3.62)		1.44 (0.72-2.89)
Cannabis before	1.75 (0.96-3.18)		1.90 (1.02-3.52)	*	1.66 (0.87-3.16)
Tobacco during	1.75 (1.16-2.64)	**	1.51 (0.98-2.32)		1.31 (0.84-2.04)
Non-use	1.0		1.0		1.0
	Externalizing problems				
Cannabis during	2.16 (1.24-3.74)	**	1.86 (1.06-3.27)	*	1.56 (0.87-2.77)
Cannabis before	1.69 (1.02 -2.82)	*	1.64 (0.98-2.74)		1.50 (0.89-2.54)
Tobacco during	1.65 (1.16-2.36)	**	1.47 (1.02-2.11)	*	1.33 (0.20-1.93)
Non-use	1.0		1.0		1.0
	Internalizing problems				
Cannabis during	0.81 (0.29-2.25)		0.74 (0.26-2.09)		0.58 (0.20-1.67)
Cannabis before	1.07 (0.49-2.36)		1.21 (0.54-2.72)		1.02 (0.44-2.35)
Tobacco during	1.49 (0.93-2.39)		1.27 (0.77-2.08)		1.09 (0.66-1.82)
Non-use	1.0		1.0		1.0
Paternal cannabis use					
	Total problems				
Cannabis use	1.69 (1.11-2.57)	*	1.43 (0.92-2.21)		1.29 (0.83-2.02)
No cannabis use	1.0		1.0		1.0
	Externalizing problems				
Cannabis use	1.54 (1.09-2.18)	*	1.33 (0.93-1.91)		1.23 (0.86-1.77)
No cannabis use	1.0		1.0		1.0
	Internalizing problems				
Cannabis use	1.33 (0.80-2.24)		1.16 (0.68-1.99)		1.06 (0.61-1.83)
No cannabis use	1.0		1.0		1.0

Table note: Confounders age and gender child, parental education, national origin and maternal alcohol use $p<0.05$, ** $p<0.01$*

Parental cannabis use and gender-specific child behavior

Because the interaction term '*gender x parental cannabis use*' was statistically significant, stratified analyses for parental cannabis use in boys and girls were performed (Table 4.1.3). These models showed that maternal cannabis use was not associated with problem behavior in boys, but it was in girls. Intrauterine cannabis-exposed daughters showed an increased risk for CBCL Total Problems compared to non-exposed daughters, even after controlling for all covariates (OR=3.07; 95%CI:1.21-7.76). They had a higher risk of Externalizing Problems (OR=3.07; 95%CI:1.40-6.79). No association was found between maternal cannabis use and Internalizing Problems in girls (OR=0.72; 95%CI:0.16-3.30). Sons and daughters of mothers who used cannabis only before pregnancy did not display an increased risk for behavioral problems. Girls exposed to maternal tobacco use during pregnancy also showed an increased risk for Total Problems (OR=2.37; 95%CI:1.33-4.23), but the risk was somewhat lower compared to the risk in girls exposed to maternal cannabis use. In contrast, these girls had an increased risk for Internalizing Problems (OR=1.96; 95%CI:1.05-3.66), but no increased risk for Externalizing Problems (Table 4.1.3). When taking into account all covariates and paternal cannabis use in the relation between maternal cannabis use and the risk of Externalizing Problems in girls, the risk remained statistically significant as well (OR=3.43; 95%CI:1.31-8.96).

Similarly, we investigated the gender-specific risk for having behavioral problems when fathers used cannabis in boys and girls separately. Significant associations were found for Externalizing Problems and Total Problems in boys and girls in the unadjusted analyses, but after adjustment for covariates these associations did not remain statistically significant (Table 4.1.3).

Table 4.1.3 Logistic regression models of the effects of parental cannabis and gender-specific child behavior

	Unadjusted model		Confounder adjusted		Confounder adjusted + psychopathology
	Risk for child problem behavior in boys (OR + 95% CI)				
Maternal cannabis use					
	Total Problems				
Cannabis during	1.04 (0.37-2.96)		0.90 (0.31-2.64)		0.66 (0.22-1.98)
Cannabis before	1.22 (0.51-2.89)		1.33 (0.54-3.24)		0.98 (0.37-2.58)
Tobacco during	0.91 (0.47-1.79)		0.78 (0.39-1.57)		0.65 (0.32-1.32)
Non-use	1.0		1.0		1.0
	Externalizing problems				
Cannabis during	1.31 (0.57-2.98)		0.99 (0.43-2.31)		0.80 (0.34-1.90)
Cannabis before	1.46 (0.72-2.94)		1.41 (0.69-2.87)		1.20 (0.57-2.52)
Tobacco during	1.40 (0.85-2.29)		1.15 (0.69-1.93)		1.03 (0.61-1.73)
Non-use	1.0		1.0		1.0
	Internalizing problems				
Cannabis during	0.66 (0.16-2.76)		0.63 (0.15-2.70)		0.45 (0.10-1.99)
Cannabis before	0.50- (0.12-2.08)		0.54 (0.13-2.30)		0.33 (0.07-1.57)
Tobacco during	0.59 (0.23-1.47)		0.55 (0.21-1.40)		0.43 (0.16-1.13)
Non-use	1.0		1.0		1.0
	Risk for child problem behavior in girls (OR + 95% CI)				
	Total Problems				
Cannabis during	3.89 (1.64-9.18)	**	3.74 (1.50-9.30)	**	3.07 (1.21-7.76)
Cannabis before	2.59 (1.13-5.94)	*	2.96 (1.25-7.03)	*	2.96 (1.25-7.00)
Tobacco during	3.11 (1.82-5.35)	**	2.65 (1.50-4.66)	**	2.37 (1.33-4.23)
Non-use	1.0		1.0		1.0
	Externalizing problems				
Cannabis during	3.64 (1.70-7.78)	**	3.57 (1.64-7.77)	**	3.07 (1.40-6.79)
Cannabis before	1.98 (0.94-4.18)		2.04 (0.96-4.34)		2.02 (0.95-4.30)
Tobacco during	1.97 (1.19-3.27)	**	1.86 (1.11-3.12)	*	1.71 (1.02-2.89)
Non-use	1.0		1.0		1.0
	Internalizing problems				
Cannabis during	1.02 (0.24-4.35)		0.88 (0.19-3.97)		0.72 (0.16-3.30)
Cannabis before	1.94 (0.74-5.02)		2.31 (0.85-6.27)		2.30 (0.85-6.24)
Tobacco during	2.79 (1.57-4.96)	**	2.19 (1.19-4.05)	*	1.96 (1.05-3.66)
Non-use	1.0		1.0		1.0

Table 4.1.3 Continued

	Unadjusted model	Confounder adjusted	Confounder adjusted + psychopathology
Paternal cannabis use			
	Risk for child problem behavior in boys (OR + 95% CI)		
	Total problems		
Cannabis use	1.60 (0.88 -2.89)	1.38 (0.75-2.55)	1.27 (0.68-2.36)
No cannabis use	1.0	1.0	1.0
	Externalizing problems		
Cannabis use	1.72 (1.09-2.71) **	1.49 (0.93-2.38)	1.37 (0.85-1.37)
No cannabis use	1.0	1.0	1.0
	Internalizing problems		
Cannabis use	1.45 (0.70-2.99)	1.22 (0.58-2.58)	1.14 (0.54-2.42)
No cannabis use	1.0	1.0	1.0
	Risk for child problem behavior in girls (OR + 95% CI)		
	Total Problems		
Cannabis use	1.80 (0.99-3.26)	1.49 (0.80-2.76)	1.32 (0.70-2.49)
No cannabis use	1.0	1.0	1.0
	Externalizing Problems		
Cannabis use	1.33 (0.77-2.28)	1.16 (0.66-2.02)	1.07 (0.61-1.88)
No cannabis use	1.0	1.0	1.0
	Internalizing Problems		
Cannabis use	1.23 (0.58-2.62)	1.12 (0.50-2.44)	0.99 (0.45-2.21)
No cannabis use	1.0	1.0	1.0

Table note: Confounders age and gender child, parental education, national origin and maternal alcohol use $p<0.05$, ** $p<0.01$*

Discussion

This population-based study investigated the relationship between parental cannabis use during pregnancy and child behavior at 18 months of age. The study showed an increased risk for Externalizing Problems in children of mothers who used cannabis during pregnancy compared to children of non-using mothers. This elevated risk remained significant after adjustment for age and gender of the child, maternal education and national origin, but was no longer statistically significant after adjustment for maternal psychopathology. We also found an elevated risk for Total Problems and Externalizing Problems in children of fathers who used cannabis during pregnancy compared to children of non-using fathers. These elevated risks were no longer statistically significant after adjustment for paternal education, national origin and psychopathology. In addition, our findings indicated gender-specific associations; we found that maternal cannabis use was significantly associated with Total Problems and Externalizing Problems in girls, but not in boys, after considering several covariates. In contrast, maternal cannabis use before pregnancy and paternal cannabis use were not significantly related to child behavioral problems after controlling for covariates, neither in boys nor in girls.

The current study reports on a population-based cohort, which was not selected from an outpatient prenatal clinic, like the Maternal Health Practices and Child Development Study (MHPCD) study [9], nor was the follow-up sample selected on the basis of their substance use, as was the case in the Ottawa Prenatal Prospective Study (OPPS) [8]. Therefore, the OPPS, the MHPCD study and the current study clearly differ in study population with a much lower prevalence of pregnant cannabis-using mothers in the current study (2.5%). Our findings are, to some extent, in agreement with the findings of the OPPS and the MHPCD Study. Our results showed that intrauterine cannabis-exposed children displayed an increased risk for Externalizing Problems at 18 months, which could be explained by covariates, such as parental education, national origin and psychopathology. In the OPPS, after adjusting for maternal education, home environment and gestational age, no association between cannabis exposure during pregnancy and neurodevelopment was observed at one year of age according to the Bayley Scales of Infant Development [24]. The MHPCD studied neurodevelopment using the Bayley Scales as well and

reported that daily maternal cannabis use during the third trimester of pregnancy was associated with lower mental scores at 9 months, but this effect was no longer found at 18 months [25]. A possible explanation for these inconsistent findings could be that in this age range a certain level of brain maturation needs to be achieved before deficits become detectable or less sensitive assessments for children in this age range were used.

The findings in our study reveal that maternal cannabis use affects boys and girls differently with a significantly greater risk of Total and Externalizing Problems in girls even after adjustment for all covariates, and no significantly increased risk for boys even in the unadjusted analyses.

Several explanations could account for the current findings. First, cannabis use is associated with an increased risk for child problem behavior due to the unfavorable environment these children grow up in. This hypothesis is supported by our findings showing that covariates explained a large part of the increased risk for problem behavior in children of cannabis-using mothers. Moreover, similar results were found in offspring when their fathers used cannabis, which points to environmental effects on behavior of parental cannabis use. In line with this suggestion are findings from a previous report on determinants of maternal cannabis use in pregnancy, which showed that maternal cannabis use was associated with multiple unfavorable environmental characteristics [26].

Second, since we found a gender-specific effect of maternal cannabis use, but not a gender-specific effect of paternal cannabis use, this finding suggests that maternal cannabis use may affect girls through a biological mechanism related to intrauterine exposure to cannabis. This explanation is supported by the finding that in the unadjusted analyses, the risk for Externalizing Problems in girls due to maternal cannabis use during pregnancy is higher than the risk for Externalizing Problems in girls due to paternal cannabis use. It is further supported by our finding that only maternal cannabis use during pregnancy, and not before pregnancy, was related to behavioral problems in girls. The mechanism by which endocannabinoids regulate emotional behavior is largely unknown. Cannabinoid receptors are distributed in key limbic regions such as the amygdala, prefrontal cortex and hypothalamus, where they regulate neurotransmission [27]. Animal studies have shown that the

endocannabinoid system in brain areas is different between the sexes from early postnatal ages [28] and that prenatal exposure to cannabinoid-like compounds affects male and female animals differently [29,30]. This mechanism may explain part of our findings, although direct evidence is not available from human studies.

Third, the maternal cannabis use could be significantly associated with behavioral problems in a different way; cannabis may not affect the child through a biological exposure, but rather by a heritable risk factor, such as transmission of a genetic risk of externalizing behavior to the offspring. Mother that use cannabis may be more likely to have an antisocial personality, which may be genetically determined. However, we have attempted to account for this by controlling for psychopathology. An alternative explanation could be that the often co-occurring tobacco exposure in cannabis-exposed children caused the behavioral problems. However, our data showed no support for this idea; tobacco exposure resulted in more Internalizing Problems rather than the increased risk for Externalizing Problems in girls.

Taken together, our results suggest that girls are more vulnerable to intrauterine cannabis exposure, or that daughters are more likely to model the behavior of their cannabis-using mother than sons [31].

The current study has both strengths and limitations. Strengths are the large and population-based cohort with information on numerous potential explaining variables, and paternal information on cannabis use. A first limitation is that we used self-reported data on substance use and mother-reported data on child behavior. Although cannabis use is not prosecuted in the Netherlands and false-negative reporting may, therefore, occur less frequently than in other countries, some residual denial cannot be ruled out. However, because urine toxicology is timeframe-specific and expensive, it has been considered acceptable to use self-reported information on prenatal drug exposure in large populations. In addition, the maternal perception of problem behavior of children might lead to misclassification, which, in theory, could be associated to substance use habits. For example, it is possible that mothers underestimate the behavior problems in boys, because they expect boys to demonstrate externalizing symptoms at 18 months. Adjustment for maternal characteristics may reduce some of the potential bias, but it would be preferred to study child behavior using multiple observers.

