# Rat offspring's microbiota composition is predominantly shaped by the postnatal maternal diet rather than prenatal diet

**Ruth Schade**[1,a], **Lin Song**[1,b,d,e], **Zachary A. Cordner**[b], **Hua Ding**[c], **Daniel A. Peterson**[c], **Timothy H. Moran**[b], **Kellie L. Tamashiro**[b,*], **Claire B. de La Serre**[a,*]

[a]Department of Nutritional Sciences, University of Georgia, Athens, GA, U.S.A.

[b]Department of Psychiatry & Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, U.S.A.

[c]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, U.S.A.

[d]Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi, China

[e]Key Laboratory of Environment and Genes Related to Diseases, Ministry of Education of China, Xi'an Jiaotong University, Xi'an, Shaanxi, China.

## Abstract

This study assessed the impact of maternal diet during pregnancy versus lactation on offspring gut microbiota. Sprague-Dawley dams were fed high fat (HF) or Chow diets during pregnancy, and their male offspring were raised by a different dam consuming the same or opposite diet (Chow-Chow, Chow-HF, HF-Chow, and HF-HF). Microbiota analysis showed that maternal lactation diet, rather than pregnancy diet, determined offspring microbiota profiles at weaning. Increased abundances of Turicibacter, Staphylococcus, and Ruminococcus were characteristic of chow lactation groups. Lactococcus, Streptococcus, and Parabacteroides were characteristic of HF lactation groups and positively correlated with offspring body weight.

Maternal obesity prevalence in the U.S. has tripled over the last 20 years and has recently been estimated at over 25% [1–3], when overweight mothers are included that number grows to 55%[2]. Maternal obesity increases future risk of metabolic disease, and other health problems for offspring [4–6]. Diet composition is a key factor contributing to maternal obesity[7]. It also shapes maternal microbiota composition [8], thus impacting which microbes colonize the offspring at birth [9] and during lactation [10].

The intrauterine and early postnatal environments are critical to the proper development of the offspring. Pregnancy and lactation are two distinct phases of development during which offspring health is directly under maternal influence. Cross-fostering rodent studies, whereby offspring are raised by surrogate mothers, have been instrumental in separating pregnancy from lactation effects and suggest that maternal status during pregnancy and

---

[*]Corresponding authors.
[1]These authors contributed equally

lactation independently impact offspring health [11, 12]. A study focusing on maternal food restriction (FR) found that offspring born to rats consuming calorically-restricted diets and then fostered by mothers consuming *ad libitum* diets (FR-AdLib) gained significantly more weight than AdLib-AdLib and AdLib-FR counterparts suggesting gestational caloric restriction had a persistent influence on offspring obesity risk [13]. In contrast, maternal high fat (HF) diet studies found that male pups fostered by rat dams consuming HF diets during the suckling period gained significantly more weight and had significantly higher fat mass than pups fostered by Chow-fed dams, independently of their birth dams' diet. These differences were maintained in adulthood suggesting that HF diet during the lactation period determined metabolic risk for offspring [14]. These data indicate that maternal diet could have differential effects on offspring phenotype depending on the nature of the diet and the timing of exposure.

Various epidemiological studies have shown that cesarean delivery is associated with increased risk of obesity, diabetes, and other inflammatory diseases [11, 15], supporting the idea that early microbial colonization plays a key role in offspring development. This current study aimed to assess the impact of maternal diet during pregnancy versus lactation on offspring gut microbiota composition at weaning. By using a cross-fostering model, we are able to differentiate the impacts of maternal HF and low-fat standard Chow feeding during pregnancy versus lactation.

Pregnant Sprague-Dawley rats (Charles River) were received on gestational day (GD) 2 and housed individually in conventional tub cages in a temperature- and humidity-controlled room with a 12-hour dark/light cycle. The rats were provided with *ad libitum* access to food and water, with 17 of the rats receiving high fat (HF) food (Research Diets, D12492, 60% kcal from fat) and 16 of the rats receiving standard Chow food (LabDiet, 5001, 13.5% kcal from fat). Diets were introduced upon arrival at the animal facility on GD2. Dams were maintained on their respective diets throughout gestation and lactation, for a total of 6 weeks. Body weight and food intake were measured daily. All animal protocols used in this study were approved by the Johns Hopkins University School of Medicine Institutional Animal Care and Use Committee.

