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Maternal exposure to Δ9-tetrahydrocannabinol impairs female offspring glucose homeostasis and endocrine pancreatic development in the rat

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ABSTRACT

Recent reports indicate that 7% of pregnant mothers in North America use *cannabis*. This is concerning given that *in utero* exposure to Δ9-tetrahydrocannabinol (Δ9-THC), the main psychoactive component in *cannabis*, causes fetal growth restriction and may alter replication and survival of pancreatic β-cells in the offspring. Accordingly, we hypothesized that maternal exposure to Δ9-THC during pregnancy would impair postnatal glucometabolic health of offspring. To test this hypothesis, pregnant Wistar rats were treated with daily intraperitoneal injections of either 3 mg/kg Δ9-THC or vehicle from gestational day 6 to birth. Offspring were subsequently challenged with glucose and insulin at 5 months of age to assess glucose tolerance and peripheral muscle insulin sensitivity. Female offspring exposed to Δ9-THC *in utero* were glucose intolerant, associated with blunted insulin response in muscle and increased serum insulin concentration 15 min after glucose challenge. Additionally, pancreata from male and female offspring were harvested at postnatal day 21 and 5 months of age for assessment of endocrine pancreas morphometry by immunostaining. This analysis revealed that gestational exposure to Δ9-THC reduced the density of islets in female, but not male, offspring at postnatal day 21 and 5 months, culminating in reduced β-cell mass at 5 months. These results demonstrate that fetal exposure to Δ9-THC causes female-specific impairments in glucose homeostasis, raising concern regarding the metabolic health of offspring, particularly females, exposed to *cannabis in utero*.

1. Introduction

Recent reports indicate that 7% of pregnant mothers in North America, and 19 % of those aged 18–24, use *cannabis* [1]. Much of this use may be motivated by the belief that *cannabis* eases nausea, vomiting, and lost appetite during pregnancy [2], in conjunction with media portrayals of *cannabis* as a natural alternative to pharmaceuticals [3,4]. A recent integrative review also concluded that women who used *cannabis* during pregnancy did so given the perception that there were no significant risks to the mother or fetus [5]. However, little is known regarding the effects of *cannabis* and its constituent compounds on postnatal outcomes.

We and others have recently demonstrated in rodents that Δ9-tetrahydrocannabinol (Δ9-THC), the main psychoactive component of *cannabis,* leads to fetal growth restriction, in part, due to placental insufficiency [6,7]. This is pertinent because since 1995, selective breeding has increased Δ9-THC content in *cannabis* from 4% to 12 % [8]. Given the strong association between impaired fetal growth and the development of type 2 diabetes [9], this raises concern regarding the metabolic health of offspring exposed to Δ9-THC *in utero*. In addition, Δ9-THC may directly influence development of the fetal pancreas, through its interaction with the cannabinoid 1 receptor (CB1R)

Abbreviations: Δ9-THC, Δ9-tetrahydrocannabinol; CB1R, Cannabinoid 1 receptor; GD, gestational day; PND, postnatal day; IP-GTT, intraperitoneal glucose tolerance test

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CB1R is expressed by both insulin-producing β-cells and glucagon-producing α-cells [13], and contributes to islet formation and organization during fetal development [10]. Moreover, CB1R activation suppresses mitogenic signaling in β-cells, while also promoting apoptosis [11,12]. In a healthy fetus, these effects of CB1R are modulated by temporally-controlled endogenous endocannabinoid levels in fetal tissue and circulation [10,14,15]. However, studies indicate that Δ9-THC readily traverses the placental barrier and 10–28 % of maternal concentrations are detected in the fetal plasma, with 2 − 5X higher concentrations in fetal tissues [16–18]. This would allow maternal Δ9-THC to potentially dysregulate physiologic control of CB1R and its downstream processes within the developing fetal pancreas. Given that the long-term effects of Δ9-THC on metabolic organs outside of the brain remain elusive, we investigated the impact of *in utero* exposure to Δ9-THC on the development of the endocrine pancreas, including its impact on whole-body glucose metabolism in the offspring.

2. Research design and methods

2.1. Animals

All procedures were performed in accordance with the guidelines of the Canadian Council of Animal Care. Female Wistar rats were purchased from Charles River (La Salle) and throughout the experimental procedure were maintained at 22 °C on a 12:12-h light-dark cycle with access to food and water *ad libitum*. Dams were randomly assigned to receive daily intraperitoneal (*i.p.*) injections of either 3 mg/kg Δ9-THC (Sigma-Aldrich, Saint Louis) or vehicle (18:1 saline:cremophor) from gestational day (GD) 6 to GD22. This dosage of Δ9-THC *i.p.* yields maternal blood concentrations of Δ9-THC (8.6–12.4 ng/mL) comparable to those seen after moderate recreational *cannabis* smoking in human adults (13 − 63 ng/mL), as well as in aborted fetal tissue (4 − 287 ng/mL) after maternal *cannabis* use [19–21]. Administration of Δ9-THC was delayed until GD6 since administration of the drug earlier in pregnancy can induce spontaneous abortions [22]. We previously demonstrated that 3 mg/kg Δ9-THC given from GD6 to GD22 does not cause fetal demise, alterations in litter size, gestational length, or maternal weight gain [7]. Dams were allowed to deliver normally, and at birth (postnatal day 1; PND1), pups were weighed, and litters randomly culled to 8 offspring. Measurements at PND1 represent mixed males and females. Offspring were killed at either PND21 or 5 months of age (5 m) by *i.p.* pentobarbital overdose (100 mg/kg). 5 m was chosen as the age for sacrifice, as this is beyond the point (130 days-of-age) when rats have been demonstrated to exhibit sexual dimorphism in glucose intolerance and β-cell mass [23]. All offspring were fasted for ~14 h prior to sacrifice, then weighed, the pancreas dissected, fixed in 10 % formalin, and embedded in paraffin. Prior to sacrifice at 5 m, glycaemia was measured with a OneTouch Ultra2 hand-held glucometer (LifeScan, Zug, Switzerland) from blood obtained by tail vein snip. Blood was also drawn by cardiac puncture for quantification of serum insulin by ELISA (ALPCO, Salem, NH, USA), as well as serum estrogen (Biovision Milpitas, CA, USA), and free testosterone (R&D Systems, Minneapolis, MN, USA). Fasting glucose (FG) and insulin (FI) levels were used to calculate HOMA1-IR by the formula HOMA1-IR = FG x FI/22.5 as a measure of peripheral insulin resistance [24].

