Combined Oral Contraceptive Treatment Does Not Alter Gut Microbiome or Serum Metabolomic Profile in Obese Girls with Polycystic Ovary Syndrome

Beza Jobira[1,5], Daniel N. Frank[2], Lori J. Silveira[3], Yesenia Garcia-Reyes[1], Charles E. Robertson[2], Diana Ir[2], Keisha L Alexander[4], Angelo D'Alessandro[4], Kristen J. Nadeau[1], and Melanie Cree-Green[1]

[1]Division of Pediatric Endocrinology; [2]Division of Infectious Diseases, [3]Department of Biostatistics and Informatics, [4]Department of Biochemistry and Molecular Genetics, [5]University of Colorado School of Medicine Anschutz Medical Campus

I. BACKGROUND

- Polycystic Ovary Syndrome (PCOS)
  - begins in adolescence
  - is commonly accompanied by obesity, and
  - is associated with metabolic disease
- The gut microbiome is altered in adolescents with PCOS and obesity, and is associated with free testosterone, metabolic markers, and insulin resistance
- Combined oral contraceptives (OCP) are a primary treatment for PCOS and lower testosterone, but it was unknown if they changed the gut microbiome in obese adolescents with PCOS

II. AIM

Assess gut microbiota, targeted serum metabolomics, hormonal and metabolic measures in adolescents with PCOS and obesity with and without OCP treatment

III. METHODS

Participants:

- Twenty-nine girls with PCOS and obesity, 8 treated with OCP and 21 without treatment
- Inclusion: Age 12-20, BMI≧90th %ile, PCOS per NIH criteria, sedentary status
- Exclusion: Hypertension, diabetes, medication affecting insulin sensitivity, antibiotics within 1 month

Study Design:

- Home stool collection
- Blood from fasting and an oral glucose tolerance test (OGTT)
- Blood for hormonal and metabolic test
- Fasting serum metabolomics with mass spectroscopy

Stool analysis:

- High-throughput sequencing of the bacterial 16S rRNA gene V3-V4 region to profile fecal bacterial communities

Calculations/Statistics:

- Insulin sensitivity per HOMA-IR for fasting from OGTT
- T-tests for group differences, Spearman's correlations between bacterial and clinical measures, FDR used for p-values with bacterial differences (R software)

IV. RESULTS

- Girls with PCOS treated with OCP compared with PCOS untreated had:
  - Lower free testosterone (p<0.001) and higher SHBG (p<0.001), and higher platelets (p<0.05), (table 1)
  - Similar fasting glucose, insulin, lipid profile, and measures of insulin resistance (HOMA-IR), (table 1)
- Bacterial α-diversity (fig.1) and β-diversity (p=0.56) were similar across groups
- Percent relative abundance (%RA) at the phylum, family and genus level (fig. 2) were similar
- Principle components analysis (fig. 3A, 3B) of serum metabolome was not different between groups
- Bacterial α-diversity was negatively associated with serum bile acids and branched chain amino acids (table 2)
- Higher %RA of family *Ruminococcaceae* was significantly associated with serum conjugated bile acids and HOMA-IR (table2)

Figure 1: Bacterial α-Diversity

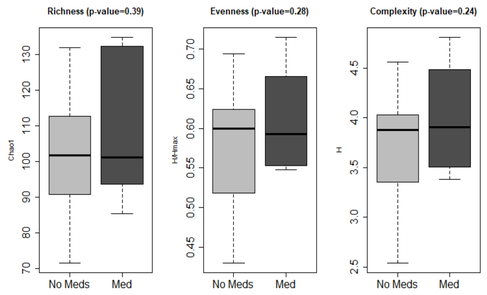

Figure 2: A. Phyla; B. Genus; C. Family Microbiome Differences

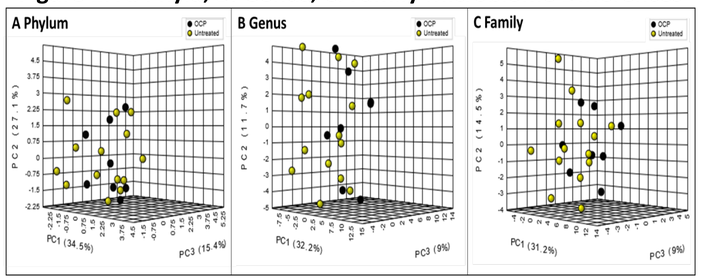

Figure 3: Principal Component Analysis of Target Metabolomics By Group

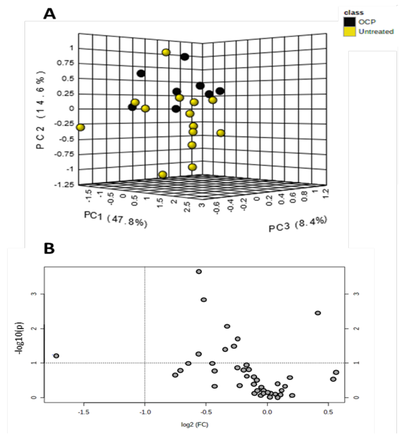

Table 2: Correlation Between Bacterial α- Diversity and %RA, and Targeted Metabolomics, Hormonal and Glucose Measure

