The impact of oral cannabis consumption during pregnancy on maternal spiral artery remodelling, fetal growth and offspring behaviour in mice

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Summary

Background The use of cannabis during pregnancy is rising following its widespread legalization. Cannabidiol (CBD) is gaining popularity due to the public perception that it is safer than the psychoactive cannabis component Δ9-tetrahydrocannabinol (THC). However, while evidence underpins the harm of THC and cannabis smoke on fetal development, there is minimal research on the safety of CBD and oral cannabis. The current study aims to decipher the safety of oral CBD and THC use during pregnancy.

Methods Using a mouse model, we directly compared the effects of oral CBD and THC oil exposure (20 mg/kg body weight) from early to mid-gestation on implantation site remodelling and fetal growth. We examined offspring behaviour and metabolic activity using both traditional and automated cage systems. Lastly, using human and mouse immune cells we assessed how CBD and THC influence angiogenic factor production.

Findings We observed impaired maternal spiral artery remodelling in cannabis exposed mice and found that CBD and THC disrupt immune cell angiogenic factor production. Oral consumption of THC or CBD oil also resulted in significant fetal growth impairment and led to long-lasting sex-dependent consequences as male offspring exhibited altered aggression and metabolic activity while females had impaired spatial learning.

Interpretation Our results show that oral consumption of either CBD or THC oil during pregnancy in mice results in harm to the developing fetus and causes behavioural changes after birth.

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Keywords: Pregnancy; Cannabis; THC oil; CBD oil; Fetal growth; Behaviour

Introduction

Cannabis use during pregnancy is gaining popularity as the widespread legalization of recreational cannabis across North America has increased access, potency, and positive perceptions surrounding its use.[1–3] The rate of maternal cannabis consumption ranges substantially from 2 to 36% with higher rates seen in young women, urban centers, and when assessing data based on toxicology compared to self-report.[4] Typically, pregnant women report using cannabis to self-medicate their pregnancy symptoms and find it more effective and natural than prescribed medications.[5,6] Thus, cannabis use is the highest in the first trimester of pregnancy and tends to decrease as the pregnancy continues.[7] While

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Research in context

Evidence before this study

Accumulating evidence from animal models and human studies outline the effects of cannabis use during pregnancy. Maternal cannabis use has been associated with several pregnancy complications such as impaired fetal growth and preterm birth, as well as long-term behavioural consequences in the child such as increased aggression and inattention. However, research has primarily focused on cannabis smoke and the psychoactive cannabis component Δ9-tetrahydrocannabinol (THC) leaving the effects of other cannabis products largely unknown.

Added value of this study

Using a mouse model, we directly compared the effect of oral cannabidiol (CBD) and THC oil on pregnancy outcome and offspring behaviour after birth. We found that exposure to either oral CBD or THC from early to mid-pregnancy leads to impairment in fetal growth. Furthermore, oral THC and CBD exposure during pregnancy led to abnormalities in offspring behaviour in adulthood. Lastly, we uncovered the ability of oral THC and CBD to disrupt vessel development at the maternal–fetal interface in early pregnancy.

Implications of all the available evidence

The potential ramifications of cannabis use during pregnancy are becoming more apparent. Our study provides the additional information that even when consumed orally CBD and THC oil can impact fetal growth and offspring behaviour as demonstrated in our mouse model.

cannabis is typically consumed via smoke-inhalation, accumulating evidence underpins the detrimental harm of cannabis smoke on lung health.[8,9] Thus, cannabis oil and other edible cannabis products are becoming popular alternatives as they provide the desired cannabis effects without the dangerous by-products of smoke exposure.[10,11] In particular, cannabidiol (CBD) oil is gaining attention due to its perceived health benefits and absence of intoxicating effects and is being used as a treatment for several conditions such as epilepsy and pain management.[10,12,13] Furthermore, individuals often make a clear distinction between the safety of CBD and the safety of the psychoactive cannabis component Δ9-tetrahydrocannabinol (THC) with perceptions around CBD use being generally positive. In two studies assessing the perceptions of cannabis use and pregnancy health by cannabis dispensary employees, employees were more likely to recommend CBD-based products over THC and suggest oral consumption rather than smoke inhalation for pregnant customers.[14,15] Additionally, pregnant individuals using cannabis perceived non-inhalation methods to be much safer in pregnancy.[16]

Despite the positive perceptions surrounding cannabis use in pregnancy, there are several reports suggesting that maternal cannabis use is associated with pregnancy complications like reduced birthweight, preterm birth, and stillbirth.[2,17–19] Cannabis also impairs fetal brain development resulting in long-lasting cognitive deficits in the child such as increased aggression, attention problems, and anxiety.[20–22] However, most animal studies focus on cannabis smoke inhalation or injection of only THC, and human research often fails to specify the method of cannabis consumption.[2,19,23,24] Thus, very little is known about the effect of CBD and oral cannabis exposure on pregnancy and long-term fetal health outcomes.

The mechanisms driving cannabis-induced pregnancy complications are still unclear. Both THC and CBD can readily cross the placenta and enter fetal circulation.[25,26] Cannabis appears to impair placental development, as THC and CBD have been shown to disrupt trophoblast cells.[23,27,28] A dysregulated immune response has also been suggested as a potential driver of cannabis-induced pregnancy complications. Analysis of placental biopsies found that many immune-related genes were substantially downregulated in cannabis users and correlated to adverse fetal outcomes.[22] The immune system at the maternal–fetal interface extensively remodels the maternal environment to support fetal growth.[29] Uterine Natural Killer (uNK) immune cells account for roughly 70% of leukocytes in the early decidua and are essential for tissue remodelling by producing angiogenic factors to remodel maternal spiral arteries, anti-inflammatory mediators to suppress immune activation against the semi-allogenic fetus, and chemokines to regulate trophoblast migration.[29–32] Preliminary work has found that THC impairs peripheral blood NK (pbNK) cell function, but no studies to date have investigated how cannabinoids affect uNK cell function during pregnancy.[33,34] Determining the effects of cannabis on uNK cell function and early pregnancy remodelling is crucial in enhancing our understanding of cannabis-induced pregnancy complications.

In this study we aimed to delineate the effect of CBD and THC from other smoke contaminants during pregnancy by exposing pregnant mice to either CBD or THC oil via an oral route. We administered cannabis oils after implantation from early to mid-gestation to uncover the direct impact of cannabis on the remodelling of the maternal–fetal interface and how any changes to this process could lead to long-term consequences in offspring growth and behaviour.

Methods

Mice

CD57BL/6J mice were obtained from the Jackson Laboratory (Strain: 000664, RRID: IMSR_JAX:000664) and bred and housed in specific pathogen-free conditions at McMaster's Central Animal Facility. A maximum of 5 mice were housed per cage in a temperature-controlled environment under a 12-h light–dark cycle. Mice had access to water and irradiated Teklad global 18% protein diet (#2918). A total of 65 dams were used throughout the entire study. For behavioural assessment, the control group had 5 dams, CBD group had 4 dams, and the THC had 2 dams which gave birth to a total of 38, 34, and 13 pups respectively. Body condition including rough coat, skin lesions, hunched posture, and loss of over 20% body weight was monitored for determination of humane endpoint.

Generation of timed pregnancies

To generate timed pregnancies, one to three reproductively mature female CD57BL/6J mice (9–14 weeks old) were paired with one male CD57BL/6J mouse (9–14 weeks old) overnight. The following morning, the presence of a hard, occlusive vaginal copulation plug indicated the mice were at gestation day (GD) 0.5.

Cannabis administration during pregnancy

Beginning on GD 6.5 mice randomly received either 100 µL of CBD oil (20 mg/kg body weight, Symbl/Redecan), 100 µL of THC oil (20 mg/kg body weight, Symbl/Redecan), or 100 µL of control medium-chain triglyceride (MCT) oil (Nutiva) daily via gavage until GD 11.5. Cannabis administration began on GD 6.5 to not interfere with the embryo implantation window (GD 4–5) as the study objective is not to understand how cannabis impacts implantation.[35,36] The CBD and THC oil were made and diluted in MCT oil. The specific concentration of CBD and THC found in the cannabis oils is outlined in Table 1. The dose of 20 mg/kg body weight was chosen as it falls within the range utilized by clinical studies investigating oral CBD use in humans.[37] Dams were weighed at GD 0.5 and then daily from GD 6.5 up until GD 15.5. A study timeline has been included to outline all experiment timepoints (Supplemental Fig. S1).

Open field and novel object recognition testing

Female mice were exposed to cannabis as described above (GD 6.5–11.5). The pups were weighed weekly from week 1–7. Starting at week 8, mice were subject to behavioural testing. For the open field testing, mice were individually placed in a square arena (50 × 50 cm) for 10 min with a camera recording from above. The following day mice were placed in the open field now with the presence of two identical objects for 10 min. Three hours later, mice were placed in the arena with one old and one new object for 10 min like previous protocols.[38,39] Video analysis was performed using BORIS (RRID: SCR_025700) with the experimenter blinded to treatment group. The number of rearing, defecation, and grooming events were counted, as well as time spent in the center area (30 × 30 cm) and time interacting with the old and new objects tracked. For novel object recognition testing, mice were excluded from analysis if they did not meet the minimum 20 s interaction criteria with the objects.

Sample ID	Description	[CBD] mg/mL	[THC] mg/mL	Avg [CBD] mg/mL	Avg [THC] mg/mL
Sample 1A	High CBD, Low THC Oil "**CBD Oil**"	17	0.53	17 ± 0.41	0.52 ± 0.02
Sample 1B		17	0.50		
Sample 1C		17	0.53		
Sample 2A	High THC, Low CBD Oil "**THC Oil**"	0.12	17	0.12 ± 0.002	17 ± 0.34
Sample 2B		0.12	18		
Sample 2C		0.12	17		

Table 1: **Quantification of THC and CBD in cannabis oils via LC-MS.**

IntelliCage behaviour tracking

Pups exposed to cannabis *in utero* were monitored in the automated IntelliCage (RRID: SCR_017404) for 15 days at either 8 weeks (males) or 11 weeks (females) post-weaning due to equipment availability. For females, all experimental groups were housed in the same cage to remove cage effect (5 control, 5 CBD, 3 THC mice per cage). However, male groups had to be housed separately (13 control and 11 CBD mice per cage) and THC male mice could not be monitored due to extensive fighting between litter mates resulting in their need to be housed independently. 5 days prior to the IntelliCage experiment, all mice were injected with a radio frequency identification (RFID) tag for mouse identification by scanners at the different drinking corners. The experiment protocol and timeline were based on previous publications.[40,41] Briefly, the acclimatization phase of the experiment consisted of free adaptation, nose poke adaptation, and temporal adaptation where mice adapted to the cage, learned to use the nose sensor panel for water access, and were exposed to the restricted water timing used in future experimental phases. Next, to assess behavioural flexibility mice had to learn a behaviour sequence to access water at diagonally placed drinking corners during the acquisition phase (4 sessions) which was then reversed in the subsequent reversal phase (4 sessions) as outlined by Endo et al. The first 100 visits during drinking sessions were analyzed to determine the error rate. Visits to the two corners not involved in water access were counted as an error. Data from the IntelliCage was analyzed using the IntelliCage® Analyzer software (IntelliCage® Plus 2.4, NewBehaviour AG, Switzerland) and FlowR (XBehaviour Gmbh, Switzerland).