2.2. Intraperitoneal glucose tolerance test (IP-GTT)

Glucose homeostasis was investigated at 5 m using an intraperitoneal glucose tolerance test (IP-GTT). 5–6 offspring per sex per treatment were fasted overnight (~14 h) then allowed to acclimate in a dark, quiet room for 2 h. Baseline blood glucose levels were obtained as above, and 2 g/kg glucose administered by *i.p.* injection. Glucose levels were taken 5, 15, 30, 60, 90, and 120 min post injection. Total glucose response to the challenge was calculated using the trapezoidal area under the curve (AUC) for each animal. Blood was collected from the tail vein at 15 and 30 min after glucose bolus for quantification of serum insulin by ELISA (Crystal Chem, Elk Grove Village, IL, USA).

2.3. Intraportal insulin challenge

To evaluate insulin sensitivity in muscle, phosphorylation of Akt was assessed in 11 female animals (6 vehicle and 5 Δ9-THC), each from a distinct dam, aged 5 m [25]. Rats were anaesthetized with isoflurane, the abdominal wall dissected, and the portal vein exposed and ligated distally. Saline was infused over 10 s and a portion of soleus muscle harvested. Insulin, 2 U/kg Humulin (Lilly, Indianapolis, IN, USA), was then infused over 10 s and a portion of soleus muscle was further harvested after 1 min. Tissue samples were snap frozen in liquid nitrogen and kept at -80 °C.

2.4. Protein extraction and western blot

Proteins were extracted from the muscle of rats subjected to intraportal insulin challenge as described previously [23]. Proteins (50 μg) were separated on an 8% SDS-PAGE, transferred to nitro-cellulose membranes, blocked for 60 min at room temperature in Tris-buffered saline containing 0.05 % Tween 20 (TTBS) and 5% non-fat dry milk or 5% BSA, then probed overnight at 48 °C with 1:1000 anti-phospho Akt (Ser 473) [pAkt (Ser 473)] mouse antibody or 1:1 000 anti-Akt2 rabbit antibody (Cell Signalling Technology, New England Biolabs, Beverly, MA, USA). Proteins were detected by enhanced chemiluminescence (Pierce, IL, USA) with horseradish peroxidase-labelled secondary antibodies (Sigma, St. Louis, MO, USA). The optical density of the bands was quantified with a Bio Imaging Gel System (Chemi Genius II, Syngene) with Gene Tools software. Each membrane was probed with an antibody against pAkt [Ser 473], then incubated in stripping buffer (Pierce, IL, USA) and re-probed with an antibody against Akt2 to normalize the results. Results are expressed as a ratio of these optical density values.

2.5. Immunohistochemistry and endocrine pancreas morphometry

Sections of 5 μm were cut from paraffin-embedded pancreata of offspring harvested at PND21 and 5 m and mounted on SuperFrost Plus glass slides (Fischer Scientific, Toronto). Two or three sections per animal, separated by at least 50 μm, were deparaffinized in xylene, rehydrated in descending ethanol series (100 %, 90 %, 70 %), then washed in tap water prior to application of rabbit anti-glucagon IgG (Novus Biologicals, Centennial, CO, USA) and mouse anti-insulin IgG (Sigma-Aldrich, Saint Louis, MO, USA) primary antibodies. ImmPRESS Duet Double Staining Kit (Vector Laboratories, Burlingame) was used to detect insulin- and glucagon-positive cells.

Analysis of pancreatic sections was performed as described previously [23]. Briefly, a composite image of the whole section was obtained using Microsoft Image Composite Editor and microphotographs taken with a 2.5x objective lens. Under 200x magnification, all individual islets were microphotographed and FIJI (http://fiji.sc) used to quantify the area occupied by β- and α-cells. β- and α-cell fractional area were calculated as the total insulin- and glucagon-positive area, respectively, divided by total pancreatic tissue area. The density of islets was calculated by counting the total number of islets present on each slide and dividing by total pancreatic tissue area. Mean islet size and mean β-cell area per islet were calculated by dividing total islet area (insulin- and glucagon-positive) or total β-cell area, respectively, by the total number of islets present on each slide. β- and α-cell mass were calculated as the product of pancreas weight and β- and α-cell fractional area, respectively. The density of extra-islet clusters (less than 500 μm^2 or ~5 endocrine cells) was also assessed as a marker of endocrine cell neogenesis [26,27]. All calculations were averaged across 2–3 slides per animal.

