Prenatal Diet Shapes Offspring Microbiome and Metabolic Health

Purpose

To examine the impact of maternal consumption of Mediterranean- and Western-style diets on the development of the offspring's gut microbiome and metabolome, and to evaluate the long-term consequences of these diets on the metabolic and intestinal health of the offspring.

Background and Context

Early-life development is the critical window in the human lifespan affected by nutritional choices. Maternal nutrition can significantly dictate the developmental origins of health and diseases (DOHaD)[1]. The theory of DOHaD posits that environmentally induced epigenetic variations in critical periods of human development of growth, metabolism, and neurodevelopment shape the later-life environments that determine the risk of developing cardiometabolic diseases[2,3]. Studies have shown how the suboptimal nutrition in early-life renders an individual susceptible to various metabolic diseases in later life[4,5].

Over the past two decades, our understanding on the convincing role of gut microbiome in influencing the host's physiology across various health and disease states has grown significantly. Within this milieu, the emerging link between the early-life gut microbiome and DOHaD theory is no exception. This is particularly significant as the early stages of infant development coincide with establishing the early-life microbiome. During this critical period, the architecture of the microbiome and its metabolites can profoundly influence metabolic, immune, endocrinological, and neurological developmental responses[3]. Studies have shown that microbial taxa identified in the infant gut microbiome often persist into childhood and adulthood[6]

Recent advancements in this continuously evolving field highlight the growing recognition of diet-microbiome-host interactions and their role in attenuating or accentuating the epidemics of chronic illness worldwide has been gaining momentum. For instance, animal-based western-style dietary patterns, which are rich sources of saturated fat and simple sugars, favor the growth of notorious microbes (e.g., *Alistipes, Bilophila*) capable of triggering inflammatory responses. In contrast, plant-based Mediterranean-style dietary patterns, which are rich sources of complex carbohydrates, mono- and poly-unsaturated fatty acids, polyphenols, and antioxidants, favor the growth of beneficial taxa (e.g., *Roseburia, Faecalibacterium*, etc). capable of resolving pro-inflammatory responses[7,8]. The positive and negative effects of plant- and animal-based diets on the human gut microbiome and their metabolites have been well documented [9,10].

Although it is acknowledged that a maternal adherence of western-style dietary patterns can lead to maternal-neonatal gut dysbiosis and impaired metabolism[11 12], research on the specific effects of maternal diets enriched with Mediterranean or Western patterns - differing in fat sources - on the offspring's gut microbiome and metabolome development, as well as their long-term metabolic and intestinal health, remains unclear.

Method

The overall study design is outlined in Figure 1. C57BL/6 mice breeders were fed on an irradiated isocaloric, and iso-nitrogenous western diet (WD) blended with either: 20% w/w anhydrous milk

fat (saturated fatty acid diet, SFA); 20% w/w corn oil (omega-6 fatty acid diet, n6); or 19% w/w olive oil plus 1% w/w fish oil (omega-3 fatty acid diet, n3); or a Mediterranean diet (MD). The offspring from these breeders were exposed to these diets only during gestation and nursing, followed by a 10-week period of WD consumption. Fecal samples from offspring were analyzed using high-throughput 16S rRNA-based microbiome sequencing and NMR-based metabolomics, at both weaning and 8-weeks. Serum lipid profiles were measured using Piccolo Lipid Panel Plus Reagent Discs (Abaxis) following the manufacturer's instructions. Real time PCR-based gene expression was performed on intestinal tissues to assess markers related to epithelial integrity and inflammation. The results were expressed as ddCt method by normalizing against the 18S housekeeping expression of the SFA control group.

Findings or Results

The prenatal diet shaped offspring microbiome, establishing distinct taxa abundances at the phyla and genus levels, even after 8 weeks of WD feeding (Figure 2). Offspring from n3- and MD-fed dams exhibited distinct microbiome beta-diversity compared to SFA-fed dams even after 8 weeks (Figure 2A). Compared to SFA group, all treatment groups reflected a higher abundance of Bacteroidota at weaning and of Bacillota at 8-week (Figure 2B). Group-specific genera that maintained higher abundance until 8-week included *Erypsilotrichaceae* [n3], *Blautia* [n6], and *Intestinomonas* [MD] (Figure 2C).