Postnatal day (PND) 0 was defined as the day on which the dams gave birth; only dams that gave birth to 10 or more pups continued to be used in the study. There were no significant differences in litter size (Chow 12.2 ± 0.5 vs. HF 12.2 ± 0.5 pups) or male:female ratio (Chow 1.4 ± 0.2 vs. HF 1.5 ± 0.2) between the diet groups. On PND1, litters were culled so that each had 5 females and 5 males. All pups from a litter were then cross-fostered to a different dam to produce 4 offspring groups based on maternal diets (gestation diet – lactation diet): Chow-Chow (n=6), Chow-HF (n=6), HF-Chow (n=6), HF-HF (n=5). One male pup from each litter was randomly selected at each timepoint (PND10 and 21) for this study (n represents number of litters).

There were limited differences in body weight between the Chow and HF fed dams. There were no differences in body weight between the dams during the first 8 days of pregnancy. By day 9, HF fed dams weighted significantly more than the Chow fed dams (Chow 247.5 ± 4.7 g vs. HF 263.5 ± 4.9 g; p < 0.05) and continued to be heavier through GD21 (Chow

367.4 ± 6.6 vs. HF 385.5 ± 6.9 g; p < 0.05) but this difference was limited to about 5 % body weight. After parturition, HF dams were no longer heavier than the Chow fed dams. HF dams were hyperphagic immediately upon access to HF diet on GD2 and consumed more calories compared to Chow dams from GD3 (Chow 63.8 ± 3.1 kcal vs. HF 88.2 ± 3.2 kcal; p < 0.05) through GD19 (Chow 75.1 ± 3.8 kcal vs. HF 92.1 ± 3.9 kcal; p < 0.05). HF fed dams continued to consume more calories than Chow dams from PND3 through PND14. After PND14 HF dam food intake continued to be higher, but we attribute part of this increase to the pups who start to consume the solid food at about PND14–15 when their eyes open.

Fresh fecal samples were collected from dams on PND21, and from the pups' colon on PND10 and PND21. Cecal samples were also collected from pups on PND21. Microbial DNA was extracted as described in [16]. The V4-V6 region of the 16S rRNA gene was amplified with the following primers: F515 (5′-GTGCCAGCMGCCGCGGTAA-3′) and RNextera (5′-CGACRRCCATGCANCACCT-3′), and targeted for sequencing by Illumina MiSeq 600. 16S rRNA gene sequences were processed with QIIME. OTUs were picked based on 97% sequence similarity via the UCLUST algorithm. OTUs were assigned to taxa through the Greengenes database.

To characterize alpha diversity, Simpson's index and the Shannon index were calculated in RStudio (RStudio Version 1.2.5042) using the package vegan v2.4-1 [17]. To assess beta diversity measures, an OTU table with taxonomic identifiers was uploaded to MicrobiomeAnalyst [18, 19]. Taxonomic abundances were then transformed based on the Relative Log Expression method proposed by Anders et al [20] for analyzing sequence counts data. Pearson's r was used in conjunction with Ward clustering to construct dendrograms and determine associations between taxa and diet groups. Partial least squares-discriminant analysis (PLS-DA) plots were generated to assess overall clustering patterns. Pearson's r was also used to assess correlations among taxa and offspring body weights, and correlations with significant p-values (p < 0.05) were used in a heatmap made in RStudio (RStudio Version 1.2.5042). To determine the impacts of maternal diet during pregnancy versus lactation, 2-way ANOVA was performed for the microbes found to significantly correlate with body weight; using Prism software (Prism 9.1.0; GraphPad Software; La Jolla, CA).

Alpha diversity in cecal and fecal samples from pups on PND21 was considered in terms of species richness and evenness. The Shannon index is more representative of richness while the Simpson index is more sensitive to evenness. Consistent with the lack of differences in alpha diversity observed in the maternal fecal samples (Figure 1A), no differences in fecal alpha diversity were present among pup dietary groups (Figure 1B). However, there were significant decreases in species richness (Shannon) and evenness (Simpson), in the cecum of pups born to HF fed dams compared to Chow offspring (Figure 1C) while postnatal maternal HF was associated with an increase in evenness. There were no interactions between the pre- and postnatal diets. A low fiber intake has previously been linked with a reduction in cecal microbial diversity [21]. The number of bacterial species in the cecum was the only microbial characteristic influenced by maternal diet during pregnancy while the species present and their relative abundance was heavily influenced by maternal diet in the postnatal period.

We used several clustering algorithms to determine overall microbiota composition patterns and assess the impact of maternal diet during pregnancy and lactation on the presence and/or abundance of certain taxa. PLS-DA (all phylogenic levels) demonstrated clear grouping in pup cecal (Figure 2A) and fecal (Figure 2B) samples at PND21 based on maternal lactation diet but not prenatal diet. Postnatal clusters overlapped with the lactating dams' fecal profile. These data are supported by heatmaps for pup cecal (Figure 2C) and fecal (Figure 2D) where phyla abundances clustered based on postnatal maternal diet. Samples from pups whose mothers consumed different diets during pregnancy are intertwined and clustered solely based on lactation diet further supporting that maternal postnatal diet is the main driver of differences in relative abundances of bacteria in pups at weaning.

