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The Fecal Fermentation Profile of Infants with Different Feeding Modalities

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Abstract

Introduction/Background

Research indicates nutrition and environment in the first year of a child's life are crucial in their development and growth and can contribute to lower chances of developing obesity and other health concerns. Key factors that can determine these outcomes include the bacteria and resulting short chain fatty acids (SCFAs) present in the gut. This composition may be affected by feeding modality (formula feeding vs breastfeeding), exposure to the mother's microbiota, weight status of the child, and type of delivery. This research aims to identify the impact of infant feeding modality on toddlers' fecal fermentation profile, and if there are associations between weight status and microbiome, fecal fermentation profile.

Methods/ Procedures

Participants (n=40) were recruited during well-child pediatric appointments at ETSU's Pediatric primary care clinic. Researchers explained the requirements of the study and participants were provided with a 90-question food frequency questionnaire (FFQ) for children ages 2-7, including 90 questions and asks about a child's typical intake over the previous 6-month period. The food list was developed from NHANES III dietary recall data. The child's history was obtained, including current age, birth length and weight, delivery type (C-section or vaginal), feeding method (breast, bottle fed, or both) and duration. The child's weight and height were obtained, and body mass index (BMI) calculated. Participant-provided stool samples were freeze-dried and ground, and SCFAs were extracted using a procedure developed by Schwiertz et al. that was modified. One mL of the SCFA extraction solution, containing Oxalic acid (0.1 mol/L), Sodium Azide (40 mmol/L), and Caproic acid (0.1 mmol/L) (internal standard) was added to 80 mg of a

freeze-dried stool sample in a 16 x 100 mm disposable culture tube, and analyzed using a Shimadzu GC2010 gas chromatograph with SigmaAldrich ZB-Wax Plus capillary column. Samples were run in duplicate, and values for each participant were averaged. Data analysis was generated using SAS software, Version 9.4 of the SAS System, Copyright © 2013 SAS Institute Inc.

Results

Initial findings showed no significant differences in the SCFA composition of obese vs non-obese toddlers in the sample. However, there were significant differences in the amount of specific SCFAs (isobutyrate, isovaleric acid, and octanoic acid) in toddlers who were formula fed as infants versus toddlers who were breastfed, and those fed a combination of breastmilk, and formula ($p < 0.05$). Further analysis will determine if these initial results may be contributed to overall dietary intake, and more specifically fiber intake.

Chapter One: Introduction

Short chain fatty acids (SCFAs) and microbiome play important roles in a child's health and development, but as this is a growing area of research, the specific mechanisms are still emerging. Fecal fermentation profiles are dependent on diet, in particular fiber intake, and the microbiota present, and reflect complex interactions. The microbiome includes a collection of microorganisms (bacteria) within the body which are determined by a number of environmental factors. The literature suggests feeding modality (breastmilk vs formula) plays a significant role when it comes to SCFA production and the diversity of the bacteria present, as well as birth method (cesarean-section vs vaginal), and complementary foods/diet, though further research is needed to determine what impact this may have on future health, and weight status. A healthy gut is thought to be an important variable. In this research, the main questions were focused on how fecal fermentation profile and the microbiome might be associated with weight status (obesity) when an infant becomes a toddler. The fecal fermentation profile (SCFAs), microbiome diversity, and food intake history are potential covariates that will give a more complete understanding of in toddlers in East Tennessee.

Chapter Two: Literature Review

Understanding the Microbiome

In recent years, the human understanding of the microbiome has grown with innovative technology and research. According to Dong and Gupta (2018), the microbiome is made up of several communities of bacteria and includes an estimated one hundred trillion microbes. The majority of these live in the human gut. The large intestine contains 30 identified genera and 500 varied species (Chong et al., 2018). The microbiome is very complex. According to Amon and Sanderson (2017), it also has several functions, such as providing immunity defense against pathogens and host nutrition (Nutrition can influence the hosts ability to cope with the consequences of parasitism and to contain and eventually to overcome parasitism), including production of short-chain fatty acids essential in host energy metabolism, synthesis of vitamins and fat storage, and influence on human behavior. According to Stiemsma and Michels (2018), the microbiome plays a valuable role in the hypothesis called the Development Origins of Health and Disease (DOHaD). This states that environmental exposure has a critical impact on long term outcomes in fetal and infant development. It suggests an infant's microbiome can grow and change the most when compared to any other age. Those benefits medicate a diverse and complex microbiome which leads to healthier adults and further developed immune systems. It also helps decrease risks of inflammatory bowel disease (IBD), obesity, allergies, and autoimmune diseases. The gut microbiome acts as a gatekeeper. It teaches immune cells (T-cells) to distinguish foreign cells from its own. According to Chong (2018), the microbiome is affected by several factors such as gestational age, mode of delivery, type of feed, maternal nutrition

status, and use of antibiotics. Only 1.9%-9% of the microbiome is affected by genetics; in comparison, 22%-36% of it is environmental effects (Dong & Gupta, 2018). This is good news because it suggests the microbiome can be changed to get the best long-term results.

