PLACENTAL ORIGIN OF FETAL SYNDROME OF ENDOCANNABINOID DEFICIENCY (FSECD)

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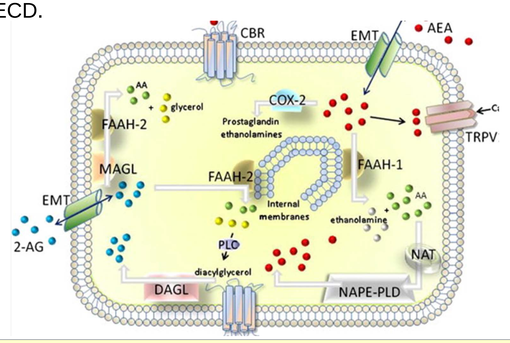
INTRODUCTION

Cannabinoids have been used for the treatment of chronic pain for millenniums [1] with documented results of numerous clinical trials in non-pregnant patients [2]. Exogenous cannabinoids act through the mechanism of "kick-starting" the components of the endogenous cannabinoid system (endocannabinoid [eCB] system, ECS) [3]. ECS is a pharmacological target for the treatment of obesity [4], inflammation [5], cardiovascular and neuronal damage [6], and pain [7, 8]. Clinical syndrome of endocannabinoid deficiency (CECD) is linked to numerous pain-related conditions in adults [9, 10]. First described by Russo in 2004 [10], the concept of CECD has been developed and applied to such conditions as irritable bowel syndrome, fibromyalgia, migraine, and autism [9]. The clinical definition of the syndrome is important, since it leads to the therapeutic application of the cannabis derivatives to its treatment.

The theory of "developmental programming" opens the venue for understanding the origin of adult diseases and their prevention at the most adaptable stage of individual's life – in the womb. In particular, maternal obesity (MO), affecting 64% of all pregnant women with linear trend toward increase between 2005 and 2014 [11] [12], is associated with significant health risks for mothers and their offspring [13-16]. However, the results of numerous maternal lifestyle changing trials (i.e. UPBEAT, LIMIT) [17, 18] and surgical interventions for weight reduction remain controversial and unable to demonstrate the benefits of such for fetal and maternal health [18]. Therefore, there is a clear need to identify novel mechanisms and pharmacological targets underlying maternal-fetal interactions under MO conditions.

Remarkably, there is experimental and epidemiological evidence that the spectrum of the diseases which are included in the definition of CECD are "programmed in utero" by MO; however there are no reports available regarding the possible mechanisms, associating MO and CECD.



MATERIALS AND METHODS

Table 1: Patients' characteristics

	Non-obese		Obese	
	Female (n=4)	Male (n=4)	Female (n=8)	Male (n=8)
Gravidity (n)	3.25 ± 1.31	3 ± 0.82	2.38 ± 0.32	3.25 ± 0.59
Parity (n)	2 ± 1.08	1.5 ± 0.65	1.14 ± 0.26	2.25 ± 0.59
Gestational age (weeks)	38.7 ± 0.37	37.63 ± 1.24	38.7 ± 0.27	38.73 ± 0.28
Maternal age (years)	28.5 ± 2.1	27 ± 3.34	25.25 ± 1.92	25.75 ± 2.34
Admission weight (kg)	76.43 ± 9.17	75.07 ± 1.55	102.63 ± 10.11	115.38 ± 9.57
Admission height (cm)	158.75 ± 8.83	226.06 ± 64.45	161.29 ± 3.33	160.97 ± 1.98
BMI (kg/m^2)	26.75 ± 0.75	24.93 ± 1.8	37.49 ± 2.58	41.5 ± 3.15

Note: All the data are presented as mean ± SEM

The data was obtained in the patient population according to an IRB-approved protocol (University of Tennessee Health Sciences Center). The results were partially presented at the Society for Maternal/Fetal Medicine meeting in February, 2014 [45].

The patients' demographics are presented in Table 1. All data were summarized as mean ± standard error of mean, categorized by MO (obese vs. non-obese) and fetal sex (female vs. male). Kruskal-Wallis ranks tests were used to assess the differences between groups. Cohen's d [46] was used as a standardized estimate of effect size. Power analyses were performed to determine the appropriate sample size after the effect size estimates. Significance level was set at 0.05.

