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# MATERNAL OBESITY REVERSES THE RESISTANCE TO LPS-INDUCED ADVERSE PREGNANCY OUTCOME AND INCREASES OFFSPRING METABOLIC ALTERATIONS IN CANNABINOID RECEPTOR 1 *KNOCKOUT* MICE

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## Highlights

- The lack of CB1 receptor did not prevent the fetal programming induced by maternal obesity
- CB1 KO mice showed resistance to LPS-induced deleterious effects on pregnancy outcome
- Maternal obesity enhanced sensibility to LPS-induced adverse pregnancy outcome in CB1 KO mice

# MATERNAL OBESITY REVERSES THE RESISTANCE TO LPS-INDUCED ADVERSE PREGNANCY OUTCOME AND INCREASES FEMALE OFFSPRING METABOLIC ALTERATIONS IN CANNABINOID RECEPTOR 1 *KNOCKOUT* MICE

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**Running Title:** Effects of obesity on pregnancy in CB1 KO mice

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**ABSTRACT**

Maternal overnutrition negatively impacts the offspring's health leading to an increased risk of developing chronic diseases or metabolic syndrome in adulthood. What we eat affects the endocannabinoid system (eCS) activity, which in turn modulates lipogenesis and fatty acids utilization in hepatic, muscle, and adipose tissues. This study aimed to evaluate the transgenerational effect of maternal obesity on cannabinoid receptor 1 knock-out (CB1 KO) animals in combination with a postnatal obesogenic diet on the development of metabolic disturbances on their offspring. CB1 KO mice were fed a control diet (CD) or a high-fat diet (HFD; 33% more energy from fat) for 3 months. Offspring born to control and obese mothers were also fed with CD or HFD. We observed that pups born to an HFD-fed mother presented higher postnatal weight, lower hepatic fatty acid amide hydrolase activity, and increased blood cholesterol levels when compared to the offspring born to CD-fed mothers. When female mice born to HFD-fed CB1 KO mothers were exposed to an HFD, they gained more weight, presented elevated blood cholesterol levels, and more abdominal adipose tissue accumulation than control-fed adult offspring. The eCS is involved in several reproductive physiological processes. Interestingly, we showed that CB1 KO mice in gestational day 15 presented resistance to LPS-induced deleterious effects on pregnancy outcome, which was overcome when these mice were obese. Our results suggest that an HFD in CB1 receptor-deficient mice contributes to a "nutritional programming" of the offspring resulting in increased susceptibility to metabolic challenges both perinatally and during adulthood.

**Keywords:** maternal obesity, high-fat diet, CB1 KO mice, nutritional programming.

## 1. INTRODUCTION

Obesity represents a major health concern whose prevalence is increasing worldwide to a pandemic level [1]. Accordingly, the number of overweight or obese women in reproductive age has also increased [2]. Maternal obesity is linked with a greater rate of early pregnancy loss, preterm birth, cesarean section, congenital anomalies, and perinatal death. Gestational diabetes, pre-eclampsia, and complicated labor and delivery are common in pregnant women with obesity [2]. Growing evidence suggests that nutritional conditions during critical stages of perinatal development could have a lifelong negative impact on the health of the offspring [3], in what has been called "nutritional programming" [4]. These changes can subsequently have long-lasting effects in postnatal metabolism and behavior, leading to increased susceptibility of adults to chronic diseases [3,5–7], as well as compulsive and hedonic eating patterns [8–13].

The endocannabinoid system (eCS) is a widespread neuromodulatory system composed of several endogenous lipid-based ligands, collectively named endocannabinoids (eCBs), being anandamide (AEA) and 2-arachidonoylglycerol (2-AG) the most important ones, their enzymes of synthesis and degradation, and two well-characterized cannabinoid receptors, CB1 and CB2. The eCS participates in multiple physiological processes through the activation of CB receptors in central and peripheral tissues. Among these processes, eCBs are involved in controlling whole-body metabolism [14]. Endocannabinoid signaling depends on its bioavailability which is under the strict control of specific catabolic enzymes. Specifically, the biological activity of AEA is terminated by its cellular uptake followed by its intracellular degradation by a fatty acid amide hydrolase (FAAH) [15,16]. Interestingly, CB1 receptors are present in tissues involved in the control of feeding behavior, and energy

homeostasis regulation such as the hypothalamus[17–19], the limbic system [20,21], adipose tissues [22,23], and liver [24]. Accordingly, CB1 receptor has been reported to be involved in food intake [25–30], energy expenditure [19,31–33] and fat metabolism [24,34–36]. Corroborating CB1 participation in these processes, it has been reported that CB1 receptor *knockout* (CB1 KO) mice have a leaner body phenotype and are resistant to weight gain when exposed to a high-fat diet compared to *wild-type* animals [37]. It has been suggested that obesity results in a dysregulation of the eCS [38] and increased circulating endocannabinoid levels have been detected in obese men and women [39,40].

Maternal obesity has been linked to offspring nonalcoholic fatty liver disease, diabetes, and obesity among other health conditions [41–43]. However, information regarding eCBs physiology in obesity associated with pregnancy and metabolic outcomes for offspring is limited. Hepatic CB1 activation has been shown to increase the *de novo* synthesis of fatty acids through the induction of lipogenic genes [24]. High-fat diets (HFD) also induce lipogenic gene expression and fatty acid synthesis [44,45]. It has been suggested that HFD increases hepatic AEA mainly due to a reduction in its degradation in adult animals. The exact mechanism involved in this effect remains to be determined since no reduction in FAAH protein expression was observed [24].

The eCS is also present in female reproductive tissues where it participates in physiological and pathophysiological processes [46]. Numerous studies have demonstrated the involvement of CB1 receptors in oviductal embryo transport [47] and uterine receptivity [48]. Whereas low levels of anandamide are required for implantation and trophoblast outgrowth, elevated anandamide concentrations lead to pregnancy failure [49,50]. We have previously shown that CB1 receptor activation mediates the deleterious effects of lipopolysaccharide (LPS) on nitric oxide and prostaglandin production, and subsequent tissue damage during early embryonic loss [51–53]. Similarly, it has been shown that the eCS

induces apoptosis in rat decidual cells [54,55]. We previously found that CB1 KO mice are resistant to LPS-induced embryotoxicity since these mice presented a lower rate of embryonic resorption after LPS administration when compared their *wild-type* counterparts [56].

Here, we show evidence that pregnant CB1 KO mice at gestational day 15 are resistant to LPS-induced pregnancy complications (preterm birth, stillbirth) although this effect is overridden when these mice are exposed to an HFD. Furthermore, the offspring from HDF-fed CB1 KO mothers show higher postnatal weight, decreased hepatic FAAH activity, and increased plasma cholesterol levels at postnatal day 1 when compared to their matched age counterparts born to control diet (CD)-fed CB1 KO mothers. After weaning, these mice were exposed to either a CD or an HFD. We found that the offspring from an HFD-fed CB1 KO mother gained weight faster, presented higher blood cholesterol levels and increased abdominal fat deposits when exposed in adulthood to an HFD when compared to those exposed to a CD. Therefore, our results suggest that CB1 KO mice are resistant to LPS-induced deleterious effects on late pregnancy outcome and that this effect is attenuated when mice are exposed to an HFD. In CB1 KO mice, maternal obesity and an obesogenic postnatal diet have deleterious effects on the offspring, predisposing them to adverse metabolic outcomes, such as the development of obesity and hypercholesterolemia in adulthood.



## 2. MATERIALS AND METHODS

### 2.1 Animals

Female CD1 *wild-type* (WT) and CB1 *knock-out* (KO) mice from our own colony were housed in a standard animal room with food and water *ad libitum*, in controlled

conditions of humidity, temperature (21 ± 2°C), and luminosity (200 lux), under a 12 h light/dark lighting schedule (lights on at 7:00 h). Animals were kindly donated by Professor Tibor Wenger from the University of Semmelweis University, Hungary. Animals were euthanized in a carbon dioxide chamber, and all efforts were made to minimize animal suffering. The experimental procedures reported here were approved by and were carried out in accordance with the Animal Care Committee of the Center for Pharmacological and Botanical Studies, National Research Council, and by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) from the School of Medicine, University of Buenos Aires (Nº 1163/2016).

**2.2 Murine model of high fat diet-induced maternal obesity**

A murine obesity model induced by a high-fat diet (HFD) intake was performed as described previously [57]. Briefly, female CD1 WT and CB1 KO mice of 21 days of age were individually housed with food and water *ad libitum* and were randomly allocated into two experimental diet groups: the first one was fed a control diet (CD), that consisted of commercial standard rodent chow (Asociación Cooperativa Argentina), whereas the second one was fed an HFD. The HFD consisted of the standard rodent chow enriched with 30% of saturated animal fat provided by manual supplementation with butter (SanCor Co., Buenos Aires, Argentina). CD provided 1,465 kJ/g while HFD provided 1,837 kJ/g., of which 11.1% and 44.3% of energy were from fat, respectively. Animals were fed the corresponding diet for 5 months and body weight was measured weekly. We validated the success of the obesity mice model according to measurement of the body weight, adipose tissue and, glucose, triglycerides and cholesterol serum levels (n= 7) as previously described [57]. Thus, we considered that animals were obese when the average body weight difference was statistically significant between CD and HFD fed mice, adipose tissue increased and we were able to

observe changes in at least one of the blood parameters measured, defining 3 months as the time needed for the treatment with the HFD diet to produce obesogenic effects. At this moment, a group of control (n=8) or obese (n=8) females were paired with control CD1 males. Time of pregnancy was determined by visual inspection of the vaginal plug, which was defined as day 0 of pregnancy. Females were maintained with the same diet all through pregnancy and switched to control diet during lactation. The litters were balanced to 7 pups (five females and two males) per mother. During weaning, all pups were weighed weekly, the weights were averaged and then the mean was used for statistical analysis (n=8 dams per group). On day 21 postnatal, 4 female pups of each mother were individually housed with food and water *ad libitum* and were randomly allocated into two experimental diet groups: CD and HFD (2 pups per dam in each group). Animals were weighed weekly; weights of 2 pups per dam were averaged and the mean was used for statistical analysis (n=8 dams per group). CD and HFD diets were the same as the ones received by the mothers.

