MATERNAL AND FETAL HEPATIC ENDOCANNABINOID SYSTEM (ECS) AND MATERNAL FAT CONSUMPTION.

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Introduction

The endocannabinoid system (ECS) is comprised of endocannabinoids (ECB's), the product of dietary fatty acids, and G-protein coupled cannabinoid receptors 1 and 2 (CBR1 and CBR2). The endogenous ECB ligands, for the cannabinoid receptors are arachidonoyl ethanolamide (anandamide or AEA) and 2-arachidonoylglycerol (2-AG). The CB1 receptor is expressed in brain and peripheral tissues, while the CB2 receptor is expressed in microglia, immune and hematopoietic tissues.

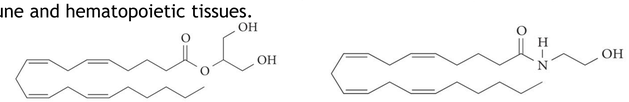

Figure 1. Structure of endogenous ECBs 2-AG and AEA) (Mechoulam, 2014).

Previous data showed a direct role of ECB's in alcoholic and obesity-related non-alcoholic fatty liver disease (NAFLD) (Fig.2). Fatty liver disease is the significant public health issue, being the major cause of liver transplantation in 21st century. Especially alarming is the fact, that 74% of obese individuals eventually develop NAFLD and NASH. The disturbing fact is that fatty liver is programmed *in utero*, contributing to epidemic of childhood metabolic disorders, however, the interventional strategies and prevention are limited. Despite the fact, that ECBs has been a pharmacological target for obesity and NAFLD treatment, no data exists regarding ECBs pharmacology in pregnancy and obesity.

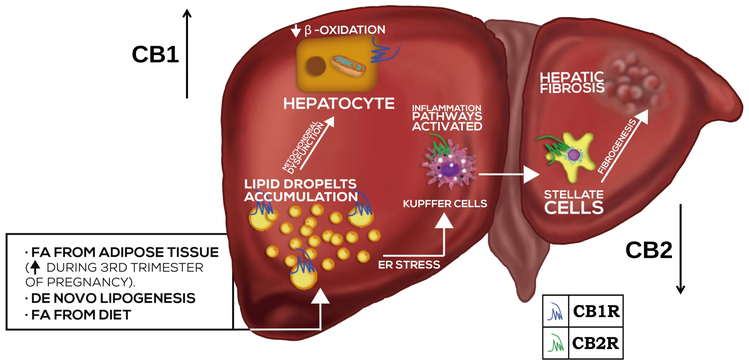

Figure 2. Endocannabinoid pathway in the pathogenesis of NAFLD (FA =fatty acid).

Objective

To evaluate the expression of CB1 and CB2 receptors in maternal and fetal liver in baboon (*Papio spp*) model of 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. Six HF and eight CTR, including archived baboon samples, livers of male and female fetuses, as well as the maternal liver, 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 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.