To assess replication in β- and α-cells, 2–3 slides per animal, separated by at least 50 μm, were incubated with mouse anti-Ki67 (Sigma-Aldrich, Saint Louis, MO, USA), guinea pig anti-insulin (Abcam, Toronto, ON, Canada), and rabbit anti-glucagon (Novus Biologicals, Centennial, CO, USA) primary antibodies. Donkey anti-mouse 488, anti-guinea pig 555, and anti-rabbit 647 fluorescent secondary antibodies (Thermofisher, Toronto, ON, Canada) were then applied, and DAPI (Thermofisher, Toronto, ON, Canada) used to counterstain nuclei. Apoptosis in β- and α-cells was examined similarly using ApopTag Fluorescein In Situ Apoptosis Detection Kit (Sigma-Aldrich, Saint Louis). At least 20 randomly-selected islets per section were micro-photographed using a Nikon Eclipse Ts2R Epifluorescence Microscope. Manual tracing was used to outline insulin- and glucagon-positive areas, and particle analysis and water shedding filters applied to find and count $DAPI^+$ nuclei within these regions [28]. $Ki67^+$ or $TUNEL^+$ nuclei were counted manually within each region and used to determine the percentage of $Ki67^+$ or $TUNEL^+$ β- and α-cells. An average of 1656 β-cells and 1201 α-cells were counted for every animal.

2.6. Statistical analysis

A sample size of 5–7 animals per sex per age group per treatment was chosen based on achieving a statistically significant difference with an expected standard deviation of 15 % or less, based on previous studies [27]. Values depicted are mean ± SEM and considered significant if $p < 0.05$. Analysis of the data was performed using Excel v16.16 (Microsoft) and GraphPad Prism v7 (GraphPad Software, Inc). Unless indicated otherwise, statistical analysis was by two-way ANOVA followed by Holm-Sidak-corrected multiple comparisons between Δ9-THC-exposed offspring and their sex-matched controls [23].

3. Results

3.1. Fetal growth restriction and postnatal catch-up growth

Offspring bodyweight and pancreatic weight were measured at birth, at weaning on PND21, and in young adulthood at 5 m. Gestational exposure to Δ9-THC significantly reduced both birthweights (Fig. 1A; 6.62 ± 0.11 g for Δ9-THC and 7.02 ± 0.11 g for vehicle, $p = 0.0135$) and pancreatic weights (Fig. 1 D; 12.18 ± 1.54 mg for Δ9-THC vs 17.33 ± 1.62 mg for vehicle, $p = 0.029$) in male and female offspring. By PND21, Δ9-THC-exposed offspring recovered in terms of both bodyweight and pancreatic weight (Fig. 1 B, E), and thereafter maintained comparable weights to that of their sex-matched controls (Fig. 1 C, F). Male did not differ from female offspring in bodyweight or pancreatic weight at PND21, therefore results are pooled.

Table 1
Fasting blood glucose, insulin, and sex steroid levels at 5 m in male and female offspring. T, $p < 0.05$ for effect of gestational Δ9-THC exposure; S, $p < 0.05$ for effect of sex by two-way ANOVA. Values are mean ± SEM.

	Female		Male		Two-way ANOVA
	Vehicle	Δ9-THC	Vehicle	Δ9-THC	
Fasting Glucose (mmol/L)	6.038 ± 0.2419	5.49 ± 0.2157	6.064 ± 0.155	5.714 ± 0.1079	T
n =	13	10	14	7	
Fasting Insulin (ng/mL)	1.335 ± 0.2164	1.473 ± 0.2115	2.208 ± 0.4498	2.253 ± 0.5146	S
n =	8	10	12	6	
Fasting Estrogen (pg/mL)	57.34 ± 5	57.75 ± 4	N/A	N/A	N/A
n =	11	10			
Fasting Testosterone (ng/mL)	3.4 ± 5	3.1 ± 0.7	N/A	N/A	N/A
n =	9	7			

3.2. Impaired glucose homeostasis

To determine the impact of *in utero* Δ9-THC exposure on glucose metabolism in postnatal life, blood glucose levels were examined after ~14 h of fasting in adulthood, and offspring underwent an IP-GTT. Exposure to Δ9-THC was associated with a decrease in fasting blood glucose at 5 m ($p = 0.0393$ for effect of Δ9-THC by two-way ANOVA), though differences did not achieve statistical significance for either males or females when evaluated independently (Table1). Fasting insulin levels, however, were not affected by gestational exposure to Δ9-THC (Table 1), and HOMA1-IR scores were also not different between vehicle and Δ9-THC-exposed offspring in males or females (data not shown). Following glucose challenge, female offspring exposed to Δ9-THC exhibited elevated blood glucose at 5 min (18.37 ± 1.74 mmol/L for Δ9-THC vs 11.48 ± 0.84 mmol/L for vehicle, adjusted p value = 0.0496), as well as an overall increased area under the curve for blood glucose (Fig. 2A). This was associated with significantly augmented serum insulin concentration compared to vehicle offspring 15 min after glucose challenge (0.47 ± 0.05 ng/mL for Δ9-THC vs 0.29 ± 0.07 for vehicle, adjusted p value = 0.0264), but which subsided to normal levels by 30 min (Fig. 2 B). No similar alterations were present in males (Fig. 2 C, D).