Second, response analysis showed that the mothers who were not in the analyses were younger, lower educated and had higher psychopathology symptom scores than the ones included in the analyses. Based on these characteristics, the excluded mothers were at higher risk for using cannabis. Likewise, their children may have been at higher risk for behavioral problems, since mothers transmit their genetic vulnerability and are part of the environment as well.

Third, in total 61% of all eligible women participated in the Generation R study and they may not be completely representative of the general Rotterdam population [18].

In conclusion, we found intrauterine cannabis exposure to be associated with problem behavior at 18 months of age in girls, but not in boys. Our findings suggest that the association between parental cannabis exposure and child behavior is partially explained by biological processes and by environmental factors such as parental education, national origin and parental psychopathology. Girls seem to be more vulnerable to the biological effects of intrauterine cannabis exposure than boys. The long-term consequences of parental cannabis use on behavioral development in later childhood and adolescence should be further studied.

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Chapter 4.2

PARENTAL CANNABIS USE AND BEHAVIOUR AND COGNITION IN PRESCHOOLERS

Abstract

The associations between parental cannabis use and child verbal and non-verbal cognitive functioning (30 months) and behavioral problems (36 months) in the general population were examined.

Within a population-based birth cohort, the Generation R Study, parents reported on their substance use habits during pregnancy. The logistic and linear regression analyses with verbal and non-verbal cognitive performance as outcomes included n=3,380 children, and for behavioral problems as outcome we included n=3,630 children in the linear regression analyses.

No significant associations between maternal cannabis use during pregnancy and child language development were found. Maternal cannabis use before pregnancy and paternal cannabis use, however, predicted a lower risk of language delay. We did not find a significant association of maternal cannabis before or during pregnancy with non-verbal cognitive performance measured. There were no statistically significant associations between maternal cannabis use before or during pregnancy and child behavioral problems after controlling for confounders. Likewise, intrauterine tobacco exposure was not associated with cognitive performance and behavior.

Our results are partly consistent with previously reported studies, suggesting no significant association between cannabis use and cognition. Probable explanations for this lack of association are (a) absence of a negative effect of intrauterine cannabis exposure on early childhood cognition and behavior; (b) presence of a negative effect of cannabis on early childhood cognition and behavior, but bias towards the null due to biased parents' reporting; and (c) presence of a (latent) negative effect of cannabis on early childhood cognition and behavior that is masked in early childhood.

Introduction

Cannabis is the most frequently used illicit substance in the world [1]. Lately, increasing concerns about the risks of cannabis use in Western countries have been expressed. The higher levels of Δ9-tetrahydrocannabinol (THC) in cannabis products [2], and the association between cannabis use and the development of schizophrenia [3] contributed to this interest. Cannabis use during pregnancy has always been condemned, but data to support this strong position have been inconsistent.

Consuming cannabis with high THC-levels during pregnancy may be harmful for the child, since cannabinoids can pass the placental barrier [4]. In-vitro studies suggest that intrauterine cannabis exposure might particularly harm the development in the offspring. First, the endocannabinoid system, present and functional in early prenatal periods [5], plays an important role in developmental processes of the central nervous system, including cell proliferation, migration and differentiation [6]. Second, intrauterine exposure may alter the expression of key genes for neural development and lead to long-lasting neurotransmitter and behavioral disturbances [7]. Findings from animal studies support the adverse influence of prenatal cannabis exposure on brain development by indicating permanent effects on functional regulation of motor behaviors [8], memory processes [9], and emotional reactivity [10] in offspring.

Whereas the influence of prenatal cannabis exposure on brain developmental processes has been the focus of several animal studies, only two longitudinal studies addressed the influence on neurodevelopmental outcomes, i.e. behavioral and cognitive functioning in human offspring [11,12]. These studies investigated cognitive and neurobehavioral development in children until adolescence. In the Ottawa Prenatal Prospective Study (OPPS), no association was found between maternal cannabis use during pregnancy and cognitive performance at the age of two [13] and three years [14] after controlling for confounding factors such as maternal age, weight, education and home environment. However, they did report associations between cannabis exposure during pregnancy and lesser/hampered cognitive performance at older ages, i.e. from four years onwards [14-17]. The Maternal Health Practices and Child Development Study (MHPCD) reported a similar

pattern of results regarding the association between maternal cannabis use during pregnancy and cognitive performance; i.e. no association at preschool age [18], but significant associations at later ages (from 3 years onwards) [19-21]. These studies also investigated maternal cannabis use and neurobehavioral development in children. Associations were found between intrauterine cannabis exposure and offspring behavioral problems at the ages of six years (i.e. attention problems and impulsivity) [22,23] and ten years (i.e. depressive symptoms and delinquency) [24,25].

Although these studies are useful in providing more insight in the association between fetal cannabis exposure and the cognitive and neurodevelopmental outcomes in offspring, there are several issues that should be considered. First, both previous studies used cohorts that started more than 25 years ago. Given the increase in THC-concentrations in cannabis in the last decade(s), we expect that the influence of intrauterine cannabis on cognitive and behavioral functioning is more pronounced in younger generations. Second, deviation from normal child development can be best assessed using general population cohorts. The previous studies, however, examined high-risk cohorts in terms of cannabis use, with 20 and 40% of the pregnant mothers using cannabis, compared to 2.8-4.5% in the general population [26,27]. Third, in order to determine whether cannabis use affects child cognition and behavior due to intrauterine influences on fetal development, the influence of confounding factors that could generate non-causal links should be considered. Moreover, comparing the associations between maternal and paternal prenatal cannabis use in relation to offspring outcome, may provide information about the potential effect of intrauterine exposure [28].

So far, none of the studies used an approach in which paternal cannabis use during their partners' pregnancy and child outcome was taken into account. In addition, to determine if exposure to cannabis has a specific intrauterine influence or not, the timing of exposure should be considered as well, i.e. a distinction between maternal cannabis use only before pregnancy and during pregnancy should be made [29].

In the current study, the primary aim was to examine the effects of prenatal parental cannabis use on cognitive and behavioral development in preschoolers in a low-risk general population sample. We examined this association by:

1. analyzing the association of maternal cannabis use during pregnancy and child outcomes separately from paternal cannabis use and child outcomes;
2. determining the influence of multiple confounding factors and parental psychopathology in the relation of prenatal parental cannabis use and child outcomes;
3. analyzing the association of maternal cannabis use prior to pregnancy only and child outcomes separately from maternal cannabis use during pregnancy and child outcomes.

Method

Setting and population

This study was conducted within the Generation R study, a prospective population-based birth cohort in Rotterdam, the Netherlands, [30,31] which follows a sample of urban parents and their newborn children from early pregnancy onwards. All children were born between April 2002 and January 2006. The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki, and has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participating parents.

For the current study, the parents of 7,893 children were approached for postnatal participation. Mothers of 598 children did not give full consent for post-natal participation. Children without information on maternal substance use habits in pregnancy were excluded (n=1490; 20.5%). If mothers used other illicit substances during pregnancy without using cannabis, they were also excluded (n=27). In order to increase the number of cannabis-exposed children in our sample, we additionally approached a selection of mothers from the pilot cohort (only prenatal participation) to fill out the postnatal questionnaire on behavioral problems at 36 months of age (n=65, response rate 69.2%). In

total, information on both maternal cannabis use and cognitive performance was available in at least n=3,380 children. And, information on both maternal cannabis use and behavioral problems was available in n=3,630 children. Urine samples to identify THC-levels were available for n=1,823 (53.9%) and n=1936 (53.3%), respectively.

Measures

Tobacco, alcohol and substance use were measured using a self-report questionnaire in the first trimester of pregnancy. Participants reported information on timing and frequency of use. A distinction was made between the use of cannabis (marijuana and hashish) and the use of other illicit drugs (cocaine, amphetamines and heroin). We explicitly asked in two separate questions whether pregnant women used drugs before pregnancy and whether they had used any of these substances in the last three months before completing this questionnaire. Because enrolment was aimed at early pregnancy, the period of last three months was chosen in this question. In the second question the answer options were: 'No', 'Yes, until I knew I was pregnant' and 'Yes, I still use substances'. Women that reported the use of cannabis during pregnancy often (85%) continued cigarette smoking as well. In order to assess the influence of cannabis over and above the influence of tobacco, we categorized intrauterine exposure in 4 groups, according to cannabis and tobacco use before or during pregnancy. Thus, our population was divided in:

1. Mothers who used cannabis during pregnancy
2. Mothers who used cannabis only before pregnancy
3. Mothers who used tobacco during pregnancy, but no cannabis
4. Mothers who did not use cannabis or tobacco during pregnancy

In a previous study, we examined the concordance between maternal report on cannabis use during pregnancy and the presence of cannabis metabolites in urine. In general, agreement between self-reported and urinalysis based cannabis use was good (Yule's Y=0.77). However, in order to reduce information bias, we used the available information on urinalysis in the formation of substance-using groups.

Children of women who had positive urine screens (without maternal report) were also categorized in the cannabis-using groups (N=14 (12.8%) for verbal cognitive outcomes, N=10 (12.0%) for non-verbal outcomes, N=9 (8.4%) for behavioral outcomes). Both mothers and fathers were questioned about paternal cannabis use habits during pregnancy of their partner (dichotomized as cannabis use versus non-use). We used maternal information on paternal cannabis use when fathers did not complete this questionnaire. Maternal report on paternal cannabis use was highly consistent with the partner's self-reported cannabis use ($r=0.83$, $p<0.001$).

Expressive language at 30 months was assessed using parent report on a Dutch translation of the Language Development Survey (LDS)[32]. The LDS contains a 310-word vocabulary checklist, with words arranged alphabetically within 14 semantic categories (e.g. animals, foods, modifiers, vehicles etc). The parent was asked to identify each word that her child uses spontaneously, yielding a total vocabulary score. For statistical analyses, LDS total vocabulary scores were z-standardized across the study sample after log transformation to improve the normality of the distribution. To determine language delay at 30 months, we converted raw total vocabulary scores into age- and gender-specific percentile scores based on the complete Generation R sample. In line with an earlier definition by Rescorla and Alley (2001), we defined a vocabulary delay at 30 months as word production scores below the 10th percentile [33]. The LDS also asks the parent to indicate whether the child has begun to combine words into phrases and, if so, to write down up to five of the child's best sentences. The parent wrote down these sentences in the child's native language. Because of the difficulty in determining the number of words in the listed sentences for some languages, the only information about sentences used in the current study was whether the child was reported to produce word combinations. Thus, expressive language delay at 30 months was operationalised as an LDS vocabulary score below the 10th percentile or no word combinations. Good reliability and validity of the LDS have been reported [32-34].

Because delays in language are often associated with delays in nonverbal cognitive ability, we also included among our measures the Dutch version of the Parent Report of Children's Abilities (PARCA) [35], which was used at 30 months. The PARCA is a non-verbal cognitive development measure obtained from parents. The parent-administered

portion of the PARCA comprises three subtests that are carried out by the parent assessing (1) matching-to-sample; (2) block building and (3) imitation. We calculated a sum score of the parent-administered part by summing the child's scores on the 22 items in these subtests. The parent-report part of the PARCA comprises 26 questions assessing quantitative skills, spatial abilities, symbolic play, planning and organizing, adaptive behaviors, and memory. The questions are formulated in terms of specific "activities," with mothers asked to report whether or not they have seen their child perform the particular activity. Each 'Yes' response was scored as 1, whereas 'No' or 'Do not know' were given a score of 0. Overall PARCA score was calculated by adding the sum scores of the parent-administered part and the sum of the 'Yes'-responses on the parent-report part. Although PARCA scores were normally distributed, they were also z-standardized across the sample of the present study to be consistent with the language measures. Previous research with the PARCA has indicated a significant and large correlation with the Mental Development Index of the Bayley Scales of Infant Development-II ($r = 0.55$) [35]. In a validation study of the original PARCA based on a sample of 107 2-year-old children, internal consistencies of the parent-administered and parent-report part were good, i.e. 0.83 and 0.74, respectively [35] .

The Child Behavior Checklist for toddlers (CBCL 1½–5 yrs) was used to acquire a standardized maternal report of children's problem behaviors. The Total Problems score consists of a sum score of the 99 problem items. The internalizing scale is the sum score of items in four syndrome scales: Emotionally Reactive, Anxious/Depressed, Somatic Complaints and Withdrawn. The externalizing scale is the sum score of Attention Problems and Aggressive Behavior. Each item is scored 0=not true, 1=somewhat or sometimes true and 2=very true or often true, based on the preceding two months. Good reliability and validity have been reported for the CBCL [34].

Demographic and obstetric information such as maternal age, ethnicity, education, and parity was assessed using self-report. Parental educational level and national origin was defined according to the classification of Statistics Netherlands [36, 37]. Educational level was categorized in three levels: primary (no or only primary education), secondary (lower and intermediate vocational education), and higher education (higher vocational education and university). Parental national

origin was classified into two categories: 1. Dutch, 2. Non-Dutch. Information on parental alcohol use was collected by self-report. Parental psychopathology was measured using the Brief Symptom Inventory (BSI), a validated 53-item (5-point scale) self-report symptom inventory outlined to ascertain the psychological state of individuals [38]. The General Symptom Index (GSI) subscale of the BSI was used to determine general psychopathology symptoms.

