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PAPIO SPP. COLON MICROBIOME AND ITS LINK TO OBESITY IN PREGNANCY

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Abstract

Introduction.—Gut microbial communities are critical players in the pathogenesis of obesity. Pregnancy is associated with increased bacterial load and changes in gut bacterial diversity. Sparse data exist regarding composition of gut microbial communities in obesity combined with pregnancy.

Material and methods.—Banked tissues were collected under sterile conditions during necropsy, from three non-obese (nOb) and four obese (Ob) near-term pregnant baboons. Sequences were assigned taxonomy using the Ribosomal Database Project classifier. Microbiome abundance and its difference between distinct groups were assessed by a nonparametric test.

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Results.—Three families predominated in both the nOb and Ob colonic microbiome: *Prevotellaceae* (25.98% and 32.71% respectively), *Ruminococcaceae* (12.96% and 7.48%), and *Lachnospiraceae* (8.78% and 11.74%). Seven families of the colon microbiome displayed differences between Ob and nOb groups.

Conclusion.—Changes in gut microbiome in pregnant obese animals open the venue for dietary manipulation in pregnancy.

INTRODUCTION

Host-bacterial symbiosis is critical for host homeostasis [1, 2]. Obesity is associated with inflammatory responses [3] and serious pathological conditions including nonalcoholic fatty liver disease (NAFLD) [4], type 2 diabetes and insulin resistance [5], and cardiovascular disorders [6, 7]. Dietary composition affects the gut microbiome [8], which in turn is connected closely to metabolic and hormonal regulations [9–12]. The microbiota of obese rodents and humans contain an abnormal ratio of *Firmicutes* to *Bacteroidetes* as compared to normal microbiota [10, 13–17]; the taxa *Bacteroidetes* is increased with weight loss [14, 18], however the reports regarding taxonomic signature of obesity in the human gut are controversial [15]. Different bacterial taxa could influence host metabolism through the production of biologically active metabolites. The prevalence of *Lactobacillus* (genus within phylum *Firmicutes*) has a negative correlation with body fat content, and the disbalance of these species can lead to an increase in propionate and acetate which are used for the *de novo* synthesis of lipids and glucose [16, 18]. As much as 95% of *Firmicutes* found in the gut belong to class *Clostridia*, (clusters IV and XIVa) which produce butyrate known to be essential for maintenance and protection of normal colonic epithelium [10, 19], but which can also be utilized for *de novo* synthesis of lipids or glucose [10, 20]. Production of ethanol by the intestinal bacteria of obese people is thought to link obesity to NAFLD [21]. Obesity in pregnancy is associated with adverse consequences for maternal and offspring health [22, 23].

Baboons (*Papio spp.*) are a well-characterized animal model for obesity and pregnancy research [24–26]. The advantages of this animal model system include its similarity to humans with respect to placental structure [27, 28] and the ability to control dietary and housing conditions [29]. Microbial gut colonization depends on diet [30], gastrointestinal tract topography [8], host morphology [31] and hormonal status [32]. The fecal microbiome in wild primates generally also varies with the host [33]. Nevertheless, no data are available regarding the microbial landscape of the large intestine in naturally obese and naturally lean pregnant animals. This study sought such data as an essential step to develop strategies targeting the obesity-derived microbiome in pregnancy and aimed to evaluate species-specific obesity-related gut microbiome in pregnancy, addressing also the question of species-specific gut topography.

MATERIALS AND METHODS

Animal housing and handling

Animals were housed in AAALAC-approved facilities and maintained in a group environment. We previously reported details of housing, handling, and dietary composition [34]. Briefly, the animals were fed commercially available LEO5 Monkey diet with the composition identical to Purina 5038 (crude protein not <15%, crude fat not <5%, crude fiber not more than 6%, ash not more than 5%, and added minerals not more than 3% Purina, MO, USA) and the absolute food consumption was increased in the obese animals as described elsewhere [34]. The dietary composition allowed daily consumption of proteins, fat, minerals and vitamins in accordance with the established for non-human primates dietary requirements [29, 35, 36].