Metabolic cage

Pups exposed to cannabis *in utero* were placed individually in the Sable Systems Promethion Comprehensive Mouse Metabolic Monitoring System to collect metabolic data for 5 day as described previously.[42] This system consists of a home-cage-like environment that contains sensors that measure food intake, water intake, animal mass, respiration rates, and animal position and activity every second as previously described.[43] Each cage is also equipped with a running wheel and distance traveled is measured. Data was processed using manufacturer supplied ExpeData and MacroInterpreter programs condensing the data to 1hr segments. Data from all days of the experiment was assessed either as a total average or average of light/dark cycles. Parameters were assessed as per Promethion guidelines. Basal metabolic rate was denoted as the lowest energy expenditure (EE) 30-min period (kcal/hr). Maximum EE was determined by the mean EE during the 15-min period with highest EE (kcal/hr). All meters traveled consisted of the distance travelled within the cage not including wheel running which was denoted as wheel running. The animals time budget was divided into different activities: eating food, touching food hopper, drinking water, touching water dispenser, wheel running, inside body mass habitat, touching body mass habitat, wandering cage >60 s (long lounge), and wandering cage <60 s (short lounge). 2 mice were excluded from the CBD male group analysis as they did not enter the cage hopper and weight could not be collected by the system which underpowered this group. Data was analyzed using GraphPad Prism 9 software (RRID: SCR_002798) and CalR Version 1.3 software (RRID: SCR_015849).

Fetal tissue processing

At GD 18.5, dams were weighed and euthanized via cervical dislocation. Their abdomens were opened, and uteri excised via cuts at the cervix and utero-tubal junction. The number of fetuses were counted. A fetus was deemed resorbed if it had black discoloration and was smaller in size. The individual implantation sites were separated, and the myometrial and placental tissue removed. The fetuses from each dam were photographed and weighed. To measure fetal brain weight, the skull cap was removed, and brain tissue was collected and weighed. Crown-rump length was measured from the superior aspect of the head to base of the tail of each fetus and fetal head length was measured from nose to posterior aspect of the head. All measurements were made using the Fiji image processing package on ImageJ (RRID: SCR_002285) with the evaluator blinded to treatment group.

H&E and PAS staining

At GD 10.5 or 12.5, dams were euthanized, and uteri excised as described above. Intact implantation sites (2–4 per dam) were fixed in 4% paraformaldehyde for 48 h and then stored in 70% ethanol. Fixed implantation sites were cut mid-sagittal and embedded cut side down in paraffin to examine the two equal halves. Cross-sections were stained with hematoxylin and eosin (H&E) or periodic acid–Schiff (PAS) and scanned with Aperia ScanScope XT (Leica Biosystems). Histology images were captured using Olympus OlyVia Software (RRID:SCR_016167). Placental area (GD 12.5) and spiral artery diameter (GD 10.5) were measured using the Fiji image processing package on ImageJ of H&E midline cross-sections (2× and 20× magnification, respectively) with the investigator blinded. Placental area was measured from the chorionic plate to the trophoblast giant cells on mid-sagittal cuts and averaged per implantation site at GD 12.5 when the mature placenta is fully functioning.

Immunofluorescent DBA staining

Paraffin-embedded implantation sites were sectioned at 4 μm using a rotary microtome and dried overnight. Slides were incubated at 65 °C for 15 min, dewaxed in Xylene, and rehydrated through a series of decreasing concentration ethanol solutions finishing in distilled water. Non-specific binding was blocked with 10% goat serum in 0.1% Tween 20 buffered PBS for 1 h. Slides were then incubated with biotinylated Dolichos Biflorus Agglutinin (DBA, Vector Laboratories #B1035-5, RRID: AB_2314288) diluted 1:200 in blocking buffer overnight at 4 °C. The following morning, sections were washed and incubated with Streptavidin-Alexa 647 (ThermoFisher #S32357, RRID: AB_2336066) 1:500 in PBS with 0.1% Tween 20 for 1 h. Sections were counterstained with DAPI for 5 min. Slides were then cover slipped with ProLong Gold Anti-Fade Mounting Media (ThermoFisher #P36934). Slides were imaged using the Nikon Eclipse Ni microscope and Nikon DS-Qi2 camera. DBA fluorescence was quantified using NIS-Elements AR image analysis software and reported as area of image DBA+ (Cy5+) divided by total image area with the investigator blinded to treatment group.

Decidual immune cell isolation and stimulation with cannabinoids

On GD 10.5, individual implantation sites were separated, myometrial tissue removed, and fetal tissue discarded. The major lymphoid aggregate of pregnancy (MLAp), decidua, and placenta from each implantation site were pooled and weighed for each dam. Tissues were homogenized with RPMI media (200 μL) and cells pelleted via centrifugation at 800×*g* for 5 min as previously described.[31] After centrifugation, the cell-free supernatants were collected and sent to Eve Technologies for a 31-plex mouse cytokine/chemokine array. The remaining cell pellet was enzymatically digested in 2.5 mL of RPMI media containing 50 μg/mL Liberase TM (Sigma–Aldrich #LIBTM-RO) and 50 μg/mL DNase I (Sigma–Aldrich #10104159001) for 30 min at 37 °C on

a ThermoMixer. After digestion, the cell suspension was passed through a 70 μm nylon cell strainer and cells pelleted via centrifugation. The cells were then placed in a 40%/80% percoll gradient to enrich decidual leukocytes and resuspended to 1×10^6 cells/mL in complete RPMI media. Cells were stimulated with 100 ng/mL of mouse IL-15 recombinant protein (Peprotech #210-15, Accession#: P48346) and 10 ng/mL of mouse IL-12 p70 recombinant protein (Peprotech #210-12, Accession#: p35: P43431 p40: P43432) for 18.5 h as previously described.[44] THC or CBD at 0.1 and 1 μg/mL were added to the cell culture for the 18.5-h incubation. 4 h before the end of the incubation, BD Golgi Stop (BD Biosciences #554715) was added. After the incubation cells were stained.

Human PBMC isolation and cannabinoid incubation

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood of healthy female volunteers (demographic information outlined in Table 2) via density centrifugation using Lymphoprep (StemCell Technologies #07861). Cells were then incubated with human IL-15 recombinant protein (100 ng/mL) (Peprotech #200-15, Accession#: P40933) for 24 h in the presence or absence of THC or CBD (0.01–10 μg/mL) in complete RPMI media. BD Golgi Stop was added 10 h prior to the end of incubation. After 24-h, cells were stained for viability, NK cell identification markers, and intracellularly for IFN-γ.

Human NK cell isolation and generation of regulatory NK cells

NK cells were isolated from female human whole blood using MACSxpress Whole Blood NK Cell Isolation Kit (Miltenyi Biotec #130-127-695). Following the isolation, NK cells were incubated at 1×10^6 cells/mL in complete RPMI media containing human IL-15 recombinant protein (10 ng/mL), human TGFβ recombinant protein (2 ng/mL, R&D Systems #240-B-002, Accession#: P01137.2) and 5-Aza-2′-deoxycytidine (Aza, 1 μM) (Sigma–Aldrich #A3656), and cultures were maintained for 3 days under hypoxia (1% O_2) as previously described.[45] To generate hypoxia, cells were kept in a plastic chamber (Stem Cell Technologies #27310) filled with a custom gas mixture of 1% O_2, 5% CO_2, and balance N_2 (Air Liquide). After 3 days, cells were counted and resuspended in the same culture conditions of IL-15, TGFβ, Aza and 1% hypoxia, but now with or without the addition of CBD or THC (0.1, 1, 5 μg/mL). After 3 days, cell-free culture supernatants were collected and stored in –80 °C until use. VEGF was quantified via ELISA according to the manufacturer's instructions (R&D Systems #DY293B).

Donor characteristics	Mean (Range) or percent
Age	26 (22–30)
Sex (self-reported)	
Female	100%
Education (highest degree earned)	
High school	20%
Bachelor's degree	80%
Race and ethnicity	
Latinx, Hispanic	20%
Asian, Chinese Canadian	20%
White, European	60%

Table 2: **Demographic information of blood donors.**

Flow cytometry staining

Mouse uNK cells were stained first with eFluor™ 780 fixable viability dye (eBioscience #65-0865-14) according to manufacturer's instructions and then incubated with anti-mouse CD16/CD32 at 4 °C (eBioscience #14-0161-82, RRID: AB_467133). To identify mouse uNK cells, cells were then stained extracellularly with the following anti-mouse monoclonal antibodies: Alexa Fluor 700 CD45.2 (eBioscience #56-0454-82, RRID: AB_657752), FITC CD3e (eBioscience #11-0031-82, RRID: AB_464882), FITC CD19 (eBioscience #11-0193-82, RRID: AB_657666), FITC F4/80 (eBioscience #11-4801-82, RRID: AB_2637191), PECF594 CD11b (BD Biosciences #562287, RRID: AB_11154216), and PerCP-eFluor 710 CD122 (eBioscience #46-1222-82, RRID: AB_11064442) for 30 min at 4 °C in FACs buffer (2% BSA in PBS). For intracellular staining, the cells were then fixed with BD Cytofix/Cytoperm Plus Fixation/Permeabilization Kit (BD Biosciences #554715, RRID: AB_2869009) for 20 min and then stained with anti-mouse monoclonal APC IFN-γ (Biolegend #505810, RRID: AB_315404) for 30 min. Human cells were first stained with eFluor™ 780 fixable viability dye (eBioscience #65-0865-14) according to manufacturer's instructions. To identify NK cells, cells were then stained extracellularly with the following anti-human monoclonal antibodies: PE-Cy7 CD14 (BD Biosciences #557742, RRID: AB_396848), FITC CD3 (Biolegend #300406, RRID: AB_314060), and PE/Dazzle™ 594 CD56 (Biolegend #398810, RRID: AB_2894511) for 30 min at 4 °C in FACs buffer. If no intracellular staining was performed, cells were then fixed for 1 h with 1% paraformaldehyde (PFA). For intracellular staining, the BD Cytofix/Cytoperm Plus Fixation/Permeabilization Kit (BD Biosciences #554715, RRID: AB_2869009) was used for 20 min followed by 30-min incubation with anti-human monoclonal antibody BV421 IFN-γ (BD Bioscience #564791, RRID: AB_2738952). All flow cytometry was performed using the BD LSRFortessa or LSRII cytometers (BD Biosciences) and analyzed using FlowJo Software (FlowJo, LLC, Ashland, OR, RRID: SCR_008520).