RESULTS

	Non-obese		Obese		
	n	M ± SEM	n	M ± SEM	p-value
Maternal					
2-AG serum (nM/L)	**5**	**481.58 ± 187.49**	**4**	**216.7 ± 35.87**	**0.462**
AEA serum (nM/L)	**5**	**7.41 ± 0.77**	**4**	**11 ± 1.54**	**0.086**
Adiponectin (pg/ml)	4	12022.5 ± 4113.62	3	7535 ± 918.17	0.479
IL-6 (pg/ml)	5	2.64 ± 0.97	11	6.94 ± 3.26	0.269
Insuline (pg/ml)	3	463.43 ± 234.18	11	940.14 ± 210.5	0.186
Leptin (pg/ml)	5	16995.59 ± 6005.9	11	32079.16 ± 7631.1	0.234
TNF-a (pg/ml)	4	23.38 ± 6.18	10	24.57 ± 2.21	0.888
Fetal					
Fetal weight (kg)	8	2.95 ± 0.17	16	3.19 ± 0.11	0.358
Fetal height (m)	4	0.49 ± 0.02	3	0.51 ± 0.02	0.479
Birth weight:length	4	56.43 ± 3.18	3	59.74 ± 1.94	0.479
Ponderal index (kg/m^3)	4	23.77 ± 1.15	3	23.35 ± 1.97	1.000
2-AG serum (nM/L)	**4**	**233.52 ± 90.35**	**4**	**80.79 ± 34.48**	**0.149**
AEA serum (nM/L)	4	12.93 ± 1.77	4	13.42 ± 1.45	0.773
Adiponectin (pg/ml)	3	6.7 ± 2.13	7	9.11 ± 3.87	0.909
IL-6 (pg/ml)	2	425.19 ± 28.05	7	1009.03 ± 241.62	**0.040**
Leptin (pg/ml)	5	9320.8 ± 3088.02	9	40435.37 ± 8910.7	**0.006**
TNF-a (pg/ml)	1	48.13 ± (–)	4	40.8 ± 10.75	–

Table 2: Selected maternal and fetal ECS, metabolic and inflammatory indices in obese and non-obese mothers.

Maternal and fetal 2-AG concentrations in women carrying male fetuses were decreased. Although no statistically significant differences were shown in ECS using nonparametric tests, large effect sizes were calculated for maternal 2-AG (d = 0.83), maternal AEA (d = -1.50), and fetal 2-AG (d = 1.16). These differences were even larger for maternal/fetal 2-AG ratio (d = 3.36). However, small effect size (d = -0.15) was found for fetal AEA. Based on maternal 2-AG effect size, assuming a significance level of 0.05 and power 0.80, the samples from 48 individuals (24 obese and 24 non-obese) would be required to detect differences between the two groups within one sex using Student's t-test.

Table 3. Placental outcomes from obese and non-obese mothers.

	Non-obese		Obese		
	n	M ± SEM	n	M ± SEM	p-value
Weight of placenta without placental membranes (g)	7	435.59 ± 28.13	16	602.13 ± 31.89	**0.002**
Cotyledons (n)	8	8.25 ± 0.59	16	9.44 ± 0.55	0.171
Longest diameter of placenta (cm)	8	15.61 ± 1.02	16	18.4 ± 0.59	**0.013**
Shortest diameter of placenta (cm)	8	15.1 ± 0.88	16	17.77 ± 0.61	**0.011**
Average diameter (cm)	8	15.36 ± 0.95	16	18.09 ± 0.59	**0.008**
Thickness mid placenta (cm)	8	2.05 ± 0.26	16	2.48 ± 0.15	0.069
Volume of placenta (cc)	8	547.23 ± 152.39	16	830.92 ± 76.59	**0.037**
Lenth of cord (cm)	8	22.43 ± 2.62	16	26.78 ± 3.03	0.480
2-AG placenta (nM/L)	4	5770.9 ± 322.1	2	3794.79 ± 260.63	0.064
AEA placenta (nM/L)	4	690.19 ± 77.19	1	577.08 ± (–)	–
Termal Villi	4	165.07 ± 26.22	3	235.83 ± 28.14	0.077
Tervillous space	4	163.29 ± 24.82	3	230.92 ± 44.54	0.157
TV core	4	104.97 ± 16.13	3	151.74 ± 21.52	0.077
Tv syntio	4	55.4 ± 10.64	3	84.73 ± 7.94	0.077
Stem villi	4	32.07 ± 9.28	3	43.44 ± 10.57	0.479
Stem villi core	4	30.38 ± 8.62	3	38.73 ± 8.25	0.479
Stem villi syntio	4	1.69 ± 0.8	2	6.52 ± 0.56	**0.064**
Basal plate	4	24.19 ± 8.81	2	24.92 ± 10.03	1.000
Chorionic plate	1	16.14 ± (–)	1	10.4 ± (–)	–
Placenta	4	405.62 ± 46.09	3	550.98 ± 11.33	**0.034**
Fetal capp	4	39.74 ± 6.4	3	53.16 ± 6.27	0.157
SA villi	4	17.43 ± 1.8	3	20.99 ± 1.96	0.157
SA fetal capp	4	28.87 ± 1.03	3	33.17 ± 1.51	0.077
Total SA (villi & capp)	4	46.3 ± 2.55	3	54.16 ± 3.2	0.157
Harmonic thickness of villous membrane	4	9.5 ± 0.64	3	9.96 ± 0.48	0.479