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC #1129). Secondary analyses of samples from descending colon (from obese (Ob, n=4) and non-obese (nOb, n=3)), collected during necropsy from near term (165 days of gestation) pregnant baboons, under aseptic conditions, flash-frozen in liquid nitrogen and stored at -80°C [37]. Specifically the sample collection was performed under aseptic conditions: the instruments were sterilized in individual sets for each tissue to be collected; sterile trays were used for weighing of tissues. The investigator was wearing a sterilized, disposable gown and sterilized gloves, the head was covered and surgical face mask was in place. Tissues were not washed.

Animal characteristics

Using a non-human primate specific obesity index [38], we defined obesity in naturally obese animals (i.e., overeating of regular monkey chow). Maternal and fetal morphometry of Ob and nOb animals has been described in details elsewhere [34, 37]. The obesity index in Ob group (n=4) was $48.9 \pm 1.4 \text{ kg/m}^2$ vs. $39.1 \pm 3.2 \text{ kg/m}^2$ in nOb group (n=3) (mean $\pm$ SEM). During necropsy, we measured lengths of components of the fetal gastrointestinal tract (GIT) as a proxy of adult GIT dimensions, specifically the distance from the cardia to pylorus (stomach), and distances to the ileocecal junction from the pylorus (small intestine) and from anus (large intestine).

DNA extraction and sequencing

DNA extraction was performed using a Qiacube® DNA isolation instrument (Qiagen, Valencia, CA, USA) and the DNeasy® Blood and Tissue Kit (Qiagen, Valencia, CA, USA) the weight of the tissue was 25 mg. Each sample was selectively amplified using the 16S Ion Metagenomics Kit™ (Life Technologies, Thermo Fisher Scientific, USA). Libraries were prepared using the NEBNext® Fast DNA Library Kit (New England Biolabs, Ipswich, MA, USA) and Ion Xpress™ Barcodes from Life Technologies (Thermo Fisher Scientific, Waltham, MA USA). Once the libraries were made, qPCR was performed and samples were pooled. Once pooled, another qPCR was performed against a standard to determine the quantity of the pool needed to prepare spheres for Ion PGM™ System. After the pooled libraries were coupled to spheres, an Ion 314 chip (Thermo Fisher Scientific, Waltham, MA USA) was loaded and run according to protocols provided by the manufacturer for 400bp

sequencing, with 30,935 –179,652 16S reads acquired per sample (Table S1). The sequences have been submitted to NCBI: accession numbers are SAMN07287352, SAMN07287353, SAMN07287354, SAMN07287355, SAMN07287356, SAMN07287357 and SAMN07287358.

Trimming and Taxonomy identification

The raw *fastq* files were trimmed using CLC Genomics workbench (CLC Bio, Qiagen, Valencia, CA, USA). For quality trimming, the Phred quality score (Q) was converted to a base-calling error probability (p) and the default parameters including the limit p value and maximal number of ambiguous nucleotides for quality trimming was maintained. For length trimming, reads below 80bp were discarded. Chimeras were removed using usearch 7.0.1090 against the ‘gold’ database [39]. 592 reads with homopolymers over the length of 8 were discarded. 0.87% of the reads matched the baboon genome [40] (*Papio Anubis*, NCBI assembly GCF_000264685.1) with bwa were discarded [41]. The Ribosomal Database Project (RDP) classifier (<http://rdp.cme.msu.edu>) was used to arrange taxonomic identification [42]. All the sequencing information could be found in Table S1.

Statistical analysis

The microbiome abundance and its difference between different colonic groups were further assessed by a nonparametric test through Metastats software (<http://metastats.cbcb.umd.edu/detection.html>) as described previously [43]. P values were adjusted by false discovery rate (FDR) correction [44]. Sigmaplot 13.0 (Systat Software Inc.) and R software were used for generating the figures, $p < 0.05$ was considered statistically significant. Alpha diversity between the groups was tested by analysis of variance, using the R function “aov” to fit linear model, computing analysis of variance for the fitted model using the function “anova”. Bray-curtis distance coupled with standard multivariate statistical techniques including principal coordinates analysis (PCoA, R function “ordination”, R-package “phyloseq” [45], was used to explain differences among microbial communities and the dissimilarity was examined by permutational multivariate analysis (PERMANOVA, R function “adonis”, R-package “vegan”) [46].