Statistical Analysis

For non-response analyses differences in maternal, paternal and child characteristics were analyzed using Chi-square tests for categorical variables and ANOVA's for continuous variables. Differences in maternal and paternal characteristics were analyzed using χ^2-tests for categorical variables and ANOVA's for continuous variables with the non-users as the reference group. Successive logistic and linear regression analyses models were performed to determine whether the association between parental cannabis use and child outcomes on language, cognition and behavior were present and whether they remained present when the following covariates were taken into account: age and gender of the child, parental education, national origin and psychopathology and maternal alcohol use. We examined the relation between paternal cannabis use during their partner's pregnancy without maternal cannabis use during or before pregnancy. For delay in language development at 30 months we used logistic regression models. For the PARCA and the CBCL data we used standardized z-scores in linear regression analysis. Because the CBCL data were not normally distributed, we first transformed the data using a square root transformation and then linear regression analyses were performed. Initially, we compared the cannabis and tobacco using groups to the non-users and in secondary analyses we compared the cannabis users to the tobacco users. When information on the covariates were missing this was handled as follows: for categorical variables an additional category 'missing' was added, and for continuous variables the data were imputed once by the Expectation-Maximization function in the Missing Value Analysis Module of Statistical Package of Social Sciences (SPSS) version 15.0.

In an alternative approach, we matched each cannabis-exposed child with a non-exposed child or tobacco-exposed child based on a propensity score. This propensity score was calculated by using the covariates. After

matching all subjects, the logistic and linear regression analyses were repeated. Because the propensity score matching procedure yielded the same results, we only present the non-matched regression analyses with the previously mentioned covariates in this study. Measures of association (Beta's and Odds Ratio's) are presented with the 95% Confidence Intervals (CIs). Statistical analyses were performed using the SPSS 15.0 for Windows (SPSS Inc. Chicago, IL).

Non-response analysis

Response analyses showed that mothers of children with information on verbal development at 30 months were older (31.4 ± 4.5 vs. 29.9 ± 5.5, $p<0.01$), higher educated (58.2% vs. 34.0% higher educational level, $p<0.01$) and more often of Dutch national origin (60.8% vs. 25.7%, $p<0.01$) as compared to mothers of children without information on verbal development. The mothers included in the analyses also had lower psychopathology scores (0.23 ± 0.28 vs. 0.32 ± 0.33, $p<0.01$) and these mothers were more likely to be non-users of cannabis and tobacco (87.0.1% vs. 79.8% non-users, $p<0.01$).

The children in the analyses had a higher mean birth weight than children not in the analyses (3459.6.2 ± 556.1 vs. 3368.1 ± 579.6, $p<0.01$). The fathers included in the analyses were older (33.9 ± 5.4 vs. 32.8 ± 5.8, $p<0.01$), higher educated (61.7% vs. 46.2% higher educational level, $p<0.01$) and more often of Dutch national origin (66.4% vs. 33.6%, $p<0.01$) as compared to fathers who were not in the analyses. The fathers included in the analyses also had lower psychopathology scores (0.13 ± 0.17 vs. 0.16 ± 0.18, $p<0.01$) and were less likely to use cannabis (91.6% vs. 86.6% non-users, $p<0.01$). Attrition analysis in the groups with and without information on behavioral development at 36 months showed the very similar differences (data not shown).

Results

Table 4.2.1 shows the descriptive statistics of the sample used in this study at 30 months. It shows that the parents were in general high educated and frequently of Dutch national origin with low psychopathology scores. The number of boys and girls was approximately the same and the mean birth weight of the samples was a healthy one (approximately 3500 grams). These characteristics were similar to the characteristics of the sample used at 36 months (data not shown).

In-depth analyses demonstrated the differences between the cannabis-using, tobacco-using and non-using groups in this sample and showed that women who used cannabis during pregnancy were significantly younger (28.7 ± 5.3 yrs) than non-users (31.4 ± 4.4 yrs) and tobacco-users (30.9 ± 5.5 yrs) and were also younger than women who used cannabis only before pregnancy (31.9 ± 5.4 yrs); overall $p<0.01$. Women who used cannabis during pregnancy had higher psychopathology scores (0.42 ± 0.43) than non-users (0.22 ± 0.27), tobacco users (0.31 ± 0.33), or women who used cannabis only prior to pregnancy (0.26 ± 0.32); $p<0.001$. Moreover, women using cannabis during pregnancy were lower educated (25.0% higher educated) than non-users (62.1% higher educated) and than women who used cannabis only before pregnancy (49.6% higher educated), but not lower educated than tobacco-users (24.7% higher educated); $p<0.01$. Children of women who used cannabis during pregnancy had lower birth weight (3228.8 ± 527.7 grams) than children of non-users (3478.2 ± 554.9 grams) or as compared to children of women who used cannabis only before pregnancy (3437.6 ± 578.8 grams); $p<0.01$.

Table 4.2.1 Parental and child characteristics at each measurement

	30 months (n=3380)
Maternal Characteristics	
Age at intake in yrs	31.4 ± 4.5
Education	
Primary	6.1
Secondary	34.6
Higher	58.4
Missing	0.9
National origin	
Dutch	61.0
Non-Dutch	35.0
Missing	4.1
Psychological symptoms	
General Symptom Index (GSI)	0.23 ± 0.27
Maternal alcohol use during pregnancy	
No use	39.3
First trimester	13.3
Continued use	43.0
Missing	4.4
Paternal Characteristics	
Age	33.8 ± 5.5
Education	
Primary	3.6
Secondary	24.5
Higher	45.4
Missing	26.5
National origin	
Dutch	61.7
Non-Dutch	31.3
Missing	7.0
Psychological symptoms	
General Symptom Index (GSI)	0.13 ± 0.17

Table 4.2.1 Continued

	30 months (n=3380)
Child Characteristics	
Child Antropometrics	
Gender (% boys)	50.5
Birth weight	3457.0 ± 559.4
Age at assessment	31.5 ± 2.2
Verbal development 30 months	
Delay in phrase development	18.5
Delay in vocabulary development	14.4
Non-verbal development at 30 months	
PARCA Total Score	47.0 ± 5.7
PARCA Parent Report	25.4 ± 4.2
PARCA Parent Administered	21.5 ± 2.9
Child Behavior Checklist at 36 months	
Total Problems Scale	20.6 ± 15.1
Externalizing Problems Scale	8.3 ± 6.3
Internalizing Problems Scale	5.0 ± 5.0

Table note: Continuous data are presented as mean ± standard deviation; Categorical data are presented as percentages

Table 4.2.2 The association between parental cannabis use and verbal cognitive performance at 30 months calculated by logistic regression analyses

	Unadjusted model	Confounder adjusted	Confounder adjusted + psychopathology
Maternal cannabis use	**Odds Ratio's + 95% Confidence Intervals**		
	Delay in phrase development		
Cannabis during pregnancy (n=69)	0.92 (0.49-1.72)	0.82 (0.43-1.58)	0.83 (0.43-1.60)
Cannabis before pregnancy (n=127)	0.59 (0.34-1.01)	0.50 (0.28-0.90) *	0.50 (0.28-0.91) *
Non-use (n=2690)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	Delay in vocabulary development		
Cannabis during pregnancy (n=51)	0.95 (0.43-2.14)	0.86 (0.38-1.97)	0.81 (0.35-1.87)
Cannabis before pregnancy (n=102)	0.95 (0.54-1.70)	0.93 (0.50-1.74)	0.92 (0.50-1.72)
Non-use (n=1994)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Paternal cannabis use			
	Delay in phrase development		
Cannabis use (n=249)	0.68 (0.47-0.99) *	0.67 (0.45-0.98) *	0.67 (0.45-0.98) *
No cannabis use (n=2743)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	Delay in vocabulary development		
Cannabis use (n=195)	0.78 (0.50-1.23)	0.77 (0.48-1.21)	0.75 (0.47-1.19)
No cannabis use (n=2033)	1.0 (ref)	1.0 (ref)	1.0 (ref)

*Table note: Confounders were age and gender child, maternal education and national origin, maternal alcohol use; Psychopathology: additional correction for General Symptom Index for psychological complaints; Delay: lowest 10% ref=reference * $p<0.05$, ** $p<0.01$*

Table 4.2.2 presents the associations between parental cannabis use and language development at 30 months of age. Maternal cannabis use during pregnancy was not associated with language development at 30 months of age. Remarkably, maternal cannabis use before pregnancy was associated with a lower risk for delay in language development (i.e. phrase development) at 30 months. Similarly, paternal cannabis use was protective with regard to delay in phrase development at 30 months. In addition, maternal tobacco use during pregnancy was not associated with a delay in phrase development (OR= 0.99; 95%CI: 0.69-1.44; $p>0.05$), nor was it related to a delay of vocabulary development (OR= 0.96; 95%CI: 0.61-1.52; $p>0.05$) at 30 months of age. Moreover, no association was found between cannabis use during pregnancy and a delay in phrase development (OR= 1.02; 95%CI: 0.46-2.28; $p>0.05$) or with a delay in vocabulary development (OR= 1.19; 95%CI: 0.42-3.33; $p>0.05$) when compared to tobacco use during pregnancy. Table 4.2.3 shows the associations between parental cannabis use and non-verbal cognitive performance at 30 months. We did not find any associations between maternal cannabis use before or during pregnancy and cognition. Moreover, paternal cannabis use was not associated with non-verbal cognitive performance of the child. Additionally, maternal smoking during pregnancy was not significantly associated with non-verbal functioning (PARCA Total Score; $\beta=0.13$; 95%CI:-0.01-0.27; $p>0.05$). Moreover, no associations were found between maternal cannabis use during pregnancy and non-verbal functioning with tobacco users as the reference (PARCA Total Score; $\beta=0.01$; 95%CI:-0.27-0.28; $p>0.05$).

Table 4.2.4 shows the associations between parental cannabis use and behavior problems at 36 months of age. These results show that children exposed to maternal cannabis use during pregnancy display higher scores on the Total Problems Scale and the Externalizing Problems Scale than children of non-using mothers in the unadjusted analysis, but after controlling for confounding and maternal psychopathology these associations are no longer statistically significant. The Externalizing Problems Scale consists of two subscales, Attention Problems and Aggressive Behavior. Additional analyses on these two subscales showed that the increased score on the Externalizing Problems Scale was due to a higher score on the Aggressive Behavior scale aggression, but not due to higher scores on the Attention Problems scale. Compared to non-users cannabis use during pregnancy was associated with a somewhat higher score on the Aggressive Behavior Scale ($\beta=0.30$; 95%CI:0.09-0.50;

p<0.01), also after adjustment for confounding (β=0.25; 95%CI:0.05-0.45; p<0.05), but not when additionally maternal psychopathology (β=0.18; 95%CI:-0.03-0.38; p<0.10) was taken into account.

For maternal cannabis use prior to pregnancy and behavioral problems no statistical significant association was observed. Paternal cannabis use was related to a lower score on the Externalizing Problems Scale and Total Problems Scale. These lower scores did not remain statistically significant after controlling for age and gender of the child, paternal education, national origin and paternal psychopathology.

Maternal tobacco smoking during pregnancy was associated with Total problems (β=0.16; 95%CI: 0.04-0.28; p<0.05) and Externalizing Problems (β=0.21; 95%CI: 0.08-0.34; p<0.01) after considering the confounders and psychopathology. Again, when comparing maternal cannabis use during pregnancy to maternal tobacco use during pregnancy, no significant associations concerning Total Problems (β=-0.07; 95%CI:-0.31-0.18; p>0.05), Externalizing Problems (β=-0.07; 95%CI: -0.30-0.17; p>0.05) or Internalizing Problems (β=-0.08; 95%CI: -0.32-0.16; p>0.05) were found.

Table 4.2.3 The association between parental cannabis use and non-verbal cognitive performance (standardized z-scores) at age 30 months calculated by linear regression analyses

	Unadjusted model	Confounder adjusted	Confounder adjusted + psychopathology
Maternal cannabis use	**Beta's + 95% Confidence Intervals**		
	Parca Total Score		
During pregnancy (n=79)	0.07 (-0.16-0.29)	0.07 (-0.15-0.30)	0.08 (-0.14-0.31)
Before pregnancy (n=127)	0.02 (-0.16-0.20)	0.05 (-0.13-0.24)	0.06 (-0.13-0.24)
Non-use (n=2742)	reference	reference	reference
	Parca Parent Administered		
During pregnancy (n=79)	0.11 (-0.11-0.34)	0.11 (-0.11-0.34)	0.11 (-0.11-0.35)
Before pregnancy (n=133)	0.03 (-0.15-0.21)	0.09 (-0.10-0.27)	0.09 (-0.10-0.27)
Non-use (n=2802)	reference	reference	reference
	Parca Parent Report		
During pregnancy (n=83)	0.01 (-0.21-0.23)	0.02 (-0.20-0.23)	0.03 (-0.19-0.25)
Before pregnancy (n=135)	0.00 (-0.17-0.18)	-0.02 (-0.20-0.16)	-0.02 (-0.19-0.16)
Non-use (n=2936)	reference	reference	reference
Paternal cannabis use			
	Parca Total Score		
Cannabis use (n=267)	0.11 (-0.02-0.23)	0.08 (-0.04-0.21)	0.09 (-0.04-0.21)
No cannabis use (n=2796)	reference	reference	reference
	Parca Parent Report		
Cannabis use (n=274)	0.08 (-0.05-0.20)	0.06 (-0.07-0.18)	0.06 (-0.07-0.18)
No cannabis use (n=2853)	reference	reference	reference
	Parca Parent Administered		
Cannabis use (n=283)	0.09 (-0.04-0.21)	0.06 (-0.06-0.18)	0.07 (-0.06-0.18)
No cannabis use (n=2994)	reference	reference	reference

*Table note: Confounders age and gender child, maternal education and national origin, maternal alcohol use; Psychopathology: additional correction for General Symptom Index for psychological complaints; ref=reference * $p<0.05$, ** $p<0.01$*