Results

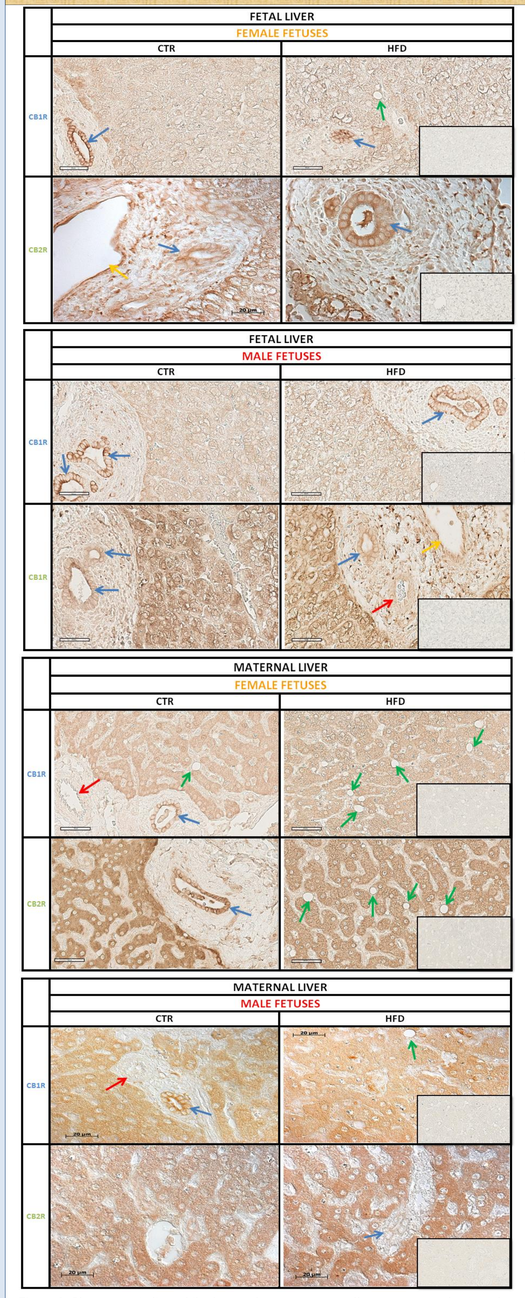

Figure 3. Blue arrows indicate bile duct, red arrows-artery, yellow arrows-portal vein and green arrows-lipid droplet.

Results

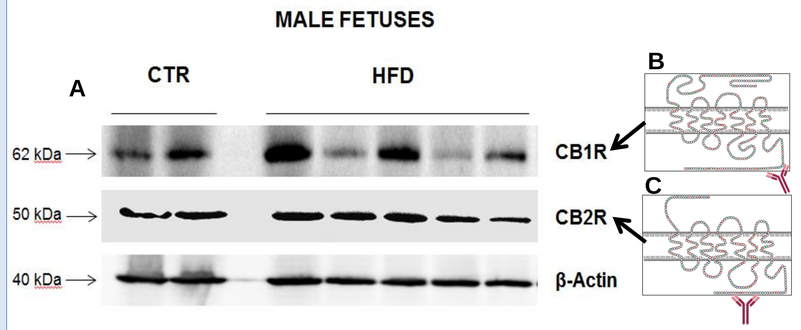

Figure 4. A. Western blot. B and C. Predicted CNR1-ORF and CNR2-ORF from the baboon (*Papio spp.*) (*Dr. J. Guindon, in Rodriquez-Sanchez et al 2016*) with the specific epitopes, recognized by antibodies.

Discussion and Conclusion

Maternal high fat diet programs offspring metabolism and intake contributes to the development of Nonalcoholic steatohepatitis (NASH) in adult offspring via the mechanism of up-regulated hepatic lipogenesis. From the other hand the activation of CB1R leads to lipogenesis and reduced fatty acid oxidation in hepatocytes. In general, increased expression of CB1R in liver of experimental, diet-induced or genetically modified model of NAFLD is a well described phenomenon in non-pregnant animals. Our funding regarding increased CB1R expression in the male offspring is in line with the results obtained in experimental models of maternal high fat diet, showing increased adiposity and hepatic steatosis in male, but in the female offspring. Our data suggests that lipogenesis, but not inflammatory responses, might be the major pathways of *in utero programming* of NAFLD by maternal consumption of high fat diet.

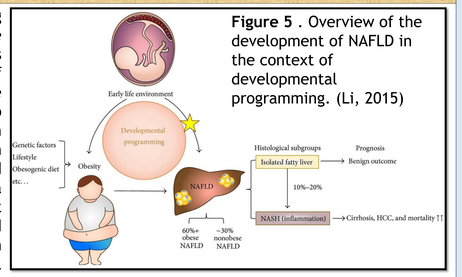

Figure 5 . Overview of the development of NAFLD in the context of developmental programming. (Li, 2015)

Our data is highly relevant in development of prevention strategies to counteract the gender-dependent effect of maternal obesity and prevent devastating effect of maternal obesity on the offspring health.

References

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Acknowledgement

The authors would like to thank the personnel of the Texas Biomedical Institute and Center for Pregnancy and Newborn Research (UTHSC—San Antonio) for their help. This study was partially supported by Texas Biomedical Research Institute Grant C06 RR013556 and NIH grant HD21350 to Dr. Peter Nathanielsz (UTHSC—San Antonio), NIH NCRR grant P51 RR013986 to the Southwest National Primate Research. This work was supported by the TTUHSC start-up funds to N.S-L.

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