We assessed whether offspring body weights differed based on pregnancy versus lactation diet, and if this correlated with specific taxa abundances. Consistent with prior reports [14], we found that maternal diet during lactation significantly impacted offspring body weights at PND21 (Figure 3A). Pups of mothers consuming a HF diet during lactation were significantly heavier than pups of dams fed a Chow diet postnatally independent of the prenatal dam's diet. There were no differences in weight between the Chow-HF and HF-HF groups or the HF-Chow and Chow-Chow groups, indicating no lasting effect of maternal HF feeding during pregnancy on offspring body weight at weaning.

To identify specific microbes that may play a role in offspring metabolic health, we performed correlation analyses and determined bacterial taxa whose abundances strongly correlated with offspring body weights (Figure 3B). We found that *Parabacteroides*, *Lactococcus*, and *Streptococcus* had the strongest positive correlations with body weight, while *Staphylococcus, Ruminococcus,* and *Turicibacter* had strong negative correlations. These taxa were previously identified as characteristics of postnatal dietary conditions (Figure 2C and 2D). Their relative abundance significantly differed based on maternal lactation diets, but not pregnancy diets (Figure 3C) and two-way ANOVA did not reveal any significant interaction between pregnancy and lactation maternal diet (Figure 3C). By PND21, rats' offspring have usually started consuming solid food and some of the differences observed between groups may be a direct consequence of diet consumption. However, the post-natal environment appears to be a strong driver of early GI bacterial colonization as differences in microbiota composition between Chow-Chow / HF-Chow and Chow-HF / HF-HF groups were evident as early as PND10, prior to food consumption. Postnatal maternal HF feeding at PND10 was associated with increased fecal abundance of the Firmicutes *Veillonella*, which has been associated with inflammation and weight gain in humans [22] and mice [23] (Figure 3D). Conversely, taxa that were strongly associated with postnatal Chow diet and/or leanness at PND21, such as the *Coriobacteriaceae* family, *Corynebacterium* and *Turicibacter* were only detected in Chow-Chow and HF-Chow pups at PND10 (Figure 3D).

Studies on the association between cesarean section and risk of metabolic diseases suggest that early microbial colonization is a major component of healthy offspring development [11, 15]. These studies also suggest that maternal influences during lactation may not be able to overcome the microbial deficit resulting from cesarean delivery but did not assess the

impacts of maternal diet during the lactation period on microbial colonization in vaginally delivered offspring.

Our results demonstrate that when offspring are born vaginally, microbiota composition at PND21 is governed by maternal diet during lactation rather than during pregnancy. The change of environment likely influenced the dams' microbiota upon arrival at the animal facility, however, diet is the main factor controlling microbiota composition [24], with changes in composition reported only days after dietary switch [25, 26]. The pups are born 19 to 20 days after diet introduction and therefore differences in microbiota composition between dams is expected to be a result of dietary manipulation.

PLS-DA and dendrograms produced via clustering algorithm found that cecal and fecal microbiota composition across taxa clustered according to maternal lactation diet regardless of pregnancy diet. Maternal HF diet during lactation resulted in significantly reduced levels of *Ruminococcus*, *Turicibacter*, and *Staphylococcus* while promoting growth of *Parabacteroides*, *Streptococcus,* and *Lactococcus* in offspring, matching the dams' microbiota at this time point. Abundances of these microbes correlated strongly with PND21 body weight and demonstrated an impact of maternal diet during lactation, not pregnancy, on offspring development. We have previously found that feeding dams a HF diet during lactation led to sustained increases in body weight and adiposity in offspring, especially in males [14]. It is possible early GI colonization with specific bacteria can protect against or predispose the offspring to obesity.

*Ruminococcus* and *Turicibacter* both produce short-chain fatty acids (SCFAs), including propionate, acetate and butyrate, that have critical roles in energy balance, immune function and inflammatory processes. The finding that *Ruminococcus* and *Turicibacter* was lower in abundance in HF offspring is consistent with a recent report showing that the same two microbes were reduced in 1-month old infants of women with obesity, which was associated with reduced infant fecal butyric acid (conjugate base of butyrate) concentration [27]. Histone acetylation is a critical epigenetic modification and butyrate acts as a histone deacetylase (HDAC) inhibitor [28]. Thus, low levels of butyrate could result in greater activity of HDACs and removal of histone acetyl groups leading to less accessible chromatin for gene transcription. Epigenetic processes, such as histone modification, are critical during development and could lead to long-term physiological and behavioral consequences for offspring.