Targeted Metabolomics	Bacterial Evenness (R, p-value)	Bacterial Complexity (R, p-value)	Ruminococcaceae (R, p-value)
Free testosterone	-0.58 (0.005)	-0.55 (0.008)	
HOMA-IR			-0.43 (0.043)
Linoleate	-0.51 (0.014)	-0.53 (0.010)	
7Z-10Z-13Z-16Z-19Z	-0.49 (0.020)	-0.51 (0.015)	
Dihomo-g-linolenic	-0.46 (0.029)	-0.50 (0.018)	-0.43 (0.044)
Ursodeoxycholic acid			-048 (0.022)
Taurocholate			0.45 (0.036)
Taurolithocholate			0.44 (0.040)
Taurodeoxycholate			0.60 (0.003)
Alanine	-0.53 (0.011)	-0.55 (0.008)	-0.52 (0.012)
L-Valine	-0.49 (0.021)	-0.50 (0.016)	
L-Isoleucine	-0.41 (0.061)	-0.40 (0.060)	
L-Leucine	-0.43 (0.042)	-0.43 (0.043)	
Glycine			-0.45 (0.033)
Acyl-C8 L-octanoyl	-0.45 (0.035)	-0.47 (0.026)	

Table 1: Cohort Characteristics

	PCOS, Untreated N=21	PCOS, OCP-Treated N=8
Demographics		
Age (years)	16 (15.5, 17)	15.5 (15, 17)
Physical Characteristics		
BMI (kg/m^2)	37 (33, 40)	33 (31, 40)
BMI (%ile)	99 (98, 99)	98 (97, 99)
BMI (Z score)	2.1 (2, 2.4)	2.0 (1.8, 2.4)
Waist-to-hip ratio	0.9 (0.86, 0.95)	0.92 (0.84, 0.98)
Systolic BP (mmHg)	120 (125,155)	117 (112, 125)
Diastolic BP (mmHg)	71 (68, 77)	71 (67, 76)
Laboratory Measurements		
Free testosterone (ng/dL)	9.2 (7.8, 13)	2.3 (1.7, 3.7)**
Total testosterone (ng/dL)	41 (33, 51)	30 (24, 49)
Estradiol (ng/dL)	53 (45, 80)	35 (28, 49)
SHBG (mmol/L)	19 (12, 22)	100 (64, 124)**
Triglycerides (mg/dL)	112 (105, 150)	144 (93, 195)
Cholesterol (mg/dL)	140 (133, 175)	165 (137, 180)
HDL (mg/dL)	35 (29, 44)	41 (38, 46)
LDL (mg/dL)	103 (91, 127)	121 (100, 137)
Free fatty acid (nmol/L)	632 (786, 574)	668 (598, 740)
Fasting glucose (mg/dL)	86 (80, 92)	88 (83, 93)
Fasting insulin (µU/mL)	23 (19, 42)	26 (18, 36)
HOMA-IR	5.1 (3.3, 7.9)	5.0 (4.6, 11.5)
HbA1c (%)	5.6 (5.3, 5.8)	5.5 (5.3, 5.7)
C-peptide (ng/mL)	2.3 (1.8, 3.4)	2.6 (2.0, 3.1)
WBC (10^9 cells/L)		
Platelets (10^8 cells/L)	310 (283, 334)	333 (323, 354)*
hs-CRP (mg/dL)	2.4 (1.1, 7.1)	9.5 (2.9, 12.7)
Adiponectin (ng/mL)	6.4 (4.9, 10.4)	5.7 (5.1, 8.0)
AST (IU/mL)	50 (38, 79)	32 (30, 48)
ALT (IU/mL)	35 (30, 46)	37 (31, 41)

*p-value <0.05; **p-value <0.001

V. CONCLUSIONS

Despite changes in free testosterone and SHBG, girls with PCOS + obesity treated with OCP had similar clinical and gut microbiome profiles compared to the untreated PCOS group, suggesting a potential role of the gut microbiome in metabolic syndrome and PCOS.

VI. FUNDING

NIDDK K23DK107871, Doris Duke Foundation 2015212, Boettcher Webb-Waring Foundation; CU GI & Liver Innate Immune Program; Center for Women's health research; NIH/NCATS Colorado CTSA Grant Number UL1 TR002535