**2.2 LPS treatment on day 15 of pregnancy**

Female CD1 WT and CB1 KO mice fed with a CD or an HFD (n WT CD= 11, n WT HFD=8, n KO CD=9, n KO HFD=6) were mated with CD1 WT or KO male mice respectively. Mating was confirmed by the appearance of a vaginal plug, which was considered the day 0 of gestation. Mice were injected i.p. on day 15 of pregnancy with a single dose of lipopolysaccharide (LPS, 0.05 µg/g body weight, *Escherichia coli*, 055:B5, Sigma). Animals were monitored twice a day for any signs of vaginal spotting or bleeding from day 15 to 19 of pregnancy. If animals delivered before day 19 of gestation it was count as preterm labor. Females undergoing preterm labor did not retain pups in their uteri. If animals delivered alive pups on day 19, it counted as term labor while the delivery of death pups was counted as stillbirth. All animals that did not deliver on day 19 were euthanized at

day 20 of gestation and intrauterine death was recorded. Adverse pregnancy outcomes such as preterm labor, intrauterine death, or stillbirth were reckoned together as "pregnancy complication". The beginning of labor was defined by the delivery of the first pup. All of the mice injected with vehicle (sterile saline solution, 100 ul i.p.) delivered at term (day 19 of pregnancy) and did not present intrauterine death nor stillbirth.

**2.3 Blood glucose levels measurement and oral glucose tolerance test**

Blood glucose levels were determined using an Accu-Chek®Performa glucometer (Roche S.A.Q. e l., Buenos Aires, Argentina). After 3 months of feeding with CD or HFD, an oral glucose tolerance test (OGTT) was performed in CB1 KO mice after 8 hours of fasting (n=7). Blood was drawn from the tail vein and glucose levels were monitored at 0, 15, 30, 60, 90 and 120 minutes after intragastric administration of 2 g/kg of dextrose.

Blood glucose levels were measured as well on postnatal day 1 in the offspring from CD (n=8) or HFD (n=8) fed mothers and in adult offspring after 3 months of feeding with the corresponding CD (n=6) or HFD (n=6).

After 8 hours of fasting, adult animals were anesthetized in a carbon dioxide chamber and decapitated to obtain whole blood. In the case of postnatal day 1 pups, the blood from two pups (from the same litter and each experimental group, CD or HFD treated mothers) were pooled. Blood samples were centrifuged at 2000 x g for 15 at room temperature to obtain the serum. Serum samples were stored at -80 C until used.

**2.4 Blood total cholesterol and triglycerides level measurement**

Blood total cholesterol and triglycerides levels were assessed using enzymatic colorimetric commercial kits following manufacturer's instructions (Wiener Lab., Rosario, Argentina). These parameters were measured in female CB1 KO mice after 3 months of

feeding with CD (n=7) or HFD diet (obese females, n=7), on postnatal day 1 in the offspring from CD (n=8) or HFD (n=8) treated mothers and in adult female offspring after 3 months of feeding with the corresponding CD (n=6) or HFD (n=6). The procedure for obtaining blood is described above.

**2.5 Protein extraction and Western Blot**

For the hepatic determination of FAAH protein levels, two livers from offspring on postnatal day 1 (from the same litter and each experimental group, CD or HFD treated mothers, n=4) were pooled and homogenized (Ultra Turrax, T25 basic, IKA Labortechnik, Staufen, Germany) in 200 µl of homogenization buffer (10 mM HEPES, 0.4 mM KCl, 1X cocktail of protease inhibitors, 10 µM DTT, 10 µM leupeptin, 0.2% v/v PMSF) and left on ice for 20 min. Next, the homogenates were centrifuged at 14000 x g for 10 min at 4°C and the supernatants were transferred to a new tube. Protein concentration was determined by Bradford assay [58] and supernatant were stored at -80°C until further use. Samples containing 40 µg of protein were loaded in each lane, separated by electrophoresis in 12% SDS–PAGE gel and transferred to a 0.45 µm nitrocellulose membrane. Non-specific binding sites were blocked by incubating the membrane with 5% skim milk in 1X phosphate-buffered saline (PBS) for 1 h. Next, membranes were incubated with the primary anti-FAAH antibody (1:500) overnight at 4°C. FAAH antibody was a kind gift from Dr. Benjamin Cravatt (The Scripps Research Institute at La Jolla, CA, USA). The next day, membranes were washed with 0.1% T-PBS (10 mM Tris, 100 mM NaCl and 0.1% Tween 20, pH 7.5) and incubated with the appropriate specie-specific HRP-conjugated secondary antibodies (1:5000; Jackson Laboratory, USA) for 1 h at room temperature. Membranes were developed by chemiluminescence. Images of immunoreactive bands were acquired using the ImageQuant blot documentation instrument (ECL Western Blotting Analysis System; GE

Healthcare, UK) and analyzed using the Image J software package. The relative protein level was normalized to β-actin (Sigma Chemical Co.; St Louis, MI, USA) and results are expressed as relative optical density (FAAH/β-actin).

**2.6 Determination of fatty acid amide hydrolase (FAAH) activity**

For the hepatic determination of FAAH activity liver from adult KO CB1 female mice fed with CD or HFD (n=6) or two livers from offspring on postnatal day 1 (from the same litter and each experimental group, CD1 WT and CB1 KO mothers fed with a CD or an HFD, n=6) were homogenized (Ultra Turrax, T25 basic, IKA Labortechnik) in Tris-HCl 20 mM (pH 7.6) buffer containing EDTA 1 mM. Protein concentration was determined by Bradford assay [58]. FAAH (EC 3.5.1.4) activity was assayed as described by Paria et al. (1996) [59]. Briefly, 100 mg of protein were incubated at 37°C for 15 min in 200 ml Tris/HCl 50 mM (pH 8.5) buffer whit 100 mM [3H]-AEA and 20 nmol AEA. The reaction was stopped adding a mix of chloroform:methanol (1:1 v/v). The aqueous phase was washed twice with chloroform. Each organic phase was dried and resuspended in chloroform and was plated on silica TLC plates Gel 60. The silica TLC plate was divided into different lanes, and each organic phase was resolved in a solvent system of ethyl acetate:hexane:acetic acid:distilled water (100:50:20:100 v v-1) mixture. The plate was exposed to iodine to identify and demark five zones, with the second and fourth corresponding to hydrolyzed arachidonic acid (AA) and AEA respectively (we selected the zones in accordance with the co-migration with AEA and AA standards). The distribution of radioactivity on the plate was counted in a scintillation counter by scraping off the corresponding five zones. The area of each radioactive peak corresponding to AA (zone 2) was calculated and expressed as a percentage of the total radioactivity of the lane (zone 1 to 5). Enzyme activity is reported as nmol [$^{3}$H]-AA/h/mg protein.

### 2.7 Data analysis and statistical procedures

In all cases the experimental unit was the mother that was assigned to each experimental group in a complete randomized manner. When the offspring from CD-fed or HFD-fed mothers was analyzed, animals were assigned to CD or HFD in a complete randomized manner. Body weight, food intake and OGTT were analyzed by two-way ANOVA (diet and time) with repeated measures. The assumptions of normality, homogeneity of variances, and sphericity were assessed by Shapiro–Wilks test, Levene test, and Mauchly test, respectively. The results of blood glucose, total cholesterol and triglycerides levels, adipose tissue weight, success rate for getting pregnant, FAAH activity, and protein levels were analyzed by one-way ANOVA (diet). The assumptions of normality and homoscedasticity were assessed by the Shapiro–Wilks test and the Levene test, respectively. Comparisons were made with the Tukey post hoc test. Means with a different letter are significantly different ($p<0.05$). Statistical differences in pregnancy complications rates between groups were analyzed by Fisher's test ($p<0.05$). Statistical analyzes were performed using the statistical program Infostat (FCA, University of Cordoba, Argentina).

## 3. RESULTS

### 3.1 HFD induces obesity in CD1 CB1 KO mice

First, we analyzed the effect of an HFD (44.3% kcal from fat) on the weight gain vs. a CD in CB1 KO mice. We observed that the HFD induced a higher weight gain than the CD from the second month onwards (Figure 1A). Since HFD-induced obesity could be related to changes in nutritional behavior, we measured the food intake during the first three months of feeding with CD or HDF of CB1 KO mice. Interestingly, mice fed with CD consumed more food (grams per day) than mice fed with HDF (Figure 1B). Paradoxically, even though HDF

has a higher caloric content than CD, mice fed with both diets consumed an equal number of kilojoules per day (Figure 1C). Since obesity has been associated with increased insulin resistance and type-2 diabetes, we proceeded to perform an oral glucose tolerance test (OGTT) to CB1 KO mice fed with CD or HDF. As shown in Figure 1D, CB1 KO mice fed with HDF presented no alterations in glucose metabolism, since their OGGT curve was similar to that obtained in CD-fed mice. Next, in order to evaluate fat tissue distribution, we weighted abdominal, gonadal, and retroperitoneal adipose tissue depots as well as the spleen as a control. A representative photograph of the increase in fat tissue is shown in Figure 1E. In addition, we evaluated blood glucose, triglycerides, and total cholesterol levels. As shown in Table 1, female CB1 KO mice showed increased blood cholesterol levels after being fed for three months with an HFD. Since HFD has been shown to increase the circulating levels of endocannabinoids [45], we next studied whether HFD-fed mice could also have an altered hepatic endocannabinoid metabolism when compared to CD-fed mice. We measured the activity of the fatty acid amide hydrolase (FAAH), a major endocannabinoid catabolic enzyme, and found no differences between CB1 KO mice fed during three months with CD or HDF (Figure 1F).