Table 4.2.4 The association between parental cannabis use and behavioral problems

	Unadjusted model	Confounder adjusted	Confounder adjusted + psychopathology
Maternal cannabis use	**Beta's + 95% Confidence Intervals**		
	Total problems		
Cannabis during pregnancy (n=107)	0.26 (0.06-0.45) **	0.20 (0.02-0.39) *	0.07 (-0.11-0.26)
Cannabis before pregnancy (n=165)	0.11 (-0.04-0.27)	0.11 (-0.04-0.26)	0.05 (-0.09-0.21)
Non-use (n=3103)	reference	reference	reference
	Externalizing problems		
Cannabis during pregnancy (n=107)	0.24 (0.05-0.43) *	0.18 (-0.02-0.37)	0.08 (-0.11-0.27)
Cannabis before pregnancy (n=165)	0.15 (0.00-0.31)	0.13 (-0.03-0.28)	0.09 (-0.07-0.24)
Non-use (n=3104)	reference	reference	reference
	Internalizing problems		
Cannabis during pregnancy (n=107)	0.14 (-0.05-0.34)	0.11 (-0.08-0.30)	-0.02 (-0.21-0.16)
Cannabis before pregnancy (n=165)	0.09 (-0.07-0.25)	0.11 (-0.04-0.27)	0.06 (-0.09-0.21)
Non-use (n=3100)	reference	reference	reference
Paternal cannabis use	**Total problems**		
Cannabis use (n=330)	-0.14 (-0.26- -0.02) *	-0.12 (-0.24-0.01)	-0.09 (-0.21- 0.04)
No cannabis use (n=3177)	reference	reference	reference
	Externalizing problems		
Cannabis use (n=331)	-0.16 (-0.29- -0.03) *	-0.13 (-0.25- -0.00) *	-0.11 (-0.24- 0.02)
No cannabis use (n=3177)	reference	reference	reference
	Internalizing problems		
Cannabis use (n=329)	-0.10 (-0.23-0.02)	-0.09 (-0.21-0.04)	-0.06 (-0.18-0.07)
No cannabis use (n=3175)	reference	reference	reference

*Table note: Confounders age and gender child, parental education and national origin maternal alcohol use; Psychopathology: additional correction for General Symptom Index for psychological complaints; * $p<0.05$, ** $p<0.01$*

Discussion

This population-based study investigated the relationship between maternal cannabis use during pregnancy and cognitive performance at 30 months and child behavior at 36 months of age. The study showed no association between maternal cannabis use during pregnancy and verbal development, nor with cognitive performance at 30 months. Unexpectedly, maternal cannabis use before pregnancy seemed to be associated with a lower risk for delayed verbal development at 30 months of age. Similarly, paternal cannabis use was also a protective factor for these outcomes. Moreover, our results demonstrated that cannabis does not influence these outcomes over and above the influence of tobacco. This combination of findings suggests that the results do not reflect a teratogenic effect of cannabis, but may be due to the influence of maternal and/or paternal environmental effects or a genetic transmission of cognitive capacities [29].

Next, we analyzed the association between parental cannabis use and child behavioral problems at 36 months of age. These analyses showed that maternal cannabis use during pregnancy was associated with increased externalizing behavior, while they showed that paternal cannabis use during his partner's pregnancy was associated with decreased externalizing behavior. The effect estimated became smaller after correcting for confounding, and particularly introducing parental psychopathology to the model seem to explain most part of these relationships. Additionally, maternal smoking during pregnancy was associated with externalizing problems after considering the confounders and psychopathology. These results suggest that the increased externalizing problems cannot be attributed to the teratogenic effect of cannabis exposure during pregnancy. The role of family environment and/or genetic transmission of behavior may be more important in predicting such behavior.

It is important to note that the current study reports on a population-based cohort, which was not selected from an outpatient prenatal clinic, like the Maternal Health Practices and Child Development Study (MHPCD) study [12], nor was the follow-up sample selected on the basis of their substance use, as was the case in the Ottawa Prenatal Prospective Study (OPPS) [11]. Therefore, the OPPS, the MHPCD study and the current study clearly differ in study population

with a much lower prevalence of pregnant cannabis-using mothers [39]. Our findings are, to some extent, in agreement with the findings of these two studies. In the OPPS, Fried and colleagues also did not find a relationship between prenatal cannabis exposure and cognitive outcomes at age 24 and 36 months after controlling for confounding [13,14]. The first significant association beyond the neonatal period reported by the OPPS was at 48 months; significantly lower scores in verbal and memory domains were then associated with maternal cannabis use after adjusting for confounding variables (not maternal psychopathology) [14]. Similarly, in the MHPCD the first significant association between intrauterine cannabis exposure and cannabis was found at 36 months of age [19]. Concerning behavioral problems our results seem to be in agreement with the OPPS and MHPCD as well. We reported that children had higher scores on the Externalizing Scale due to Aggression, but this association disappeared after correcting for maternal psychopathology. The MHPCD Study reported that at 6-year follow up, children were rated as showing more delinquent and impulsive behavior by their teachers, corrected for confounding, including maternal hostility [23]. The OPPS reported more maternal-rated impulsive and hyperactive behavior at 6 years of age in intrauterine cannabis-exposed children, corrected for several confounders but not for maternal psychological symptoms [22].

Explanations

Several explanations may contribute to the lack of association between prenatal cannabis exposure and cognitive performance. First, it may be that in reality there is no effect of intrauterine cannabis exposure on early childhood cognition and behavior. Second, intrauterine cannabis exposure may affect child cognition and behavior, but in this study this effect is biased towards the null due to biased parents' reporting on cognition and behavior of their offspring. Finally, it may be that at this age intrauterine cannabis exposure is not yet reflected by changes in cognition and that at later ages the effects of cannabis upon more developed and complex behavior may become more evident. Therefore, it may be that latent differences between cannabis-exposed and non-exposed children become present and/or detectable when cognitive development reaches a higher level of maturation and the demand on the cognitive system becomes higher. Given the finding that no effect of tobacco-exposure was present for the cognitive outcomes and only small

effects on behavior outcomes were demonstrated, the latter explanation may be right, but needs further follow-up of this cohort. In addition to this, our findings may also reflect the general trend of prenatal biological determinants to have more clear effects later in life [40].

Unexpected is the finding that maternal cannabis use before pregnancy and paternal cannabis are associated with a better verbal performance. It may be that the mothers of these children evaluate their children as 'smart', while in reality these children may be just performing on average. Indeed, a recent study that examined the association between quitting smoking and child outcomes suggested that mothers who quit smoking during pregnancy have heritable, stable personality traits, such as flexibility, regularity and positive mood. These maternal characteristics could influence the child in two ways; firstly, by genetic transmission of these favorable traits and secondly this favorable personality may be associated with a positive rating bias [41]. A second explanation that account for the finding of paternal cannabis use during pregnancy is that mothers (who did not use cannabis) compensate for their partner's cannabis use, because they may stimulate their child and positively influence their children's cognitive performance.

We expected that maternal cannabis use during pregnancy would be associated with a higher score for child problem behavior, either due to the teratogenic effects of intrauterine cannabis exposure or due to the unfavorable environment these children grow up in. The latter hypothesis is supported by our findings showing that covariates explained the increased risk for problem behavior in children of cannabis-using mothers and by the finding that cannabis exposure does not have an additional effect over and above that of tobacco use. In line with this suggestion are findings from a previous report on determinants of maternal cannabis use in pregnancy, which showed that maternal cannabis use was associated with multiple unfavorable environmental characteristics [39]. This weak association between maternal cannabis use during pregnancy and externalizing behavior may be the first sign of stronger associations with behavioral problems later in life in children of mothers using cannabis during pregnancy (delinquency, impulsivity and depressive symptoms) [22-24]. Surprisingly, we found that paternal cannabis use is associated with lower scores on the Externalizing Problem Scale. There is no clear explanation for this finding. Perhaps, cannabis-using partners have more disruptive behavior problems (e.g. delinquency) [42],

which may lead mothers to underreport their child behavior problems because of habituation to such behavior.

Limitations

The current study has both strengths and limitations. Strengths are the large and population-based cohort with information on numerous potential confounders and the ability to compare the effects of maternal and paternal cannabis use.

A first limitation is that we used mother-reported data on child behavior. The maternal perception of problem behavior of children may have lead to misclassification, which, in theory, could be associated to substance use habits. For example, it is possible that mothers underestimate the behavioral problems in their children, because they expect their children to demonstrate externalizing behavior at 36 months. Adjustment for maternal characteristics may reduce some of the potential bias, but it would be preferred to study child behavior using multiple sources of information including ratings by researchers and teachers blinded to the cannabis use of the mother during pregnancy.

Second, response analysis showed that the mothers who were not in the analyses were younger, lower educated and had higher psychopathology symptom scores than the ones included in the analyses. Based on these characteristics, the excluded mothers were at higher risk for using cannabis. Likewise, their children may have been at higher risk for behavioral problems, since mothers transmit their genetic vulnerability and are part of the environment as well.

Third, in total 61% of all eligible women participated in the Generation R study and they may not be completely representative of the general Rotterdam population [30].

Fourth, data on verbal and nonverbal cognitive development were also based on maternal report. However, all parent-based measure of cognitive development, i.e. LDS and PARCA, have been shown to be reliable and valid measures of cognitive functioning in early childhood [32,33,35,43]. Furthermore, these instruments have been shown to predict language and language-related problems later in life [44,45]. Nevertheless, structured testing and/or observation of cognitive abilities may have clear methodological advantages in addition to parental reports.

Recommendations for further research

Because this study raised some questions about the consequences of maternal cannabis use during pregnancy and its possible effects on children, we encourage further investigation of this topic. Furthermore, observational data on cognitive performance may be less biased than maternal reported data, and we therefore suggest following this group of children across a longer period and investigating the effects of maternal cannabis use during pregnancy and child academic achievement by using results from official school-exams. In addition, like the OPPS and MHPCD Study, we suggest using different age-appropriate test-batteries to measure cognitive performance. Moreover, in this study, we collected data on cognitive performance in the preschool children, it may be that in the school-period (after 48 months of age) children are more cognitively challenged and latent effects of prenatal cannabis exposure may be more easily noticed.

Concerning data on behavioral problems, we suggest multiple observers including maternal, paternal and teacher reports. Additionally, when children become older, they can report about their own behavior and emotions. For instance, for preschoolers, the Berkeley Puppet Interview, which is a semi-structured interview measure of young children's (aged 4.5 to 7.5 years) perceptions about family environment, school contexts, social skills and behavior, would be a suitable observational measurement for examining behavioral and emotional problems.

Conclusions

In conclusion, this study provides little evidence for a clear teratogenic effect of cannabis on child cognitive and behavioral development. The data showed that intrauterine cannabis exposure was not associated with verbal and cognitive performance at 30 months of age, nor was it associated with child behavioral problems at 36 months of age, after taking into account general confounders and parental psychopathology. Surprisingly, we found that maternal cannabis use before pregnancy and paternal cannabis were protective for delay in verbal development. These findings may be best explained by heritable, stable personality traits of the mother and/or by reporting bias. Our findings suggest that the association between parental cannabis exposure and child behavior is explained by environmental factors such as parental education, national origin and parental psychopathology. Because maternal report may be biased, the long-term consequences of parental cannabis use on behavioral development in later childhood and adolescence should be further studied using multiple sources of information.

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Chapter 5

SUMMARY OF THE RESULTS AND GENERAL DISCUSSION

Abstract

This thesis aimed to extend existing knowledge on cannabis use in pregnant women and the effects of intrauterine cannabis exposure on foetal and infant development. All studies in this thesis were conducted within the framework of the Generation R study, a population-based cohort study among pregnant women and their children, which offered a unique opportunity to investigate the early and late effects of intrauterine environmental factors on child growth and development. The Generation R cohort provided us more recent data on maternal and paternal cannabis use, its determinants and the long-term effects of intrauterine exposure to cannabis as compared to previous cohorts that started more than 25 years ago. The main aims of this thesis were:

1. To explore the psychosocial characteristics that are associated with maternal cannabis use before and during pregnancy;
2. To assess the agreement between maternal self-report on cannabis use during pregnancy and the presence of cannabis metabolites in maternal urine;
3. To test the hypothesis that intrauterine cannabis exposure in humans has adverse effects on foetal growth trajectories and foetal circulatory redistribution;
4. To investigate the association between parental cannabis use and behavioural problems of their children at 18 and 36 months of age;
5. To study the relation between prenatal parental cannabis use and cognitive development of their children at the age of 30 months;

In the current chapter the main findings will be summarized, the interpretation of the results and some methodological aspects will be discussed. Finally, the implications for clinical practice and future research will be addressed.

Summary of the main findings

The studies presented in this thesis describe the determinants of maternal cannabis use during pregnancy, the agreement between self-reported cannabis use and urinalysis, and the effects of intrauterine cannabis exposure on offspring in pre- and postnatal life. Below, a summary of the main results from these studies is presented.