SCFA (Short Chain Fatty Acids)

Short- chain fatty acids are the main metabolites produced in the colon by bacterial fermentation of fibers and resistant starch. These are thought to play a key role in neuro-immunoendocrine regulation. (Silva et al., 2020). The SCFAs acetate, propionate, and butyrate are the most common. They also have crucial physiological effects on organs, including the brain. This indicates that many gut microbiota have a role in behavioral and neurologic pathologies, such as depression, Alzheimer's and Parkinson's diseases and autism spectrum disorder. Furthermore, SCFA microbiota manipulation could be used as treatment appropriate for such diseases (Silva et al., 2020).

Microbiota and SCFA manipulation explored associations between infant's anthropometrics and short-chain fatty acids (SCFAs) (Prentice et al., 2019). This was done since SCFA are known to be found in human milk and linked to energy metabolism. Findings showed peaks for human milk butyrate, acetate, and formic acid, but not propionate. Butyrate peaks were 17.8% higher in human milk from solely breastfeeding mothers (Prentice et al., 2019). Human milk butyrate peak values were negatively associated with increases in infants' weight and BMI between 3 and 12 months. These effects on infant adiposity were clear at age 24 months because of positive associations between human milk butyrate peak values and changes in infant BMI and skinfold thickness between 12 and 24 months (Prentice et al., 2019). Human milk formic acid peak values showed a constant negative association with infant BMI at all time points.

These suggest that human milk SCFAs play a beneficial role in weight gain during infancy. SCFAs butyrate, formic acid, and acetate are all in human milk and have negative associations with measure of infant adiposity. This suggest that breastmilk has concentrations of SCFAs that can provide early protection against excess weight gain (Prentice et al., 2019). With this fecal fermentation profiles are used to investigate the influence of the substrate presented to fecal microbiota.

Mothers Milk

The composition of breast milk is primarily water and then lipids. Lipids make up the largest source of energy in breastmilk. Most are triacylglycerides (an ester derived from glycerol and three fatty acids), While also containing diacylglycerides, monoacylglycerides, free fatty acids, phospholipids, and cholesterol. There are also around 200 fatty acids but in lower concentrations (Andreas et al., 2015). Short-chain fatty acids are essential as a source of energy and necessary for the normal maturation of the GI tract. Next in concentration are proteins, of which there are about 400 diverse types that go into three groups: caseins, whey, and mucin (Andreas et al., 2015). Some of their functions include providing nutrition, possessing antimicrobial and immunomodulatory activities, and stimulating the absorption of nutrients (Andreas et al., 2015). Also, in breastmilk, some antibodies help provide immunological protection to the infant. The baby's immune system is fragile; it has not been developed enough yet. The milk provides protection during this vulnerable time. Next is the carbohydrate component of breastmilk, which are complex, with lactose being the most abundant. Finally, human milk oligosaccharides (HMOs) are essential since they function as a prebiotic, (a form of

dietary fiber that feeds healthy gut bacteria), helping the growth of certain bacteria strains. The infant cannot digest them, but they help nourish the gastrointestinal microbiota.

The composition of breastmilk evolves through three different stages as time goes on: colostrum, transitional milk, and mature milk (Le Doare et al., 2018). These represent the change in the content of the milk. Colostrum's main role is promoting growth and immunologic function. It protects the baby from the outside environment—the composition changes during nursing from start to end. Factors that can affect the mother's milk concentration are diet, ethnicity, weight gain during pregnancy, and birth weight of the mother. Overall, breastmilk is a complex system critical to the infant's development (Le Doare et al., 2018).

Overall, breastmilk is a complex system, including bacteria, vital to the infant and its development. The baby gets a variety of bacteria, as many as 800,000 bacteria daily (Le Doare et al., 2018). Furthermore, there is a direct link between breastmilk and infant fecal makeup of *Bifid bacterium, Lactobacillus, Enterococcus*, and *Staphylococcus* species (Le Doare et al., 2018). As the baby receives more breastmilk, the microbes increase, and there is evidence that the baby's microbes closely resemble that of the mothers. Some of the roles these bacteria play are expressing antimicrobial properties against pathogenic bacteria, reducing infections, and developing the immune system.

