MATERNAL AND FETAL HEPATIC ENDOGENOUS CANNABINOIDS RESPONSE TO MATERNAL HIGH FAT DIET

Kushal Gandhi, PhD[1], Cun Li, MD[2, 3], Marcel Chuecos, BS[1], Maira Carrillo, PhD[1], Stacy Martinez, MS[1], Charles Burns, MD[4], Moss Hampton, MD[1], Peter Nathanielsz, MD, PhD [2, 3], and Natalia Schlabritz-Lutsevich, MD, PhD [1]

[1] Department of Obstetrics and Gynecology, Texas Tech University Health Sciences Center at the Permian Basin, Odessa, TX, USA
[2] Animal Science, University of Wyoming, Laramie, Wyoming, USA
[3] Exp. Physiology, SNPRC, San Antonio, TX, USA
[4] Department of Pathology, Medical Center Hospital, Odessa, TX, USA

INTRODUCTION

- Fatty liver disease is the major cause of liver transplantation in 21st century.
- **Maternal BMI:** influences fetal liver and brain development and is the strongest predictor of fetal fat accumulation and offspring adiposity (PMID: 24740157).
- **Maternal obesity (MO):** links to autism spectrum disorders, non-alcoholic fatty liver diseases (NAFLD), diabetes and other life-threatening diseases.
- **Endogenous cannabinoid (eCB) system (ECS):** family of the biologically active lipids – derivatives of omega-3 fatty acids, which regulate vascular tone, metabolic rate, inflammatory and stress responses – all hallmarks of MO and play vital role in obesity, appetite regulation and lipogenesis (Fig. 1).

Information regarding eCBs physiology in obesity, associated with pregnancy, is sparse. The different patterns of maternal obesity: pre-pregnancy obesity vs. pregnancy-related weight gain, over-eating vs. high - fat - high calorie diet make studies of mechanisms of developmental programming challenging. Animal models represent an opportunity to dissect specific mechanisms associated with dietary patterns and provide important data for development of interventional strategies in humans.

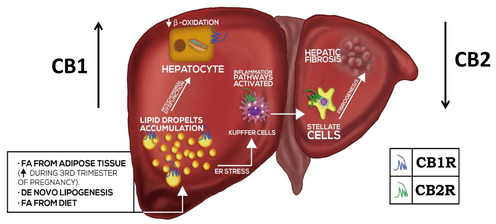

Figure 1: Endocannabinoid pathway in the pathogenesis of NAFLD (FA = Fatty Acid)

OBJECTIVE

To describe the influence of a maternal high fat diet on the feto-maternal hepatic axis in baboon (*Papio* spp) model of obesity.

MATERIALS AND METHODS

Baboons (*Papio* spp) were fed a diet of 45% fat (HFD, n=11), while controls (CTR, n=9) ate 12% fat from at least 9 months prior to conception. Fetal and maternal serums samples were collected at term Cesarean Section. qRT-PCR, immunohistochemistry (IHC) and western blot analysis were performed to quantify expression of CB1 and CB2 receptors and the central enzyme regulating eCBs tone - Fatty Acid Amid Hydrolase (FAAH) in maternal and fetal liver. Data were presented as mean ± SEM (Standard Error of Mean) and statistical analysis was performed using Mann-Whitney test.

RESULTS

Fetal and maternal hepatic gene expression for CB1R, CB2R and FAAH did not differ between two groups. CB1R protein expression (full length receptor, ~ 55 kDa), was increased in HFD dams, carrying male fetuses by 40% and decreased in their fetuses by 75%. Full length hepatic CB1R was not detectable in the HFD female fetuses and their mothers (Fig. 2 and Fig. 3). Maternal hepatic CB2R and FAAH protein expressions did not differ between groups. Fetal hepatic expression of CB2R and FAAH were decreased in both – HFD male and female fetuses compared to CTR (Fig. 4 and Fig. 5).