RESULTS

Morphometry

The length of the fetal stomach was 3.3 ± 0.3 cm (Ob) vs. 4.3 ± 0.25 cm (nOb). Lengths of the small intestine (68 ± 7.8 cm vs. 78.5 ± 8.2 cm) and large intestine (32 ± 3.2 cm vs. 34.5 ± 1.2 cm) did not differ between Ob and nOb groups respectively.

Microbial niches

Figure 1 represents the relative abundance of the identified colon microbiome assigned at the phylum (Figure 1 A) and family (Figure 1B) level with the highest occurrence (the alpha diversity and beta diversity curves are presented on Fig 1C and Fig 1D).

At the phylum level, *Firmicutes* and *Bacteroidetes* were predominant in nOb (Figure 1 Aa) and Ob (Figure 1 Ab) colonic samples, which accounted for 80% of all bacteria detected in

the gut, followed by *Proteobacteria*, *Actinobacteria*, *Spirochaetes*, *Verrucomicrobia*, and samples that had sequences unmatched with those of the database. Colonic *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* abundances were similar in nOb and Ob groups, while *Verrucomicrobia* and *Spirochaetes* were different.

At the family level, the predominant families in the nOb colon microbiome (Figure 1 Ba) were *Prevotellaceae* (25.73%), followed by *Ruminococcaceae* (12.8%) and *Lachnospiraceae* (9.1%). For the colon microbiome in the Ob group (Figure 2 Bb), *Prevotellaceae* (32.86%) was the predominant family followed by the *Lachnospiraceae* family (12.9%). Among the *Firmicutes* phylum, the families *Veillonellaceae*, *Ruminococcaceae*, and *Lactobacillaceae* comprised 5.9%, 8.1% and 6.6%, respectively. Obesity resulted in a somewhat but not significantly lower colonic microbial community (Figure 1C). The PCOA plot for seven colonic samples based on Bray-curtis distance is presented on Figure 1D.

Comparison of colonic microbiome in obese vs non-obese animals

Family level.—Seven families within the colon microbiome displayed significant changes in the Ob group compared to nOb (* $p < 0.05$, ** $p < 0.01$) (Figure 2).

The abundance of one family was increased in the Ob compared to the nOb group: *Streptococcaceae*, which displayed a 10-fold increase in the Ob group (0.28% vs. 3.003%). The relative abundance of *Oligosphaeraceae* and *Flavobacteriaceae* showed the biggest difference, and was approximately 46-fold lesser in the Ob group. Detailed information can be found in the supplementary material (Table S2). Among the seven families, *Syntrophaceae* demonstrated statistically significant difference assessed by both p and q values ($p = 0.0003$, $q = 0.017$), with 10-fold reduction in the relative abundances in Ob groups (to 0.003%),

Genus level.—Sixteen colonic genera with significant changes between the Ob and nOb groups were identified (* $p < 0.05$, ** $p < 0.01$) (Figure 3).

In the Ob group, 13 genera were less abundant (vs. the nOb group), while three genera were more frequent (*Peptococcus*, *Lachnospira*, and *Streptococcus*). Genus *Streptococcus* increased nearly 11-fold in the Ob group. Relative abundances of *Lachnospira* and *Peptococcus* were about 3-fold higher compared to the nOb group. The relative abundance of *Oligosphaera* was almost 50-fold decreased in Ob group. The genera *Saccharofermentans*, *Subdivision5_genera_incertain_sedis*, *Fibrobacter*, *Sphaerochaeta*, and *Oscillibacter* were almost 10-fold increase in the Ob group. Supplementary material presents detailed information (Table S3).

DISCUSSION

Intestinal microbiota are linked to different physiological and pathological conditions. It has been suggested, that the gut microbiome plays an important role in maternal adaptation to pregnancy [47].

Pregnancy is associated with an increased bacterial biomass and changes in gut bacterial β -diversity [48, 49] which may contribute to the epigenetic programming of metabolism. Limited data are available on the composition of gut microbial communities in obesity combined with pregnancy, and on the extent of their commonality with the placental microbiome. Here we present a novel comparison of the colonic microbiomes in Ob and nOb pregnant baboons.