While we did not assess microbiota composition in milk of dams consuming Chow versus HF diets, Pocheron *et al.* [29] found that obesity prone Sprague Dawley rats had different breast milk microbiota profiles compared to obesity resistant rats. The *Staphylococcaceae* family was found in higher abundance in milk from obesity resistant dams compared to obesity prone dams. *Staphylococci* are capable of utilizing human milk oligosaccharides [12], so the differences in *Staphylococcus* abundance between maternal Chow and HF lactation groups may be related to differences in milk oligosaccharide levels in addition to the increased presence of *Staphylococcus* in the gut of Chow fed dams. *Streptococcus* is similar to *Staphylococcus* in that it is a facultative anaerobe associated with breastfeeding and one of the first microbes to colonize the neonatal gut [30, 31].

*Lactococcus* has been shown to increase with HF diet consumption, and this genus is positively correlated with body fat in HF fed mice [32]. Our study reflects these findings since *Lactococcus* was elevated in the HF lactation diet groups, which were heavier and likely fed more frequently. HF-fed dams nurse their offspring more often than dams on standard Chow diet [33]. A recent meta-analysis identified *Lactococcus* as a signature of HF diets across studies, which may be related to the way casein is processed in these diets [32]. While the increased *Lactococcus* may be derived from the food and not reflect a stable host microbiome phenotype in the mothers, it is interesting to note that it is being transmitted from mother to offspring.

*Parabacteroides* is a Bacteroidetes and is characteristic of the "Bacteroides enterotype" associated with HF-high protein western diets identified by Wu *et al.* [10]. A recent study assessing the impact of maternal HF diet on offspring metabolic health and memory function found that at one month of age, *Parabacteroides* in the colonic microbiome was a signature of offspring with obese mothers, and at six months of age, *Parabacteroides* was negatively associated with memory and exploratory behavior [34].

The central role of the postnatal environment in shaping offspring gut microbiota composition and influencing body weight is supported by our results. Several environmental factors controlled by the lactation diet could influence the offspring microbiota composition including diet consumption by the offspring, milk composition [33], environmental exposure to the dams' intestinal and skin microbiota and feces consumption. At PND10, pups exclusively consumed milk, ruling out direct consumption of the dams' diet or feces as driving factors for these early differences; they are however exposed to the feces present in the home cage. Dietary intake strongly modulates microbiota composition, suggesting the possible presence of important differences in milk composition and nursing patterns between chow- and HF-fed dams. This is supported by Purcell *et al.*'s [33] findings that HF-fed dams nurse their offspring more, and that their milk has significantly higher lipid content than their Chow-fed counterparts. Analyses of breast milk from women consuming different diets have been less conclusive regarding total lipid content, but maternal obesity has been associated with differences in the fatty acids present in breast milk, which could impact microbial populations in infants [35–37]. A limitation of our study is that milk composition was not analyzed, so differences in milk oligosaccharides, fatty acids, hormones, and immune complexes resulting from maternal diet are unknown, and these could have key roles in mediating offspring metabolic health and gut microbiota composition during the lactation period. At PND21, the offspring microbiota may be influenced by a combination of milk and diet consumption as well as exposure to (and consumption of) the dams' feces. Offspring begin to consume some of the dam's diet at about PND15 with HF offspring reported to spend more time feeding than Chow offspring [38], diet consumption by offspring could contribute to some of the differences we observed at PND21. Overall, we found limited differences in body weight between dams despite an increase in intake in HF fed dams. In previous cohorts, we had found no differences in body weight between Chow and HF fed dams during gestation [14, 39–41] and lactation [41] and differences in intake in early but not late gestation [14, 39, 40]. We did not assess body composition for the dams in this study. However, we previously reported that the HF dams have significantly greater plasma leptin levels by GD10 and this remains elevated through PND21 suggesting greater

adiposity in the HF dams [40, 42]. Body composition measures on GD21 confirmed greater adiposity at that timepoint [40]. Metabolic outcomes in the offspring are similar between our cohorts whether or not we observed differences in body weight between dams, therefore maternal body weight does not appear to be a key predictor of offspring risk for metabolic disease. Diet composition and/or excess caloric intake in HF fed dams may have a direct effect on milk composition and GI bacterial make-up in the offspring.

