BABOON PLACENTAL ENDOCANNABINOID RESPONSES TO MATERNAL HIGH FAT DIET

TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER™ *at the* Permian Basin

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Introduction

Maternal obesity (MO) affects fetal development, which in turn, influences individual's life trajectory. Specifically, MO has been linked to autism spectrum disorders, inflammatory bowel syndrome, fibromyalgia, asthma, non-alcoholic fatty liver diseases (NAFLD), diabetes and obesity in the offspring. While the suggested mechanisms, linking obesity to the offspring health, are varying from the oxidative stress, inflammation, fatty acid transport and stress-related pathways, the common mechanisms remain obscure. Endogenous cannabinoid (eCB) system (ECS) is involved in the appetite regulation and lipogenesis (Cota et al., 2003). While the spectrum of offspring health conditions, programmed by MO, is broad, it could be brought under the umbrella of Clinical Endocannabinoid deficiency syndrome (CECDS), described by *Russo* in 2004 (Russo, 2004; Schlabritz-Loutsevitch, German, Ventolini, Larumbe, & Samson, 2016).

ECS is the family of the biologically active lipids – derivatives of omega-3 fatty acids, which regulate vascular tone, metabolic rate, neurogenesis, inflammatory and stress responses – all hallmarks of MO. We recently suggested the existence of Fetal Syndrome of Endocannabinoid deficiency in MO, based on the preliminary data, obtained in human population (Schlabritz-Loutsevitch et al., 2016) and in natural model of obesity in the baboons (*Papio spp.)*(B. Brocato et al., 2013). The different patterns of MO: pre-pregnancy obesity vs. pregnancy-related weight gain, over-eating vs high-fat –high calorie diet (HFD)-are making the studies of the mechanism of developmental programming by MO challenging. Animal models represent the opportunity to dissect specific mechanisms of the dietary patterns and provide important data for development of interventional strategies in humans.

Objective

To evaluate placental expression of ECS receptors CB1R and CB2R in an experimental model of a maternal high fat diet.

Materials and Methods

Baboons (*Papio spp*) were fed a diet of 45% fat, called high fat diet (HFD) while controls (CTR) ate a 12% fat diet from at least 9 months prior to conception through pregnancy until 0.9 gestation. Eleven HF and nine CTR placental samples, from male and female fetuses, were evaluated using immunostaining. Commercially available CB1R (CB1 monoclonal primary antibody, Immunogenes; Budakeszi, Hungary. Cat# Img-CB1r-mab001) and CB2R (CB2 mouse monoclonal primary antibody, Novus Biologicals; Littleton, Co, USA. Cat# H00001269-M01) antibodies were applied for immunohistochemistry, and the secondary antibody was included in the Vectastain ABC kit (Vector laboratories; Burlingame, CA. Cat# PK 4002). The slides were scanned using the NanoZoomer SQ (Hamamatsu; Middlesex, NJ), quantification was performed using ImageScope™ v11.1.2.752 by Aperio (Leica Biosystems; Buffalo Grove, IL).

Western blot was also performed with the same primary antibodies, secondary antibody (Jackson Immuno Research Laboratories, Inc. USA. Cat. #: 715-035-150), and ß-Actin antibody (Monoclonal Anti ß-Actin peroxidase antibody clone AC-15. St. Louis, MO, USA. Cat# A3854). The dilution used for the CB1 primary antibody was 1:1000 in 5% BSA (3 hour incubation at 40C), for the CB2 primary antibody 1:2000 in 5% BSA (3 hour incubation at 40C), and ß-Actin primary antibody 1:20,000 in 5% BSA.

The TRIzol method was used to isolate RNA from tissue samples (Life Technologies, USA), and cDNA was synthesized according to the manufacturer's instructions (Applied Biosystems/ Roche, USA). qPCR was performed using Fast start Essential DNA Probe Master Mix (Roche, USA), and TaqMan Gene Expression Assay Probes (Life Technology, USA) for CB1. The TaqMan probes used were CB1 (Hs01038522). The housekeeping gene used for these genes was 18S (Hs99999901). The CB2 gene was detected using FastStart Essential DNA Green Master Mix (Roche, USA).