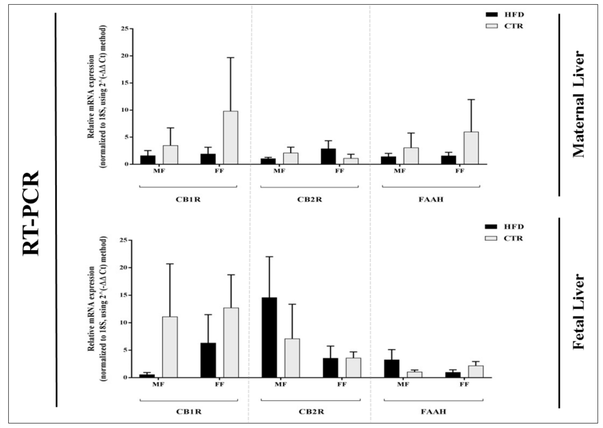

Figure 2: mRNA expression of CB1R, CB2R and FAAH in the maternal and fetal livers of HFD and CTR animals.
Analysis of the mRNA expression levels of cannabinoid receptors (*CB1R* and *CB2R*) and fatty acid amide hydrolase (FAAH) by reverse transcription real-time quantitative PCR (qRT-PCR) using specific primers in the maternal liver (n=3 to 6) and fetal liver (n=4 to 6). The results are shown as ($2^{-\Delta\Delta Ct}$), normalized to levels of control 18S mRNA expression.

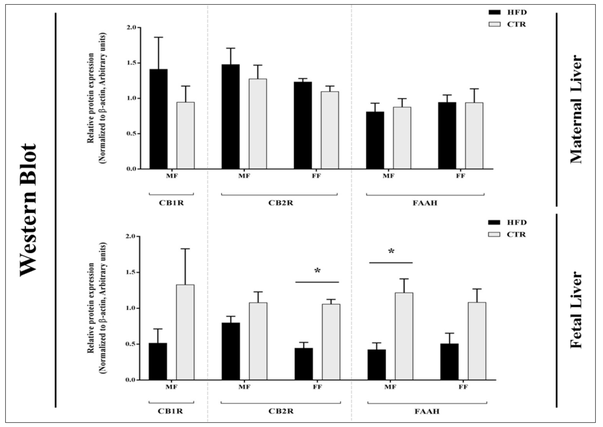

Figure 3: Protein expression of CB1R, CB2R and FAAH in the maternal and fetal livers of HFD and CTR animals.
Protein expression levels of cannabinoid receptors (CB1R and CB2R) and FAAH were analyzed by Western Blot in the maternal liver (n=3 to 6) and fetal liver (n = 4 to 5). The bar diagram shows relative band intensity, quantified using ImageJ software, normalized to control β-actin expression. $p < 0.05$ indicates a significant difference between groups. (*$p < 0.05$)

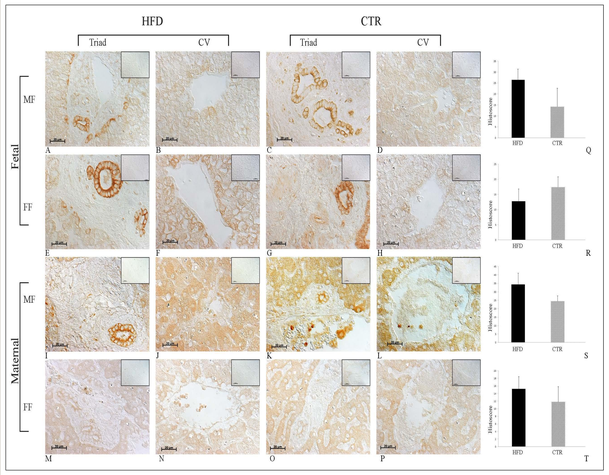

Figure 4: CB1R protein expression in the maternal and fetal livers of HFD (n=11) and CTR (n=10) animals.
CB1R protein expression in the maternal and fetal livers of HFD (n=11) and CTR (n=10) animals. **A to D:** Representative images of fetal livers from male fetuses in the CTR (n=3) and HFD (n=6) groups; **Q:** quantitative analyses. **E to H:** The fetal livers from female fetuses in the CTR (n=6) and HFD (n=3) groups; **R:** quantitative analyses. **I to L:** Maternal livers of dams with male fetuses in the CTR (n=4) and HFD (n=5) groups; **S:** quantitative analyses. **M to P:** Maternal liver sections from dams carrying female fetuses in the CTR (n=5) and HFD (n=4) groups; **T:** quantitative analyses.
[**Blue arrows:** bile ducts, **green arrows:** arteries, **pink arrows:** central veins, and **yellow:** portal vein. **Negative controls:** omission of primary antibodies (shown in upper right corner of all panels). **Scale bars:** 20 µm. **Data:** presented as the mean ± SEM. (CTR: Control; HFD: High-fat Diet; MF: Male Fetus; FF: Female Fetus; IHC: Immunohistochemistry; CV: Central Vein)].