### 3.2 Obesity alters CB1 KO mice resistance to LPS-induced pregnancy complications

In order to explore the consequences of obesity in CB1 KO pregnant mice, we firstly explored the weight gain rate of pregnant animals fed with either CD or HFD. As shown in Figure 2A, CB1 KO mice were fed for three months with CD or HFD before mating and they continue with the same diet throughout the course of pregnancy. Despite the initial difference in weight at day 0 of pregnancy (the day of appearance of a vaginal plug), the weight gain rate was similar between pregnant CB1 KO mice fed with CD or HDF on days 7, 14 and 18

of pregnancy. Furthermore, we did not find differences in success rate for getting pregnant (CD-fed mice: 88 ± 5%; HFD-fed mice: 81 ± 5%; $p>0.05$), pregnancy length (19 ± 0 days for both groups) and litter size (CD-fed mice: 11 ± 2; HFD-fed mice: 11 ± 1) between control and obese mothers.

Since previous reports from our lab showed that CB1 KO mice are more resistant to LPS-induced embryonic resorption on day 7 of pregnancy [48], we sought to compare the effects of the endotoxin on pregnancy outcome on WT and CB1 KO mice fed with CD. As shown in Figure 2B, when CD-fed WT mice were exposed to a dose of LPS (0.05 µg/g) on day 15th of gestation, 73% of them presented pregnancy complications (preterm delivery and/or fetal death). However, only 22% of LPS-treated CD-fed CB1 KO mice presented these complications, suggesting that CB1 KO mice are resistant to LPS-induced deleterious effects on pregnancy outcome. Next, we evaluated the effects of obesity on LPS-induced preterm birth, stillbirth, or intrauterine death to WT and CB1 KO mice fed with HFD. As shown in Figure 2B, LPS-treated HFD-fed WT mice presented a similar percentage of pregnancy complications (75%) than their lean counterpart. Interestingly, LPS-treated HFD-fed CB1 KO mice showed an increase in pregnancy complications (50%) when compared to their CD-fed KO counterpart (22%), suggesting that obesity negatively alters the susceptibility to the endotoxin in CB1 KO mice. In fact, when the statistical analysis was performed, HFD-fed CB1 KO mice complication rate were similar to the observed in WT CD mice, (50% vs 75%, respectively, $p>0.05$), meaning that HFD feeding reversed the resistance to LPS-induced adverse pregnancy outcome found in mice lacking CB1 fed with a CD.

### 3.3 Maternal obesity increases weight gain and produces metabolic changes in the offspring

It has been previously shown that maternal obesity has a negative impact on the offspring. Therefore, our next aim was to evaluate the changes induced by maternal obesity on metabolic and physiological parameters in the offspring. First, we studied the weight gain rate in the offspring from CB1 KO mice fed with CD or HFD. We found that pups from HFD-fed mice weighed more than those from CD-fed mothers since postnatal day-1 and the difference remained up until weaning (Figure 3A). When metabolic parameters were measured at postnatal day-1, we found that pups from HFD-fed CB1 KO mice presented higher blood cholesterol levels than those from CD-fed CB1 KO mothers (Table 2). Since the eCS has been shown to play an important role in the metabolism of lipids, we decided to analyze the FAAH protein expression in the liver of pups from CD-fed or HDF-fed CB1 KO mothers and we did not find differences (Figure 3B). Furthermore, we analyzed FAAH activity in the liver of pups from CD-fed or HDF-fed born to WT and CB1 KO mothers to evaluate not only the possible effect of the mother's diet but also the effect of the lacking the CB1 receptor on this parameter. Interestingly, WT animals born to control mothers presented lower levels of FAAH activity when compared to CB1 KO from CD-fed mother (66.28 ± 10.05 vs. 129.8 ± 6.43 nmol [3H]-AA/h/mg protein, respectively; $p<0.05$). Although in WT animals we did not observe differences regarding mother's diets (66.28 ± 10.05 vs. 72.51 ± 8.44 nmol [3H]-AA/h/mg protein, CD and HFD respectively; $p>0.05$), CB1 KO mice FAAH activity was lower in the liver from pups from HFD-fed mothers compared to pups from CD-fed CB1 KO mothers (Figure 3C), suggesting that the lack of CB1 receptor influences the offspring eCS changes that can be provoked by maternal obesity.

**3.4 Maternal obesity has long-lasting effects on the offspring weight gain after they are exposed to an HFD.**

In order to study the effects of maternal obesity on later stages of life, we fed adult offspring from CD-fed or HFD-fed mothers for three months with the same CD or HFD. We found that when fed with CD, adult offspring from CD-fed and HFD-fed CB1 KO mother did not differ in the weight gain rate (Figure 4A). However, when fed with HFD, adult offspring from HDF-fed CB1 KO mothers showed an increased weight gain rate when compared to those from CD-fed CB1 KO mothers (Figure 4B). Although we did not find differences on this parameter between offspring born to CD-fed mothers after 3 months of feeding with a CD or an HFD (35.69 ± 0.51 g vs. 37.34 ± 2.56 g, respectively; $p>0.05$) when we calculated the weight gain relative to the offspring weight on day 21 (i.e. when animals were randomly allocated into the two experimental diet groups) we observed a difference statistically different between these groups (142.91 ± 4.44 % vs. 184.56 ± 16.56 %, respectively: $p<0.05$), demonstrating that the HFD had an effect on the weight on offspring born to a CD-fed mother. We believe that we could not observe differences in the weight at 3 months since animals presented differences in the weight on day 21 (14.72 ± 0.35 g vs. 13.17 ± 0.52 g, respectively; $p<0.05$). This increase in weight gain was accompanied by an increase in abdominal adipose tissue (Figure 4C). We compared the adiposity between offspring born to CD-fed mothers after 3 months of feeding with a CD or an HFD (0.95 ± 0.29 g vs. 1.15 ± 0.26 g; $p>0.05$; respectively) and we did not find statically significant differences. Since we did not analyze other fat pads, such as gonadal and retroperitoneal adipose tissues, we believe that we may be missing other changes provoked by HDF on adiposity. Furthermore, when fed with HFD, adult offspring from HFD-fed CB1 KO mother showed higher blood cholesterol levels than those from CD-fed mother (Table 3). Overall, these results suggest that maternal obesity has long-lasting effects on the offspring's response to exposure to an HFD in CB1 KO mice.

## 4. DISCUSSION

In this study, we investigated the effect of HFD-induced maternal obesity on gestation and on the offspring in CB1 KO mice. We found that obese CB1 KO mice were more susceptible to LPS-induced pregnancy complications than CB1 KO mice fed with a control diet. Interestingly, CB1 KO mice were resistant to LPS-induced pregnancy complications when compared to their *wild-type* counterparts. Furthermore, the offspring from HFD-fed CB1 KO mothers were more susceptible to metabolic changes in adulthood when exposed to an HFD, suggesting that the lack of CB1 receptor does not prevent the fetal programming induced by maternal obesity.

Even though C57BL/6N CB1 KO mice have been reported to be resistant to developing obesity when exposed to a high-fat highly palatable [34], we found that our HFD-fed CD1 CB1 KO mice showed increased weight gain when compared to the CD-fed CB1 KO mice. A possible explanation for this discrepancy could be due to the murine strains used. Whilst mice used in Cota et al. (2003) were in a mixed genetic background [34,60], with a predominant C57BL/6N contribution, the strain used in this work, CD1, is not a hybrid strain. These differences in the genetic background could explain the fact that we observed weight gain in CB1 KO mice fed with a HFD together with an increase in fat tissue distribution and blood cholesterol levels. It is important to point out that not only the murine strain used was different but also the diet treatment and the mice sex chosen for this study. Interestingly, we did not observed changes in gestational parameters (such as pregnancy length and litter size) between CD-fed and HDF-fed CB1 KO mice. We reported a similar observation with CD1 WT mice [49], which contrasted with the results from Kamimae-Lanning et al., [61] who found that C57BL/6J CD45.2 mice presented a reduced litter size when exposed to an HFD.

Differences in genetic background and diet composition could account for these discrepancies [62][54].

As we mentioned previously CB1 receptors participate in key physiological events [46]. Furthermore, CB1 receptors have been reported to mediate LPS-induced tissue damage [51–55]. Accordingly, we have previously shown that CB1 protein levels were upregulated in a murine model of preterm labor induced by LPS [63] and that CB1 KO mice were resistant to LPS-induced early pregnancy loss [56]. Therefore, we hypothesized that CB1 KO would be resistant to LPS-induced pregnancy complications. Indeed, when compared to their CD-fed WT counterparts, CD-fed 15-days-pregnant CB1 KO mice showed a lower percentage (73% vs. 23% respectively, $p<0.05$) of pregnancy complications (stillbirth, preterm delivery) after a systemic administration of LPS. However, when CB1 KO mice at gestational day 15 were exposed to an HFD, the protective effect of lacking CB1 receptor was partially lost and the percentage of pregnancy complications, when exposed to LPS, increased to 50%. Despite the fact that we did not find a statistically significant difference between CD-fed CB1 KO mice at gestational day 15 when compared to HFD-fed CB1 KO mice at gestational day 15 regarding pregnancy complications rate, we believe that this difference is nevertheless biologically relevant. It is important to highlight that, in CB1 KO mice, the high fat diet reversed the resistance to LPS-induced adverse pregnancy outcome, which is indeed supported by the statistical analysis of results. It is well known that obesity is associated with a state of chronic low-level inflammation [56]. Based on our results, we speculate that the inflammatory state of CB1 KO HFD-fed mothers due to obesity could be impairing the immune system. In this sense, Miranda et al. [2019] [64] have shown that male C57Bl/6J mice fed with an HFD present more pro-inflammatory macrophages infiltration in epididymal fat compared to the animals fed with the control diet, as well as increased plasma levels of IFN-γ, an important inflammatory mediator. These observations highlight that a proper