References

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Results

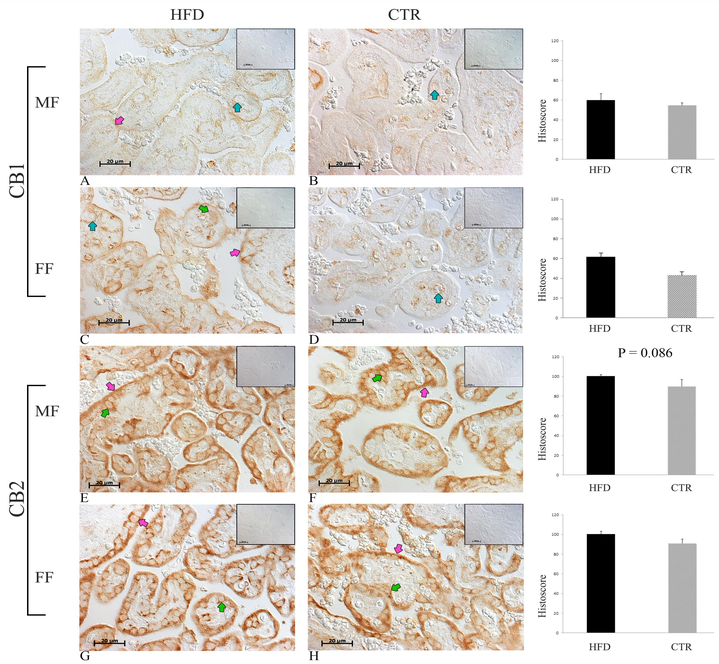

Figure 1. Protein expression level of CB1R and CB2R using IHC in placenta samples. A and B panels show the placenta sections from male fetuses in CTR and HFD animals. C and D panels show the placenta sections from female fetus in CTR and HFD animals. E and F panels show the placenta sections from male fetuses in CTR and HFD animals. G and H panels show the placenta sections from female fetuses in HFD and CTR animals. In all panels blue arrows point to fetal endothelium, green arrows point to Cytotrophoblast, and pink arrows point to Syncytiotrophoblast. Negative control figures are shown in small boxes in all panels (upper right corner). I to L panels show the quantification of IHC figures in HFD and CTR groups. The figures were taken using a 20 X magnification. Scale bar, 20 µm. Data were represented as the mean ± SEM.

[CTR: Control; HFD: High-Fat Diet; MF: Male Fetus; FF: Female Fetus; IHC: Immunohistochemistry]