Note: p-values were calculated using Kruskal-Wallis rank tests; statistically significant values are bolded

Table 4 Pearson product-moment correlation matrix of endocannabinoid concentrations.

	1.		2.		3.		4.		5.	
	(n)	r	(n)	r	(n)	r	(n)	r	(n)	r
1. 2-AG mother	(8)	—								
2. 2-AG fetus	(7)	.56	(7)	—						
3. 2-AG placenta	(5)	-.07	(4)	.23	(5)	—				
4. AEA mother	(8)	-.39	(7)	-.11	(5)	-.90**	(8)	—		
5. AEA fetus	(7)	.49	(7)	.21	(4)	-.90*	(7)	.50	(7)	—
6. AEA placenta	(5)	-.58	(4)	-.68	(5)	.65	(5)	-.53	(4)	-.75

DISCUSSIONS

Selected phenotypes shared between CECD and programmed by MO conditions.

Irritable Bowel Syndrome (IBS) involves chronic constipation/diarrhea with painful abdominal spasms. Based on the presence of both cannabinoid receptors and their ligands in the GIT, it has been suggested that cannabinoid derivatives could be used to treat IBS [31], which is CECD [9]. Chronic bowel inflammation, associated with IBS, has been documented in an experimental animal model of MO [32], suggesting fetal *in utero* programming of this condition.

Autism spectrum disorders (ASD) have affected families, society, and individuals with wide clinical manifestations and a dramatically-increased prevalence during the last decade [33]. ASD have been linked to the mechanism of neuro-inflammation through CBR dysregulation [9, 34]. Multiple systematic reviews and meta-analyses demonstrated *in utero* programming of mental disorders, including ASD, by maternal obesity [15, 35].

Asthma has been successfully treated with cannabis derivatives [36] and the therapeutic usefulness of marijuana has been extensively explored in bronchial function [37], pointing out possible underlining ECS deficiencies. Maternal obesity is associated with an increased risk of respiratory tract infections, asthma, and wheezing in post-natal life [38, 39]. Fetal lung development depends (among other factors) on maternal lipid transfer [38]. The mechanisms linking maternal obesity and an offspring's bronchopulmonary health are unknown [40].

Insulin resistance and pancreatic function have been tightly linked to endocannabinoid function [41], 2-AG–induced CB1R activity is a survival signal for β but not α cells during fetal pancreatic development [42]. Numerous human and experimental animal studies linked maternal obesity to metabolic programming, including the programming of diabetes [43, 44].

CONCLUSIONS

The decrease in 2-AG is in agreement with our previous observations of decreased placental 2-AG concentrations in the baboon model of obesity and decreased ECS receptors expression in maternal obesity in humans and baboons *(IFPA, 2016, P.2.18).* Fetal Syndrome of Endocannabinoid Deficiency (FSECD) might be considered as a clinical diagnosis in maternal obesity.

ACKNOWLEDGEMENTS

This authors are grateful to start-up funds from University of Tennessee Health Science Center and Texas Tech University Health Sciences Center support. The authors are thankful to Dr. Sonali Gupta and the personnel of labor and delivery for their help with specimens' collection.