Comparative gut topography

The relative size of the various segments of the gastrointestinal tract for each species depend on dietary composition [50]. Baboons belong to the *Cercopithecidae* family and consume an omnivorous diet [51]. The diet of baboons in the wild includes leaves, fruits, and other vegetation; wild baboons may occasionally eat meat and also insects, while diet of baboons in captivity differ in the source of nutrients and microbial composition [52–54]. The anatomy and some functional aspects of the gut differ between baboons and anthropoid primates. For example, the size and structure of the cecum and the presence of an *appendix vermiformis* is characteristic only of apes and humans [55–57]. The appendix of these species is considered to be place of biofilm formation, protecting and preserving the commensal bacteria [58] [59]. The cecum of the Old World non-human primates is shorter relative to the total length of the large intestine, compared to anthropoids and the baboon cecum contains a different biofilm than the human appendix [60]. Interestingly, gut proportions differ between human and other primates, including apes [61]. Humans have longer small intestines [62], whereas chimps and orangutans have larger colons. Savanna baboons might represent an exception to this observation with the similar relative volume of the small intestine in both species [61] (Figure 4). In the fetal baboon near term, we observed that the colon length was 18.6–26.2% of gut length, which is within the range reported for humans [63], where the colon represents 17–23% of total gut volume [64].

Comparative colon microbiome in obesity

The dominant bacterial phyla in the nOb animals of our study were *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Spirochaetes* and *Verrucomicrobia*. These data agree with reports that the majority of microbiome sequences in the human gut microbiome belong to *Firmicutes* (65.7%) and *Bacteroidetes* (16.3%) [65] as well as the universal abundance of gut *Firmicutes* across mammalian species [66]. However, the total relative abundance of *Firmicutes* and *Bacteroidetes* in the baboon gut was smaller than in the human gut. As previously published by Brinkley and Mott [67], anaerobic gut bacteria in the baboon were predominantly *Lactobacillus* (45%), *Eubacterium* (16.7%), *Streptococcus* (enterococci) (11.9%) and *Bacteroides* (10.3%) [67]. Baboons reported by those authors were housed in the same facility as in the present study and specifically were male pre-pubertal animals on a regular chow diet with 20% saturated fat and 0.4% cholesterol. Interestingly, while *Lactobacilli* were predominant in baboon vs. human feces, *Lactobacilli* were sparse in baboon vagina, as compared to human vagina [68]. In feces, obligate anaerobes greatly outnumber the facultative bacteria. In our present study, we did not observe differences in *Bacteroides* and *Firmicutes* in Ob animals (vs. nOb). These findings oppose results in humans showing a decrease in *Bacteroides* in obese compared with lean subjects [10, 15]. In general, studies of various gut microbial families affected by obesity are

controversial. One reason could be differences in sampling site. The fecal, luminal, and intestinal wall represent related but potentially distinct anatomic locations, enabling the microbial communities of the gut lumen, colonic mucus layers, and colonic crypts to form “discrete bacterial communities” [8]. The microbiota observed in our study most likely represent combined communities of the mucus layers and crypts.