This study provide further support for the role of maternal health in shaping offspring development and health outcomes. The degree to which maternal health and behavior impacts offspring during pregnancy versus lactation requires further characterization. The cross-fostering design used in this study allowed us to assess the relative contributions of pregnancy versus lactation and our results suggest that certain effects of poor maternal diet during pregnancy may be overcome by a healthy diet during lactation.

## Acknowledgements:

## References:

1. Chu SY, Kim SY, and Bish CL, Prepregnancy obesity prevalence in the United States, 2004–2005. Matern Child Health J, 2009. 13(5): p. 614–20. [PubMed: 18618231]

2. Wang MC, et al. , Trends in Prepregnancy Obesity and Association With Adverse Pregnancy Outcomes in the United States, 2013 to 2018. Journal of the American Heart Association, 2021. 10(17): p. e020717. [PubMed: 34431359]

3. Driscoll AK and Gregory ECW, Increases in Prepregnancy Obesity: United States, 2016–2019. NCHS Data Brief, 2020(392): p. 1–8.

4. Yu Z, et al. , Trends in overweight and obesity among children and adolescents in China from 1981 to 2010: a meta-analysis. PLoS One, 2012. 7(12): p. e51949. [PubMed: 23284829]

5. Drake AJ, et al. , An unbalanced maternal diet in pregnancy associates with offspring epigenetic changes in genes controlling glucocorticoid action and foetal growth. Clin Endocrinol (Oxf), 2012. 77(6): p. 808–15. [PubMed: 22642564]

6. Drake AJ and Reynolds RM, Impact of maternal obesity on offspring obesity and cardiometabolic disease risk. Reproduction, 2010. 140(3): p. 387–98. [PubMed: 20562299]

7. Murtaugh MA, et al. , Diet Composition and Risk of Overweight and Obesity in Women Living in the Southwestern United States. Journal of the American Dietetic Association, 2007. 107(8): p. 1311–1321. [PubMed: 17659896]

8. Cabrera-Rubio R, et al. , The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. The American journal of clinical nutrition, 2012. 96(3): p. 544–551. [PubMed: 22836031]

9. Bäckhed F, et al. , Dynamics and stabilization of the human gut microbiome during the first year of life. Cell host & microbe, 2015. 17(5): p. 690–703. [PubMed: 25974306]

10. Wu GD, et al. , Linking long-term dietary patterns with gut microbial enterotypes. Science, 2011. 334(6052): p. 105–108. [PubMed: 21885731]

11. Hansen S, et al. , Birth by cesarean section in relation to adult offspring overweight and biomarkers of cardiometabolic risk. International journal of obesity, 2018. 42(1): p. 15–19. [PubMed: 28757643]

12. Hunt K, et al. , Human milk oligosaccharides promote the growth of staphylococci. Applied and environmental microbiology, 2012. 78(14): p. 4763–4770. [PubMed: 22562995]

13. Desai M, et al. , Programmed obesity in intrauterine growth-restricted newborns: modulation by newborn nutrition. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2005. 288(1): p. R91–R96. [PubMed: 15297266]

14. Sun B, et al. , Maternal High-Fat Diet During Gestation or Suckling Differentially Affects Offspring Leptin Sensitivity and Obesity. Diabetes, 2012. 61(11): p. 2833–2841. [PubMed: 22751689]

15. Jakobsson HE, et al. , Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. Gut, 2014. 63(4): p. 559–566. [PubMed: 23926244]

16. Ley R, et al. , Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A, 2005. 102(31): p. 11070–11075. [PubMed: 16033867]

17. Oksanen J, et al., Package 'vegan'. Community ecology package, version, 2013. 2(9): p. 1–295.

18. Chong J, et al. , Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. Nature Protocols, 2020. 15(3): p. 799–821. [PubMed: 31942082]

19. Dhariwal A, et al. , MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic acids research, 2017. 45(W1): p. W180–W188. [PubMed: 28449106]

20. Anders S and Huber W, Differential expression analysis for sequence count data. Nature Precedings, 2010: p. 1–1.

21. Li Y, et al. , Effects of dietary fiber levels on cecal microbiota composition in geese. Asian-Australasian journal of animal sciences, 2018. 31(8): p. 1285–1290. [PubMed: 29381893]

22. Palmas V, et al. , Gut microbiota markers associated with obesity and overweight in Italian adults. Scientific Reports, 2021. 11(1): p. 5532. [PubMed: 33750881]

23. Zhao H, et al. , Dynamic Alteration of the Gut Microbiota Associated with Obesity and Intestinal Inflammation in Ovariectomy C57BL/6 Mice. International Journal of Endocrinology, 2022. 2022: p. 6600158. [PubMed: 35103060]

24. Klingbeil E and Serre C.B.d.L., Microbiota modulation by eating patterns and diet composition: impact on food intake. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2018. 315(6): p. R1254–R1260. [PubMed: 30230934]

25. Hamilton MK, et al. , Changes in intestinal barrier function and gut microbiota in high-fat diet-fed rats are dynamic and region dependent. American Journal of Physiology - Gastrointestinal and Liver Physiology, 2015. 308(10): p. G840–G851. [PubMed: 25747351]

26. Graf D, et al. , Contribution of diet to the composition of the human gut microbiota. Microbial Ecology in Health and Disease, 2015. 26: p. 10.3402/mehd.v26.26164.