balance between metabolism and the immune response is crucial for health. Interestingly, obesity has been recently reported to be associated with intestinal barrier disruption and altered gut microbiota [65,66]. Changes in microbial community and increased permeability to bacterial metabolic products and/or endotoxins results in a chronic inflammatory status with higher levels of circulating LPS and pro-inflammatory cytokines, a phenomenon known as “metabolic endotoxemia” [67–69]. Furthermore, it has been demonstrated that obesity is also associated with increased levels of endocannabinoids in plasma and adipose tissue together with changes in CB1 receptor expression [39,70]. Interestingly, Mehrpouya-Bahrami et al. (2017) [71] have recently reported that CB1 receptor blockade with SR14178A ameliorated diet-induced obesity by attenuating inflammation and restoring gut microbiota in mice. One caveat of our study is that we did not assess the possible changes in tissue or blood inflammatory markers, nor in gut microbiota when CB1 KO mice are exposed to an HFD compared to CD-fed CB1 KO mice. We could speculate that exposure to an HFD alters gut microbiota in CB1 KO mice, which triggers metabolic endotoxemia and a systemic pro-inflammatory status. When these mice are subsequently exposed to an injection of LPS, the putative resistance to the endotoxin in CB1 KO mice is overcome and pregnancy complications occur at higher rates. Furthermore, we speculate that CB1 independent mechanisms may influence inflammation and gut barrier dysfunction in the mice used in this work, which lacks CB1 receptors. Deveaux et al. (2009) [72] have revealed that CB2 potentiates obesity-associated comorbidities via an impact on inflammation, insulin resistance, and fatty liver. Given the complementary functions of CB1 and CB2, we cannot discard the existence of compensation mechanisms through CB2 against the lack of CB1 receptors. Moreover, CB2 is expressed by immune and epithelial cells in the gastrointestinal tract [73]. In addition, it has been shown that chronic stress and antibiotic-induced dysbiosis up-regulated CB2 mRNA levels in the gastrointestinal tract [74].

*In utero* exposure to an adverse environment programs changes in the offspring that may be the origin of several diseases later in life, including metabolic alterations and proneness to obesity. Recently, Chang et al. (2019) [75] have demonstrated that prenatal and pregnancy windows have independent programming effects on offspring. However, both affect descendant health, either changing body composition and adiposity, or producing effects on the metabolism and tissue immune cell phenotypes. Interestingly, we found that the offspring from HFD-fed CB1 KO mothers presented increased weight and higher serum levels of cholesterol. The liver plays a pivotal role in all metabolic processes in the body, especially in fat metabolism. There is evidence that maternal high-fat diet increases susceptibility to the development of steatosis in the offspring [76] . On the other hand, eCBs appear to be involved in several aspects of acute and chronic liver disease [77]. Furthermore, an HFD increases hepatic levels of the endocannabinoid anandamide (AEA) [24]. A delicate balance between AEA synthesis and degradation (mainly regulated by the FAAH enzyme) is necessary to ensure an appropriate 'AEA tone' in tissues [78] . We observed a decreased liver FAAH activity, without changes in its protein expression at postnatal day 1 when compared to those born to CD-fed CB1 KO mothers. This change could impact key metabolic pathways which could have severe short- and long-term consequences in hepatic metabolic functions. It is important to highlight that the changes observed on FAAH activity on livers from CB1 KO offspring on postnatal day 1 not only are influenced by the maternal diet but also by the lacking of the CB1 receptor since in WT animals we did not find differences regarding diets but WT animals presented lower levels of FAAH activity when compared to CB1 KO mice. These observations are intriguing since it has been reported that AEA decreases fatty acid synthesis in rat hepatocytes through a cannabinoid receptor-independent mechanism [79]. It remains to be determined whether the lower enzymatic activity of FAAH in the liver of the offspring from HDF-fed CB1 KO mothers has a negative impact later on during adulthood.

Nevertheless, this change could impact key metabolic pathways which could have severe short- and long-term consequences during development.

Maternal overnutrition during the gestational period has been associated with deleterious metabolic outcomes in the offspring in several animal models [9,80–82], and epidemiological data strongly suggest that something similar occurs in humans [83]. One of the long-term negative consequences of maternal obesity in the metabolism of the offspring is a greater susceptibility to high-fat diets in adulthood [9]. When we exposed, after weaning, the offspring from CD-fed or HDF-fed CB1 KO mothers to a control diet, we found that both groups gained weight in a similar fashion. However, when the offspring from CD-fed or HDF-fed CB1 KO mothers were exposed, after weaning, to a high-fat diet, those born to an HDF-fed CB1 KO mother showed a significantly higher weight gain than those born to a CD-fed CB1 KO mother. Furthermore, they also presented increased blood cholesterol levels and the abdominal adipose tissue accumulation was also significantly higher. Collectively, our data suggest that the long-term adverse metabolic changes produced in the offspring due to maternal obesity could be triggered by an obesogenic diet. In animal models of fetal programming, maternal nutrition impact on the offspring's food preference in adulthood [9,12,13]. Clinical evidence suggests that early life nutrition have an impact later on food preference, with maternal diet contributing to predispose their children to highly palatable, high-energy foods [10,11].

There are several reports that show that the effects of fetal programming against harmful stimuli differ depending on the biological sex of the offspring [84–86]. Furthermore, it has been shown that AEA levels vary between sexes in rodents. Specifically, female mice have been shown to have higher brain AEA levels than males [87,88], although these differences seem to be brain region-specific [89]. However, this situation changes depending on the tissue evaluated since, in the liver, there are lower AEA levels but higher CB1 protein

expression in female mice than in male mice [90]. Additionally, diet-induced obesity models also seem to differently affect male and female mice, with an *ad libitum* HFD showing a profound impact on brain AEA levels in males but not in females [91]. Further characterization of sex differences of the eCS and a better understanding of the mechanism underlying sexual dimorphism regarding obesity is required in the next years. Since we only evaluated female offspring during adult life in the present work, we cannot rule out the possibility of male offspring presenting a different programming effect.

Any stimulus or insult at a critical period of embryonic and fetal development can result in developmental adaptations that produce permanent structural, physiological and metabolic changes, thereby predisposing an individual to cardiovascular, metabolic and endocrine disease in adult life. Our results in CB1 KO mice provide evidence linking the maternal obesity with metabolic disorders in offspring. In the absence of the CB1 receptor, obese mothers have obese offspring when prenatal and postnatal nutritional supply is high in fat. Moreover, our work contributes to demonstrate that the eCS may influence the outcome induced by an obesogenic environment. Specifically, maternal obesity changed the liver FAAH activity only in the newborn offspring lacking CB1, which may impact the endocannabinoid levels in certain organs. This reinforces the hypothesis of "nutritional programming" or obesity "programmed'" by early life insults. Understanding the role and mechanism of fetal programming, which has a negative impact on future health, has important academic value in finding intervention strategies.



**DECLARATIONS OF INTEREST**

None.

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## AUTHOR CONTRIBUTIONS

MVB contributed to conceptualization, methodology, investigation, formal analysis, writing-original draft preparation, editing and visualization. FC provided resources, contributed to writing- original, review, editing and visualization. APDR contribute to formal analysis. JAS contributed to review, editing and visualization. MLW, and MC contributed to investigation. JA and AMF contributed to conceptualization, methodology, supervision and visualization. AMF provided resources and funding acquisition.

## REFERENCES

[1] Blüher M. Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol 2019;15:288–98. https://doi.org/10.1038/s41574-019-0176-8.

[2] Poston L, Caleyachetty R, Cnattingius S, Corvalán C, Uauy R, Herring S, et al. Preconceptional and maternal obesity: epidemiology and health consequences. Lancet Diabetes Endocrinol 2016;4:1025–36. https://doi.org/10.1016/S2213-8587(16)30217-0.

[3] Godfrey KM, Reynolds RM, Prescott SL, Nyirenda M, Jaddoe VW V, Eriksson JG, et al. Influence of maternal obesity on the long-term health of offspring. Lancet Diabetes Endocrinol 2017;5:53–64. https://doi.org/10.1016/S2213-8587(16)30107-3.

[4] Langley-Evans SC. Nutritional programming of disease: Unravelling the mechanism. J. Anat., vol. 215, 2009, p. 36–51. https://doi.org/10.1111/j.1469-7580.2008.00977.x.

[5] Luzzo KM, Wang Q, Purcell SH, Chi M, Jimenez PT, Grindler N, et al. High Fat Diet Induced Developmental Defects in the Mouse: Oocyte Meiotic Aneuploidy and Fetal Growth Retardation/Brain Defects. PLoS One 2012;7:e49217. https://doi.org/10.1371/journal.pone.0049217.

[6] Sloboda DM, Hickey M, Hart R. Reproduction in females: the role of the early life environment. Hum Reprod Update 2011;17:210–27. https://doi.org/10.1093/humupd/dmq048.

[7] Cinquina V, Calvigioni D, Farlik M, Halbritter F, Fife-Gernedl V, Shirran SL, et al. Life-long epigenetic programming of cortical architecture by maternal 'Western' diet during pregnancy. Mol Psychiatry 2020;25:22–36. https://doi.org/10.1038/s41380-019-0580-4.

[8] Rivera HM, Kievit P, Kirigiti MA, Bauman LA, Baquero K, Blundell P, et al. Maternal high-fat diet and obesity impact palatable food intake and dopamine signaling in nonhuman primate offspring. Obesity 2015;23:2157–64. https://doi.org/10.1002/oby.21306.

[9] Dias-Rocha CP, Almeida MM, Santana EM, Costa JCB, Franco JG, Pazos-Moura CC, et al. Maternal high-fat diet induces sex-specific endocannabinoid system changes in newborn rats and programs adiposity, energy expenditure and food preference in adulthood. J Nutr Biochem 2018;51:56–68. https://doi.org/10.1016/j.jnutbio.2017.09.019.

[10] Rising R, Lifshitz F. Relationship between maternal obesity and infant feeding-interactions. Nutr J 2005;4:17. https://doi.org/10.1186/1475-2891-4-17.

