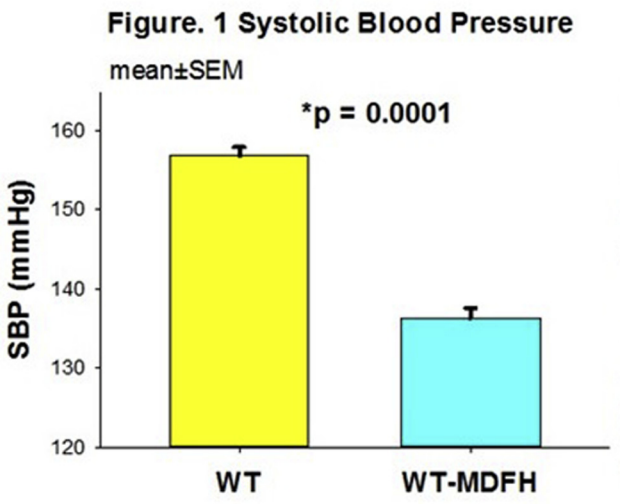

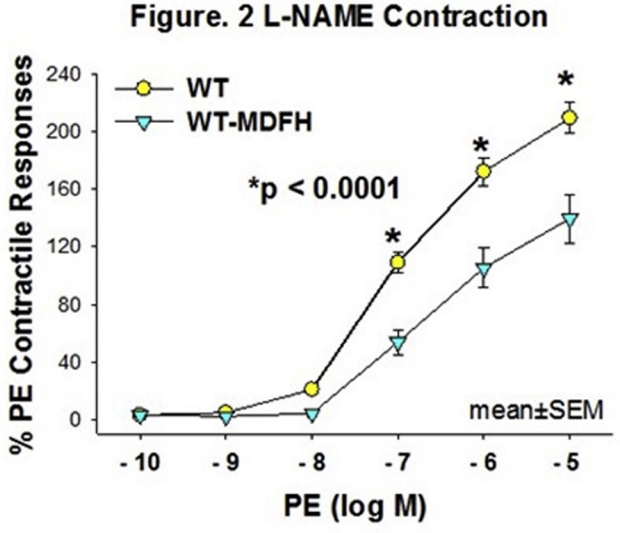

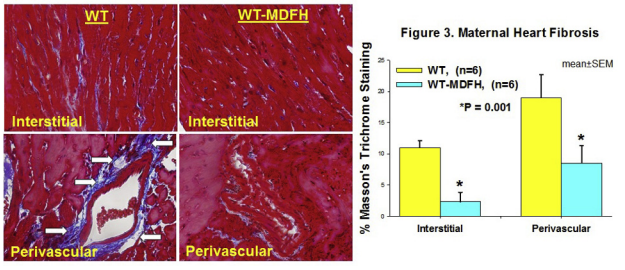

29 Exposure to a high fat diet is associated with persistent alterations in behavior and the gut microbiome in juvenile offspring primates

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OBJECTIVE: Prior work from our consortium shows that maternal high-fat diet (HFD) consumption is associated with modifications in behavior and the central serotonergic system in juveniles at 1 year of age. Additionally, we have described dysbiosis of the gut microbiome occurring at this age with exposure to maternal HFD. Intriguingly, ~90% of serotonin (5HT) is made in the gut, and microbes and their metabolites function as important regulators of 5HT production. Here, we aimed to determine the importance of exposure to a HFD as a persistent behavioral mediator in juvenile offspring by examining the associations between diet, behavior, and the gut microbiome.

STUDY DESIGN: Using our Japanese macaque model of maternal HFD exposure, we performed 16S or whole genome shotgun (WGS) sequencing on stool samples (n=15) collected at 1 & 3 years of age. WGS was performed on the Illumina HiSeq platform and resultant sequences were trimmed/quality filtered using KneadData. Taxonomic classification and functional annotation of species-specific (MetaPhlAn2) and community level metabolic pathways (HUMAnN2) was performed. Stool 5HT levels were assayed by ELISA. Behavioral testing (i.e. novel objects, repetitive behavior) occurred to associate with 5HT and microbiome data.

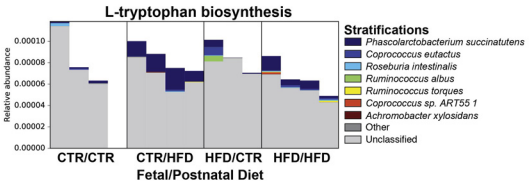
RESULTS: Exposure to HFD in gestation is associated with persistent behavioral modifications (increased anxiety) in offspring at 3 years of age (Fig A). Additionally, we found alterations in metabolic pathways by metagenomic analysis (0.9 billion reads, average 62 million reads/sample) that have been previously shown to modulate 5HT; this includes L-tryptophan biosynthesis, of which *Phascolarctobacterium succinatutens* was identified as a primary contributor (Fig B). Furthermore, we detected significant inverse correlations with specific taxa, including *Phascolarctobacterium* and 5HT at 3 years of age (Fig C).