Given the alterations in glucose tolerance and serum insulin concentration in Δ9-THC-exposed female offspring, peripheral insulin sensitivity in the soleus muscle was examined after insulin challenge in

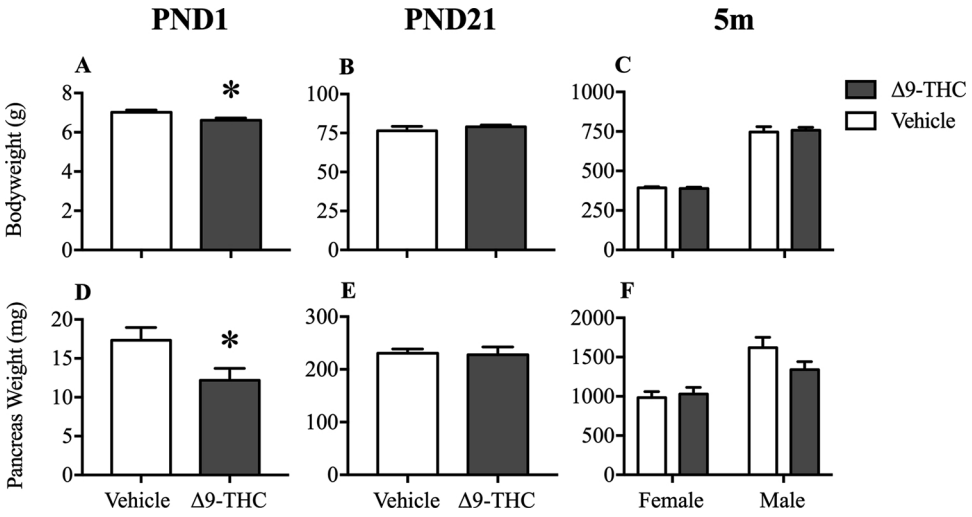

Fig. 1. Gestational exposure to Δ9-THC reduces birthweight and pancreatic weight which is followed by catch-up growth. Bodyweight (A, B, C) and pancreatic weight (D, E, F) at PND1, PND21, and 5 m. *, $p < 0.05$ for vehicle vs Δ9-THC assessed by Student's *t* test. Values are mean ± SEM. *n* = mean of 4-5 litters per treatment (PND1), 16-23 individuals per treatment (PND21), 7-8 individuals per treatment per sex (5 m).

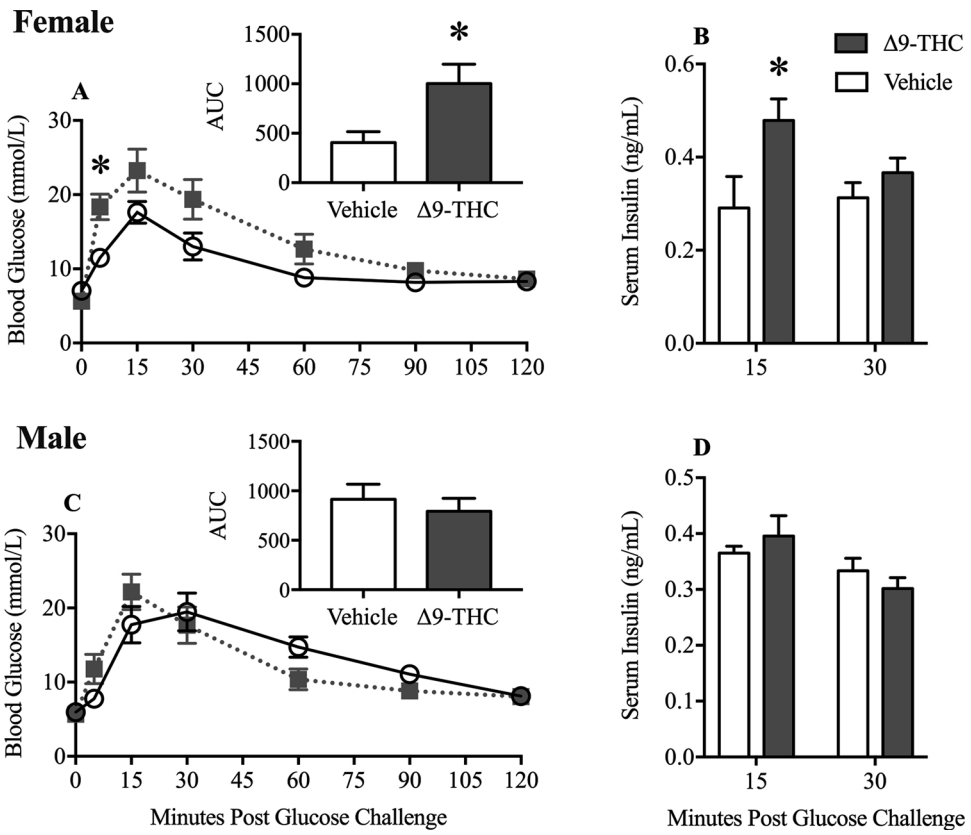

Fig. 2. Gestational exposure to Δ9-THC causes glucose intolerance exclusively in female offspring at 5 months of age associated with increased serum insulin 15 min after glucose challenge. Blood glucose levels and integrated area under the curve for blood glucose tracked for 120 min following 2 g/kg glucose challenge in female and male offspring aged 5 months (A, C). Serum insulin concentrations 15 and 30 min after glucose challenge (B, D). *, $p < 0.05$ for vehicle vs Δ9-THC assessed by repeated measures two-way ANOVA and Holm-Sidak-corrected post-hoc tests, or Student's *t* test (AUC). Values are mean ± SEM. *n* = 4-6 per treatment per sex.