[11] De Cosmi V, Scaglioni S, Agostoni C. Early taste experiences and later food choices. Nutrients 2017;9. https://doi.org/10.3390/nu9020107.

[12] Peleg-Raibstein D, Sarker G, Litwan K, Krämer SD, Ametamey SM, Schibli R, et al. Enhanced sensitivity to drugs of abuse and palatable foods following maternal overnutrition. Transl Psychiatry 2016;6:e911. https://doi.org/10.1038/tp.2016.176.

[13] Sarker G, Litwan K, Kastli R, Peleg-Raibstein D. Maternal overnutrition during critical developmental periods leads to different health adversities in the offspring : relevance of obesity , addiction and schizophrenia. Sci Rep 2019;9:1–17. https://doi.org/10.1038/s41598-019-53652-x.

[14] Ruiz de Azua I, Lutz B. Multiple endocannabinoid-mediated mechanisms in the regulation of energy homeostasis in brain and peripheral tissues. Cell Mol Life Sci 2019;76:1341–63. https://doi.org/10.1007/s00018-018-2994-6.

[15] Bisogno T, Petrocellis L, Marzo V. Fatty Acid Amide Hydrolase, an Enzyme with Many Bioactive Substrates. Possible Therapeutic Implications. Curr Pharm Des 2002;8:533–47. https://doi.org/10.2174/1381612023395655.

[16] Maccarrone M, Finazzi-Agró A. The endocannabinoid system, anandamide and the regulation of mammalian cell apoptosis. Cell Death Differ 2003;10:946–55. https://doi.org/10.1038/sj.cdd.4401284.

[17] Kirkham TC, Williams CM, Fezza F, Di Marzo V. Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: Stimulation of eating by 2-arachidonoyl glycerol. Br J Pharmacol 2002;136:550–7. https://doi.org/10.1038/sj.bjp.0704767.

[18] Dore R, Valenza M, Wang X, Rice KC, Sabino V, Cottone P. The inverse agonist of CB1 receptor SR141716 blocks compulsive eating of palatable food. Addict Biol 2014;19:849–61. https://doi.org/10.1111/adb.12056.

[19] Cardinal P, Bellocchio L, Guzmán-Quevedo O, André C, Clark S, Elie M, et al. Cannabinoid type 1 (CB1) receptors on Sim1-expressing neurons regulate energy expenditure in male mice. Endocrinology 2015;156:411–8. https://doi.org/10.1210/en.2014-1437.

[20] Soria-Gómez E, Matias I, Rueda-Orozco PE, Cisneros M, Petrosino S, Navarro L, et al. Pharmacological enhancement of the endocannabinoid system in the nucleus accumbens shell stimulates food intake and increases c-Fos expression in the hypothalamus. Br J Pharmacol 2007;151:1109–16. https://doi.org/10.1038/sj.bjp.0707313.

[21] Amancio-Belmont O, Romano-López A, Ruiz-Contreras AE, Méndez-Díaz M, Prospéro-García O. From adolescent to elder rats: Motivation for palatable food and cannabinoids receptors. Dev Neurobiol 2017;77:917–27. https://doi.org/10.1002/dneu.22472.

[22] Matias I, Bisogno T, Di Marzo V. Endogenous cannabinoids in the brain and peripheral tissues: Regulation of their levels and control of food intake. Int J Obes 2006;30:S7–12. https://doi.org/10.1038/sj.ijo.0803271.

[23] Karaliota S, Siafaka-Kapadai A, Gontinou C, Psarra K, Mavri-Vavayanni M. Anandamide increases the differentiation of rat adipocytes and causes PPARλ and cB1 receptor upregulation. Obesity 2009;17:1833–8. https://doi.org/10.1038/oby.2009.177.

[24] Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Bátkai S, et al. Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. J Clin Invest 2005;115:1298–305. https://doi.org/10.1172/JCI23057.

[25] Williams CM, Kirkham TC. Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. Psychopharmacology (Berl) 1999;143:315–7. https://doi.org/10.1007/s002130050953.

[26] Freedland CS, Poston JS, Porrino LJ. Effects of SR141716A, a central cannabinoid receptor antagonist, on food-maintained responding. Pharmacol Biochem Behav 2000;67:265–70. https://doi.org/10.1016/s0091-3057(00)00359-2.

[27] Gómez R, Navarro M, Ferrer B, Trigo JM, Bilbao A, Arco I Del, et al. A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. J Neurosci 2002;22:9612–7. https://doi.org/10.1523/jneurosci.22-21-09612.2002.

[28] Hildebrandt AL, Kelly-Sullivan DM, Black SC. Antiobesity effects of chronic cannabinoid CB1 receptor antagonist treatment in diet-induced obese mice. Eur J Pharmacol 2003;462:125–32. https://doi.org/10.1016/S0014-2999(03)01343-8.

[29] Ravinet Trillou C, Arnone M, Delgorge C, Gonalons N, Keane P, Maffrand J-P, et al. Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. Am J Physiol Integr Comp Physiol 2003;284:R345–53. https://doi.org/10.1152/ajpregu.00545.2002.

[30] Di Marzo V, Matias I. Endocannabinoid control of food intake and energy balance. Nat Neurosci 2005;8:585–9. https://doi.org/10.1038/nn1457.

[31] Verty ANA, Allen AM, Oldfield BJ. The effects of rimonabant on brown adipose tissue in rat: Implications for energy expenditure. Obesity 2009;17:254–61. https://doi.org/10.1038/oby.2008.509.

[32] Quarta C, Bellocchio L, Mancini G, Mazza R, Cervino C, Braulke LJ, et al. CB1 Signaling in Forebrain and Sympathetic Neurons Is a Key Determinant of Endocannabinoid Actions on Energy Balance. Cell Metab 2010;11:273–85. https://doi.org/10.1016/j.cmet.2010.02.015.

[33] Cardinal P, Bellocchio L, Clark S, Cannich A, Klugmann M, Lutz B, et al. Hypothalamic CB1 cannabinoid receptors regulate energy balance in mice. Endocrinology 2012;153:4136–43. https://doi.org/10.1210/en.2012-1405.

[34] Cota D, Marsicano G, Tschöp M, Grübler Y, Flachskamm C, Schubert M, et al. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest 2003;112:423–31. https://doi.org/10.1172/JCI17725.

[35] Bensaid M, Gary-Bobo M, Esclangon A, Maffrand JP, Le Fur G, Oury-donat F, et al. The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. Mol Pharmacol 2003;63:908–14. https://doi.org/10.1124/mol.63.4.908.

[36] Boon MR, Kooijman S, Van Dam AD, Pelgrom LR, Berbée JFP, Visseren CAR, et al. Peripheral cannabinoid 1 receptor blockade activates brown adipose tissue and diminishes dyslipidemia and obesity. FASEB J 2014;28:5361–75. https://doi.org/10.1096/fj.13-247643.

[37] Ravinet Trillou C, Delgorge C, Menet C, Arnone M, Soubrié P. CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. Int J Obes Relat Metab Disord 2004;28:640–8. https://doi.org/10.1038/sj.ijo.0802583.

[38] Matias I, Di Marzo V. Endocannabinoids and the control of energy balance. Trends Endocrinol Metab 2007;18:27–37. https://doi.org/10.1016/j.tem.2006.11.006.

[39] Engeli S, Böhnke J, Feldpausch M, Gorzelniak K, Janke J, Bátkai S, et al. Activation of the peripheral endocannabinoid system in human obesity. Diabetes 2005;54:2838–43. https://doi.org/10.2337/diabetes.54.10.2838.

[40] Blüher M, Engeli S, Klöting N, Berndt J, Fasshauer M, Bátkai S, et al. Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. Diabetes 2006;55:3053–60. https://doi.org/10.2337/db06-0812.

[41] Alfaradhi MZ, Ozanne SE. Developmental Programming in Response to Maternal Overnutrition. Front Genet 2011;2. https://doi.org/10.3389/fgene.2011.00027.

[42] Dearden L, Ozanne SE. Early life origins of metabolic disease: Developmental programming of hypothalamic pathways controlling energy homeostasis. Front Neuroendocrinol 2015;39:3–16. https://doi.org/10.1016/j.yfrne.2015.08.001.

[43] Zambrano E, Nathanielsz PW. Mechanisms by which maternal obesity programs offspring for obesity: evidence from animal studies. Nutr Rev 2013;71:S42–54. https://doi.org/10.1111/nure.12068.

[44] Gregoire FM, Zhang Q, Smith SJ, Tong C, Ross D, Lopez H, et al. Diet-induced obesity and hepatic gene expression alterations in C57BL/6J and ICAM-1-deficient mice. Am J Physiol - Endocrinol Metab 2002;282. https://doi.org/10.1152/ajpendo.00072.2001.

[45] Kuipers EN, Kantae V, Maarse BCE, van den Berg SM, van Eenige R, Nahon KJ, et al. High Fat Diet Increases Circulating Endocannabinoids Accompanied by Increased Synthesis Enzymes in Adipose Tissue. Front Physiol 2018;9:1913. https://doi.org/10.3389/fphys.2018.01913.

[46] Correa F, Wolfson ML, Valchi P, Aisemberg J, Franchi AM. Endocannabinoid system and pregnancy. Reproduction 2016;152:R191–200. https://doi.org/10.1530/REP-16-0167.

[47] Wang H, Guo Y, Wang D, Kingsley PJ, Marnett LJ, Das SK, et al. Aberrant cannabinoid signaling impairs oviductal transport of embryos. Nat Med 2004;10:1074–80. https://doi.org/10.1038/nm1104.

[48] Paria BC, Song H, Wang X, Schmid PC, Krebsbach RJ, Schmid HHO, et al. Dysregulated Cannabinoid Signaling Disrupts Uterine Receptivity for Embryo Implantation. J Biol Chem 2001;276:20523–8. https://doi.org/10.1074/jbc.M100679200.