Ethics

All work involving mice was approved and conducted in accordance with guidelines provided by the McMaster University Animal Research Ethics Board (21-04-12). The study adhered to the ARRIVE guidelines. All research utilizing human samples was approved by the Hamilton Integrated Research Ethics Board in Hamilton Ontario (13-813T-Ashkar) and all human samples were collected after written informed consent.

Statistics

All statistical analysis was performed using the GraphPad Prism 9 software. To check for normal distribution of the data the Shapiro–Wilk test was performed. Homoscedasticity of the data was determined by the Brown–Forsythe test. Comparisons made between two conditions were analyzed using an unpaired t-test. A one-way ANOVA followed by a Tukey's multiple comparisons test was used for more than two conditions. For data of three-group comparisons not meeting normality, the non-parametric Kruskal–Wallis test with the Dunn's post-hoc test for multiple comparisons was used. Analysis of two independent variables was conducted using a two-way ANOVA with Dunnett correction for multiple comparisons. A chi-square test was used to compare dichotomous outcomes. Significance was defined as $P < 0.05$. For the a priori sample size calculation we chose to use the resource equation approach given this is an exploratory animal study and our outcome measures have not been previously done in offspring exposed to this dose of THC or CBD *in utero* making it not possible to assume effect size and standard deviation from previous work. This method assumes that an acceptable sample size will have a degree of freedom (E) between 10 and 20, whereby E is the total number of mice minus the total number of groups. As we have 3 treatment groups, an adequate sample size for our study would be between 5 and 7.

Role of funders

The funding source of this study had no role in the design, data collection, data analysis, writing of the text, or in the decision to submit the work for publication.

Results

Oral consumption of CBD causes abnormalities in placental and decidual structure

While accumulating evidence suggests that cannabis use during pregnancy is associated with several pregnancy complications it is not clear whether all types of cannabis and all methods of consumption cause harm. A large majority of current animal studies focus on either cannabis smoke inhalation, which contains over 2500 confounding compounds, or injection of only THC which leaves CBD largely unstudied.[9,23,24] Thus, we chose an oral cannabis consumption model to directly compare the effects of CBD and THC on pregnancy without the additional contaminants of cannabis smoke. We administered commercially available cannabis oils via oral gavage from early to mid-gestation as cannabis use typically drops after the first trimester.[7] The concentration of cannabinoids within the commercial products was first confirmed via high-performance liquid chromatography coupled to a mass spectrometer (LC-MS) (Table 1). We also confirmed the gavage technique alone does not induce stress causing fetal resorption (Supplemental Fig. S2a–d).

Early in pregnancy, the maternal endometrium is extensively remodelled to support fetal development and disruption of this highly regulated process can lead to fetal growth restriction.[46] As the mechanism driving cannabis-induced pregnancy complications is largely unknown, we first sought to examine whether consumption of cannabis oil is disrupting implantation site remodelling early in the pregnancy. Pregnant mice received either THC, CBD, or control medium chain triglyceride oil (MCT) oil via gavage from gestation day (GD) 6.5–11.5 and were then euthanized at GD 12.5 which corresponds to when the placenta is fully mature (Fig. 1a).[47] We first assessed placental area in mid-sagittal cross sections from the THC and CBD oil treated mice. While there is a slight trend for lower placental area in the THC oil treated mice, there is a significant reduction in placental area in the mice treated with CBD oil (Fig. 1b and c).

Next, we assessed decidual morphology in the cannabis treated mice. Pregnant mice exposed to CBD oil appeared to have a high density of cells located within their decidua compared to control or THC treated mice (Fig. 1d–f). In early pregnancy, the decidua is largely populated by immune cells, with uNK cells comprising nearly 70% of leukocytes in the first trimester decidua.[32] To decipher whether the dense collection of cells within the decidua of CBD oil treated mice were uNK cells we performed staining with Periodic Acid Schiff's (PAS) reagent and immunofluorescent *Dolichos biflorus* agglutinin (DBA) staining on GD 12.5 implantation sites. The high density of cells within the decidua of CBD treated mice were positive for PAS and DBA identifying them as uNK cells (Fig. 1g–l). Quantification of DBA fluorescence (Area DBA+/Total Area) shows there is a significant elevation in DBA + staining in the deciduae of CBD oil treated mice indicative of increased uNK cell number whereas the THC treated group was closer to control (Fig. 1m).

Interestingly, cytokine analysis of decidua supernatants showed a trend for elevated levels of MIP-1α and IL-15, both of which have been implicated in uNK cell recruitment, in CBD treated mice (Fig. 1n and o).[48,49] Thus, the slightly elevated levels of IL-15 and MIP-1α may possibly explain the elevated number of uNK cells we observe in pregnant mice exposed to CBD oil. Overall, CBD oil seems to substantially disrupt the

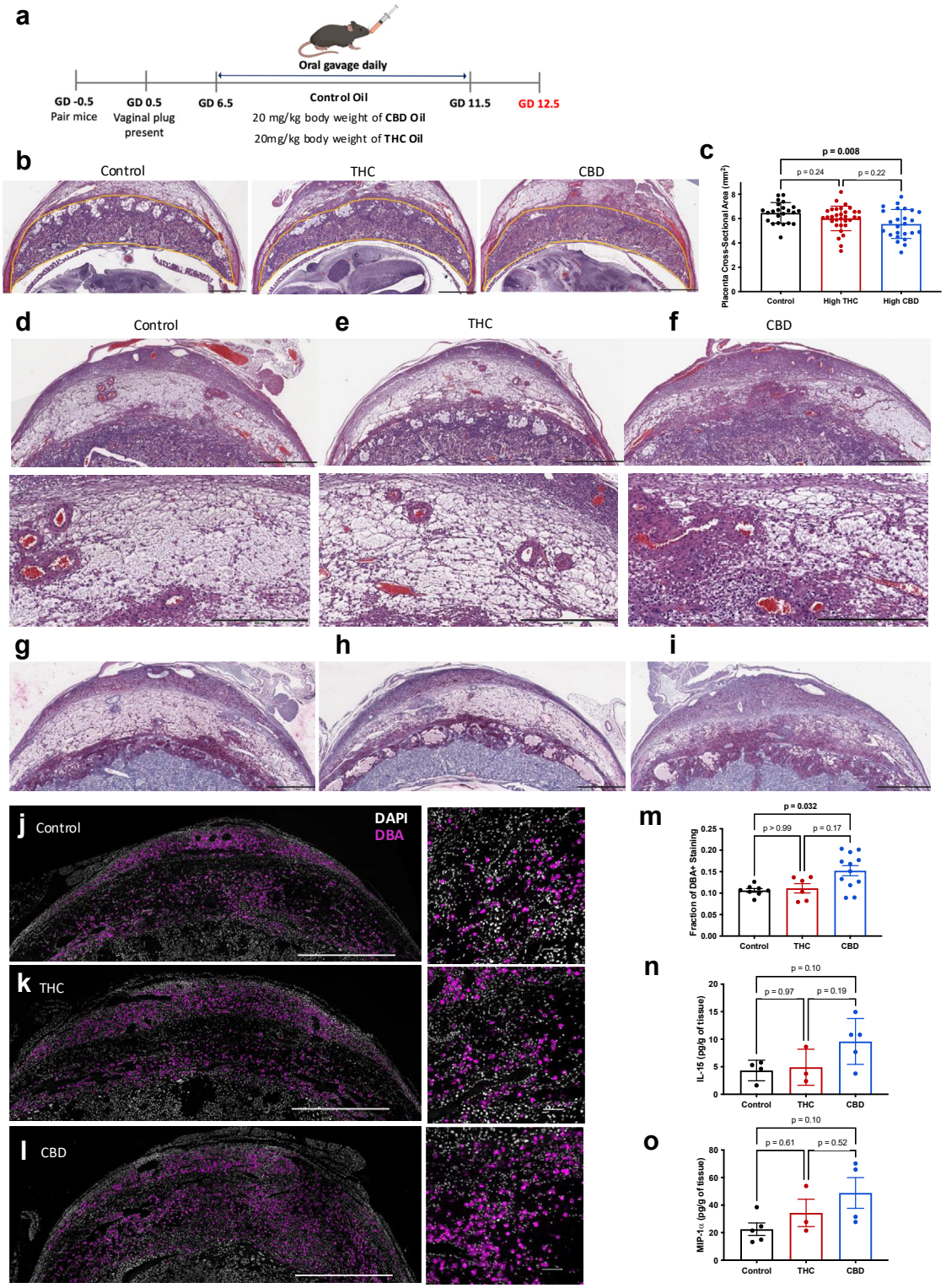

Fig. 1: **Consumption of CBD oil disrupts the placenta and uNK cell population within the decidua**. Implantation sites were collected on GD 12.5 following gavage of CBD, THC, or control oil from GD 6.5–11.5 and then fixed in paraformaldehyde and embedded in paraffin. **a**: Schematic illustrating experimental design. **b**: Representative H&E images of GD 12.5 placenta at 2× magnification with placenta outlined. **c**: Quantification

maternal–fetal interface by affecting placental size and uNK cell number.

Cannabis oil impairs spiral artery remodelling and uNK cell IFN-γ production

As we observed higher levels of uNK cells within the decidua of CBD-treated pregnant mice, we next sought to determine whether uNK cell function is impacted by cannabis oil. uNK cells are a central driver of spiral artery remodelling during pregnancy to create dilated, low resistance vessels that support fetal growth.[50] Both THC oil and CBD oil treated mice had significantly increased vessel to lumen diameter in decidual spiral arteries indicating an abnormally thick vessel wall and reduced vessel remodelling (Fig. 2a and b). The vessels also appeared circular and less elongated in appearance indicative of poor remodelling.

uNK cells promote vascular remodelling via production of angiogenic factors and secretion of matrix metalloproteinases (MMPs).[51] Interferon (IFN)-γ is a key player in uNK cell-mediated vascular remodelling in both mice and humans.[30,52] Interestingly, THC has been shown to reduce the production of IFN-γ from murine NK cells, but no studies have assessed this effect during pregnancy.[34] We next sought to determine whether CBD and THC can disrupt uNK cell production of IFN-γ. Uterine immune cells were isolated from non-treated GD 10.5 implantation sites and then stimulated with IL-15 and IL-12 in the presence or absence of THC or CBD (Fig. 2c). Neither THC nor CBD had an impact on cell viability (Fig. 2d). While THC reduced the expression of IFN-γ in murine uNK cells compared to media only control, CBD seemed to increase it (Fig. 2e–g). Thus, the under-developed vessels in the THC oil treated mice may be a result of THC impairing uNK cell production of IFN-γ.