27. Gilley SP, et al. , Associations between maternal obesity and offspring gut microbiome in the first year of life. Pediatr Obes, 2022: p. e12921. [PubMed: 35478493]

28. Chriett S, et al. , Prominent action of butyrate over β-hydroxybutyrate as histone deacetylase inhibitor, transcriptional modulator and anti-inflammatory molecule. Scientific Reports, 2019. 9(1): p. 742. [PubMed: 30679586]

29. Pocheron A. l., et al. , Maternal microbiota transfer programs offspring eating behavior. Frontiers in Microbiology, 2021. 12: p. 1272.

30. Moore RE and Townsend SD, Temporal development of the infant gut microbiome. Open biology, 2019. 9(9): p. 190128. [PubMed: 31506017]

31. Chu DM, et al. , Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. Nature medicine, 2017. 23(3): p. 314–326.

32. Bisanz JE, et al. , Meta-analysis reveals reproducible gut microbiome alterations in response to a high-fat diet. Cell host & microbe, 2019. 26(2): p. 265–272. e4. [PubMed: 31324413]

33. Purcell RH, et al. , Maternal stress and high-fat diet effect on maternal behavior, milk composition, and pup ingestive behavior. Physiology & behavior, 2011. 104(3): p. 474–479. [PubMed: 21605577]

34. Sanguinetti E, et al. , Microbiota signatures relating to reduced memory and exploratory behaviour in the offspring of overweight mothers in a murine model. Scientific reports, 2019. 9(1): p. 1–12. [PubMed: 30626917]

35. Álvarez D, et al. , Impact of maternal obesity on the metabolism and bioavailability of polyunsaturated fatty acids during pregnancy and breastfeeding. Nutrients, 2021. 13(1): p. 19.

36. Enstad S, et al. , The impact of maternal obesity and breast milk inflammation on developmental programming of infant growth. European journal of clinical nutrition, 2021. 75(1): p. 180–188. [PubMed: 32814855]

37. de la Garza Puentes A, et al. , The Effect of Maternal Obesity on Breast Milk Fatty Acids and Its Association with Infant Growth and Cognition—The PREOBE Follow-Up. Nutrients, 2019. 11(9): p. 2154. [PubMed: 31505767]

38. Kojima S, Catavero C, and Rinaman L, Maternal high-fat diet increases independent feeding in pre-weanling rat pups. Physiology & behavior, 2016. 157: p. 237–245. [PubMed: 26873412]

39. Sun B, et al. , Early postweaning exercise improves central leptin sensitivity in offspring of rat dams fed high-fat diet during pregnancy and lactation. Am J Physiol Regul Integr Comp Physiol, 2013. 305(9): p. R1076–84. [PubMed: 24026073]

40. Song L, et al. , Prenatal high-fat diet alters placental morphology, nutrient transporter expression, and mtorc1 signaling in rat. Obesity, 2017. 25(5): p. 909–919. [PubMed: 28332771]

41. Sun B, et al. , Large litter rearing improves leptin sensitivity and hypothalamic appetite markers in offspring of rat dams fed high-fat diet during pregnancy and lactation. Endocrinology, 2014. 155(9): p. 3421–33. [PubMed: 24926823]

42. Tamashiro KL, et al. , Prenatal stress or high-fat diet increases susceptibility to diet-induced obesity in rat offspring. Diabetes, 2009. 58(5): p. 1116–25. [PubMed: 19188431]

Author Manuscript

**Highlights**

- Postnatal maternal dietary environment determines offspring microbiota composition