[49] Paria BC, Dey SK. Ligand-receptor signaling with endocannabinoids in preimplantation embryo development and implantation. Chem Phys Lipids 2000;108:211–20. https://doi.org/10.1016/S0009-3084(00)00197-3.

[50] Habayeb OMH, Taylor AH, Finney M, Evans MD, Konje JC. Plasma anandamide concentration and pregnancy outcome in women with threatened miscarriage. JAMA - J Am Med Assoc 2008;299:1135–6. https://doi.org/10.1001/jama.299.10.1135.

[51] Vercelli CA, Aisemberg J, Billi S, Cervini M, Ribeiro ML, Farina M, et al. Anandamide regulates lipopolysaccharide-induced nitric oxide synthesis and tissue damage in the murine uterus. Reprod Biomed Online 2009;18:824–31.

[52] Vercelli CA, Aisemberg J, Billi S, Wolfson ML, Franchi AM. Endocannabinoid system and nitric oxide are involved in the deleterious effects of lipopolysaccharide on murine decidua. Placenta 2009;30:579–84. https://doi.org/10.1016/j.placenta.2009.04.003.

[53] Vercelli C a., Aisemberg J, Cella M, Salazar AI, Wolfson ML, Franchi AM. Opposite effects of methanandamide on lipopolysaccharide-induced prostaglandin E2 and F2α synthesis in uterine explants from pregnant mice. PLoS One 2012;7. https://doi.org/10.1371/journal.pone.0039532.

[54] Fonseca BM, Correia-da-Silva G, Taylor AH, Konje JC, Bell SC, Teixeira N a. Spatio-temporal expression patterns of anandamide-binding receptors in rat implantation sites: evidence for a role of the endocannabinoid system during the period of placental development. Reprod Biol Endocrinol 2009;7:121. https://doi.org/10.1186/1477-7827-7-121.

[55] Fonseca BM, Correia-Da-Silva G, Almada M, Costa M a., Teixeira N a. The endocannabinoid system in the postimplantation period: A role during decidualization and placentation. Int J Endocrinol 2013;2013. https://doi.org/10.1155/2013/510540.

[56] Wolfson ML, Correa F, Leishman E, Vercelli C, Cymeryng C, Blanco J, et al. Lipopolysaccharide-induced murine embryonic resorption involves changes in endocannabinoid profiling and alters progesterone secretion and inflammatory response by a CB1-mediated fashion. Mol Cell Endocrinol 2015;411:214–22. https://doi.org/10.1016/j.mce.2015.04.032.

[57] Bariani M V., Correa F, Domínguez Rubio AP, Marvaldi C, Schander JA, Beltrame JS, et al. Maternal obesogenic diet combined with postnatal exposure to high-fat diet induces metabolic alterations in offspring. J Cell Physiol 2020:jcp.29482. https://doi.org/10.1002/jcp.29482.

[58] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.

[59] Paria BC, Deutsch DD, Dey SK. The uterus is a potential site for anandamide synthesis and hydrolysis: differential profiles of anandamide synthase and hydrolase activities in the mouse uterus during the periimplantation period. Mol Reprod Dev 1996;45:183–92. https://doi.org/10.1002/(SICI)1098-2795(199610)45:2<183::AID-MRD11>3.0.CO;2-2.

[60] Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, et al. The endogenous cannabinoid system controls extinction of aversive memories. Nature 2002;418:530–4. https://doi.org/10.1038/nature00839.

[61] Kamimae-Lanning AN, Krasnow SM, Goloviznina NA, Zhu X, Roth-Carter QR, Levasseur PR, et al. Maternal high-fat diet and obesity compromise fetal hematopoiesis. Mol Metab 2015;4:25–38. https://doi.org/10.1016/j.molmet.2014.11.001.

[62] Pellizzon MA, Ricci MR. The common use of improper control diets in diet-induced metabolic disease research confounds data interpretation: The fiber factor. Nutr Metab 2018;15. https://doi.org/10.1186/s12986-018-0243-5.

[63] Bariani MV, Domínguez Rubio AP, Cella M, Burdet J, Franchi AM, Aisemberg J. Role of the endocannabinoid system in the mechanisms involved in the LPS-induced preterm labor. Reproduction 2015;150:463–72. https://doi.org/10.1530/REP-15-0211.

[64] Miranda K, Mehrpouya-Bahrami P, Nagarkatti PS, Nagarkatti M. Cannabinoid Receptor 1 Blockade Attenuates Obesity and Adipose Tissue Type 1 Inflammation Through miR-30e-5p Regulation of Delta-Like-4 in Macrophages and Consequently Downregulation of Th1 Cells. Front Immunol 2019;10. https://doi.org/10.3389/fimmu.2019.01049.

[65] Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027–31. https://doi.org/10.1038/nature05414.

[66] Turnbaugh P, Backhed F, Fulton L, Gordon J. Marked alterations in the distal gut microbiome linked to diet-induced obesity. Cell Host … 2008;3:213–23. https://doi.org/10.1016/j.chom.2008.02.015.Marked.

[67] Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761–72. https://doi.org/10.2337/db06-1491.

[68] Cani PD, Possemiers S, Van De Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009;58:1091–103. https://doi.org/10.1136/gut.2008.165886.

[69] Ghoshal S, Witta J, Zhong J, de Villiers W, Eckhardt E. Chylomicrons promote intestinal absorption of lipopolysaccharides. J Lipid Res 2009;50:90–7. https://doi.org/10.1194/jlr.M800156-JLR200.

[70] Izzo AA, Piscitelli F, Capasso R, Aviello G, Romano B, Borrelli F, et al. Peripheral endocannabinoid dysregulation in obesity: Relation to intestinal motility and energy processing induced by food deprivation and re-feeding. Br J Pharmacol 2009;158:451–61. https://doi.org/10.1111/j.1476-5381.2009.00183.x.

[71] Mehrpouya-Bahrami P, Chitrala KN, Ganewatta MS, Tang C, Murphy EA, Enos RT, et al. Blockade of CB1 cannabinoid receptor alters gut microbiota and attenuates inflammation and diet-induced obesity. Sci Rep 2017;7. https://doi.org/10.1038/s41598-017-15154-6.

[72] Deveaux V, Cadoudal T, Ichigotani Y, Teixeira-Clerc F, Louvet A, Manin S, et al. Cannabinoid CB2 receptor potentiates obesity-associated inflammation, insulin resistance and hepatic steatosis. PLoS One 2009;4:e5844. https://doi.org/10.1371/journal.pone.0005844.

[73] Wright KL, Duncan M, Sharkey KA. Cannabinoid CB 2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation. Br J Pharmacol 2008;153:263–70. https://doi.org/10.1038/sj.bjp.0707486.

[74] Aguilera M, Vergara P, Martínez V. Stress and antibiotics alter luminal and wall-adhered microbiota and enhance the local expression of visceral sensory-related systems in mice. Neurogastroenterol Motil 2013;25:e515–29. https://doi.org/10.1111/nmo.12154.

[75] Chang E, Hafner H, Varghese M, Griffin C, Clemente J, Islam M, et al. Programming effects of maternal and gestational obesity on offspring metabolism and metabolic inflammation. Sci Rep 2019;9. https://doi.org/10.1038/s41598-019-52583-x.

[76] Thompson MD, Cismowski MJ, Trask AJ, Lallier SW, Graf AE, Rogers LK, et al. Enhanced Steatosis and Fibrosis in Liver of Adult Offspring Exposed to Maternal High-Fat Diet. Gene Expr 2016;17:47–59. https://doi.org/10.3727/105221616X692135.

[77] Gabbay E, Avraham Y, Ilan Y, Israeli E, Berry EM. Endocannabinoids and liver disease - review. Liver Int 2005;25:921–6. https://doi.org/10.1111/j.1478-3231.2005.01180.x.

[78] Deutsch DG, Ueda N, Yamamoto S. The fatty acid amide hydrolase (FAAH). Prostaglandins, Leukot Essent Fat Acids 2002;66:201–10. https://doi.org/10.1054/plef.2001.0358.

[79] Guzmán M, Fernández-Ruiz JJ, Sánchez C, Velasco G, Ramos JA. Effects of anandamide on hepatic fatty acid metabolism. Biochem Pharmacol 1995;50:885–8. https://doi.org/10.1016/0006-2952(95)00198-9.

[80] Franco JG, Fernandes TP, Rocha CPD, Calviño C, Pazos-Moura CC, Lisboa PC, et al. Maternal high-fat diet induces obesity and adrenal and thyroid dysfunction in male rat offspring at weaning. J Physiol 2012;590:5503–18. https://doi.org/10.1113/jphysiol.2012.240655.

[81] de Oliveira JC, Gomes RM, Miranda RA, Barella LF, Malta A, Martins IP, et al. Protein Restriction During the Last Third of Pregnancy Malprograms the Neuroendocrine Axes to Induce Metabolic Syndrome in Adult Male Rat Offspring. Endocrinology 2016;157:1799–812. https://doi.org/10.1210/en.2015-1883.

[82] M.M. A, C.P. D-R, C.F. R-G, H. W, G.C. A, A. C, et al. Maternal high-fat diet impairs leptin signaling and up-regulates type-1 cannabinoid receptor with sex-specific epigenetic changes in the hypothalamus of newborn rats. Psychoneuroendocrinology 2019;103:306–15. https://doi.org/10.1016/j.psyneuen.2019.02.004 LK - http://huji-primo.hosted.exlibrisgroup.com/openurl/972HUJI/972HUJI_SP?sid=EMBASE&sid=EMBASE&issn=18733360&id=doi:10.1016%2Fj.psyneuen.2019.02.004&atitle=Maternal+high-fat+diet+impairs+leptin+signaling+and+up-regulates+type-1+cannabinoid+receptor+with+sex-specific+epigenetic+changes+in+the+hypothalamus+of+newborn+rats&stitle=Psychoneuroendocrinology&title=Psychoneuroendocrinology&volume=103&issue=&spage=306&epage=315&aulast=Almeida&aufirst=Mariana+M.&auinit=M.