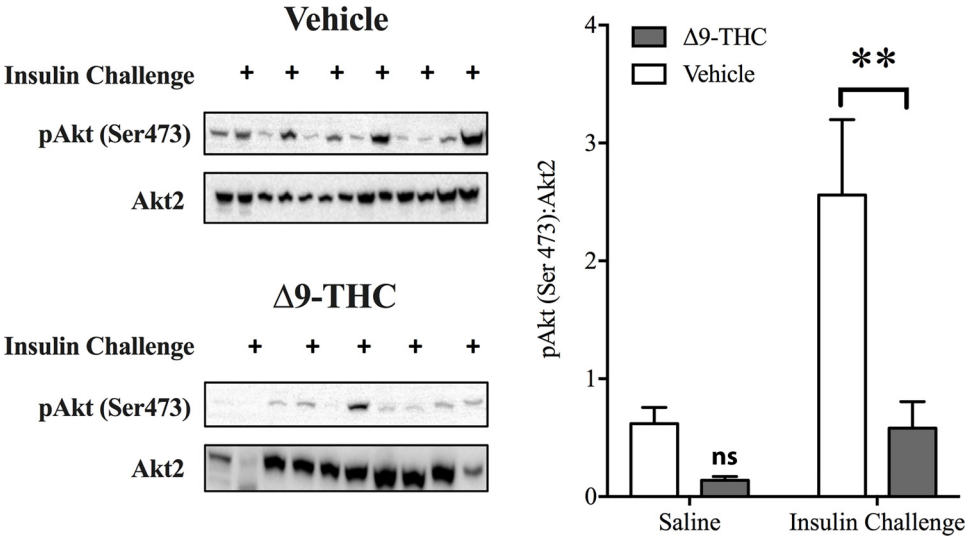

Fig. 3. Gestational exposure to Δ9-THC impairs insulin response in muscle in female offspring aged 5 months. Ratio of phosphorylated Akt [Ser 473] relative to total Akt2 protein levels. **, $p < 0.01$; ns, not significant for vehicle vs Δ9-THC assessed by two-way ANOVA and Holm-Sidak-corrected post-hoc tests. Values are mean ± SEM. *n* = 5-6 per treatment, where each *n* value represents one offspring per dam.

these animals via phosphorylated Akt [Ser 473], a hallmark of insulin receptor activation and sensitivity [25]. Female Δ9-THC-exposed offspring exhibited a significantly reduced ratio of pAkt [Ser 473] relative to total Akt2 protein expression after insulin challenge (Fig. 3; 0.58 ± 0.22 for Δ9-THC vs 2.56 ± 0.64 for vehicle, adjusted p value = 0.0031).

Given the importance of estrogen and testosterone for glucose tolerance and preservation of β-cells [29–31], we also quantified fasting estrogen and testosterone levels at 5 m. However, analysis revealed that estrogen and testosterone were unaltered in female offspring (Table 1), and both levels obtained were similar to those previously reported in young adult Wistar rats [23].

3.3. Altered endocrine pancreatic development

In female offspring, gestational Δ9-THC exposure reduced the total density of islets at both PND21 (2.23 ± 0.17 islets/mm^2 for Δ9-THC vs 2.78 ± 0.19 islets/mm^2 for vehicle, adjusted p value = 0.0247) and 5 m (1.32 ± 0.10 islets/mm^2 for Δ9-THC vs 1.94 ± 0.22 islets/mm^2 for vehicle, adjusted p value = 0.0002), attributable to a specific decrease in the density of small islets (Fig. 4A, G). By 5 m, this decrease in total islet density culminated in a reduction in β-cell mass in female Δ9-THC-exposed offspring (Fig. 4 H; 6.68 ± 0.71 mg for Δ9-THC vs 11.32 ± 1.76 mg for vehicle, adjusted p value = 0.0332). No equivalent alterations were apparent in male offspring (Fig. 4). This reduction in β-cell mass in female Δ9-THC-exposed offspring was attributable