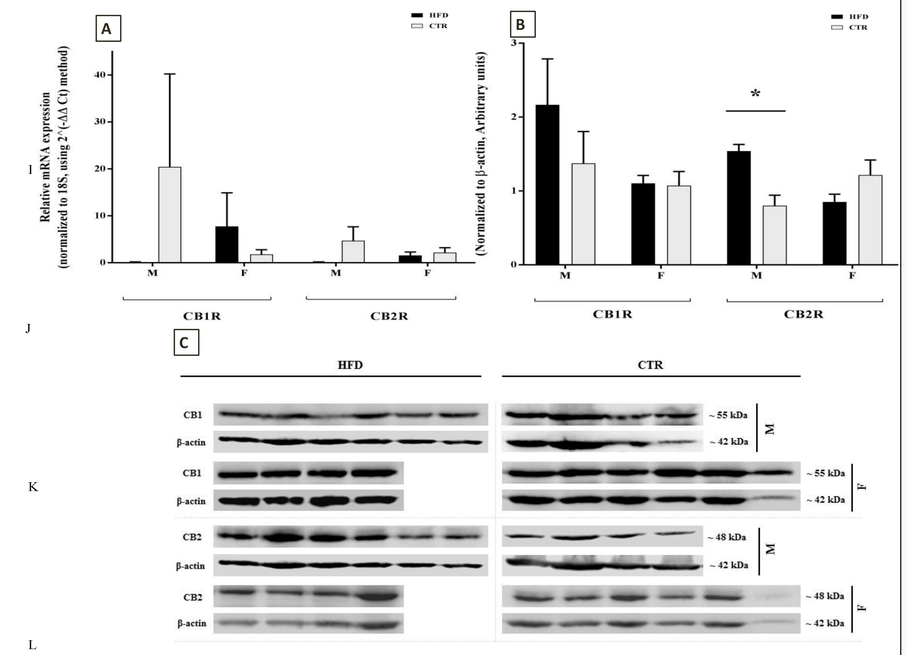

Figure 2. A) Expression of mRNA for cannabinoid receptors (CB1 and CB2) by Reverse Transcription Real Time quantitative PCR analysis (q RT-PCR) using specific primers, in maternal liver (n = 3 to 6), fetal liver (n = 4 to 6), and placenta (n = 4 to 6). Results were shown as fold change (2^{-}(-ΔΔCt) method), normalized to levels of control 18S mRNA expression. Data were represented as the mean ± SEM.

B) Protein expression for cannabinoid receptors (CB1 and CB2) by western blot (n = 4 to 6). Graphs depict relative band intensity, quantified using Image J software, and normalized to control ß-actin expression. Data were represented as mean ± SEM, $p < 0.05$ was considered as statistically significant difference between the groups (*$p < 0.05$).

C) Representative images of western blot analysis of CB1R and CB2R expressions detected in placenta (n = 4 to 6).

Discussion and Conclusion

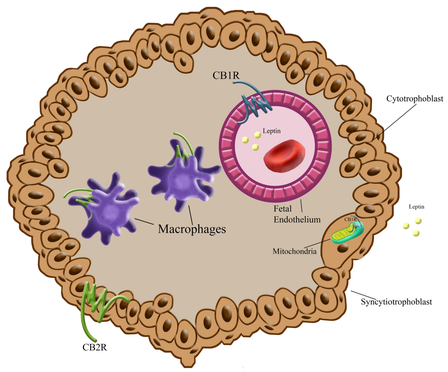

Figure 3. Graphic representation of a transverse section of a chorionic villus exposed to a control diet.

Our finding regarding increased CB2 expression in the placenta of animals fed HFD is in agreement with our previous observation in the placenta of naturally obese baboons (Brocato, B., et al., 2013) and placental macrophages infiltration in obesity (Challier et al., 2008).

The endothelial dysfunction is the hallmark of pregnancies complicated by maternal obesity and excessive gestational weight gain (Pardo et al., 2015; Spradley, Palei, & Granger, 2015). Location of CB1R in the fetal placental endothelium is in agreement with previously published data in the baboon (B. Brocato et al., 2013) and human placenta (Fugedi et al., 2014). In the present study the expression of CB1R was mostly located in the fetal vascular endothelium of CTR animals and maternal feeding with the HFD shifted this expression toward ST.

Endothelium-dependent CB1R-mediated vascular relaxation is the phenomenon described in mesenteric arteries (Stanley, Hind, Tufarelli, & O'Sullivan, 2016). The CB1R expression in the outer layer of the ST might be associated with increased Lipoprotein lipase activity in the ST in maternal obesity (Qiao et al., 2015), since LPL activity is regulated by eCBs through CB1R related mechanism (Cota et al., 2003). Thus in HFD eCB effect could be shifted from vascular to metabolic responses.

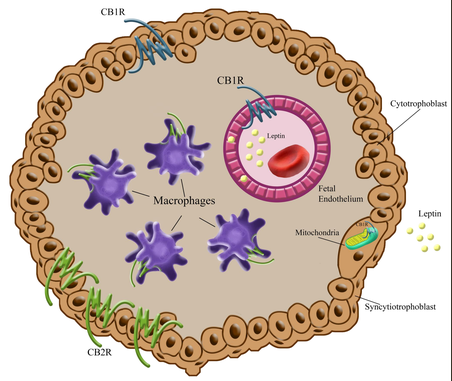

Figure 4. Graphic representation of a transverse section of a chorionic villus exposed to a high fat diet.

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