[83] Gaillard R. Maternal obesity during pregnancy and cardiovascular development and disease in the offspring. Eur J Epidemiol 2015;30:1141–52. https://doi.org/10.1007/s10654-015-0085-7.

[84] Vyas AK, Veiga-Lopez A, Ye W, Abi Salloum B, Abbott DH, Yang S, et al. Developmental programming: Sex-specific programming of growth upon prenatal bisphenol A exposure. J Appl Toxicol 2019:jat.3836. https://doi.org/10.1002/jat.3836.

[85] Dudele A, Hougaard KS, Kjølby M, Hokland M, Winther G, Elfving B, et al. Chronic maternal inflammation or high-fat-feeding programs offspring obesity in a sex-dependent manner. Int J Obes 2017;41:1420–6. https://doi.org/10.1038/ijo.2017.136.

[86] Dahlhoff M, Pfister S, Blutke A, Rozman J, Klingenspor M, Deutsch MJ, et al. Peri-conceptional obesogenic exposure induces sex-specific programming of disease susceptibilities in adult mouse offspring. Biochim Biophys Acta - Mol Basis Dis 2014;1842:304–17. https://doi.org/10.1016/j.bbadis.2013.11.021.

[87] Martin GG, Chung S, Landrock D, Landrock KK, Huang H, Dangott LJ, et al. FABP-1 gene ablation impacts brain endocannabinoid system in male mice. J Neurochem 2016;138:407–22. https://doi.org/10.1111/jnc.13664.

[88] Martin GG, Chung S, Landrock D, Landrock KK, Dangott LJ, Peng X, et al. Female Mice are Resistant to Fabp1 Gene Ablation-Induced Alterations in Brain Endocannabinoid Levels. Lipids 2016;51:1007–20. https://doi.org/10.1007/s11745-016-4175-4.

[89] Rubino T, Parolaro D. Sexually Dimorphic Effects of Cannabinoid Compounds on Emotion and Cognition. Front Behav Neurosci 2011;5. https://doi.org/10.3389/fnbeh.2011.00064.

[90] Martin GG, Landrock D, Chung S, Dangott LJ, McIntosh AL, Mackie JT, et al. Loss of fatty acid binding protein-1 alters the hepatic endocannabinoid system response to a high-fat diet. J Lipid Res 2017;58:2114–26. https://doi.org/10.1194/jlr.M077891.

[91] Martin GG, Landrock D, Chung S, Dangott LJ, Seeger DR, Murphy EJ, et al. Fabp1 gene ablation inhibits high-fat diet-induced increase in brain endocannabinoids. J Neurochem 2017;140:294–306. https://doi.org/10.1111/jnc.13890.

## FIGURE AND TABLE LEGENDS

**Figure 1. Model of high fat diet-induced obesity in CB1 KO mice. A)** Body weight of CB1 KO mice fed with CD or HFD during 5 months (n=7). Two-way ANOVA with repeated measures ($p < 0.05$). Means with different letters are significantly different (Interaction between time and diet). **B)** Food intake of CB1 KO mice fed with CD or HFD during 3 months by grams per day (n=7). Two-way ANOVA with repeated measures ($p < 0.05$). Means with different capital letters are significantly different between times and means with different lower-case letters are significantly different between diets. **C)** Food intake of CB1 KO mice fed with CD or HFD for 3 months by kilojoules per day (n=7). Two-way ANOVA with repeated measures ($p < 0.05$). Means with different capital letter are significantly different between times. **D)** OGTT of CB1 KO mice (n=7). Two-way ANOVA with repeated measures ($p < 0.05$). Means with different capital letters are significantly different between times. **E)** Weight of abdominal, gonadal, and retroperitoneal adipose tissue and spleen of CB1 KO mice (n=7) and representative images of abdominal adipose tissue accumulation. **F)** Hepatic FAAH activity of CB1 KO mice (n=6). Measurement in **D) E)** and **F)** were performed after 3 months of feeding with CD or HFD. One-way ANOVA ($p < 0.05$). Means with different letters are significantly different. Data are shown as mean ± S.E.M. WT: wild

type mice. CB1 KO: cannabinoid receptor 1 *knock-out* mice. CD: control diet. HFD: high-fat diet. pnd: postnatal day. AA: arachidonic acid.

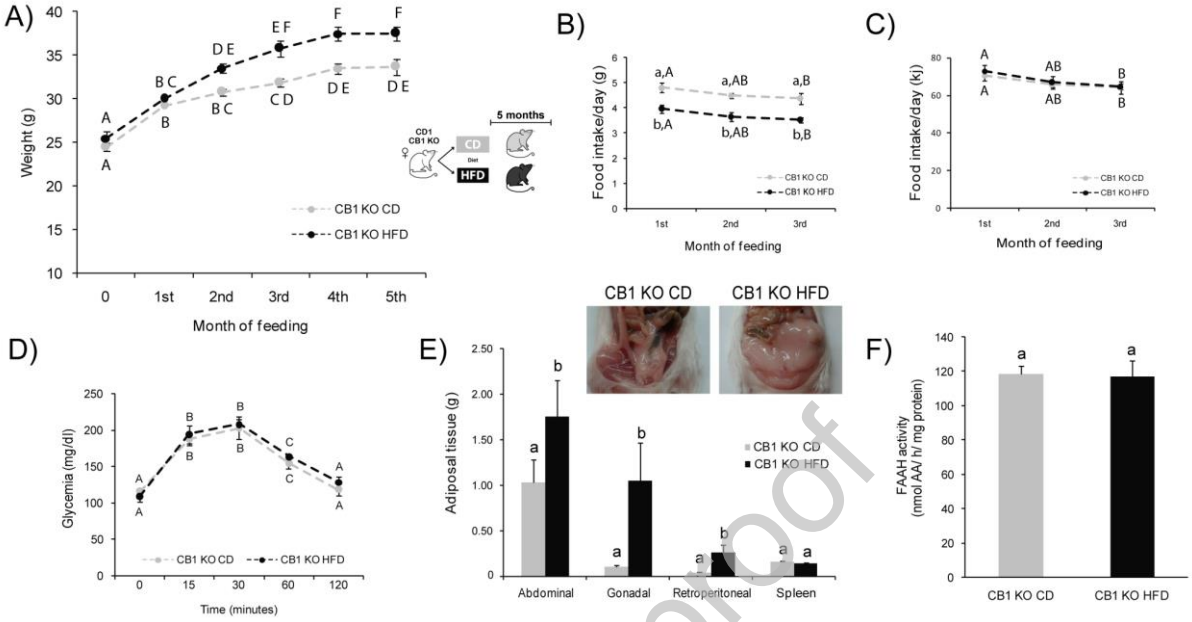



**Figure 2. Effect of maternal obesity and inflammation on pregnancy. A)** Body weight of CB1 KO mice fed with CD or HFD during pregnancy (n=6). Data are shown as mean ± S.E.M. Two-way ANOVA with repeated measures ($p < 0.05$). Means with different letters are significantly different. **B)** Percentages of term labor and pregnancy complications after an LPS injection (i.p., 0.05 µg/g body weight) at gestational day 15 (WT CD n = 11, WT HFD n =8, KO CD n =9, KO HFD n =6). *Indicates statistically significant differences ($p < 0.05$), Fisher test. WT: wild type mice. CB1 KO: cannabinoid receptor 1 *knock-out* mice. CD: control diet. HFD: high-fat diet. pnd: postnatal day. n: total sample size.

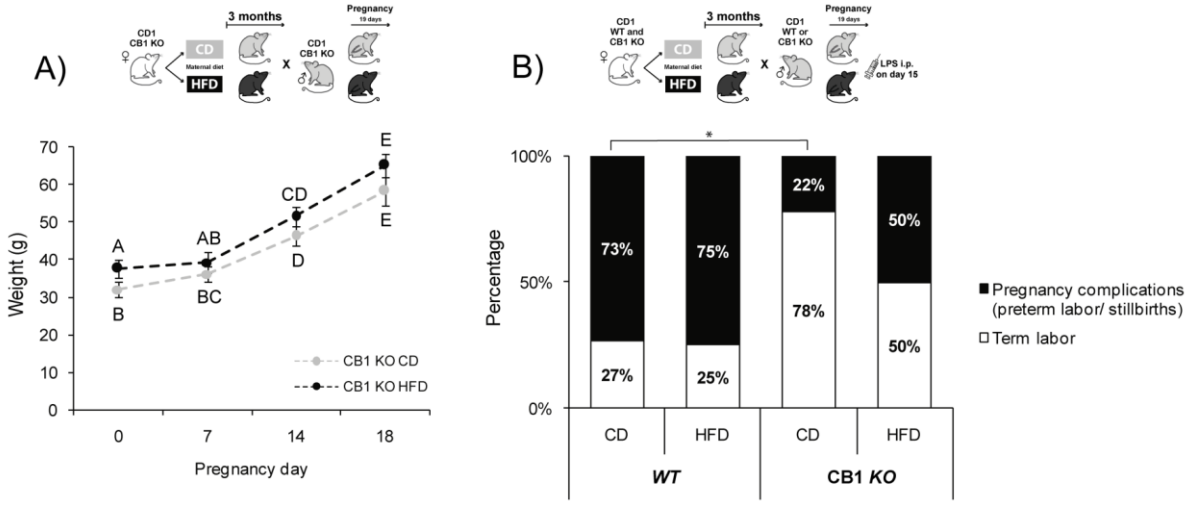



**Figure 3. Effect of maternal obesity on offspring weight and on hepatic FAAH protein levels and activity. A)** Body weight on postnatal days 1, 7, 14, and 21 from offspring born to control or obese CB1 KO mothers (n=8 dams per group). Data are shown as mean ± S.E.M. Two-way ANOVA with repeated measures ($p < 0.05$). Means with different capital letters are significantly different between times and means with different lower-case letters are significantly different between. **B)** Representative gel and densitometry analysis of western blot of FAAH protein (n=4) and **C)** FAAH activity on livers from postnatal day 1 offspring born to control or obese CB1 KO mothers (n=6 animals per group). Data are shown as mean ± S.E.M. One-way ANOVA ($p < 0.05$). Means with different letters are significantly different. CB1 KO: cannabinoid receptor 1 *knock-out* mice. CD: control diet. HFD: high-fat diet. pnd: postnatal day. AA: arachidonic acid.