Oral consumption of THC or CBD leads to impaired fetal development

As we see significant irregularities in implantation site remodelling in mice exposed to THC oil or CBD oil, we next questioned whether these impairments in remodelling would affect fetal development. To do this, pregnant mice received either CBD, THC, or control oil from GD 6.5 to 11.5 via gavage and were then euthanized at GD 18.5 one day prior to term (Fig. 3a). We saw no difference in maternal weight gain throughout the pregnancy or in the number of fetuses that appeared viable at term (Fig. 3b and c). However, there was a trend for a higher number of resorbed fetuses and higher resorption rate in dams receiving CBD oil (Fig. 3d–f).

We next measured fetal weight and crown-rump length, as fetuses exposed to THC oil *in utero* appeared smaller (Fig. 3g). Dams exposed to THC oil had significantly lower fetal weights at GD 18.5 with a 4.9% reduction in mean weight (1.16 ± 0.08 in THC group vs. 1.22 ± 0.07 in controls) which corresponded to a reduced crown-rump length (Fig. 3h and i). While the mice receiving CBD oil did not have significantly lower mean fetal weight compared to controls, there was a high degree of variability in fetal weights (Fig. 3h). Given this, we quantified the percent of fetuses that would fall below the 10th percentile of control weights. Strikingly, exposure to either THC or CBD oil doubled the percent of fetuses that fall below the 10th percentile of control weights (Fig. 3j).

Previous studies also report that maternal cannabis use is associated with abnormal brain development and reduction in fetal head circumference.[17,53] Thus, we next assessed whether oral exposure to CBD or THC oil would alter brain weight and head size at GD 18.5. While there appears to be a trend for decreased fetal brain weight and head length in mice treated with CBD oil, the fetal head length was significantly smaller in mice exposed to THC oil compared to controls (Fig. 3k and l). Altogether, these results highlight that oral consumption of either THC or CBD during early to mid-pregnancy can cause fetal growth impairments.

Exposure to CBD or THC oil *in utero* disrupts early postpartum development

To explore whether the significant fetal growth impairments we see in the THC and CBD exposed dams results in perinatal mortality or pup development problems postpartum, we observed the pups at birth and tracked their development for several weeks (Fig. 4a). Interestingly, while all dams that appeared pregnant at GD 11.5 based on weight gain and physical appearance in the control and CBD-exposed groups had live pups at term, only 2/5 in the THC group had live pups at term (Fig. 4b and c). This suggests either late term fetal resorption, stillbirth, or infanticide by the mother shortly after birth. There were comparable number of pups in each litter between the control, THC, and CBD oil exposed groups as well as number of male and

of placental cross-sectional area (mm^2) of each mid-sagittal section averaged per implantation site (n = 23–32 per group). **d–f**: Representative H&E images of GD 12.5 decidua at 2× and 10× magnification from **d** control, **e** THC and **f** CBD oil treated mice. **g–i**: Representative PAS images of GD 12.5 decidua at 2× magnification from **g** control, **h** THC, and **i** CBD oil treated mice. **j–l**: Representative DBA/DAPI immunofluorescence images from GD 12.5 decidua at 10× and 20× magnification from **j** control, **k** THC and **l** CBD oil treated mice. **m**: Quantification of DBA + area/whole area averaged per dam (n = 6–12 per group). **n:** Amount of IL-15 (pg/g of tissue) in decidua cell-free supernatants averaged per dam (n = 3–5 per group). **o:** Amount of MIP-1α (pg/g of tissue) in decidua cell-free supernatants averaged per dam (n = 3–5 per group). H&E/PAS images: 2× scale bar represents 1 mm and 10× scale bar represents 500 μm. DBA images: 10× scale bar represents 1 mm and 20× represent 100 μm. Data are means ± SEM, *P < 0.05, **P < 0.01 (c, n, o one-way ANOVA, m Kruskal–Wallis test).

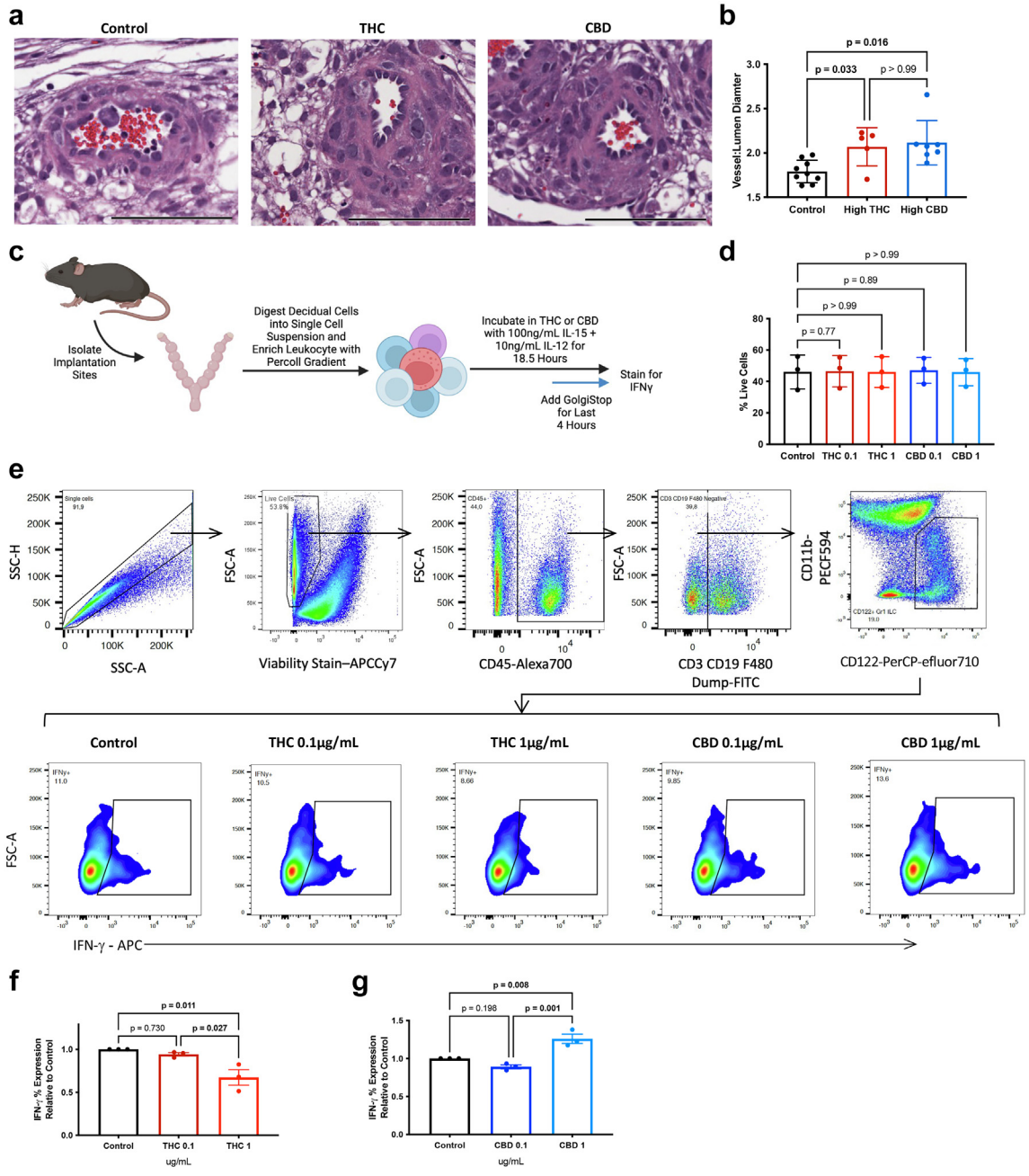

***Fig. 2:* CBD and THC disrupts decidual vascular remodelling and uNK cell angiogenic factor production**. **a and b**: Mice were treated with or without CBD or THC oil from GD 6.5–9.5 and implantation sites isolated, fixed, and embedded. **a**: Representative images of spiral arteries from H&E-stained GD 10.5 implantation sites, scale bar represents 100 µm. **b**: Quantification of vessel to lumen diameter of spiral arteries from GD 10.5 mice averaged per implantation site (n = 5–9 per group). **c–g**: GD 10.5 implantation were isolated and digested into single cell suspension. Cells were then stimulated and incubated with various doses of THC or CBD. **c**: Schematic of experimental design. **d**: Viability of cells after incubation (n = 3 per group). **e**: Representative flow cytometry gating strategy to identify uNK cells and representative flow plots of uNK cell IFN-γ expression. IFN-γ percent expression relative to control following **f** THC or **g** CBD exposure (n = 3 per group). Data are means ± SEM, *P < 0.05, **P < 0.01 (b Kruskal–Wallis test, d, f & g one-way ANOVA).

females (Fig. 4d and e). Interestingly, there was a repetitive trend for lower weight in the pups exposed to THC or CBD throughout the follow-up period with significantly lower weights seen especially in female pups suggesting that the growth restriction we see at term may persist into postnatal life (Fig. 4f–h).