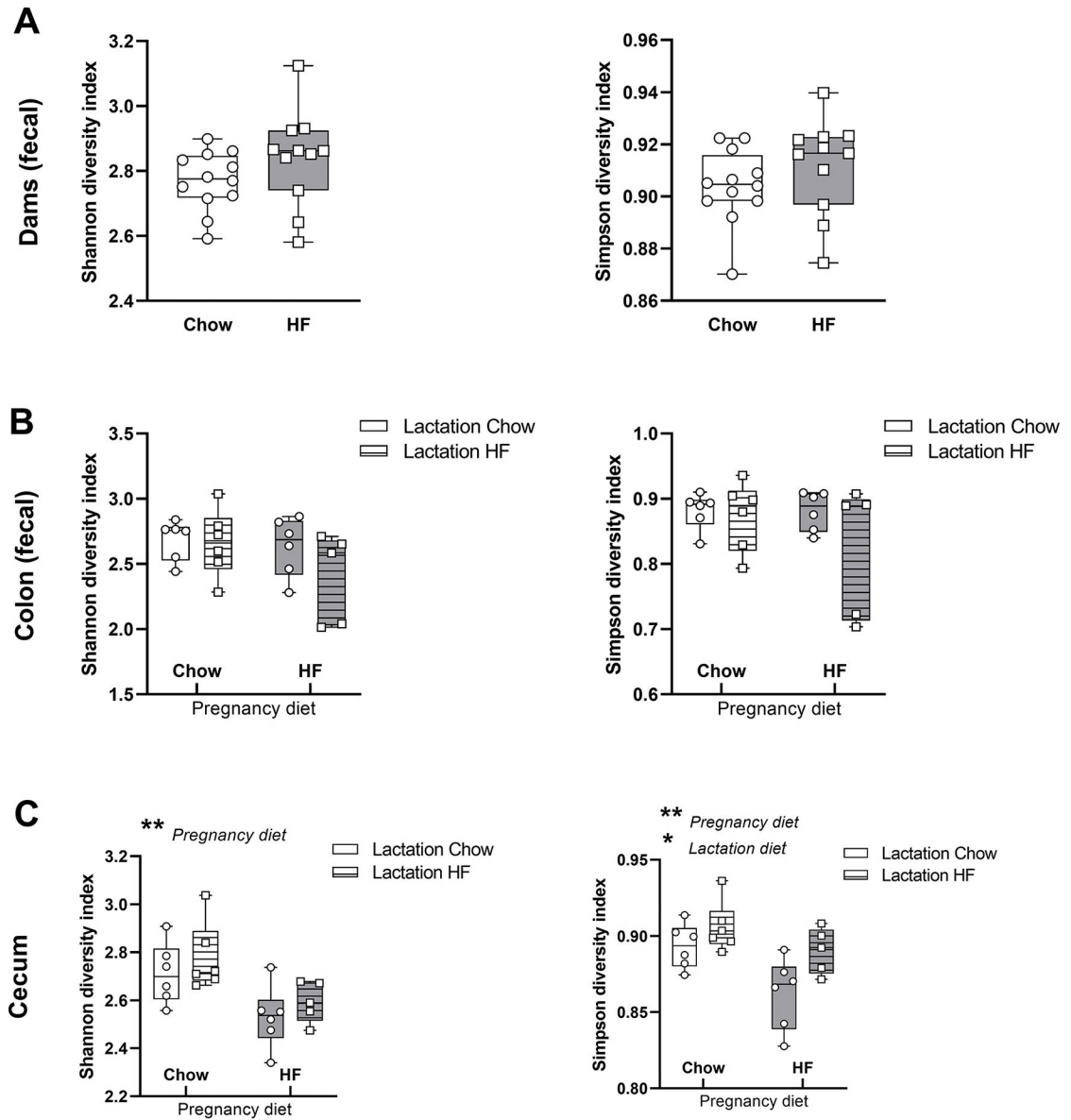- Fecal bacterial abundances at weaning correlates with body weight