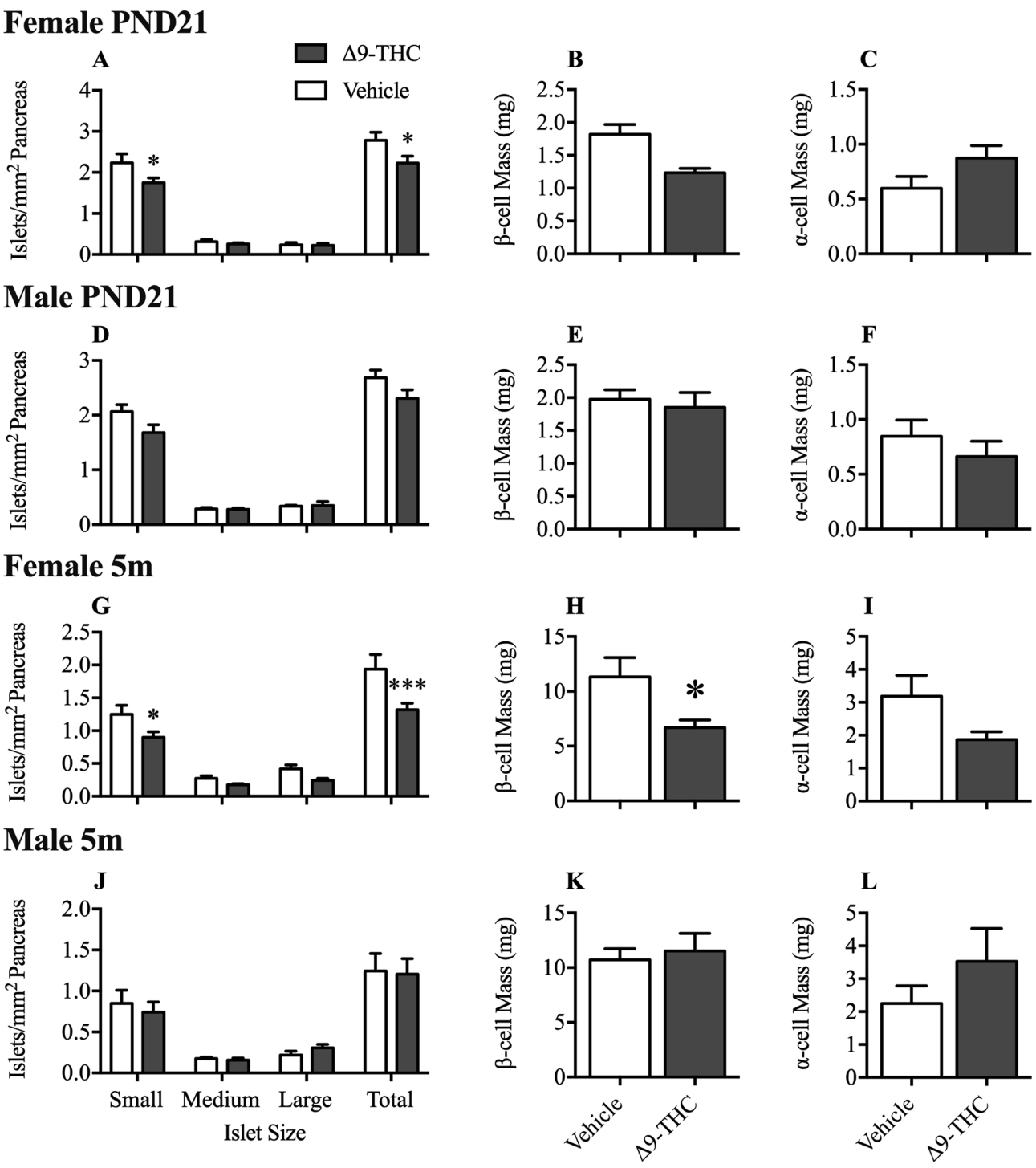

Fig. 4. Gestational exposure to Δ9-THC reduces small islet and total islet density at PND21 and 5 m, and reduces β-cell mass at 5 m exclusively in female offspring. Density of small (500-5 000 μm^2), medium (5 000 - 10 000 μm^2), large (> 10 000 μm^2), and total islets for female (A, G) and male (D, J) offspring at PND21 and 5 m. β-cell mass (B, E, H, K) and α-cell mass (C, F, I, L) in female and male offspring at PND21 and 5 m. *, $p < 0.05$; ***, $p < 0.001$ for vehicle vs Δ9-THC assessed by repeated measures two-way ANOVA and Holm-Sidak-corrected post-hoc tests. Values are mean ± SEM. n = 4-7 per treatment per sex per age (2-3 technical replicates per animal), where each n value represents one offspring per dam.

exclusively to a reduction in the total number of islets, as mean islet size and mean β-cell area per islet were found to be unchanged at both weaning and in adulthood (Table 2).

To gain insight into possible mechanisms involved in the observed reductions in islet density and β-cell mass in Δ9-THC-exposed females, endocrine cell neogenesis, as well as β- and α-cell replication and apoptosis were examined. Neogenesis was assessed via the density of extra-islet clusters (< 500 μm^2) [26,27], and was found to be unaltered at either PND21 or 5 m (Table 2). Pancreatic islet replication and apoptosis were examined in PND21 female offspring by immunohistochemistry with Ki67 antibody and TUNEL assay, respectively (Fig. 5A (Ki67) and B (TUNEL)). PND21 was chosen given the reduction in islet density at this age and that replication and apoptosis in α- and β-cells declines markedly after this age [32,33]. Neither the percentage of β-cells (Ins$^+$) or α-cells (Glucagon$^+$) positive for Ki67 (Fig. 5 C, D), nor the percentage of β-cells (Ins$^+$) positive for TUNEL (Fig. 5 E) were

Table 2
Endocrine pancreas morphometry in female and male offspring at PND21 and 5 m. S, $p < 0.05$ for effect of sex by two-way ANOVA; ns, not significant. Values are mean ± SEM. n = 4-7 per treatment per sex (2-3 slides per animal).

		Female		Male		Two-way ANOVA
		Δ9-THC	Vehicle	Δ9-THC	Vehicle	
Mean Islet Size (μm^2)	PND21	3627 ± 272.5	3592 ± 349.5	4537 ± 471.3	3995 ± 147.4	ns
	5 m	6322 ± 533.2	7341 ± 514.2	8394 ± 861	7228 ± 236.4	ns
Mean β-cell Area per Islet (μm^2)	PND21	2226 ± 117.8	2750 ± 381.6	3326 ± 243.9	2812 ± 163.5	S
	5 m	4999 ± 488.1	5781 ± 453.1	6691 ± 538.8	6000 ± 34.89	ns
Clusters per mm^2 Pancreas	PND21	4.092 ± 0.5584	3.256 ± 0.6557	2.603 ± 0.1859	2.852 ± 0.1772	S
	5 m	0.795 ± 0.1543	0.802 ± 0.0814	0.644 ± 0.1359	0.689 ± 0.134	ns