CONCLUSION: Altogether, our data suggest HFD exposure leads to persistent gut microbial dysbiosis associated with reductions in serotonergic signaling mediators and 5HT synthesis that are detectable at 3 years. The interplay between the gut microbiome and serotonergic signaling potentially contribute to behavioral modifications observed with HFD exposure during fetal life, even with consumption of a postnatal control diet.

A

Anxiety			
	Responders% (n)	Non-responders% (n)	χ^2 (p value)
CTD/CTD	80.0% (4)	20.0% (1)	63.3 ($1.2e^{-13}$)
CTD/HFD	33.3% (2)	66.7% (4)	
HFD/CTD	36.4% (4)	63.6% (7)	
HFD/HFD	33.3% (3)	66.7% (9)	
Repetition			
	Stereotypy % (n)	No Stereotypy % (n)	χ^2 (p value)
CTD/CTD	40.0% (2)	60.0% (3)	17.4 (0.0006)
CTD/HFD	66.7% (4)	33.3% (2)	
HFD/CTD	63.6% (7)	36.4% (5)	
HFD/HFD	55.6% (5)	44.4% (4)	

B



C

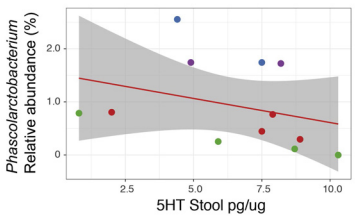


Figure 1. High fat diet exposure alters the gut-brain axis in a non-human primate model.
A) Chi-squared analysis of 3-year-old juvenile offspring demonstrate that exposure to a HFD significantly decreases the percent responders when presented a novel object (touch latency) or increases repetitive behavior (stereotypy). **B)** *Phascolarctobacterium succinatutens* was identified as one of the largest contributors to microbial L-tryptophan biosynthesis as measured by HUManN2. *Phascolarctobacterium spp.* have been previously identified with an increased abundance in the feces of patients with major depressive disorder compared to healthy controls. **C)** Stool 5HT levels at three years of age negatively correlated with the abundance of *Phascolarctobacterium* genera (spearman r = -0.6, p = 0.03). Diet cohorts are notated as gestational diet / post weaning diet: CTD/CTD, control/control in red; CTR/HFD, control/high fat in blue; HFD/CTR, high fat/control in green; and HFD/HFD, high fat/high fat in purple.

30 Diversity of the vaginal virome is associated with preterm birth

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OBJECTIVE: Culture-independent, sequence-based analysis of vaginal bacterial communities demonstrate patterns associated with preterm birth (PTB). However, little is known about the eukaryotic viral ecology of the vaginal niche in normal or abnormal pregnancies. We aimed to describe the vaginal viral community longitudinally over pregnancy and tested the hypothesis that presence of virus and higher viral diversity is associated with PTB

STUDY DESIGN: Serial vaginal swabs were obtained in each trimester and pregnancies were followed prospectively for outcomes. DNA was extracted with the MoBio PowerSoil DNA Isolation Kit (MoBio). Sequencing libraries were constructed with the Swift Biosciences Accel NGS kit with dual indexes (Swift Biosciences). Vertebrate viruses were enriched with ViroCap, our custom panel of targeted sequence capture probes designed to comprehensively enrich the genomes of vertebrate viruses prior to sequencing (Roche Nimblegen). Sequence data were generated on the Illumina HiSeq 2500 1T instrument. Viral sequences were identified using our computational pipeline for viral genomic analysis. Genomic and clinical data were analyzed and plotted using the R statistical language.

RESULTS: We analyzed 128 samples from 60 subjects, of whom 24 (38%) delivered preterm. 80% of women were positive for virus at some point during pregnancy. We detected a broad range of viruses in vaginal swabs from pregnant women, including papillomaviruses (high-risk and low-risk subtypes), herpesviruses (including HSV-2 and CMV), molluscum contagiosum viruses, and others. In some cases, the same viruses were detected in longitudinal samples, and in other cases we observed dynamic changes in the composition of viruses in a patient throughout pregnancy. No viral taxa associated with preterm birth. However, women who delivered preterm had significantly higher viral diversity than those who delivered full term (Figure 1).

CONCLUSION: The vaginal viral community over pregnancy appears unique to individual women. Although a single viral community does not predict PTB, increased viral diversity is associated with PTB. These findings suggest that viral community dynamics in addition to bacterial communities should be considered in investigations of microbial associations with PTB.