Determinants of cannabis use during pregnancy

The results described in Chapter 2.1 demonstrate that cannabis use during pregnancy is associated with multiple characteristics that may by themselves also influence foetal growth and offspring outcomes. The strongest predictor for cannabis use before and during pregnancy was cannabis use by the biological father. Positive correlations between spouses for cannabis use, abuse and dependence are well known [1]. We further found that childhood maltreatment and delinquent behaviour of pregnant women also seem to be significant independent predictors for using cannabis before and during pregnancy. Previous research reported that childhood maltreatment is related to cannabis use [2] and it is known that substance use increases the risk of committing antisocial, aggressive and delinquent acts [3]. Additionally, lower educational level is an important determinant for continued cannabis use in ever-users. Women using cannabis were also more likely to be single. This is in line with the marijuana cohort of the Maternal Health Practices and Child Development (MHPCD) Study; the MHPCD study examined the consequences of cannabis (and tobacco use) during pregnancy in a high-risk cohort of low socioeconomic status. They reported that 71% of the cannabis-using women were single at the beginning of pregnancy and only 4% were married [4]. Moreover, it has previously been reported that young married women used cannabis or substances less often [5]. Furthermore, being religious is a significant protective factor for cannabis use before or during pregnancy. This may result from most religions' disapproval of psychotropic substances use [6,7]. In addition, based on the existing literature we expected that long-lasting difficulties (social stress), psychological difficulties and unhealthy family functioning would be significant independent predictors of cannabis use in pregnancy [8-10]. In the final model, however, these predictors no longer reached significance. A plausible explanation for the non-significance of psychopathology in

the full model is that childhood trauma accounted for the effect of psychopathology. Although psychopathology was not significant in the full model, it remains an essential confounding factor because our results also show that it is a significant predictor for cannabis use in the univariate model. Finally, it seemed that cannabis use during pregnancy might reflect addictive behaviour, because women using cannabis during pregnancy were more likely to be frequent users and women with a history of cannabis dependence continued using cannabis during pregnancy more often.

Agreement between self-report and urinalysis of cannabis use women

The study described in Chapter 2.2 showed that both self-report and urinalysis provide important but different information about cannabis use during pregnancy: self-report provides a higher estimate of cannabis use during pregnancy (2.3%) than urinalysis (1.8%), but urinalysis provides additional cases of women using cannabis during pregnancy. Based on a combination of positive self-report and positive urinalysis data, at least 130 of the 3997 pregnant women used cannabis during pregnancy (3.3%), i.e. 1.43 times the prevalence of cannabis use during pregnancy based on self-reported data only and 1.83 times the prevalence based on urinalysis data only. Interestingly, positive urine screens were mainly in the group that reported cannabis use before pregnancy (7.6%) and in the group that refused to report on their cannabis use (2.6%). Only a small proportion of positive urine screens was present in the non-users group (0.4%). Therefore, it is important to consider women who reported to use cannabis only before pregnancy (i.e. past users) as a separate group, and not just regard these women as non-users during pregnancy. The measure of agreement Yule's Y amounted to 0.77, indicating substantial agreement between self-report and urinalysis. Thus, this study demonstrates that reliance on self-reported cannabis alone underestimates the prevalence of cannabis use during pregnancy even in a country where neither cannabis possession nor cannabis use is prosecuted. Reliance on urinalysis only results in an even stronger underestimation of the prevalence of cannabis use during pregnancy and may be biased toward long-term or heavy users, because they are more likely than occasional users to be detected through urinalysis.

Maternal cannabis use and foetal growth and foetal blood redistribution

Chapter 3.1 showed that exposure to potent cannabis in utero is related to a reduced foetal growth and smaller foetal head size, which are risk factors for neurodevelopment and behavioural problems, while intrauterine exposure to cannabis is not related to gestational duration. Importantly, we found that cannabis use during pregnancy, often combined with tobacco, has a stronger effect on intrauterine growth when compared to the effect of prenatal tobacco exposure only. This may be especially true for those women who continued their cannabis use throughout their pregnancy. Importantly, even short-term intrauterine cannabis exposure, i.e. in the first trimester of pregnancy, was associated with impaired foetal growth. These associations between maternal cannabis use and foetal growth were independent of lifestyle and socio-economic factors. Interestingly, paternal cannabis use during pregnancy was not associated with foetal growth restriction, which suggests that the negative association between maternal cannabis use and foetal growth is due to intrauterine exposure and not to confounding by the family environment. Finally, no effects of cannabis or tobacco use in pregnancy were found on cerebellar size; this is consistent with the fact that the cerebellum is the least affected in growth restriction [11].

Chapter 3.2 described the effect of maternal cannabis use during pregnancy on foetal hemodynamic adaptations. Because it has been shown that endogenous cannabinoid system plays an important neuromodulatory role in cardiovascular regulation, prenatal exposure to cannabis in early foetal life could result in hemodynamic adaptations, such as a reduction of vascular resistance and an increase in vascular flow. Our results demonstrate that cannabis use during pregnancy is associated with adaptations in foetal placental and cardiac blood flow, but not with foetal cerebral blood flow. However, the observed associations could be explained by the co-occurrence of tobacco use during pregnancy in this group as we have found that the blood flow parameters did not significantly differ between cannabis-exposed foetuses and tobacco-exposed foetuses. Against the expectation, we found statistically significant specific associations between maternal cannabis use and the uterine resistance indices, including a higher uterine pulsatility index and a higher uterine resistance index in cannabis-exposed foetuses, which remained present after taking into account maternal tobacco use.

Maternal cannabis use and child behavioural development

Chapter 4.1 described the relationship between parental cannabis use during pregnancy and child behaviour at 18 months of age. The study showed an increased risk for externalizing problems in children of mothers who used cannabis during pregnancy compared to children of non-using mothers. This elevated risk remained significant after adjustment for age and gender of the child, maternal education and national origin, but was no longer statistically significant after adjustment for maternal psychopathology. We also found an elevated risk for total problems and externalizing problems in children of fathers who used cannabis during pregnancy compared to children of non-using fathers, but these elevated risks were no longer statistically significant after adjustment for paternal education, national origin and psychopathology. Our findings indicated gender-specific associations; we found that maternal cannabis use was significantly associated with total problems and externalizing problems in girls, but not in boys, after considering several covariates. In contrast, maternal cannabis use before pregnancy and paternal cannabis use were not significantly related to child behavioural problems after controlling for covariates, neither in boys or girls.

This effect of cannabis exposure on behaviour was not a consistent one; the association was weaker at 36 months of age, as is described in Chapter 4.2. In this chapter we found that children who were exposed to cannabis during pregnancy showed an increased score for total problems and externalizing problems compared to children of non-using mothers. However, this elevation did not remain statistically significant after adjustment for age and gender of the child, maternal education and national origin and maternal psychopathology. In contrast to the positive associations between maternal cannabis use and behavioural problems, we found a lower score for total problems and externalizing problems in children of fathers who used cannabis during pregnancy compared to children of non-using fathers. These lower scores were no longer statistically significant after adjustment for age and gender of the child, paternal education, national origin and psychopathology. A possible explanation for this contrast may be that mothers, who do not use cannabis while their partner does, tend to underrate their children's problems at the age of 36 months, because they become 'less sensitive' to

problem behaviour of the child due to their partner's cannabis use which may be associated with disruptive behaviour [12].

Maternal cannabis use and child cognitive development

The study described in Chapter 4.2 concerns the relationship between maternal cannabis use during pregnancy and cognitive performance at 30 months, and child behavior at 36 months of age. The study showed no association between maternal cannabis use and verbal or non-verbal cognitive performance at 30 months. Unexpectedly, maternal cannabis use before pregnancy was a protective factor for delay in verbal development at 30 months of age. A possible explanation for this finding may be that women who use cannabis are more likely to be single mothers (Chapter 2.1) and partners do not play an active role in raising the child. Therefore, these mothers may want to prove that they do well with their child and this may result in better parent ratings on verbal performance. Another possible explanation for this finding may be that intentionally discontinuing cannabis use before getting pregnant reflects more positive persistent maternal attributes and these attributes may also have implications for postnatal caregiving styles.

In Chapter 2.1 we described that the ever-users (cannabis use before pregnancy) have a higher educational level than women that use cannabis during pregnancy, so this may contribute in an accelerated verbal cognitive performance in these children. In addition, it appeared that paternal cannabis use was also a protective factor for delay in verbal development at 30 months of age. This positive association may also be explained by a combination of selection bias and information bias as described above.

Main findings described in this thesis

Figure 5.1 represents the main findings of all chapters in this thesis. The boxes with the straight lines represent the statistical significant associations tested in this manuscript, and the dotted boxes represent the non-significant findings. Summarized, this figure shows that maternal cannabis use during pregnancy is associated with prenatal outcomes, such as foetal growth and blood redistribution, while in the postnatal period these associations become weaker with the increasing age of the child. At 18 months, we still found an association between maternal cannabis use and child behavioural problems in girls, but not in boys.

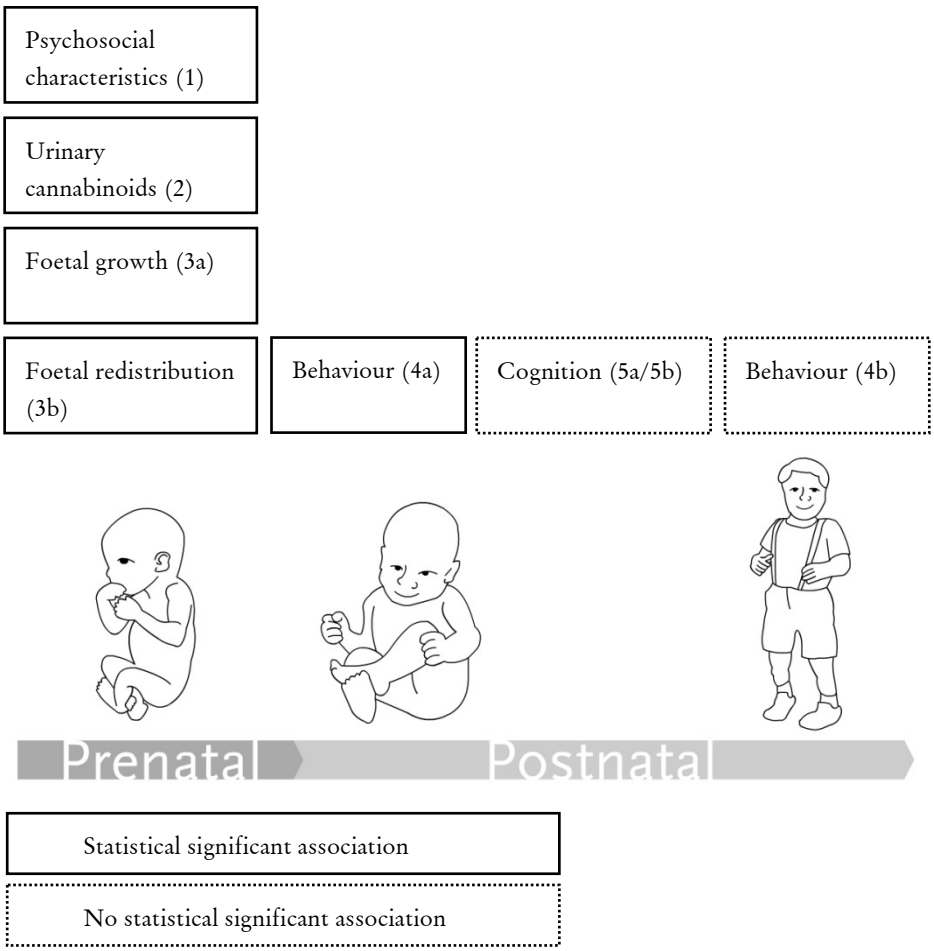

Figure 5.1 Schematic presentation of the main findings in this thesis

However, as the children become older, no statistically significant associations were found between maternal cannabis use and behavioural problems or poor verbal and non-verbal cognitive performance. Previous studies (the OPPS and MHPCD Study) demonstrated similar effects of maternal cannabis use during pregnancy showing mild consequences of cannabis use on neurobehaviour in the neonatal period, no behavioural consequences of cannabis exposure in children between 6 months and 3 years after controlling for confounding, followed by clear negative effects of maternal cannabis use during pregnancy on neurobehavioral outcomes from approximately 4 years onwards [13-16].

Interpretation of the results

In each chapter, the results were interpreted and possible mechanisms were addressed. Below, we attempt to explain the findings in a broader view, because combined the results showed that maternal cannabis use during pregnancy was associated with prenatal outcomes, such as foetal growth, while in the postnatal period these associations become weaker with the increasing age of the child (Figure 5.1). Several explanations may underlie our pattern of results.

First, our findings may be (partly) explained by the fact that the number of participants decreases over time and this decrease seemed to be selective (i.e. more healthy children remain active participants in the study). Indeed, attrition analyses consistently showed that mothers included in the analyses were older, higher educated and more often of Dutch origin compared to excluded mothers. The mothers included in the analyses had lower psychopathology scores. Additionally, tobacco- and cannabis-using women were more likely to drop out of the study than non-using women. Moreover, children included in the analyses had a higher mean birth weight than children who were not included in the analyses.

Second, in the postnatal period we made use of maternal report on child behaviour and cognition which may be biased, while in the prenatal period we made use of observational data. This may have contributed to our findings that in the postnatal period we found no clear effects of intrauterine cannabis exposure on behaviour and cognition. Therefore, it is desirable to make use of researcher-based measurements and/or multiple informants when using reported information. When children become older and reach school-age, we could also make use of teacher ratings of child behaviour by using the Teacher Report Form and teacher ratings of cognitive performances.

Third, it may be that the brain needs to mature until a certain level before cognitive deficits become detectable. A small delay in development during early infancy may persist and slow down further child development, which may result in larger differences later on, and these functional impairments then become detectable. For example, according to the concept of 'developmental origins of health and disease' small differences in birth weight are associated with cardiovascular disease, type 2 diabetes and their risk factors in adult life [17]. Therefore, it

is important to further monitor the development of these cannabis-exposed children and continue collecting information.