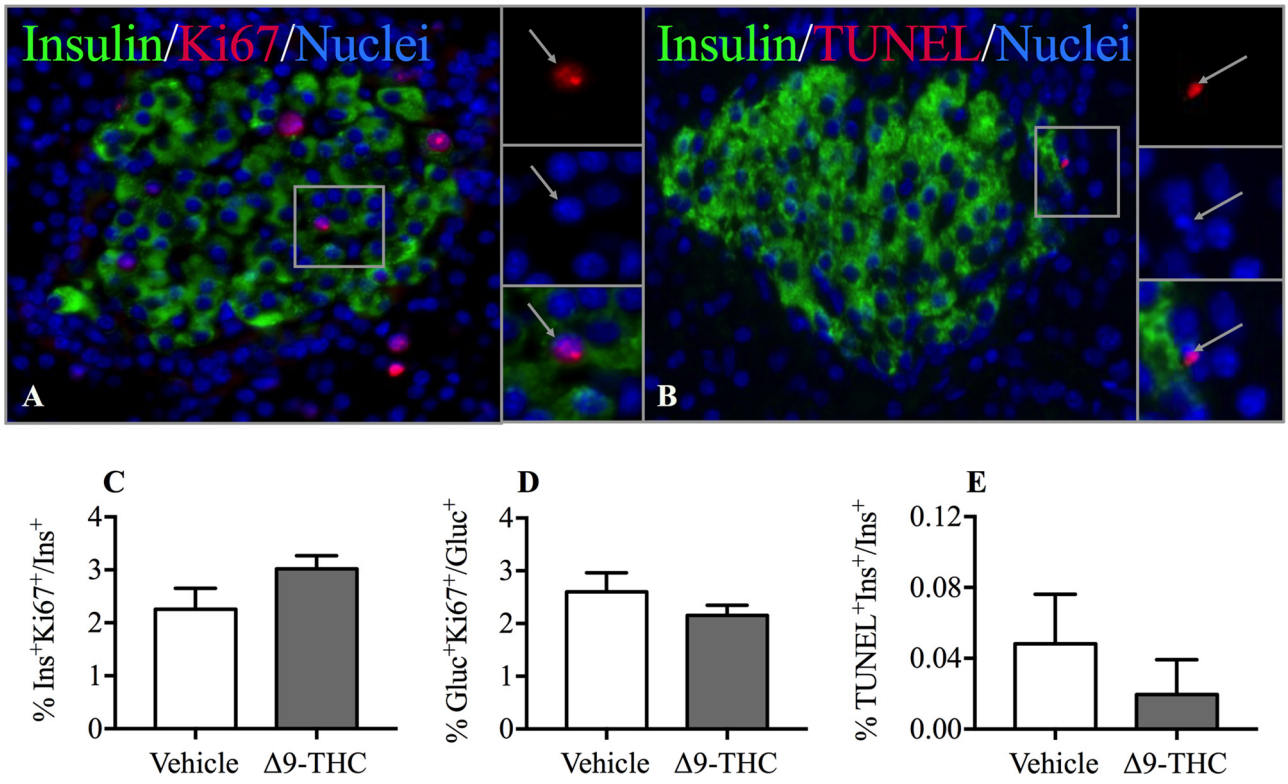

Fig. 5. Gestational exposure to Δ9-THC does not alter replication or apoptosis in β- or α-cells in females at PND21. Representative immunohistochemistry of an islet immunostained for Ki67 (A) and TUNEL (B). Percentage of β-cells positive for Ki67 (C); percentage of α-cells positive for Ki67 (D); percentage of β-cells positive for TUNEL (E). Values are mean ± SEM. n = 4-5 per treatment (2-3 technical replicates per animal), where each n value represents one offspring per dam.

altered following Δ9-THC exposure. No TUNEL-positive α-cells were observed in either group.

4. Discussion

Considering the increased popularity of *cannabis* in pregnancy [1], and the rising concentration of Δ9-THC in *cannabis* over the last two decades [8], it is essential to understand the long-term metabolic effects of gestational exposure to this specific cannabinoid. In the current study, we and others utilized a physiologically-relevant dosage of Δ9-THC (3 mg/kg) which yields maternal blood concentrations of Δ9-THC (8.6–12.4 ng/mL), which are comparable to those seen after moderate recreational *cannabis* smoking in human adults (13 − 63 ng/mL), as well as in the tissue of aborted fetuses after maternal *cannabis* use (4 − 287 ng/mL) [19–21,34–36]. Previous studies in humans as well as animals have demonstrated that Δ9-THC readily crosses the placenta, and that 10–28 % of maternal concentrations are detected in the fetal plasma, with 2 − 5X higher concentrations in fetal tissues [16–18]. However, given evidence that fetal plasma concentrations of Δ9-THC may depend on the route of maternal exposure [16,37–39], quantification of Δ9-THC in fetal circulation after maternal *i.p.* Δ9-THC exposure would add insight to the results obtained in the present study. Nevertheless, this lower dosage of Δ9-THC (3 mg/kg) led to fetal growth restriction, and unlike other studies employing 5 mg/kg [6], it did so *without* fetal demise [7]. Additionally, it should be noted that in same cohort of vehicle and Δ9-THC offspring, we have published that Δ9-THC exposure in pregnancy did not lead to changes in maternal food intake, maternal weight gain, or gestational length [7]. In this cohort, we now demonstrate alterations in endocrine pancreatic development culminating in decreased β-cell mass, impaired glucose tolerance, and aberrant insulin response in adult female Δ9-THC-exposed offspring.