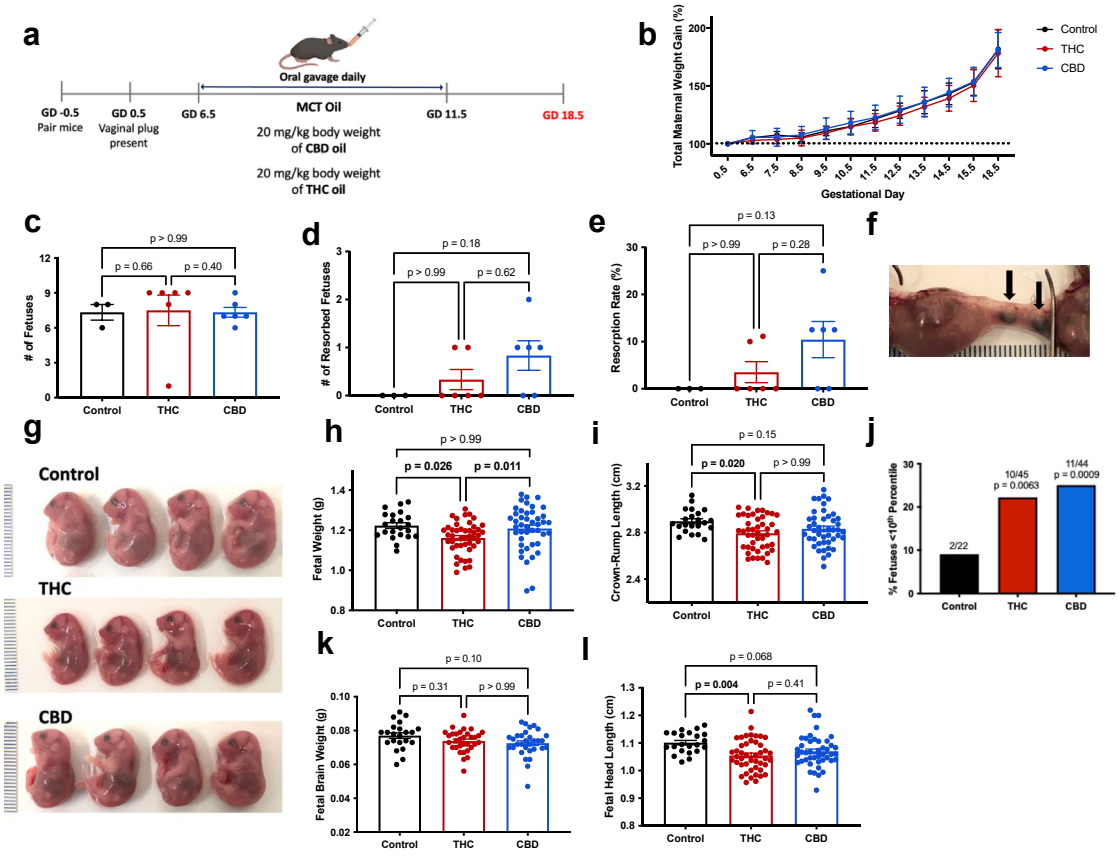

***Fig. 3:* Oral consumption of CBD or THC oil from early to mid-gestation affects fetal development at term**. Mice received gavage of CBD, THC, or control MCT oil from GD 6.5–11.5 and were euthanized on GD 18.5 to assess fetal growth. **a:** Schematic illustrating experimental design. **b:** Maternal weight gain throughout the pregnancy (% of pre-pregnancy weight) (n = 3–6 per group). **c:** Number of fetuses appearing viable (n = 3–6 per group). **d:** Number of resorbed fetuses (n = 3–6 per group). **e:** Resorption rate shown as percent of fetuses resorbed out of total fetuses (n = 3–6 per group). **f:** Image showing two resorbed embryos from a CBD-exposed dam. **g:** Representative image of fetuses. **h:** Fetal weight (n = 22–45 per group). **i:** Crown-rump length (n = 22–45 per group). **j:** Percent of fetuses that fall below the 10th percentile of control weights. **k:** Fetal brain weight (n = 22–32 per group). **l:** Fetal head length (n = 22–45 per group). Data are means ± SEM, *P < 0.05, **P < 0.01 (b two-way ANOVA; c–e, h, i, k Kruskal–Wallis test, l one-way ANOVA; j chi-square test).

THC or CBD exposure *in utero* results in behavioural changes in offspring

Several studies have suggested that prenatal cannabis exposure predisposes a child for cognitive differences later in life such as increased aggression, attention difficulties, and impaired executive functioning.[20–22,54] As we saw growth restriction in the fetuses exposed to CBD or THC oil, we next aimed to determine whether oral cannabis consumption during pregnancy can lead to long-term cognitive impairments after birth. Using the same protocol for cannabis use during pregnancy, we performed a series of behavioural tests on the pups birthed from dams exposed to THC, CBD or control oil using traditional video methods and the automated IntelliCage system (Fig. 5a).

We found that males exposed to THC exhibited heightened exploratory behaviour with elevated number of rearing events in the open field compared to both control and CBD groups (Fig. 5b). However, they exhibited comparable numbers of grooming and defecation events, and similar time spent in the center of the field (Fig. 5c–e). All groups of female mice exhibited comparable rearing, grooming, defecation, and time spent in the center of the field (Fig. 5f–i). During novel object recognition testing, male mice from the CBD group showed very slight trends for less preference of the novel object and appeared to split their time more evenly between the new and old object suggesting possible impaired memory (Supplemental Fig. S3a and b). Again, there was no difference seen with the female mice (Supplemental Fig. S3c and d).

To further assess cognitive function, we utilized the automated IntelliCage system which allows us to perform behavioural phenotyping in a social home cage

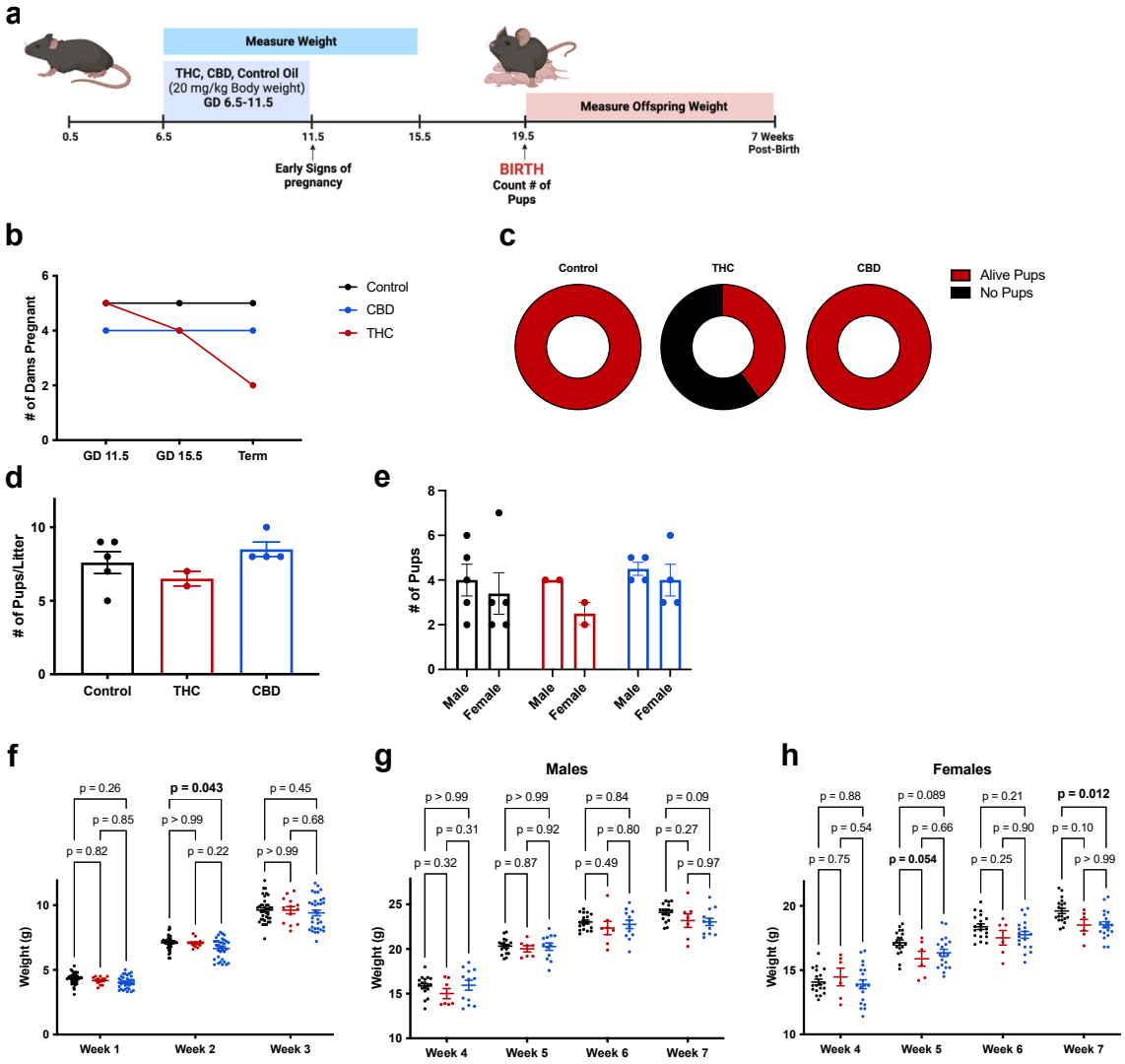

Fig. 4: **Exposure to CBD or THC oil *in utero* disrupts early postpartum development**. Pregnant mice received control, CBD, or THC oil via gavage from GD 6.5 to 11.5 after birth, pups were counted and weighed weekly. **a:** Schematic of experimental design. **b:** Number of dams that appeared pregnant on GD 11.5 and 15.5 that had live births at term (n = 4–5 per group). **c:** Pie chart indicating the number of dams that appeared pregnant at GD 11.5 based on weight gain and physical appearance with live pups at term (n = 2–5 per group). **d:** Number of pups per litter (n = 2–5 per group). **e:** Number of male and female pups per litter (n = 2–5 per group). **f:** weight of pups at week 1, 2, and 3 with female and male combined (n = 13–37 per group). **g and h:** weekly weight of pups after weaning (week 3) of **g** male (n = 7–18 per group) and **h** female pups (n = 6–19 per group). Data are means ± SEM, *P < 0.05, **P < 0.01 (f-h two-way ANOVA).

setting. Unfortunately, male THC-exposed mice could not be observed in the IntelliCage system due to heightened aggression and extensive fighting between the mice. Only male mice from CBD and control cohorts could be assessed and males were placed in two separate IntelliCages divided by treatment group to avoid potential fighting (13 control males and 11 CBD males). No fighting was observed in the female cohorts, which allowed all three female cohorts to be housed together (5 control, 5 CBD and 3 THC females per IntelliCage). Mice were monitored in the IntelliCage for a period of 15 days (Fig. 5j).