Alternatively, it could be that the contribution of the relatively short period of intrauterine cannabis exposure becomes smaller in time, because postnatal environments become more important as life proceeds. Given that important developmental events in the brain during the early prenatal period which need to be properly organized to ensure appropriate brain patterning, the introduction of cannabis during this critical period has the potential to change neuronal connectivity, the idea of a decreasing relative contribution seems unlikely. In-vitro studies with human foetal brains showed that maternal cannabis use was associated with a reduction of mRNA expression of the dopamine receptor type 2 in the amygdala [18]. Moreover, this reduction was directly correlated with the degree of maternal cannabis intake. In addition, studies have shown that cannabis exposure during early ontogeny influences specific components of the endogenous opioid system, especially within limbic structures, and that these disturbances that may have long-term effects on cognitive and emotional behaviours [19]. We have grounded our studies in the theory that disturbances caused by cannabis exposure during foetal life, may yield so-called 'prenatal programming' effects, resulting in long-term effects on multiple outcomes in offspring.

Methodological issues

The strengths and limitations of each study in this thesis have been described in each of the chapters. In this chapter, we will discuss some general methodological issues related to study design, measurement of cannabis exposure and the outcome variables, and statistical matters, which should be considered in human studies regarding the association of prenatal maternal cannabis use and child physiological, neurobehavioral and cognitive outcomes.

Study design

The Generation R Study is a cohort study in which subjects are categorized according to their exposure and are followed over time to determine disease incidence. This design is prospective and allows us to monitor the child in a systematic manner and it provides the opportunity to assess temporal relationship between cannabis exposure and several child outcomes. Moreover, recall bias with regard to cannabis use is minimized. Moreover, an advantage of a prospective design is the possibility to determine the degree and the timing of drug exposure during pregnancy, to include a biochemical verification of self-report on cannabis use and to accurately estimate other covariates. Usually, population-based studies focus on exposures that a large proportion of the general population experiences [20]. Such long-term studies are needed to assess behavioural and cognitive development until children reach adolescence. However, such long-term follow-up studies are difficult to conduct, are very expensive, and may suffer from selective drop-out. The studies discussed in this thesis showed some selective loss to follow-up. For example, attrition analysis showed that mothers with missing data were in general lower educated, younger and had higher psychopathology scores. Moreover, of the women who used cannabis during pregnancy (n=220), only half or less than half filled out postnatal questionnaires. The women that participated after pregnancy delivered significantly healthier children (in terms of birth weight) than children of women who stopped participation. Assuming that the association between intrauterine cannabis exposure and behavioural problems is larger among non-participants, it would mean that the reported association in this thesis is an underestimation of the true effect in the general population.

A possible alternative design is the cross-sectional study, in which children are examined at a certain developmental stage or age. These cross-sectional studies may be designed as case-control studies, in which highly-exposed subjects are compared to non-exposed controls. Often, clinical populations (e.g. hospital-based) are used in these cross-sectional studies. It may be that factors, such as maternal and foetal malnutrition, inadequate prenatal medical care, and exposure to sexually transmitted diseases and other infectious diseases, polydrug use, or postnatal drug use may confound the association in these clinical populations [21]. Moreover, clinical samples are generally not completely representative for all cases, but are likely to be the more complex [22]. Even though these cross-sectional and case-control study designs have provided fundamental information on consequences of prenatal substance exposure, the prospective longitudinal design is methodologically to be preferred. However, given the observational nature of the current prospective study (no randomization and no blinding) and the subjective nature of the outcome variables, some information bias, selection bias and residual confounding can still not be fully excluded [23].

Exposure variables

Information bias in relation to prenatal cannabis and tobacco exposure is a threat to the validity of the results. In this thesis, the main source of information on maternal cannabis use during pregnancy is based on self-report, which could be a potential source of information bias. It may be that pregnant women underreport their actual cannabis use and, since people use cannabis voluntarily, the dose administered or the pattern of use was not experimentally controlled. Indeed, we have shown in Chapter 2.2 that reliance on self-report alone underestimates the true prevalence, especially in women who refused to report on their substance use and in women who reported to have used cannabis only before pregnancy. Thus, it is complicated to acquire a reliable and valid measure of prenatal cannabis use. Given that women may underreport cannabis use when asked during pregnancy attributable to stigma associated with this particular period in life, some researchers have suggested that retrospective reporting may obtain more valid information. Particularly, when there are no distinct consequences on the child after pregnancy, the mother may be more honest in reporting cannabis use [24,25]. Of course, a

retrospective design has other limitations and is no perfect solution to the issue under study.

Additionally, it is important to take the categorization of exposure measures into account. Dichotomization of cannabis exposure may hide a true association, and may limit the detection of a possible dose-response effect, because information about the frequency or intensity is lost. A possible answer to these methodological problems is a combination of self-report and a direct measurement of cannabis and/or it metabolites at regular intervals during pregnancy (e.g. all three trimesters). A limitation of the Generation R study is that for the major part of the cohort (women who reported not to have used cannabis and women who did not report at all) prenatal urinalysis on cannabinoids was only performed in early pregnancy and not in mid- and late pregnancy.

Outcome variables

Measurements during the early (foetal) life of the offspring (foetal growth, birth weight) may present a clear picture of the immediate health or nutritional status of the infant, but are inadequate in determining long-term consequences in the neurobehavioral and cognitive domain. Moreover, the neurobehavioral and cognitive outcomes may be instable from infancy to childhood and adolescence, because the child is continuously in development and different competencies are demanded of children at different ages.

In this study, the quality of the different measurements may have influenced our findings. First the prenatal outcome measurements, foetal growth and redistribution assessed with ultrasound measurements and birth weight, are reliable, precise and objective measures. In the postnatal period, we made use of questionnaire information, which may be biased and of lesser quality as compared to the prenatal measurements. Possibly, the differences in quality of measurements on the outcome variables may have contributed to our findings. An alternative explanation may be that the possible effect of teratogenic compounds (e.g. cannabis) is not constant over age [15] and may cause transitory effects due to an immature central nervous system. For this reason, long-term studies are needed to assess behavioural and cognitive development until children reach adolescence.

Statistical issues

One of the important strengths of the Generation R study is its impressive sample size, which is population based. A large cohort is needed when either the outcomes are rare, or the expected effect is relatively small. Moreover, it is sometimes impossible to identify the main determinant that predicts the outcome of interest, because in human studies different exposure variables are highly correlated (e.g. cannabis and tobacco use). To test the separate effects of cannabis, or rather the effects of cannabis over and above that of tobacco, a large total sample size is needed, in which multiple exposure-subgroups can be formed.

Another important strength of the Generation R study is the availability of information on many potential confounding variables. It is always a concern in what degree statistical associations between maternal cannabis use during pregnancy and later outcomes reveal cause-and-effect associations or non-causal, or spurious association that arise as a consequence of the different demographics, social background and behavioural characteristics of mothers who choose to use cannabis during pregnancy. Moreover, postnatal environmental factors may account for problem behaviour and poor cognitive function in infants. For example, spurious relations between prenatal cannabis use and neurobehavioral and cognitive outcomes in offspring may be caused by malnutrition, lower social background and more problematic parental and family functioning, i.e. variables associated with maternal substance use during pregnancy and predictive of neurobehavioral and cognitive functioning [26-28]. Thus, in determining the possible influence of environment during the development of the child, it is essential to statistically account for social, emotional and demographic factors of mother and child. In our studies, we took into account several of these factors, but it cannot be excluded that we have missed possible other confounders, e.g. malnutrition during pregnancy.

A related issue is the likelihood that women who used cannabis during pregnancy will continue doing so after the birth of the child. Although it is not very likely that passive inhalation of cannabis will affect the child (unless levels are very high) [29], but since cannabis goes together with tobacco use in 85% of the users, it is possible that tobacco exposure affects the child's health and development. Indeed, effects of postnatal passive smoking on child behaviour have been found; children

exposed to environmental tobacco exposure display more externalizing problem behaviour [30-33]. Therefore, tobacco exposure should be taken into account when interpreting the results. So, from now on, it would be preferable to take into consideration that postnatal exposure to tobacco is an important confounder as well. However, such analysis may be prone to statistical limitations, including co-linearity between prenatal and postnatal tobacco exposure.

Causal inference

Causal inference is a topic that may pose problems in observational studies, and thus in the studies described in these thesis as well. The causal criteria of Hill accumulate along 9 domains: temporality, strength, biological gradient, plausibility, consistency, coherence, specificity, experimental evidence and analogy [20].

First, temporality is the only essential criterion; it is clear that maternal cannabis exposure always needs to precede the outcome. This is no problem in our study.

Second, the strength of the association is important; the stronger the association the more likely it is that the relation of maternal cannabis exposure to child outcomes is causal. For example, maternal cannabis use during pregnancy was strongly associated with a reduction in birth weight, while paternal cannabis use during the partners' pregnancy was not.

Third, most of data about the biological gradient and plausibility originated from animal research, which has shown that cannabis and its metabolites pass the placental barrier [34] and by entering the foetal circulation may affect the developing foetus. Findings from animal studies show adverse effects of prenatal cannabis exposure on birth weight [35,36] and on brain development by indicating permanent effects on functional regulation of motor behaviours [37], memory processes [38], and emotional reactivity [39] in offspring. Moreover, the temporal sequence of cannabis exposure coming prior to outcome and experimental evidence is also clearly evident in animal models. In addition, some in-vitro human studies showed that prenatal cannabis exposure was related to foetal brain differences in the limbic system [18,40]. Other experimental evidence from human studies is not available, because it would not be ethically accepted to perform randomized trials on cannabis use among pregnant women and examine the effects of maternal cannabis use during pregnancy using

an experimental design. In this observational study, we relied on data involving the possibility that the exposure to cannabis in the prenatal period is not random. Indeed, we have consistently shown in each chapter that pregnant women who use cannabis are younger, more likely to be unmarried, lower educated and have more psychological problems. Such factors are known risks for the development of behavioural problems in children.

Consistency in findings may reflect causality, but exceptions are possible [20]. Indeed, in this thesis, we found that although maternal cannabis use was related to intrauterine growth retardation and behavioural problems in girls at 18 months of age, but it was not related to cognitive performance at 30 months and behavioural problems at 36 months of age. Further studies on the association between intrauterine cannabis exposure and school performance in children studies may provide additional information.

The associations found should be compatible with the existing theory and knowledge (coherence); the studies described in this thesis are. A next criterion based on Hill's is that the studies described in this thesis should be in line with previous animal and human studies. In-vitro studies have shown that prenatal cannabis exposure results in changes in the brain, which as a consequence may affect child behaviour and cognitive functioning [41].

Finally, specificity is established when a single putative cause produced a specific effect. This criterion is considered to be one of the weaker criteria [20], because it may misleadingly suggest that a relation is more likely to be causal when exposure is related to a single outcome rather than to several outcomes. Indeed, as the endocannabinoid system is involved in multiple processes, it would be extraordinary if prenatal cannabis exposure would produce a single specific effect. In our studies, we found that cannabis exposure was associated with multiple outcomes in the offspring, including foetal growth and child behaviour.

In judging whether a reported association is causal, it is necessary to determine the extent to which other possible explanations were taken into account. For example, the association between maternal cannabis use during pregnancy and foetal growth reduction may have been partially caused by maternal malnutrition, and the association between maternal cannabis use and behavioural outcome in the offspring may well be directly associated with the psychopathology of the mother before the

pregnancy. Another alternative explanation may be that cannabis use during pregnancy may be derived from heritable maternal behaviour, and because mothers transmit genes to their offspring, there is the possibility that the associations arise through mothers and offspring sharing some of their genome rather than because of a true prenatal risk effect [42]. However, in our studies we took into account multiple confounding factors to deal with this possibility. Yet, it remains challenging to draw firm conclusions about the effects of intrauterine cannabis exposure, because it is possible that unmeasured residual confounding may still be present. Therefore, to provide a solution for this problem, we investigated the effects of paternal cannabis use as well. In general, similarity between partners is common and may results from processes such as assortative mating and behaviour contagion [43]. Consequently, if the link of maternal exposure with offspring outcomes is much stronger than that of paternal exposure, intrauterine exposure plays a more important role in the effect on offspring outcome [44].

Moreover, it is also important to consider effect sizes and clinical relevance instead of statistical significance only. Based on the effect sizes discussed in this thesis, we conclude that maternal cannabis use during is associated with severe growth reduction in late pregnancy and at birth, and is significantly associated with behavioural problems in girls (but not in boys) at 18 months of age, however effects in the third year of life on behavioural and cognitive development were not found.

In conclusion, it is not straightforward to determine whether an observed association is causal, but the causal criteria of Hill are useful for inference of causality.

Implications and recommendations for future research

Clinical implications

Our findings have important implications for clinicians and public health workers. First, they facilitate the recognition of women at risk for using cannabis in pregnancy. In chapter 2.1 we showed that the most important predictors for maternal cannabis use during pregnancy were cannabis use of the biological father of the child, maternal delinquency, being a single mother and having a history of childhood trauma (physical or sexual abuse and/or neglect). Ideally, this recognition could improve education

and prevention of cannabis use in pregnant women, which could start even before pregnancy, because often, pregnancy was unplanned in cannabis users. Such preconception and prenatal care directed at young women with a partner using cannabis and at women with a history of emotional and social difficulties might be an efficient approach to reduce the exposure in utero to cannabis, as well as to tobacco and alcohol. However, the reality is different; a major difficulty is that both mothers and fathers need to participate in such approach. Moreover, it is probable that cannabis use in these women is hard to influence or reduce, because it has become a habit, which is hard to overcome.