At birth, both male and female offspring exposed to Δ9-THC had reduced bodyweight and pancreatic weight. We have previously demonstrated that Δ9-THC-exposed offspring also exhibit significant decreases in brain to bodyweight ratio, and liver to bodyweight ratio, indicative of symmetrical intrauterine growth restriction [7]. Both male and female Δ9-THC-exposed offspring underwent catch-up growth by PND21, but only females were glucose intolerant at 5 m. Specifically, 5-month-old females exposed to Δ9-THC exhibited elevated blood glucose 5 min after glucose challenge and overall elevated area under the curve

for blood glucose. Interestingly, this occurred in association with increased serum insulin 15 min after glucose challenge, suggesting that peripheral insulin resistance, leading to impaired glucose disposal, contributes to the observed glucose intolerance. To explore this further, we challenged vehicle and Δ9-THC-exposed female offspring with insulin and found that Δ9-THC-exposed offspring demonstrated blunted pAkt [Ser473] activation, a hallmark of insulin receptor activation and sensitivity [25], in the soleus muscle.

In addition to the observed impairments in downstream insulin signaling, Δ9-THC-exposed females also exhibited altered development of the endocrine pancreas. Specifically, these Δ9-THC female offspring had decreased total and small islet density at both PND21 and 5 m, along with a significant reduction (41 %) in β-cell mass at 5 m. Despite this decrease in β-cell mass, fasting serum insulin levels were maintained, suggesting some degree of β-cell compensation [40]. Other studies have shown that this type of β-cell compensation is implicated in eventual β-cell exhaustion [41], which along with chronic glucose intolerance, culminates in β-cell death [42], or dedifferentiation [43] seen in the progression from prediabetes to overt type 2 diabetes. It will therefore be interesting to explore the future metabolic health of these offspring in later life (*i.e.* > 6 months of age).

The sexual dimorphism observed in this study is noteworthy as it has previously been demonstrated that female offspring exposed to Δ9-THC *in utero* exhibit neurodevelopmental abnormalities pertaining to addiction, while their male counterparts do not, suggesting that female offspring may be more sensitive to the effects of Δ9-THC *in utero* [44]. Based on the collective results of this current study, the mechanisms responsible for the reductions in islet density and β-cell mass in female Δ9-THC-exposed offspring remain elusive. While decreases in estrogen are associated with β-cell apoptosis [30], no changes were apparent in either estrogen or testosterone levels, and no alterations were apparent in postnatal (PND21) β- or α-cell replication, neogenesis, or apoptosis. This contrasts with what is observed in offspring exposed to another psychoactive compound, nicotine. When administered during gestation, nicotine-exposed offspring exhibit reduced β-cell mass at four weeks of age, but not 26 weeks of age, due to compensatory increased islet cell proliferation at four weeks [45]. However, this compensatory mechanism is not seen when nicotine exposure extends through lactation, and islet cell apoptosis is now increased at 4 weeks of age, resulting in reduced β-cell mass from 4 to 26 weeks [45]. Despite lacking any such alterations in islet cell proliferation, apoptosis, or neogenesis, we still observed reduced islet density in both PND21 and 5 m female Δ9-THC exposed offspring. It is therefore likely that the glucometabolic effects of Δ9-THC incurred earlier during prenatal development. For example in a model of maternal malnutrition (total caloric intake reduced to 50 % of controls), fetal growth restricted offspring exhibited reduced β-cell mass at birth (PND1) and PND21, attributable to reduced islet density and β-cell size, with no alterations present in β-cell replication at PND1 or PND21 [46,47]. Δ9-THC may be particularly deleterious to β-cell development during prenatal life, first due to the effects of Δ9-THC on placental insufficiency leading to less maternal transfer of oxygen and nutrients for fetal pancreatic development [6,7]. This is consistent with the thrifty phenotype hypothesis, in which adaptations suited to malnutrition *in utero* contribute to metabolic disease in later life [48]. Noteworthy for the present study are adaptations concerning the endocrine pancreas, namely reduced β-cell mass and reduced β-cell number at 90 % gestation, as observed in growth-restricted fetal sheep [49]. Similar adaptations may contribute to the altered endocrine pancreatic development as seen in our Δ9-THC-exposed female offspring, warranting further investigation related to perinatal windows of Δ9-THC exposure. Secondly, Δ9-THC *in utero* may have direct adverse glucometabolic effects on the developing fetal pancreas via its interaction with CB1R [11,12]. In particular, *in vitro* studies have shown that CB1R activation suppresses mitogenic signaling in β-cells via decreased expression of cyclin D2 and impaired downstream insulin signaling, and promotes apoptosis through decreased expression of Bcl-2 [11,12]. As such, there is potential for a direct impact of Δ9-THC on development of the fetal endocrine pancreas, but further work is needed to investigate if such alterations are apparent in the fetal pancreas after Δ9-THC exposure *in vivo*.

5. Conclusion

In summary, though to date no associations have yet been found between maternal *cannabis* use and offspring development of type 2 diabetes in humans, future prospective clinical studies are warranted given that the major increases in Δ9-THC content in *cannabis* have only occurred over the past two decades. This is the first study to demonstrate a link between maternal Δ9-THC exposure and predisposition to develop glucose intolerance in female offspring. This raises concern about the future metabolic health of offspring exposed to Δ9-THC *in utero,* particularly as this study examined the effects of Δ9-THC at levels which are conservative estimates of what is available in recreational *cannabis* today.

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Author contribution

R.G. and S.V. were involved in design, execution, and interpretation of experiments. K.L. was involved with implementing the animal model. D.B.H. and E.A. were involved in the design of this project and interpretation of results. S.R.L. contributed to the design of the animal experiments and in the preparation and dosing of vehicle and Δ9-THC *in vivo*. A.C.H. played a role in the design of the animal experiments and in the analysis of metabolic outcomes. R.G. wrote the first draft of the manuscript; all authors edited drafts of the manuscript.

Data availability

The datasets generated and analyzed in the current study are available from the corresponding author upon reasonable request.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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