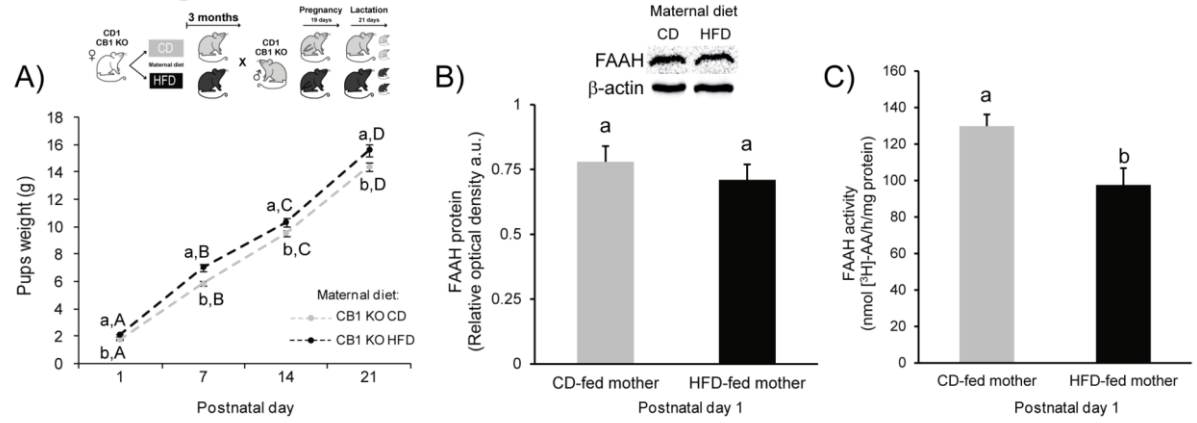
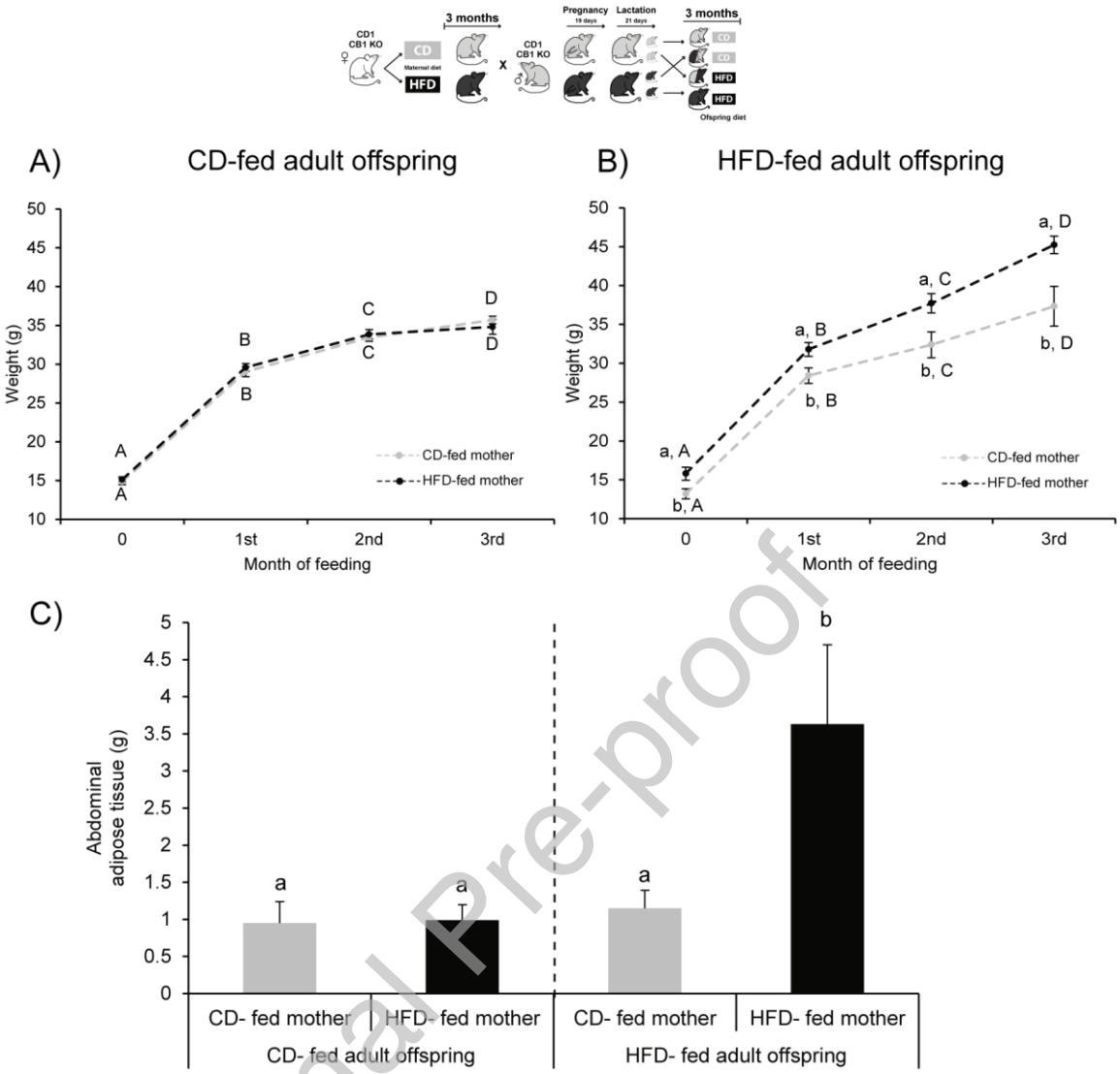


**Figure 4. Effect of maternal obesity on CB1 KO adult offspring fed with CD or HFD for 3 months. A)** Body weight of CD-fed adult offspring born to control or obese CB1 KO mothers (n=6 dams per group). Data are shown as mean ± S.E.M. Two-way ANOVA with repeated measures ($p < 0.05$). Means with different capital letters are significantly different between times. **B)** Body weight from HFD-fed adult offspring born to control or obese CB1 KO mothers (n=6 dams per group). Data are shown as mean ± S.E.M. Two-way ANOVA with repeated measures ($p < 0.05$). Means with different capital letters are significantly different between times and means with different lower-case letters are significantly different between diets. **C)** Weight of abdominal adipose tissue from CB1 KO adult offspring (born to control or obese CB1 KO mothers) after 3 months of being fed with CD (n=5) or HFD (n=6). Data are shown as mean ± S.E.M. One-way ANOVA ($p < 0.05$). Means with different letters are significantly different. CB1 KO: cannabinoid receptor 1 *knock-out* mice. CD: control diet. HFD: high-fat diet. pnd: postnatal day.




**TABLES**

| | | Blood parameter | | |
|---|---|---|---|---|
| | | **Glucose** | **Triglycerides** | **Cholesterol** |
| Female CB1 KO mice | **CD** | 114.3 ± 8.5 | 83.6 ± 7.7 | 79.4 ± 5.5 [a] |
| | **HFD** | 127.8 ± 5.9 | 99.1 ± 7.5 | 109.8 ± 8.7 [b] |

**Table 1. Blood metabolic parameters measured in CB1 KO mice fed with CD or HFD for 3 months.** Blood glucose, triglycerides, and total cholesterol levels (n=7). Data are shown as mean ± S.E.M. One-way ANOVA ($p < 0.05$). Means with different letters are significantly

different. CB1 KO: cannabinoid receptor 1 *knock-out* mice. CD: control diet. HFD: high-fat diet.

| | Maternal diet | Blood parameter | | |
|---|---|---|---|---|
| | | **Glucose** | **Triglycerides** | **Cholesterol** |
| Postnatal day 1 offspring | **CD** | 73.8 ± 4.8 | 119.4 ± 8.9 | 65.4 ± 2.1 [a] |
| | **HFD** | 81.0 ± 2.0 | 151.4 ± 20.1 | 79.8 ± 2.6 [b] |

**Table 2. Blood metabolic parameters measured in CB1 KO pups on postnatal day 1 born to control or obese mothers.** Blood glucose, triglycerides, and total cholesterol levels (n=8 dams per group). Data are shown as mean ± S.E.M. One-way ANOVA ($p < 0.05$). Means with different letters are significantly different. CB1 KO: cannabinoid receptor 1 *knock-out* mice. CD: control diet. HFD: high-fat diet.

| | | Maternal diet | Blood parameter | | |
|---|---|---|---|---|---|
| | | | **Glucose** | **Triglycerides** | **Cholesterol** |
| **A** | CD-fed adult offspring | **CD** | 120.7 ± 10.7 | 81.1 ± 8.4 | 95.1 ± 11.7 |
| | | **HFD** | 114.2 ± 6.3 | 75.0 ± 5.2 | 94.1 ± 8.7 |
| **B** | HFD-fed adult offspring | **CD** | 130.6 ± 8.6 | 133 ± 12 | 104.0 ± 4.9 [a] |
| | | **HFD** | 123.2 ± 5.2 | 138.4 ± 12.3 | 133.8 ± 4.1[b] |

**Table 3. Blood metabolic parameters measured in adult offspring.** Blood glucose, triglycerides, and total cholesterol levels from mice born from control or obese mothers after 3 months of being fed with **A)** CD (n=6 dams) and **B)** HFD (n=6 dams). Data are shown as mean ± S.E.M. One-way ANOVA ($p < 0.05$). Means with different letters are significantly different. CB1 KO: cannabinoid receptor 1 *knock-out* mice. CD: control diet. HFD: high-fat diet.