An important strategy for preconception and prenatal care should be to provide comprehensive education about cannabis and its effects on the unborn child, because future mothers might not be aware of the fact that cannabis can affect their child by passing the placental barrier and by breast-feeding milk. It should be noted, however, that many women in our study who used cannabis in early pregnancy already tried to reduce the risk of exposing their child to cannabis by changing their use of hashish (more potent) to marijuana (less potent) and by using cannabis less often than before pregnancy. We prefer preconception care because our results show that cannabis exposure at any time during pregnancy already affects foetal growth, for example. The knowledge and attitudes on preconception wellbeing of potential parents could be improved by increasing community consciousness via schools and media. Furthermore, as we have shown that cannabis use during pregnancy often goes together with multiple adverse factors, such as psychiatric symptoms and a low educational level, it suggests a need for counselling for parents and children at risk on various domains.

We recommend providing preconception visits for couples planning a pregnancy as a constituent of maternity care. Certain couples and their children may need additional counselling or intervention therapies or extensive follow-up by medical professionals such as gynaecologists, paediatricians and psychologists or psychiatrists. Our findings suggest that such interventions or treatments are especially needed in the prenatal period and to a lesser extent in the postnatal period.

Recommendations for future research

Several recommendations for future research may be inferred from our findings described in this thesis. First, when investigating maternal cannabis use we should not only make use of self-reported information on substance use during pregnancy. Self-report alone underestimates the true prevalence for maternal cannabis use in a cohort study, especially in women that reported to have used cannabis only before pregnancy and in women with missing information on substance use. In order to improve the quality of maternal cannabis use data, we suggest a two-step approach, starting with self-reported information and followed by urinalysis in the two groups mentioned above. Moreover, with the purpose of improving the quality of maternal cannabis use data, we recommend to assess maternal cannabis use in each trimester in pregnancy and in the postnatal periods as well using both self-reported information and urinalysis. Improvement of the quality of these data is especially needed to avoid misclassification when studying potential dose-response associations.

Second, when performing studies on maternal cannabis use and long-term consequences in children, one should take into account multiple confounding factors, as we have shown in this thesis that cannabis goes together with demographic, psychosocial and emotional adverse circumstances.

Third, the study on intrauterine growth showed clear differences in foetal and birth weight between cannabis-, tobacco- and non-exposed children. It would be very interesting to examine whether the lower weight in cannabis-exposed children will persist in childhood, or whether the affected children catch up in growth. In addition, we performed a pilot study on foetal redistribution and intrauterine cannabis exposure; this pilot indicated that a larger-scale study in order to increase the statistical power is needed to elucidate the differences between cannabis-exposed and non-exposed children concerning foetal blood flow.

Fourth, for maintaining the study on consequences of maternal cannabis use within the Generation R Study, we advise to focus on possible selective loss to follow-up. Mothers who stop participating in the Generation R Study should be re-approached to keep the sample as large as possible (increase in power) and as representative as possible (reduce selective loss to follow-up). Moreover, high-risk groups (such as the pregnant cannabis users) may be motivated and stimulated for

participation by a more personal approach and small rewards including money, discount-coupons or positive feedback of results. Additionally, we suggest re-approaching the Pilot group (South) for all future measurements. This pilot group consisted of a larger proportion of cannabis-using mothers (5.9% in pilot South as compared to 5.2% in cohort North) and cannabis-using fathers (7.7% in pilot South as compared to 10.8% in cohort North).

Furthermore, in terms of future measurements, we propose to make use of observational data combined with reported data. We suggest making use of data on school performance (results from school-exams, levels of reading, such as CITO and AVI-levels). Based on the existing literature, we would expect that the association between intrauterine cannabis exposure and behavioural and cognitive outcomes in children will become more evident when they become older and when reliable assessments based on multiple informants, such as the child itself, mothers, fathers, and teachers, become available. We also recommend in-depth studies in subgroups, instead of examining the whole cohort; such in-depth study might concern an imaging study. There is little data documenting structural brain changes as a result of prenatal cannabis exposure. A recent MRI study of 10-14 year old children showed that cortical gray matter was reduced in marijuana exposed children [45]. Since we have shown that foetal growth of the head circumference lagged in cannabis-exposed foetuses, it is of interest to perform similar imaging studies to investigate whether differences in brain volumes are present in childhood and whether these differences persist into adolescence and adulthood.

Finally, in Europe cannabis use during pregnancy generally goes together with tobacco use during pregnancy (85%), therefore the studies in this thesis mainly reflect the effects of cannabis over and above that of tobacco. We would, therefore, recommend performing similar studies in a population of pregnant women in other countries where cannabis use (during pregnancy) is not combined with tobacco in order to better delineate the potential detrimental effects of cannabis use from those of tobacco use during pregnancy.

In conclusion, this thesis extended existing knowledge about foetal consequences of cannabis use during pregnancy. The Generation R cohort provided and is currently still providing more recent data on the associations between parental cannabis use during pregnancy and long-term outcomes. Summarized, our results demonstrated strong associations between intrauterine cannabis exposure and foetal growth reduction. However, no substantial effects were found on postnatal outcomes from 18 until the age of 36 months, in spite of the higher THC levels than those in previous studies. We believe that it is important to further monitor the development of these children and continue collecting information.

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CHAPTER 6

SUMMARY/SAMENVATTING

LIST OF ABBREVIATIONS

DANKWOORD

ABOUT THE AUTHOR

PHD PORTFOLIO

Summary

Among illicit drug use of pregnant women, cannabis is most often consumed. Additionally, the potency of cannabis products has increased in the last decade due to development of cultivation techniques. Intrauterine exposure to cannabis may result in a long-term risk for the developing child; the results of previous research concerning the effect of maternal cannabis use during pregnancy on child behaviour and cognition in early childhood were ambiguous. This thesis, therefore, aimed to extend existing knowledge on cannabis use in pregnant women and the effects of intrauterine cannabis exposure on foetal and infant development. All studies in this thesis were conducted within the Generation R study, a population-based cohort study among pregnant women and their children in Rotterdam, the Netherlands. The studies presented in this thesis describe the determinants of maternal cannabis use during pregnancy, the agreement between self-reported cannabis use and urinalysis, and the effects of intrauterine cannabis exposure on offspring in pre- and postnatal life. Below, a brief summary of the main results from these studies is presented.

In Chapter 2.1, we studied the determinants of cannabis use during pregnancy. The results demonstrated that maternal cannabis use was associated with multiple characteristics that may influence foetal growth and offspring outcomes. The strongest predictor for cannabis use before and during pregnancy was cannabis use by the biological father. We further found that childhood maltreatment and delinquent behaviour of pregnant women also seem to be significant independent predictors for using cannabis before and during pregnancy. Women using cannabis were also more likely to be single. Furthermore, being religious is a significant protective factor for cannabis use before or during pregnancy. Additionally, lower educational level is an important determinant for continued cannabis use in ever-users. Finally, women using cannabis during pregnancy were more likely to be frequent users and women with a history of cannabis dependence continued using cannabis during pregnancy more often.

The study described in Chapter 2.2 showed that self-report of cannabis use provides a higher estimate than urinalysis, but urinalysis provides additional cases of women using cannabis during pregnancy. Interestingly, positive urine screens were mainly in the group that

reported cannabis use before pregnancy (7.6%) and in the group that refused to report on their cannabis use (2.6%). Only a small proportion of positive urine screens was present in the non-users group (0.4%). The measure of agreement (Yule's Y) indicated substantial agreement between self-report and urinalysis. Thus, this study demonstrates that reliance on self-reported cannabis alone underestimates the prevalence of cannabis use during pregnancy even in a country where neither cannabis possession nor cannabis use is prosecuted.

Chapter 3.1 showed that exposure to potent cannabis in utero was related to a reduced foetal growth and smaller foetal head size, while intrauterine exposure to cannabis is not related to gestational duration. Importantly, we found that cannabis use during pregnancy, often combined with tobacco, has a stronger effect on intrauterine growth when compared to the effect of prenatal tobacco exposure only. Importantly, even short-term intrauterine cannabis exposure, i.e. in the first trimester of pregnancy, was associated with impaired foetal growth. These associations between maternal cannabis use and foetal growth were independent of lifestyle and socio-economic factors. Interestingly, paternal cannabis use during pregnancy was not associated with foetal growth restriction, which suggests that the negative association between maternal cannabis use and foetal growth is due to intrauterine exposure and not to confounding by the family environment.

In Chapter 3.2 the effect of maternal cannabis use during pregnancy on foetal hemodynamic adaptations was described. Because it has been shown that endogenous cannabinoid system plays an important neuromodulatory role in cardiovascular regulation, prenatal exposure to cannabis in early foetal life could result in hemodynamic adaptations, such as a reduction of vascular resistance and an increase in vascular flow. Our results demonstrate that cannabis use during pregnancy is associated with adaptations in foetal placental and cardiac blood flow, but not with foetal cerebral blood flow. However, the observed associations could be explained by the co-occurrence of tobacco use during pregnancy in this group as we have found that the blood flow parameters did not significantly differ between cannabis-exposed foetuses and tobacco-exposed foetuses. Against the expectation, we found statistically significant specific associations between maternal cannabis use and the uterine resistance indices, including a higher uterine pulsatility index and

a higher uterine resistance index in cannabis-exposed foetuses, which remained present after taking into account maternal tobacco use.

Chapter 4.1 described the relationship between parental cannabis use during pregnancy and child behaviour at 18 months of age. The study showed an increased risk for externalizing problems in children of mothers who used cannabis during pregnancy compared to children of non-using mothers. This elevated risk remained significant after adjustment for age and gender of the child, maternal education and national origin, but was no longer statistically significant after adjustment for maternal psychopathology. We also found an elevated risk for total problems and externalizing problems in children of fathers who used cannabis during pregnancy compared to children of non-using fathers, but these elevated risks were no longer statistically significant after adjustment for paternal education, national origin and psychopathology. Our findings indicated gender-specific associations; we found that maternal cannabis use was significantly associated with total problems and externalizing problems in girls, but not in boys, after considering several covariates.

This effect of cannabis exposure on behaviour was not a consistent one; the association was weaker at 36 months of age, as was described in Chapter 4.2. In this chapter we found that children who were exposed to cannabis during pregnancy showed an increased score for total problems and externalizing problems compared to children of non-using mothers. However, this elevation did not remain statistically significant after adjustment for age and gender of the child, maternal education and national origin and maternal psychopathology. In contrast to the positive associations between maternal cannabis use and behavioural problems, we found a lower score for total problems and externalizing problems in children of fathers who used cannabis during pregnancy compared to children of non-using fathers. These lower scores were no longer statistically significant after adjustment for age and gender of the child, paternal education, national origin and psychopathology. In Chapter 4.2, we also investigated the association between intrauterine cannabis exposure and cognitive performance at 30 months. The study showed no association between maternal cannabis use and verbal or non-verbal cognitive performance at 30 months. Unexpectedly, maternal cannabis use before pregnancy was a protective factor for delay in verbal development at 30 months of age. In addition, it appeared that paternal

cannabis use was also a protective factor for delay in verbal development at 30 months of age.

Summarized, this thesis showed that maternal cannabis use during pregnancy is associated with prenatal outcomes, such as foetal growth and blood redistribution, while in the postnatal period these associations become weaker with the increasing age of the child.

Samenvatting

Cannabis wordt in vergelijking met andere drugs vaak gebruikt; ook onder zwangere vrouwen is dit het geval. Bovendien is de concentratie van het psychoactieve bestanddeel THC door de huidige teeltechnieken veel hoger geworden. Intra-uteriene blootstelling aan cannabis kan mogelijk leiden tot een langdurig risico in het ontwikkelende kind; de resultaten van voorgaand onderzoek met betrekking tot het effect van moederlijk cannabisgebruik tijdens de zwangerschap op het gedrag en de cognitieve van het kind in de peutertijd zijn niet altijd eenduidig. Dit proefschrift tracht, daarom, de huidige kennis over cannabisgebruik in zwangere vrouwen en de mogelijke effecten op de foetus en het kind uit te breiden. Alle studies beschreven in dit proefschrift zijn uitgevoerd binnen de Generation R studie, een longitudinale bevolkingsstudie onder zwangere vrouwen en hun kinderen in Rotterdam. Het onderzoek in dit proefschrift beschrijven de determinanten van cannabisgebruik, de overeenkomsten tussen zelfrapportage en aanwezigheid van cannabis in urine, en het effect van cannabisblootstelling op de nakomelingen in de prenatale en postnatale periode. Hieronder is een korte samenvatting van de hoofdzakelijke bevindingen van deze onderzoeken beschreven.

In hoofdstuk 2.1 hebben we de determinanten van cannabisgebruik tijdens de zwangerschap bestudeerd. De resultaten toonden aan dat moederlijk cannabisgebruik samenhangt met verschillende kenmerken die foetale groei en kind-uitkomsten mogelijk beïnvloeden. De sterkste voorspeller van cannabisgebruik voor en tijdens de zwangerschap was het cannabisgebruik van de biologische vader van het kind. We vonden ook dat jeugdtraumatische ervaringen, zoals mishandeling en verwaarlozing, en delinquent gedrag belangrijke significante onafhankelijke voorspellers van het cannabisgebruik was. De vrouwen die cannabis gebruikten tijdens de zwangerschap waren vaker alleenstaand. Echter, religie bleek een significante beschermende factor tegen cannabisgebruik. Verder, bleek

dat een laag opleidingsniveau een belangrijke determinant was voor doorgaand gebruik in ooit-gebruikers. Ten slotte bleek uit de gegevens dat vrouwen die cannabis gebruikten tijdens de zwangerschap vaker frequent cannabis gebruikten en dat deze vrouwen ook vaker een voorgeschiedenis had met cannabisverslaving.