Early in the adaptation phase, female THC-exposed mice showed lower levels of general activity, but this phenotype was restored by the end of the adaptation period (Fig. 5k and l). Strikingly, the female THC mice also showed higher error rates on reward corner recognition both in the learning and reversal stages (Fig. 5m–p). This indicates impaired spatial learning and cognitive flexibility in the female THC mice, respectively. In the male mice, the CBD-exposed male mice exhibited lower levels of general activity that remained throughout the adaptation phase but there were no differences in spatial learning or cognitive

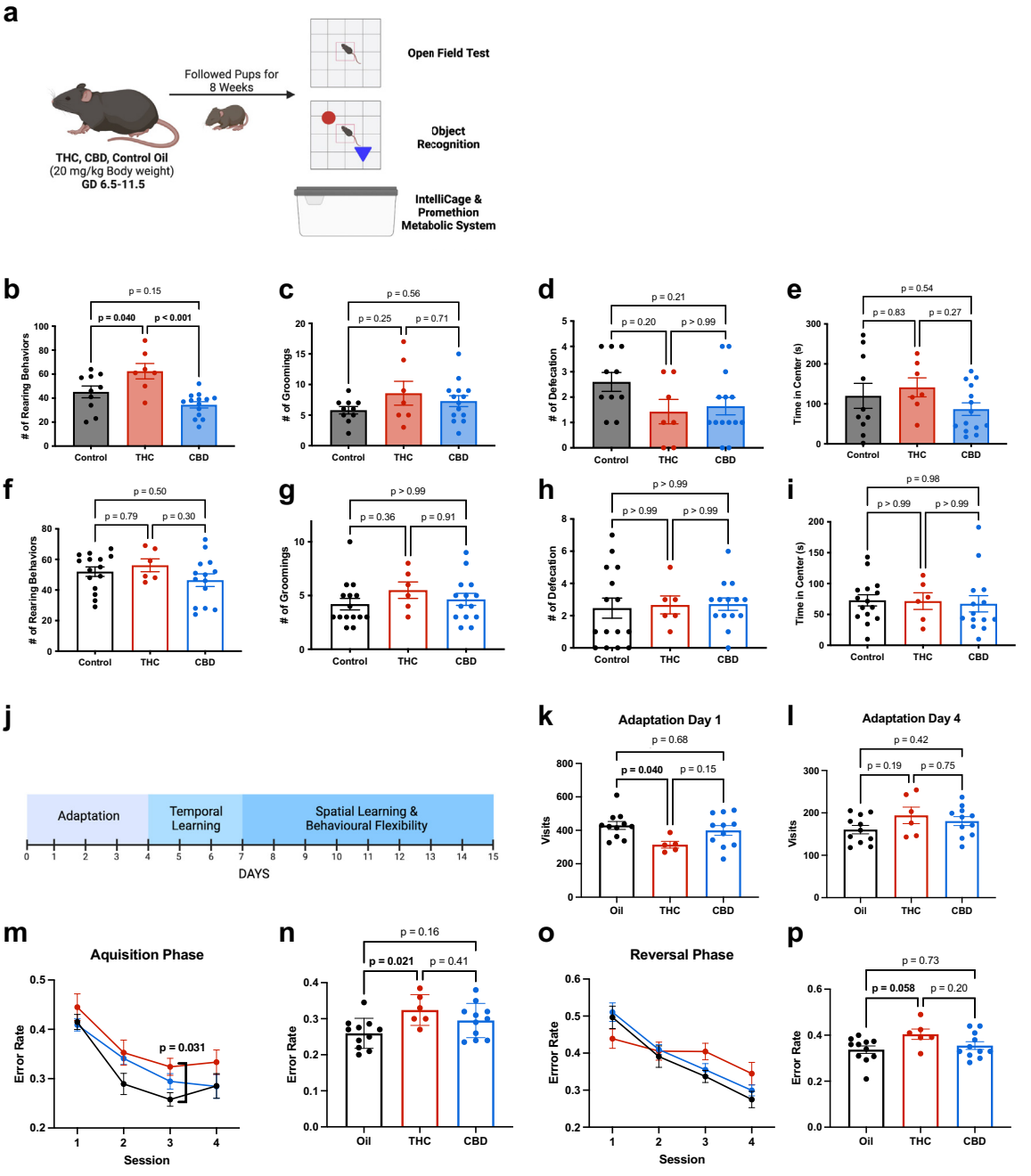

***Fig. 5:* Exposure to cannabis oil *in utero* causes sex-dependent behavioural differences**. Pregnant mice received THC, CBD, or control oil from GD 6.5–11.5. The dams gave birth, and their pups were followed for 8 weeks before beginning behavioural assessment. **a:** schematic illustrating experiment timeline. **b–e:** Open field testing of adult male mice exposed to THC, CBD or control oil *in utero* assessing **b** number of rearing events, **c** number of grooming events, **d** number of defecation events and **e** time spent in center (seconds) over of 10-min assessment period (n = 7–14 per group). **f–i:** Open field testing of adult female mice exposed to THC, CBD or control oil *in utero* assessing **f** number of rearing events, **g** number of grooming events, **h** number of defecation events and **i** time spent in center (seconds) over of 10-min assessment period (n = 6–15 per group). **j–p:** Female mice exposed to THC, CBD or control oil *in utero* were monitored in the IntelliCage system for 15 days (n = 5–11 per group). **j:** Schematic of experimental design. **k:** number of visits during adaptation phase day 1. **l:** number of visits on adaptation phase day 4. **m:** Error rate over the four-day acquisition phase. **n:** Error rate on day 3 of acquisition phase. **o:** Error rate over the four-day reversal phase. **p:** Error rate on day 3 of reversal phase. Data are means ± SEM, *P < 0.05, ***P < 0.001 (b, c, e and f, k and l, n, p one-way ANOVA; d, g–i Kuskal–Wallis test; m, o two-way ANOVA).

flexibility (Supplemental Fig. S4a–d). However, there may be a possible cage effect as male groups had to be separated due to risk of fighting. Altogether, our results indicate sex-dependent behavioural changes following oral exposure to THC or CBD during pregnancy resulting in changes in aggression, spatial learning, and cognitive flexibility.

THC exposure during pregnancy alters activity level and energy expenditure in offspring

In addition to cognitive impairments, early exposure to THC in adolescence has been shown to cause metabolic changes in adulthood.[55] However, no one to our knowledge has explored whether this phenomenon also occurs if the child is exposed to cannabis during pregnancy. To assess if cannabis exposure during pregnancy results in altered energy metabolism later in adulthood we analyzed our *in utero* exposed THC, CBD, and control mice in the Promethion metabolic cage for several days. THC exposed male mice had significantly decreased basal metabolic rate, maximal energy expenditure, oxygen consumption, and carbon dioxide production, all indicative of reduced metabolic activity (Fig. 6a–e). In contrast, the female mice exposed to cannabis during pregnancy showed no differences in their basal metabolic rate, maximal energy expenditure, oxygen consumption, or carbon dioxide production (Fig. 6f–i).

All experimental groups in both male and female mice showed comparable food intake throughout the experiment (Fig. 6j and k). However, the male THC-exposed mice drank less water compared to the control group which corresponded to a reduced rate of water vapor loss (Fig. 6l and n). There were no differences in water consumption or water vapor loss in the female mice (Fig. 6o and p).

As we observed behavioural changes as well as differences in energy expenditure in the cannabis exposed offspring, we next wanted to assess their overall activity level. During open field testing we observed a significant increase in the distance travelled by the male THC-exposed mice over the 10-min assessment period (Fig. 7a). However, when looking at the first 1 h in the Promethion metabolic cage, there is a trend for less distance travelled by the male THC-exposed mice and overall, no change in activity level during the entire metabolic cage experiment (Fig. 7b–e). This suggests that the male THC mice may be slower to adapt to a new environment as they are very active with lots of rearing behaviour during a 10-min period in the open field (Figs. 5b and 7a). Interestingly, the female THC mice had increased levels of movement throughout the entire experiment that seemed to begin within the first hour and persist (Fig. 7f–j).

When looking at their time budget, both THC and CBD exposed male mice seemed to spend less of their time moving and more time at their habitat compared to controls (Fig. 7k and l). This potential decrease in activity may correspond to the decrease in reduced metabolic rate. However, the female THC mice had a time budget comparable to the control and CBD-exposed mice (Fig. 7m and n).

Ultimately, these results show that exposure to THC oil from early-to mid-gestation not only impedes fetal growth but results in long-lasting sex-specific changes in metabolic rate and activity level.

THC and CBD reduce angiogenic factor production in human NK cells

We lastly sought to uncover whether the anti-angiogenic effect of cannabinoids we saw with mouse uNK cells is also observed in human cells. We first isolated peripheral blood mononuclear cells (PBMCs) from healthy human whole blood and stimulated them with IL-15 for 24 h to induce IFN-γ expression and incubated these cells in the presence or absence of THC or CBD (Fig. 8a). THC significantly reduced IFN-γ expression in human peripheral blood NK (pbNK) cells relative to control by more than half without impacting cell viability (Fig. 8b and Supplemental Fig. S5a and b). CBD also reduced IFN-γ expression, but a higher dose was needed to see similar affects as THC (Fig. 8c and Supplemental Fig. S5c).

Since human NK cells from peripheral blood differ significantly from uNK cells in terms of both their phenotype and function we aimed to assess the effects of THC and CBD on a model of human uNK cells. We first generated regulatory NK (NKreg) cells that closely mimic uNK cells using a protocol developed by Cerdeira et al. and then incubated them with THC or CBD (Fig. 8d).[45] We assessed vascular endothelial growth factor (VEGF), which like IFN-γ, is another crucial angiogenic factor that is significantly implicated in human uNK cell-mediated vascular remodelling.[56] Strikingly, we found that THC significantly reduces production of VEGF by human NKreg cells compared to the media only control, without affecting cell viability, and there is a trend for reduction following CBD (Fig. 8e and f and Supplemental Fig. S5d). Overall, these results suggest that the impaired spiral artery remodelling and anti-angiogenic effect of THC and CBD that we see in pregnant mice may also translate to humans.