Subsequent investigations were conducted to determine the differential effects of prenatal diet on the fecal metabolome (Figure 3). Maternal MD diet considerably modulated the fecal metabolome including higher level of glutamate (at weaning) and propionate (at 8-week) compared to SFA group. No significant differences were observed for the n3 group at weaning; however, certain downstream metabolites of choline, such as trimethylamine and N-nitrosodimethylamine, were found in higher abundance in the n3 group compared to SFA. In the n6 group, the metabolite N-nitrosodimethylamine, which was initially present in higher abundance in the SFA group at weaning, was significantly increased in the n6 group after 8 weeks, along with another metabolite, isoleucine.

Prenatal diet exposure uniquely influenced lipid metabolism, liver function, ileal barrier integrity, and inflammation-related gene expression (Figure 4). The serum levels of circulating lipoproteins - cholesterol (CHOL), non-high-density lipoprotein cholesterol (nHDLc), and low-density lipoprotein (LDL), were increased in both the n6 and MD groups, while high-density lipoprotein (HDL) levels only increased in the MD group compared to the SFA group (Figure 4A). The n3 group exhibited lower levels of LDL compared to SFA group, but this was non-significant. The markers of hepatic dysfunction - alanine aminotransferase (ALT) and aspartate aminotransferase (AST) - were reduced in all groups, with only the MD group showing a significant reduction compared to the SFA group (Figure 4B). Interestingly, the ileal gene expression for tight-junction proteins, including claudins (CLDN-2, 12, 15), zonulins (ZO-1, 2), occludin (OCCL), and junctional adhesion molecule 3 (JAM3) was downregulated in all the treatment groups, with significant reached for at least one of them compared to the SFA group (Figure 4C). The inflammatory gene expression of cytokines (IL-10 and IL-17a) were relatively higher across all treatment groups compared to SFA group, with significance observed for the n3 group (Figure 4D).

Conclusions and Implications

The study concludes that prenatal exposure to different dietary patterns distinctly modulates later-life health by shaping early-life gut microbiome and metabolome. Maternal nutrition during critical windows of neonatal development i.e., in-utero (gestation) and weaning (lactation), had a lasting impact on the gut microbiome diversity and composition, even when offspring were fed WD post-weaning through adulthood. The MD-exposed pups exhibited more pronounced beneficial effects. Furthermore, maternal MD intervention protect the offspring's liver from WD-induced harmful effects. Overall, the study highlights the importance of adhering to or incorporating Mediterranean-style patterns during pregnancy can deliver transgenerational benefits by nurturing a healthier neonatal gut microbiome and supporting cardiometabolic health development.

Appendix A - References

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1 Alves, J. G. B. & Alves, L. V. Early-life nutrition and adult-life outcomes. *J. Pediatr. (Rio J.)* (2023).

2 Aagaard-Tillery, K. M. *et al.* Developmental origins of disease and determinants of chromatin structure: maternal diet modifies the primate fetal epigenome. *J. Mol. Endocrinol.* **41**, 91 (2008).

3 Sarkar, A., Yoo, J. Y., Valeria Ozorio Dutra, S., Morgan, K. H. & Groer, M. The association between early-life gut microbiota and long-term health and diseases. *Journal of Clinical Medicine* **10**, 459 (2021).

4 Barker, D. J. *et al.* Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* **36**, 62-67 (1993).

5 Tarry-Adkins, J. L. & Ozanne, S. E. Nutrition in early life and age-associated diseases. *Ageing research reviews* **39**, 96-105 (2017).

6 Yee, A. L. *et al.* Longitudinal microbiome composition and stability correlate with increased weight and length of very-low-birth-weight infants. *MSystems* **4**, 10.1128/msystems. 00229-00218 (2019).

7 Nagpal, R., Shively, C. A., Register, T. C., Craft, S. & Yadav, H. Gut microbiome-Mediterranean diet interactions in improving host health. *F1000Research* **8** (2019).

8 David, L. A. *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559-563 (2014).

9 O'Keefe, S. J. *et al.* Fat, fibre and cancer risk in African Americans and rural Africans. *Nature communications* **6**, 1-14 (2015).

10 Rampelli, S. *et al.* Metagenome sequencing of the Hadza hunter-gatherer gut microbiota. *Curr. Biol.* **25**, 1682-1693 (2015).

11 Zou, J., Ngo, V. L., Wang, Y., Wang, Y. & Gewirtz, A. T. Maternal fiber deprivation alters microbiota in offspring, resulting in low-grade inflammation and predisposition to obesity. *Cell Host Microbe* **31**, 45-57. e47 (2023).

12 Grant, E. T., Boudaud, M., Muller, A., Macpherson, A. J. & Desai, M. S. Maternal diet and gut microbiome composition modulate early-life immune development. *EMBO Mol. Med.* **15**, e17241 (2023).