Het onderzoek beschreven in hoofdstuk 2.2 toonde dat zelfrapportage van cannabisgebruik een hogere schatting geeft, dan urinalyse, maar dat urinalyse wel additionele casussen van cannabisgebruikende vrouwen oplevert. Interessant hierbij was dat de positieve urinemonsters voornamelijk te vinden waren in de groep vrouwen die rapporteerde dat ze alleen voorafgaand aan de zwangerschap hadden gebruikt (7.6%) en in de groep vrouwen die de vragen over middelen gebruik niet hadden beantwoord (2.6%). Slechts een klein deel van de cannabinoid-positive urinemonsters was te vinden in de niet-gebruikers (0.4%). De maat voor overeenkomst (Yule's Y) gaf aan dat er substantële overeenkomst was tussen zelfrapportage en urinalyse. Dus, deze studie toont aan dat het vertrouwen op alleen zelfrapportage de prevalentie van het cannabisgebruik onderschat, zelfs in een land waarin noch het cannabisbezit noch het cannabisgebruik wettelijk wordt veroordeeld.

Hoofdstuk 3.1 toont aan de intra-uteriene blootstelling aan potente cannabis gerelateerd was aan een verminderde foetale groei en een kleinere foetale hoofdomtrek, terwijl deze blootstelling niet gerelateerd bleek te zijn aan de zwangerschapsduur. Deze studie toonde ook aan dat cannabisgebruik tijdens de zwangerschap, dat vaak samengaat met tabaksgebruik, een sterker effect op intra-uteriene groei heeft dan prenatale tabaksblootstelling alleen. Zelfs kortdurende intra-uteriene cannabisblootstelling, d.w.z. alleen in het eerste trimester van de zwangerschap, was gerelateerd aan een verminderde foetale groei. Deze associaties tussen moederlijk cannabisgebruik en foetale groei waren onafhankelijk van andere levensstijl factoren en sociaaleconomische factoren. Van belang was ook de bevinding dat vaderlijk cannabisgebruik tijdens de zwangerschap geen associatie had met een verminderde foetale groei, deze bevinding suggereert dat de negatieve associatie tussen moederlijk cannabisgebruik en foetale groei wordt bepaald door de intra-uteriene blootstelling en niet zo zeer door mogelijke gezinsomgevingsfactoren.

In hoofdstuk 3.2 is het effect van moederlijk cannabisgebruik tijdens de zwangerschap op foetale bloedvoorziening beschreven. Aangezien eerder is aangetoond dat het endocannabinoide systeem een rol speelt in de cardiovasculaire regulatie, kan prenatale blootstelling aan cannabis in het vroege foetale leven mogelijk leiden tot hemodynamische adaptaties, zoals een vermindering van de vaatresistentie en een toename in de vaatdoorstroming. Onze resultaten toonden aan dat cannabisgebruik tijdens de zwangerschap geassocieerd is met veranderingen in de doorstroming van de placenta en de doorstroming van het hart in de late zwangerschap, maar niet er was geen associatie met de cerebrale bloeddoorstroming in de foetus. Echter, de geobserveerde associaties konden worden verklaard door het tabaksgebruik tijdens de zwangerschap, want we vonden geen verschillen in bloeddoorstroming tussen foetussen van de cannabisgebruikers en tabaksgebruikers. Tegen de verwachting in, vonden we een specifiek statistisch significant verband tussen moederlijk cannabisgebruik en de baarmoederlijke resistentie indices, inclusief een hogere uteriene pulsatiliteits index en een hogere uteriene resistentie index in cannabisblootgestelde foetussen, welke niet toe te schrijven was aan het tabaksgebruik.

Hoofdstuk 4.1 beschrijft de relatie tussen ouderlijk cannabisgebruik tijdens de zwangerschap en het gedrag van het kind op de leeftijd van 18 maanden. Deze studie toonde aan dat kinderen van cannabisgebruikende moeders een hoger risico hadden op externaliserend probleemgedrag. Dit verhoogde risico bleef statistisch significant na het corrigeren voor leeftijd en geslacht van het kind, moederlijk opleidingsniveau en de etnische afkomst in het model. Echter na het corrigeren voor moederlijke psychopathologie verdween deze relatie. Ook vonden we een verhoogd risico op (externaliserend) probleemgedrag in kinderen van vaders die cannabis gebruikten tijdens de zwangerschap van hun partner, ten opzichte van niet-gebruikende vaders. Echter, dit verhoogde risico was toe te schrijven aan factoren zoals, vaderlijk opleidingsniveau, etnische afkomst en psychopathologie. De resultaten van het onderzoek toonde een seksspecifiek verband aan: moederlijk cannabisgebruik was gerelateerd aan (externaliserend) probleemgedrag in meisjes, maar niet in jongens, zelfs na beschouwing van de andere bovengenoemde factoren.

Het effect van cannabisblootstelling op probleemgedrag was niet consistent; the associatie bleek zwakker op de leeftijd van 36 maanden, zoals beschreven in hoofdstuk 4.2. In dit hoofdstuk vonden we dat

kinderen met prenatale cannabisblootstelling een hogere score hadden op (externaliserende) problemen in vergelijking met niet-blootgestelde kinderen. Echter, dit verband was niet meer statistisch significant wanneer andere verklarende factoren, zoals leeftijd en geslacht van het kind, het opleidingsniveau van moeder, etnische afkomst en psychopathologie, in het model werden meegenomen. In tegenstelling tot de positieve associaties (hogere score op probleemgedrag) tussen moederlijk cannabisgebruik en gedragsproblemen, vonden we een lagere score op (externaliserend) probleemgedrag voor kinderen van vaders die cannabis gebruikten. Deze lagere scores bleven niet meer statistisch significant nadat we in het model corrigeerden voor leeftijd en geslacht van het kind, en vaderlijk opleidingsniveau, etnische afkomst en psychopathologie. In hoofdstuk 4.2 was ook onderzocht of intrauteriene cannabisblootstelling een relatie had met cognitief functioneren op de leeftijd van 30 maanden. Deze studie toonde aan dat er geen verband was tussen moederlijk cannabisgebruik en (non)verbaal cognitief functioneren. Onverwacht, bleek moederlijk cannabisgebruik voorafgaand aan de zwangerschap een beschermende factor op een vertraagde verbale ontwikkeling. In toevoeging hierop bleek dat vaderlijk cannabisgebruik een beschermende factor was voor vertraging in de verbale ontwikkeling van het kind.

Samengevat toont dit proefschrift dat moederlijk cannabisgebruik tijdens de zwangerschap sterk geassocieerd is met prenatale uitkomsten, zoals foetale groei en foetale bloedvoorziening, maar in de postnatale periode worden deze associaties zwakker naarmate de kinderen ouder worden.

List of Abbreviations

ANOVA	Analysis of Variance
BSI	Brief Symptom Inventory
CBCL	Child Behavior Checklist
CB	Cannabinoid
CI	Confidence Interval
CTQ	Childhood Trauma Questionnaire
EMBU	'Own memories on parenting'
FAD	Family Assessment Device
FHR	Foetal Heart Rate
GF	General Functioning
GSI	Global Severity Index/ General Symptom Index
IGF	Insulin-like Growth Factor
LDS	Language Development Survey
LLD	Long Lasting Difficulties
MHPCD	Maternal Health Practices and Child Development Study
NIDA	National Institute on Drug Abuse
OPPS	Ottawa Prenatal Prospective Study
OR	Odds Ratio
PARCA	Parent Report of Children's Abilities
PI	Pulsatility Index
PSV	Peak Systolic Velocity
RI	Resistance Index
SD	Standard Deviation
SPSS	Statistical Package of Social Sciences
THC	Δ9-tetrahydrocannabinol
TLFB	Time-Line Follow-Back Interview

Dankwoord

Hora est. Het is tijd. Het is tijd om iedereen te bedanken die direct of indirect heeft bijgedragen aan dit proefschrift en het ontstaan hiervan.

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Hora Est. Het is tijd. Het is tijd om een nieuw onderzoek te starten.

About the author

Hanan el Marroun is the daughter of Mr. Alami El Marroun en Mrs. Fatima Jabroun; she is the third out of three children, born on 15th of June in 1981 in The Hague, the Netherlands.

In 1999 she passed secondary school (Athenaeum) at the Aloysius College in The Hague. In the same year she started her study Biology and Medical Laboratory Research at the higher professional school in Rotterdam (1999-2002). She became intrigued by the brain and it's functioning at the department of Genetics and Cell Biology (Erasmus MC) where she studied the functioning of proteins involved in the ticking of the biological clock located at the suprachiasmatic nucleus. After obtaining her Bachelor Degree (with animal care annotation) Hanan chose to continue studying instead of working as a lab-technician. She completed the Bachelor Biomedical Science (Free University, Amsterdam) within one year. Then, she followed her passion for understanding the functioning of the brain and started the 2-year Master of Neuroscience (2003-2005), where she completed two internships. First, she studied relapse in alcohol-addicted rats and mice at the department of Neuro- & Psychopharmacology (Free University). Then, she studied the functioning of the autonomic nervous system during stress in children with externalizing problems as compared to healthy children (Sophia Children's Hospital; Child and Adolescent Psychiatry). During the summer of 2005 she started as a PhD student at Generation R with the department of Child and Adolescent Psychiatry. While studying the association of maternal cannabis use during pregnancy on infant development, she followed and obtained the Master of Epidemiology (2006-2008). Next to her work, she volunteered for the PhD association ProMEras as a board member (2005-2007). In December 2009, the Sophia Foundation for Scientific Research granted her research proposal "Early and late effects of antidepressants use during pregnancy". In January 2010, she started this post-doctoral fellowship at the department of Child and Adolescent Psychiatry.

Hanan El Marroun is married with Amin Baameur and they are proud parents of Cherine (born on 20th June in 2008).

PhD Portfolio

1. PhD training	Year	Workload (ECTS)
General academic skills		
Biomedical English Writing and Communication, Erasmus MC	2007	1.1
Sollicitatietraining, Loopbaancentrum, Erasmus MC	2009	0.3
Research skills		
MSc Epidemiology,NIHES:	2006-2008	
Principles of Research in Medicine	2006	0.7
Methods of Public Health Research	2006	0.7
Health Economics	2006	0.7
Case-Control Studies	2006	0.7
Introduction to Public Health	2006	0.7
Prevention Research	2006	0.7
Study Design	2006	4.3
Classical Methods for Data Analysis	2006	5.7
Public Health Research: Analysis of Population Health	2006	1.4
Public Health Research: Analysis of Determinants	2006	1.4
Public Health Research: Intervention Development and Evaluation	2006	1.4
Methodological Topics in Epidemiologic Research	2007	1.4
Modern Statistical Methods	2007	4.3
In-depth courses		
MSc Epidemiology, short courses, NIHES		
Psychiatric Epidemiology	2007	1.1
Advances in Clinical Neuro-epidemiology	2007	0.7
Paediatric Drug Research	2007	0.9
Maternal and Child Health	2007	0.9
Ethnicity, Health, and Health Care	2007	1.1
National and International conferences, seminars, meetings and workshops		
Jaarcongres Jeugdgezondheidszorg, Soesterberg, Nederland	2006	0.3
Dag van de Promovendus, PNN i.s.m. ABVAKABO FNV, Utrecht	2006	0.3
Programmadag ZonMW, Verslaving, Den Haag	2006	0.6
Forum Alcohol en Drugs Onderzoek, Trimbos Instituut, Utrecht	2007	0.6
Geestkracht ZonMW projectleiderbijeenkomst, Nieuwegein	2007	0.3
Programmadag ZonMW, Verslaving, Tropenmuseum, Amsterdam	2007	0.6
PhD day, Erasmus MC Rotterdam, The Netherlands	2008	0.3
Prenatal and early Postnatal Brain development, Symposium Generation R, Rotterdam, Nederland (gepresenteerd door A.C. Huizink)	2008	0.3
Forum Alcohol en Drugs Onderzoek, Trimbos Instituut, Utrecht	2008	0.6
Biennial Meeting of Society Research in Child Development, Denver, USA	2009	0.6
Geestkracht ZonMW projectleiderbijeenkomst, Utrecht	2009	0.6
ZonMW Bessensap, Wetenschap ontmoet Pers, NEMO, Amsterdam	2009	0.6
Item in Noorderlicht Nieuws, Wetenschapjournaal van de VPRO	2009	-
Forum Alcohol en Drugs Onderzoek, Trimbos Instituut, Utrecht	2009	0.6

Grant applications & Reviewing papers	Year	Workload (ECTS)
SSWO: Early and late effects of antidepressants use during pregnancy	2009	3.5
Review paper for Neuropsychology	2009	0.3
Review paper for Journal of Child Psychology and Psychiatry	2009	0.2

2. Teaching Activities		
Supervising practical session and/or excursions		
Supervising practical, Course Study Design, NIHES	2007	0.7
Supervising practical/lecture, Child Development, Child- and Adolescent Psychiatry	2008	0.6
Lecturing students, Erasmus University, Department of Psychology	2009	0.6
Lecturing students, University of Amsterdam, Department of Educational Sciences	2009	0.6
Supervising practical/lecture, Child Development, Child- and Adolescent Psychiatry	2009	0.6
Supervising Master's theses		
Supervised Marvin van der Krogt, student Master Criminology, EUR Thesis topic: Determinants of delinquency in pregnant mothers and their partners	2007/8	2.6

1 ECTS (European Credit Transfer System) is equal to a workload of 28 hrs