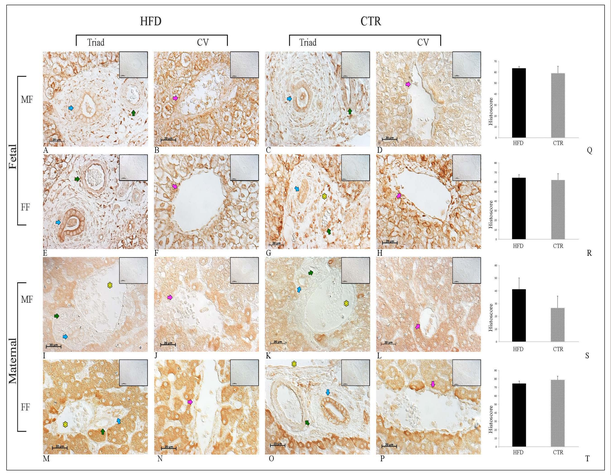

Figure 5: CB2R protein expression in the maternal and fetal livers of HFD (n=11) and CTR (n=10) animals
CB2R protein expression in the maternal and fetal livers of HFD (n=11) and CTR (n=10) animals. **A to D:** Representative images of fetal livers from male fetuses in the CTR (n=3) and HFD (n=6) diet groups; **Q:** quantitative analyses. **E to H:** The fetal livers from female fetuses in the CTR (n=4) and HFD (n=4) groups; **R:** quantitative analyses. **I to L:** Maternal livers of dams with male fetuses in the CTR (n=3) and HFD (n=5) groups; **S:** quantitative analyses. **M to P:** Maternal liver sections from dams carrying female fetuses in the CTR (n=5) and HFD (n=4) groups; **T:** quantitative analyses.

RESULTS

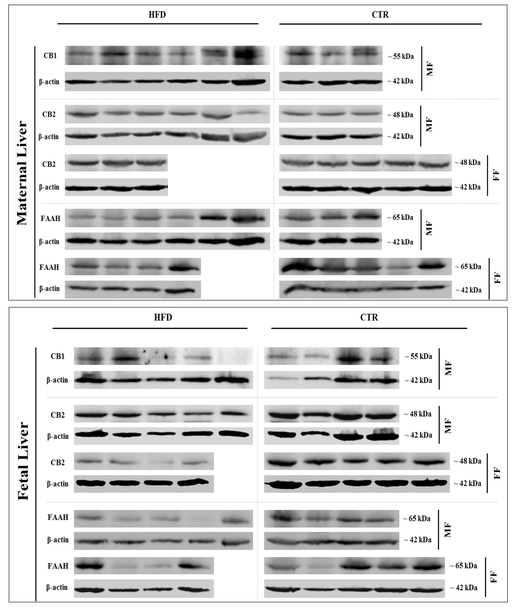

Figure 6: Representative image of western blot analysis. [FAAH: Fatty Acid Amide Hydrolase; CTR: Control; HFD: High-Fat Diet; MF: Male Fetus; FF: Female Fetus]

DISCUSSION

To our knowledge this is the first report of decreased fetal systemic ECS concentration in response to a HFD. HFD stimulated maternal eCBs excess and was associated with hepatic and placental eCB changes which suggest compensatory peripheral mechanisms to alleviate systemic eCB deficiency.

CONCLUSION

High fat diet consumption in pregnancy decreases the abundance of the main enzyme degrading endogenous cannabinoids and decreased ability of fetal liver to counteract changes, associated with hepatic fat deposition.

REFERENCES

- Brocato B. et al. 2013
- Cota D. *et al.* 2003
- González-Mariscal I. *et al.* 2016
- Rodriguez-Sanchez I. P. *et al.* 2016

ACKNOWLEDGEMENTS

- We acknowledge the help of Mr. E. Arzate with images, and Melissa Waggoner with the editing. The authors are thankful to Dr. Charles Burns (Pathology, MCH) and his team for their continuous support. We would like to acknowledge generosity of Dr. Raul Martinez-Zaguilan, who provided essential for this study equipment. This study was supported by NIH HD 21350 to PWN and TTUHSC start-up funds to NSL.
- Funding for this conference was made possible in part by grant R13DA016280 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.