Introduction

Maternal obesity (MO) affects fetal development, which in turn, influences individuals’s life trajectory. Schematically, MO has been linked to increases in maternal gestational diabetes mellitus, hypertensive disorders of pregnancy, fibromyalgia, asthma, non-alcoholic fatty liver diseases (NAFLD), diabetes and obesity in the offspring. While increased maternal body mass index (BMI) is a risk factor for these adverse outcomes, the mechanistic pathways 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 (CB) 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 (CECS), described by Russo in 2004 (Russo, 2004, Schlabritz-Loutevitch, German, Ventolini, Lombar, & 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 populations (Loutsevitch et al., 2016) and in normal model of obesity in the baboons (Pappio 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.

Materials and Methods

Baboons (Pappio 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 6.9 gestation. Eleven HF and nine CTR placental samples, from male and female fetuses, were evaluated using immunohistology. Commercially available antibodies to CB1 (CB1 monoclonal primary antibody, Immunogen, Budeksee, Hungary), CBM (Img-Cbr-mab001) and CB2 (CB2 mouse monoclonal primary antibody, Novus Biologicals); Littleton, CO, USA. CbrM (M0001246-M01) antibodies were applied for immunofluorescent staining, 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, Japan). Digital images were acquired using ImageScope (v11.2.752 by Perpico (Leica Biosystems; Buffalo Grove, IL).

Western blot was also performed with the same primary antibodies, secondary antibody (Jackson Immuno Research Laboratories, Inc.), and anti-β-Actin antibody (Cell Signaling Technology). The β-Actin antibody was used to show the loading of the samples. The images were analyzed using the Image J program (National Institutes of Health). The western blot was repeated for two independent experiments.

Figure 1. Protein expression level of CB1 and CB2 using IHC in placental 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 fetuses in CTR and HFD animals. E and F panels show the placenta sections from male fetuses in CTR and HFD animals. Graphs depict relative band intensity of CB1 and CB2 protein expression (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 mean ± SD.

Discussion and Conclusion

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 (Challier 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, Pain, & Granger, 2015). Location of CB1R in the fetal placental endothelium is in agreement with previously published data in the baboon (B. Brocata 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, Hendy, Tufarelli, & O’Sullivan, 2016). The CB1R expression in the outer layer of the ST might be associated with increased L-arginine l-ipoasipate activity in the ST in maternal obesity (Saal 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.

References