Recent data confirm microbial biodiversity and also production of certain proteins (e.g., ClpB mimicking hormones) by gut commensal bacteria (e.g., *E. coli* or *Enterobacteriaceae*) that influence host appetite regulation and meal pattern by exerting specific biologic actions [11]. Indeed, the intestinal microbiome has been implicated to regulate progression of type 1 diabetes in the NOD model [69]. Transplantation of gut microbiota from normal mice to germ-free mice increased their body fat without any increase in food consumption [70]. The baboons in our study were spontaneously obese; likely due to excess food intake [34]. The major products of microbial carbohydrate fermentation are short chain fatty acids (SCFA: acetate, propionate, butyrate) [71]. SCFA concentrations in baboon colon range from 90–150 nM [66, 71], which is lower than SCFA levels in stools from humans (450–900 nM total) [72], and chimpanzees, or gorillas [66]. These differences could be explained by dietary composition and other variables [73]. The biggest changes in the colon of Ob compared to nOb animals in our study occurred in the reduction of the carbohydrate-fermenting family, *Oligosphaeria* (Phylum *Lentisphaerae*). This phylum was also detected in fecal samples of non-human wild primate [33] and might be a marker of nutritional status. Another major family that was reduced in the colon of obese animals was *Syntrophaceae*—butyrate-fermenting and fatty-acid-metabolizing bacteria [74]. This finding agrees with the results of experimental studies in rodent, showing that SCFAs regulated gut hormones [75] and protected from diet-induced obesity and insulin-resistance [76]. Interestingly, the genus *Ruminococcaceae*—acetate-producing cellulolytic bacteria described originally in the ruminant and detected in colon of primates [66]—were decreased in the colon of Ob animals in our cohort (*Oscillibacter* and *Pseudoflavonifractor*). This observation agrees with decreased *Ruminococcaceae* and *Oscillibacter* abundance in obese patients with NAFLD [77] and in a rodent high protein diet model [78]. The abundance of the family *Peptococcaceae* was greater in the colon of Ob animals. *Peptococcaceae* species, which produce urease [79], were elevated in a model of intestinal pathology induced by prenatal stress [80], which might link epigenetic mechanisms of prenatal stress to programming of obesity. Importantly, urea transport is decreased in patients with NAFLD [81], thus the increased abundance of *Peptococcaceae* might represent a physiological adaptation to the obese / dysmetabolic state. The more than 10-fold increase in *Lactobacillales* (family *Streptococcaceae*) in Ob animals in our study is remarkable, because anti-streptococcal antibodies have been associated with metabolic disturbances and insulin resistance [82]. Dietary modulation of other *Lactobacilli* strains has been reported to restore metabolic balance and attenuate inflammatory responses [83, 84], hence might be a target for microbiome-related intervention in pregnancy. One could speculate that the reported obesity-driven changes in the microbial landscape of pregnancy may represent a basis for epigenetic programming of obesity in offspring.

In summary

Reported here, the obesity-driven microbial landscape in pregnant *Papio spp.* has similar to human microbial changes, despite differences in the diet.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The dominant phylum-level (A) and family-level (B) abundance of colonic microbiome: Aa and Ba. non-obese colonic microbiome, Ab and Bb. obese colonic microbiome. Each color represents individual bacterial phylum or family. C. Alpha diversity curves. D. Beta diversity curves.

Figure 2.

The relative abundance of colon microbiome at family level with significant changes ($p < 0.05$) and their change folds (obese ($n=4$) vs non-obese ($n=3$) animals).

colon obese vs non-obese at genus level

Root;k_Bacteria;p_Spirochaetes;c_Spirochaetia;o_Spirochaetales;f_Spirochaetaceae;g_Sphaerochaeta;
 Root;k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Hallella;
 Root;k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Flavonifractor;
 Root;k_Bacteria;p_Lentisphaerae;c_Oligosphaeria;o_Oligosphaerales;f_Oligosphaeraceae;g_Oligosphaera;
 Root;k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Acetanaerobacterium;
 Root;k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Peptococcaceae;g_Peptococcus;
 Root;k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Saccharofermentans;
 Root;k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Marinilabiaceae;g_Anaerophaga;
 Root;k_Bacteria;p_Spirochaetes;c_Spirochaetia;o_Spirochaetales;f_Spirochaetaceae;g_Treponema;
 Root;k_Bacteria;p_Fibrobacteres;c_Fibrobacteria;o_Fibrobacteriales;f_Fibrobacteraceae;g_Fibrobacter;
 Root;k_Bacteria;p_Verrucomicrobia;c_Verrucomicrobiae;o_Verrucomicrobiales;f_Subdivision5_genera_incertae;
 Root;k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Pseudoflavonifractor;
 Root;k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillibacter;
 Root;k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Lachnospira;
 Root;k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Sutterellaceae;g_Parasutterella;
 Root;k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae;g_Streptococcus;

Figure 3.

The relative abundance of colon microbiome at genus level with significant changes ($p < 0.05$) and their change folds (obese ($n=4$) vs non-obese ($n=3$) animals).

Figure 4.
Comparative anatomy of digestive tract in humans and baboons (from (61)).