Discussion

Maternal cannabis use during pregnancy has drastically increased in recent years despite clear evidence that cannabis is associated with serious pregnancy complications.[1–3] Thus far, research has focused on the effects of cannabis smoke exposure and the use of psychoactive THC demonstrating a clear association with fetal growth restriction, preterm birth, and neurobehavioural differences in the offspring.[2,17–19] However, limited studies have explored the safety of oral cannabis

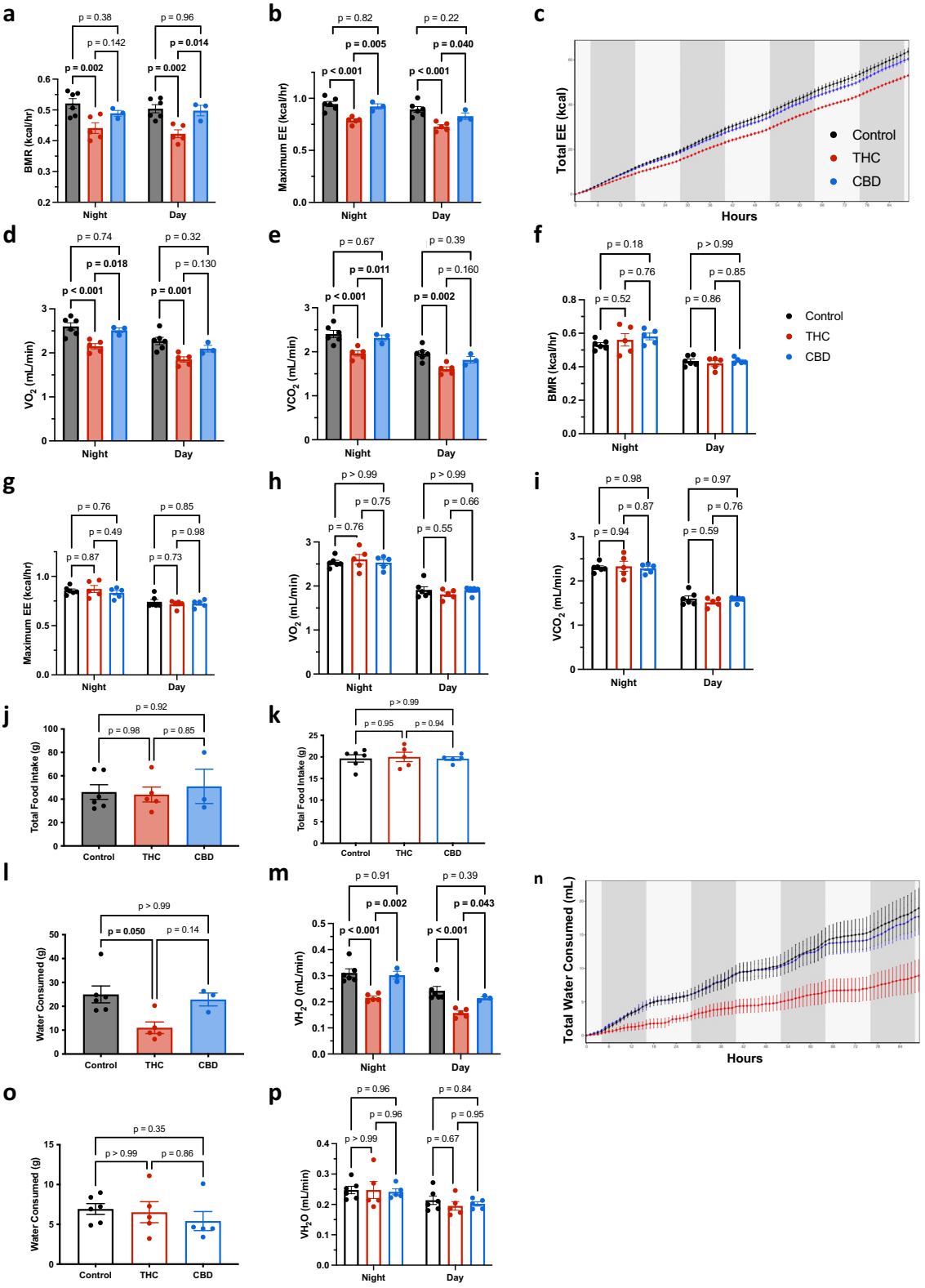

***Fig. 6: In utero* cannabis exposure results in long-term metabolic adaptations in offspring**. Mice were monitored in the Promethion metabolic cage for 5 days. **a–e:** Male mice. **a:** Average basal metabolic rate during the night and day cycle. **b:** Average maximum energy

and CBD products in pregnancy. Since CBD and other oral forms of cannabis are becoming increasingly popular due to the perception that these products are safer to use in pregnancy there is an urgent need to understand their effects on pregnancy outcomes.[14–16] Here, using a murine model, we show that oral consumption of either THC or CBD at the dose of 20 mg/kg bodyweight leads to negative consequences both during pregnancy and later in the offspring's life. Overall, we find that oral CBD and THC use disrupts early pregnancy tissue remodelling, impairs fetal growth, and leads to altered activity and metabolic rate in the offspring after birth.

While several studies have uncovered that cannabis disrupts fetal growth, the mechanism driving this is largely unknown. Both THC and CBD can cross the placenta and enter fetal circulation hinting at a possible direct effect of cannabinoids on fetal development.[25,26] Additionally, past work has demonstrated that THC and CBD disrupt placental development, and our work furthers this by demonstrating that oral consumption of CBD oil reduces placental area.[23,27] However, no research to our knowledge has explored how cannabis affects the maternal side of the implantation site. Early in pregnancy, extensive remodelling of the maternal endometrium is required to create an environment suitable for fetal growth and disruption of this process has been shown to impair fetal development.[46] uNK cell-mediated remodelling of maternal spiral arteries is crucial during this period to allow sufficient circulation to the placenta.[30] Abnormal NK cell function and number has been linked to pregnancy complications so we sought to explore how THC and CBD impact uNK cell number and vessel-remodelling ability.[57,58] In the present study, CBD exposure leads to an increase in the number of uNK cells in the decidua at GD 12.5 but THC does not. Increased levels of uNK cells in the decidua has been associated with pregnancy complications like preeclampsia in past work.[59] Furthermore, we also demonstrate that THC and CBD can disrupt both mouse uNK cell and human NK cell production of the angiogenic factors IFN-γ and VEGF in vitro. This impairment of NK cell angiogenic ability may potentially explain the poor spiral artery remodelling seen in both the CBD and THC exposed dams. Ultimately, we hypothesize that these underdeveloped vessels cannot adequately support the extensive growth demands of late-stage pregnancy and thereby contribute to the fetal growth restriction occurring following both THC and CBD exposure.

Fetal growth restriction is a common complication observed in animal models of *in utero* cannabis exposure and in human studies evaluating cannabis use during pregnancy.[2,17,24] However, research to date has focused primarily on smoke exposure or exposure to only the psychoactive component of cannabis THC. Accumulating evidence illuminates the clear distinction pregnant individuals make between the safety of cannabis smoke and edible products, as well as THC and CBD.[14,16] Here, we show the detrimental consequences of oral THC and CBD exposure on murine fetal development in the absence of the numerous harmful by-products of smoke. Oral THC exposure from early-to mid-gestation leads to a significant reduction in fetal weight and both CBD and THC increase the number of fetuses falling below the 10th percentile of control weights. This growth restriction may predispose the fetus to long-term adverse health outcomes such as chronic metabolic and cardiovascular disease as well as neurobehavioural impairment.[60–62]

A major concern regarding the use of cannabis during pregnancy is its association with cognitive impairments as several reports demonstrate prenatal cannabis exposure results in long-term behavioural consequences in the child after birth.[20,21] Recurring findings demonstrate that cannabis leads to increased aggression, hyperactivity, and inattention.[21] In the present study, we demonstrate that even oral consumption of CBD and THC leads to significant sex-dependent changes in behaviour. Using both traditional methods and new automated cage systems, we find that oral consumption of THC results in increased aggression and exploratory behaviour in male mice, poor spatial learning in female mice, and changes in activity level in both sexes. Meanwhile, oral CBD exposure does not affect the assessed behaviour to the extent of THC but does still result in changes in activity specifically in male mice. Our findings align with previous work that has shown THC exposure increases exploratory and motor activity in the open field particularly in male mice.[63] In past publications, male offspring tend to exhibit more pronounced changes in cognition following prenatal THC exposure than females but unfortunately due to fighting between THC-exposed male pups we were

expenditure during the night and day cycle. **c:** Total energy expenditure over the entire experiment. **d:** Average rate of oxygen consumption during the night and day cycle. **e:** Average rate of carbon dioxide emission over the night and day cycle. **f–i:** Female mice. **f:** Average basal metabolic rate during the night and day cycle. **g:** Average maximum energy expenditure during the night and day cycle. **h:** Average rate of oxygen consumption during the night and day cycle. **i:** Average rate of carbon dioxide emission over the night and day cycle. Total food intake over experiment in **j** male and **k** female mice. **l:** Total water consumed over experiment by male mice. **m:** Average water vapor lost over night and dark cycle in male mice. **n:** Water consumed over experiment by male mice. **o:** Total water consumed over experiment by female mice. **p:** Average water vapor lost over night and day cycle by female mice. Data are means ± SEM. a–e, j, l–n: n = 3–6 per condition; f–i,k,o,p: n = 5–6 per group, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (a and b, d–i, m, p two-way ANOVA, j and k one-way ANOVA, l, o Kruskal–Willis test).