Appendix B - Tables and Figures

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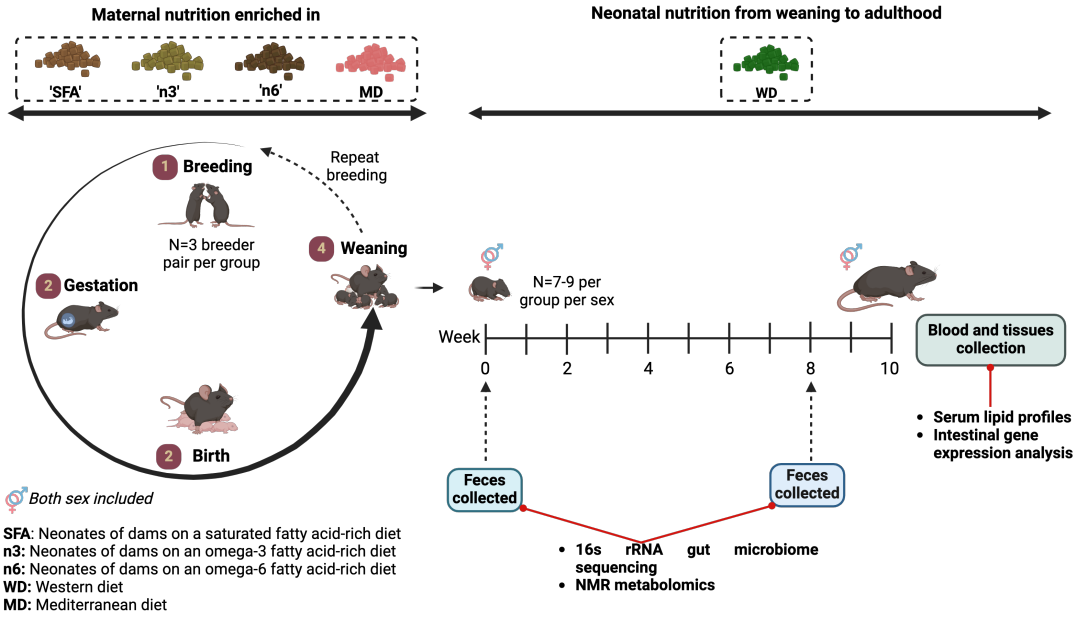