**Fig. 1.**
Alpha diversity indices for dams fecal (A) and pups' fecal (B) and cecal (C) microbiome samples at PND21. The Shannon diversity index indicates the extent of species richness and evenness, while Simpson's diversity index is another measure of evenness. Data are presented as box plots with medians (middle lines), 25th to 75th percentiles (box boundaries), and minimum and maximum values (whiskers). Two-way ANOVA or T-test (C) were performed to assess significance. * p < 0.05 and ** p < 0.01 indicate significant differences.
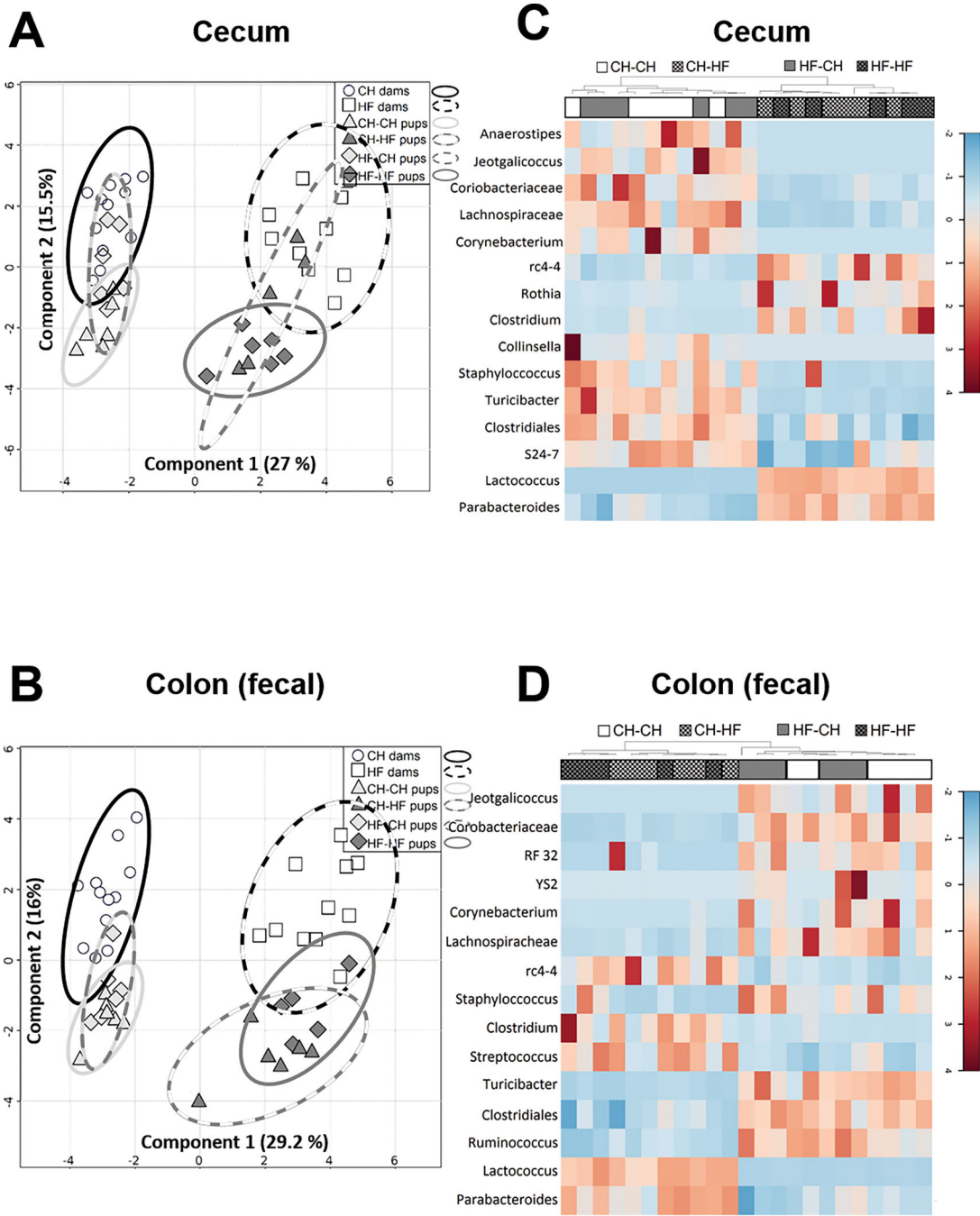
**Fig. 2.**
Microbiota clustering patterns in pups and dams at PND21. Partial least squares-discriminant analysis (PLS-DA) of pup cecal (A) and fecal content (B) alongside dam fecal samples. PLS-DA was determined using bacterial taxa normalized abundances at all phylogenic levels. Ellipses indicate the 95% confidence interval. Heatmaps with ward's clustering algorithm organizing the samples and Pearson's r indicating association between taxa and diet group for pups cecal (C) and fecal content (D), top 15 discriminative features were included.

**Fig. 3.**

Body weight correlations with specific taxa in pup fecal samples at PND21 and taxa differences in pups fecal samples at PND10. Pup body weights (A) were measured on PND21 and Pearson's R indicates significant correlations between body weight and specific genera present in fecal samples (B). Correlations among fecal sample genera and body weight are presented as a heatmap with color indicating the extent to which the correlation is positive or negative and size indicating the correlation p-value with larger circles indicating smaller p-values. Relative abundances of these taxa in the colon were plotted and

demonstrated lactation diet-driven influence (C). (D) shows that some of these differences among other differences in taxa abundances were already present at PND10. Data in (C) and (D) are presented as means ± SEM and two-way ANOVA was performed to assess significance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ indicate significant differences.