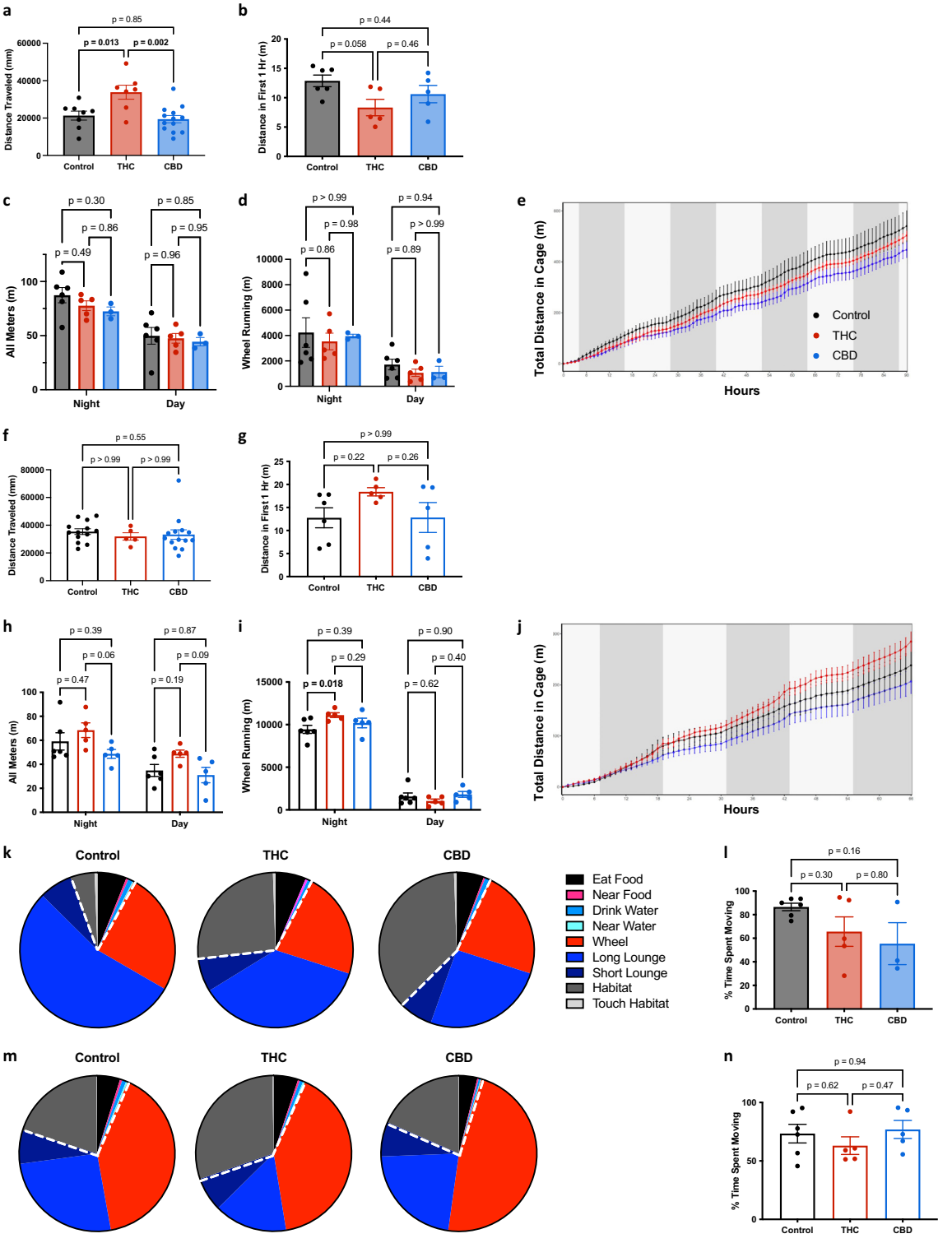

***Fig. 7*: THC exposure *in utero* results in sex-dependent changes in offspring activity level.** **a–e:** Male mice exposed to THC, CBD, or control oil *in utero*. **a:** Total distance travelled by male mice during the 10-min assessment period in the open field (n = 7–13 per group). **b:** Distance travelled by male mice in first 1 h in the Promethion metabolic cage (n = 5–6 per group). **c:** All meters travelled not including meters run on the wheel during the night and day cycle (n = 3–6 per group). **d:** Average meters travelled on the wheel during the night and day cycle (n = 3–6 per

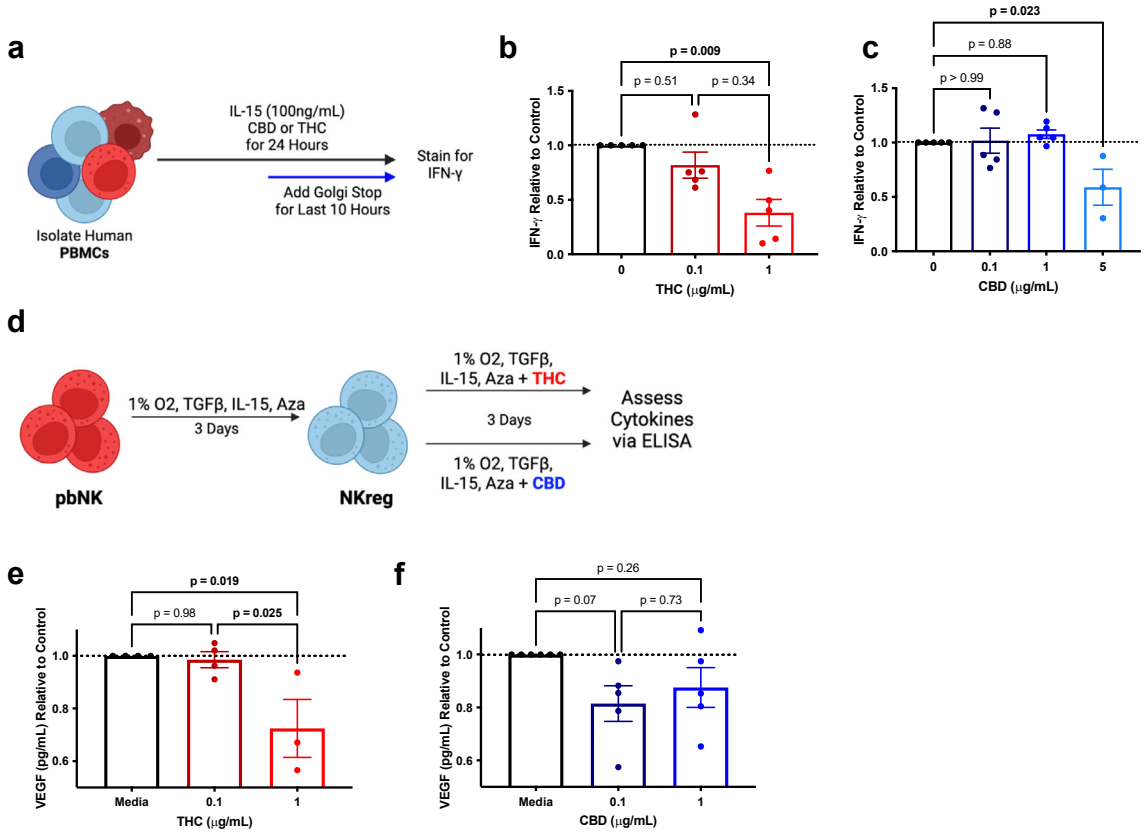

Fig. 8: **Cannabinoids decrease IFN-γ expression in human NK cells**. **a–c:** PBMCs were isolated from healthy donor blood and incubated in the presence of absence of THC or CBD for 24 h and then stained for IFN-γ. **a:** Schematic illustrating experimental design. **b:** IFN-γ expression in NK cells relative to control following incubation in THC (n = 5 per group). **c:** IFN-γ expression in NK cells relative to control following incubation in CBD (n = 3–5 per group). **d–f:** Isolated pbNK cells were converted to NKreg cells and then incubated with THC or CBD. **d:** Schematic illustrating experimental design. **e:** VEGF amount relative to control following THC incubation (n = 3–4 per group). **f:** VEGF amount relative to control following CBD incubation (n = 5 per group). Data are means ± SEM, *P < 0.05, **P < 0.01 (b Kruskal–Wallis test, c, e and f one-way ANOVA).

unable to examine their behaviour in the IntelliCage system.[64,65] However, we see significant impairments in spatial learning and behavioural flexibility in the female pups exposed to THC. Many studies do not stratify cannabis behaviour outcomes by sex despite evidence that THC and CBD impact brain structure differently in males and females.[66,67] Thus, our work supports previous studies outlining the impact of cannabis on offspring behaviour but also adds the additional context that it may impact male and female offspring differently.

Lastly, an emerging area of research explores how cannabis exposure may lead to altered energy metabolism and adipose organ function. One study examining adolescent THC exposure in young male mice found that THC altered energy expenditure both during adolescence and into adulthood.[55] Additionally, prenatal cannabis exposure has been associated with increased fat mass and fasting glucose in childhood.[68] In the present study, we show that oral exposure to THC *in utero* results in significant sex-dependent changes in energy expenditure with male mice exhibiting reduced metabolic activity and females remaining unchanged. The reduction in metabolism in male mice exposed to THC *in utero* may predispose the offspring for weight gain or development of metabolic syndrome.[69]

group). **e:** Total distance travelled in the metabolic cage, not including wheel running, over the entire experiment. **f–j:** Female mice exposed to THC, CBD, or control oil *in utero*. **f:** Distance travelled by female mice during the 10-min period in the open field (n = 5–14 per group). **g:** Distance travelled by female mice in first 1 h in the metabolic cage (n = 3–6 per group). **h:** All meters travelled not including meters run on the wheel during the night and day cycle (n = 5–6 per group). **i:** Average meters travelled on the wheel during the night and day cycle (n = 5–6 per group). **j:** Total distance travelled in the metabolic cage, not including wheel running, over the entire experiment (n = 5–6 per group). **k:** Time distribution spent doing various activities by male mice (n = 3–6 per group). **l:** Percent of time male mice spent moving (n = 3–6 per group). **m:** Time distribution spent doing various activities by female mice (n = 5–6 per group). **n:** Percent of time female mice spent moving (n = 5–6 per group). Data are means ± SEM, *P < 0.05, **P < 0.01 (a and b, g, l, n one-way ANOVA; c and d, h and i two-way ANOVA; f Kruskal–Wallis test).

Typically, animal models of maternal cannabis use administer cannabis throughout the entirety of pregnancy until term.[24] However, maternal cannabis use is typically highest in the first trimester and drops substantially afterwards.[7] Our study administered CBD and THC oil from early to mid-gestation in mice and demonstrated altered fetal growth and offspring behaviour, therefore, suggesting that even without late-pregnancy exposure, CBD and THC may have detrimental consequences. Thus, future work is needed to explore how cannabis use during different timepoints in pregnancy could affect fetal outcomes.

Ultimately, despite oral cannabis consumption and CBD being perceived as safer, our work demonstrates that oral consumption of THC and CBD during early-to mid-gestation in mice results in significant pregnancy complications and behavioural consequences in the offspring. Our data contributes to the growing paradigm that cannabis exposure during pregnancy results in detrimental effects to the developing fetus and suggests that oral consumption cannot be considered safe.

Limitations

This study aimed to decipher whether CBD and THC can impact maternal–fetal interface remodelling, fetal growth, and offspring behaviour when consumed orally. We administered cannabis oil via gavage to model cannabis edibles to ensure mice were given the same concentration of CBD and THC in each group. However, we recognize that cannabis oil is not typically consumed by pregnant individuals like cannabis edibles and has a different bioavailability than edibles which may impact translatability. Additionally, we used a high dose of CBD and THC that has been used previously in clinical studies to first clarify if there is any effect of oral cannabis on pregnancy. Future work is needed to understand whether a similar phenotype is observed at lower doses more frequently used by pregnant individuals. This study also used human pbNK cells to model uNK cells to study the effect of THC and CBD on angiogenic factor production. Given the inherent differences between these NK cell populations, additional work is needed to explore the effect of cannabinoids on human uNK cells from diverse pregnant individuals. Ultimately, this work highlights negative consequences of oral CBD and THC oil exposure on fetal growth and child development in mice but future work is necessary to determine whether these consequences translate to humans.

Contributors

TMR and AAA conceived the project, designed the experiments, and accessed and verified the underlying data. TMR, EF, FV, SE, and ED performed experiments. ALP, SMP, LC, and SME contributed to performing experiments. TMR and SE formally analyzed the data. CB, DMS, and DMEB provided intellectual and experimental input. TMR and AAA wrote the manuscript. EF, SE, and ALP edited the manuscript. AAA secured funding. AAA supervised the project. All authors read and approved the final manuscript.

Data sharing statement

All data is available in the main text or supplementary materials. Raw data will be shared for research purposes upon request to the corresponding author after publication via email (ashkara@mcmaster.ca) with no restriction. In addition to raw data, any request for information that is not in the materials and methods section can also be requested via email to the corresponding author.

Declaration of interests

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105572.

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