Figure 1. Study design

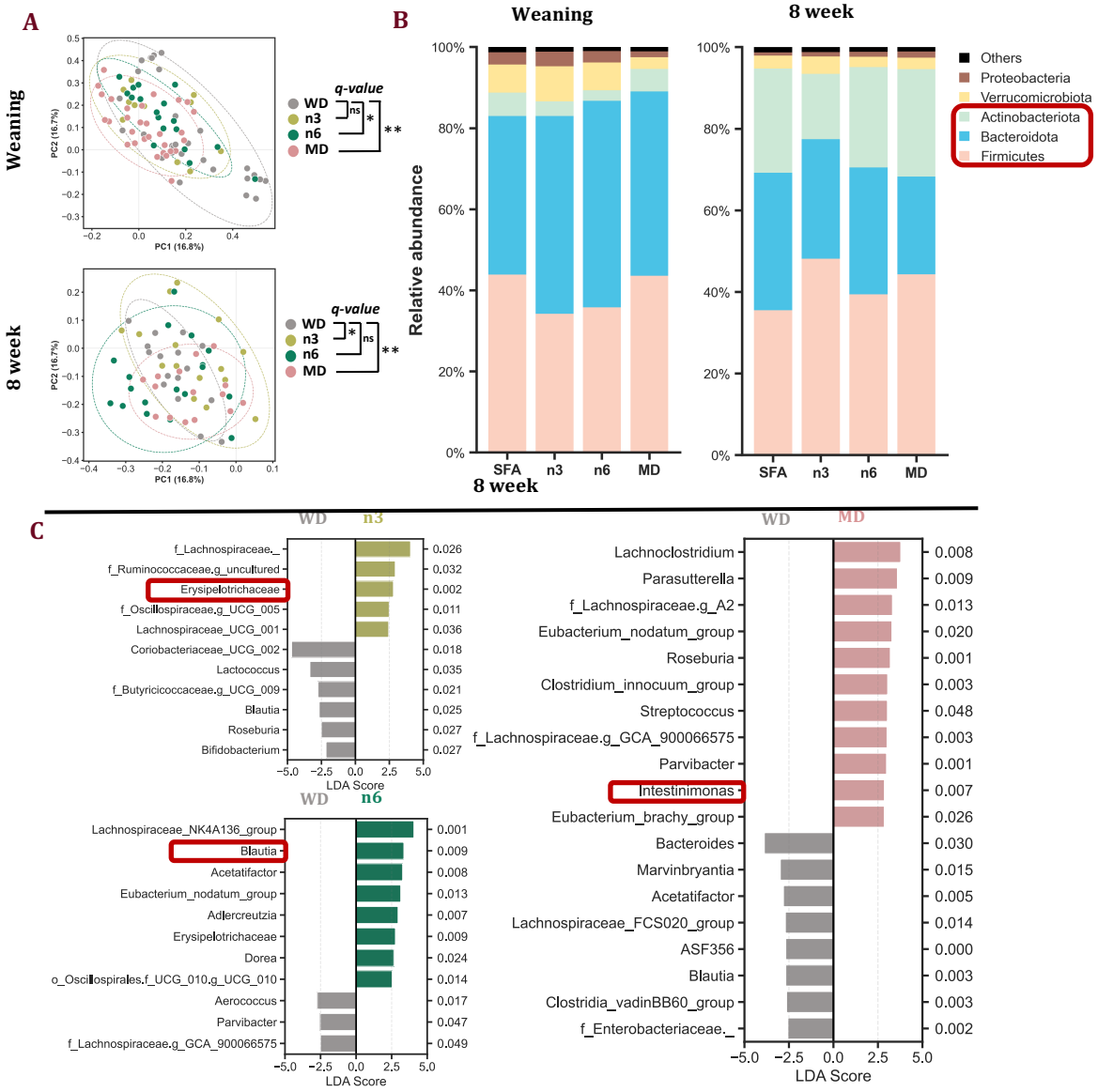

Figure 2. (A) Beta diversity evaluated using Bray-Curtis principal coordinate analysis at weaning and 8 week. **(B)** Gut microbiome composition at phylum level. **(C)** LEfSe analysis showing group-specific discriminative taxa at 8 week (LDA>2.0, p<0.05). *p<0.05, **p<0.01

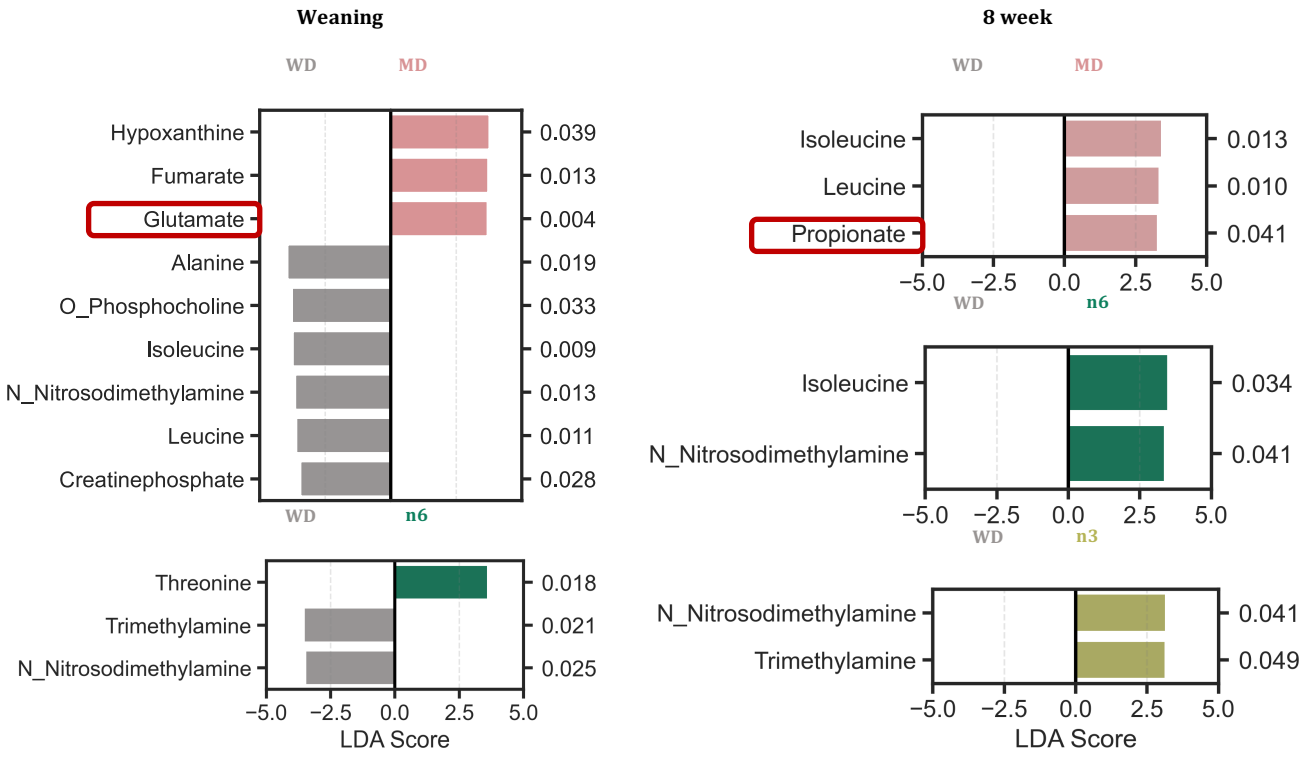

Figure 3. LEfSe analysis showing group-specific changes in fecal metabolome at weaning and 8-week (LDA>3.0, p<0.05).

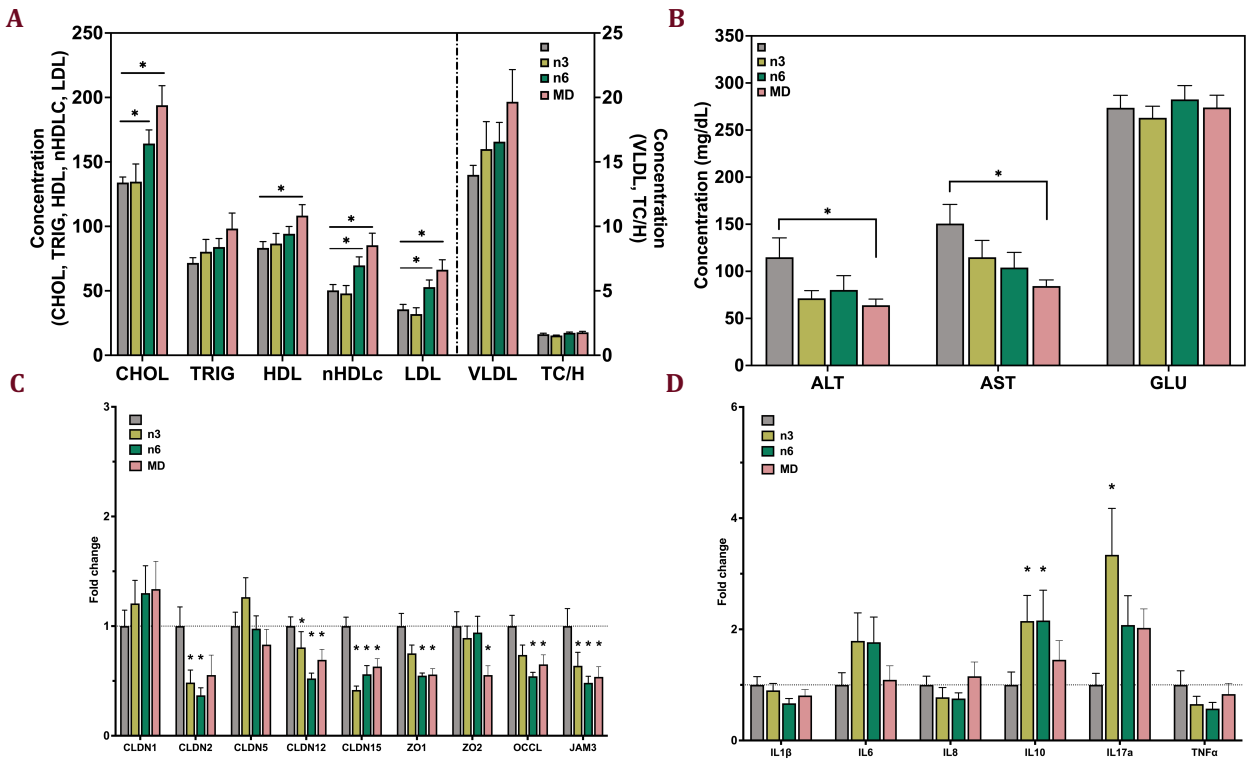

Figure 4. (A) Serum lipid profiles. **(B)** Serum liver enzymes and glucose concentration. **(C)** Gene expression of tight-junction proteins in the ileum. **(D)** Gene expression of inflammatory cytokines in the ileum *p<0.05