Breastfeeding vs. Formula Feeding/Combined Feeding

Breastfeeding has been the recommended source of nutrition in full-term infants for decades. There are many health benefits, but still, breastfeeding rates are low. Those first years of life help determine if the child will develop diseases or become obese later depending on how

they are fed as an infant. According to Lemaire et al. (2018), breastfed infants have 3% lower insulin levels in later life (childhood and adulthood) than formula-fed infants. These higher insulin levels in formula-fed infants may explain more significant deposition of subcutaneous adipose tissue. Also, formula-feeding could be associated with higher blood pressure levels in childhood than breastfeeding. This is thought to be the case because of the differing composition of breast milk and formula milk, particularly the sodium and fatty acid content. Formula-feeding is associated with a less stable microbiota and different overall bacterial make up (Lemaire, 2018). The infants fed with formula had higher levels of SCFAs (Propionate, butyrate, acetate, 5-aminovalerate, and free amino acids). In comparison, breastfed infants had higher levels of fucosylated oligosaccharides and lactic acid, resulting in a higher fermentation of HMOs (Human Milk Oligosaccharides). The Centers for Disease Control (CDC, 2021) promote breastfeeding to help the mother reduce high blood pressure, type two diabetes, ovarian cancer, and breast cancer. For baby, they agree with researchers that it can reduce asthma, obesity, and diabetes. This new research offers another reason breastfeeding is so crucial for the infant.

Pannaraj et al. (2017) completed a study that looked at 107 healthy mother-infant pairs over the first year of the infant's life. They found 27.7% of infants gut bacteria was from breast milk and 10.4% from areolar skin. Also, finding several bacterial communities were identified in milk, areolar skin, and in the stool. They discovered that the bacteria from the mother's milk and skin are most prominent in the infant's gut for the first month, at 40%, this which would available without that exposure to breastfeeding. The skin contact alone has bacteria that cannot be duplicated from bottle feeding. Savage and colleagues explored (2018) associations between the mother’s diet during pregnancy and infancy, including breastfeeding versus formula feeding and the impact on microbiome in the infants’ stool. They learned that breastfeeding was associated

with infant intestinal microbial diversity when comparing the two among the (323) participants. Breastfeeding also had the most associations with individual taxa(a term for in which microorganism groups (taxa) that have been previously linked to early-life diet and health outcomes such as *Bifidobacterium*. While researchers have seen differences in the intestinal microbiome and body growth between breastfed versus formula-fed infants, little is known about the effects of mixed feeding either briefly or long term on these health outcomes. Because breastfeeding leave an impact, it is essential to discuss the barriers to breastfeeding and why only one in four women breastfeed (CDC, 2021). Common issues are problems latching, concerns about babies' weight/ nutrition, mother's diet, and medication while breastfeeding, lack of support, and cultural norms. They also looked at common breastfeeding issues and found the majors one to be lack of experience, lack of support, lack of up-to-date info from health care workers, lacking hospital practices, and workplace not supporting. These can lessen with education with a lactation specialist and establishing a solid support system. O'Sullivan and colleagues (2015) demonstrated the importance of early breastfeeding and how brief, or long-term use of formula influences the health benefits of exclusive breastfeeding.

C-Section vs. Vaginal Birth

Delivery mode is the primary determinant of neonatal intestinal microbiome establishment. C-sections have been found to limit the infant from gaining beneficial bacteria. Due to the infant not receiving maternal vaginal and fecal bacteria during birth (Kim et al., 2020). In numerous studies, birth by C-section has been associated with allergic outcomes in children. Rutayisire et al. (2016) used fecal samples to look at gut microbiome relating to

delivery mode. They found that C-sections were associated with low abundance/diversity of the *phyla Actinobacteria* and *Bacteroidetes*. While higher in *phylum Firmicute* from birth to three months of age. Also, according to Jakobsson et al. (2014) *Bifid bacterium, Bacteroides, Clostridium,* and *Lactobacillus genera* were greater in vaginal births. They concluded that delivery mode is essential to developing the microbiome in the first three months of life, but differences are lesser after six months of life. Kim et al. (2020) found C-sections can lead to dysbiosis (imbalanced gut bacteria) in newborn gut microbiota, and noted decreased abundance of *Bifid bacterium*, *Bacteroides*, and *Lactobacillus* in infants born by C-section. Levin et al. (2016) found similar results in their research. They found mice born via C-section had changes in their immune function, such as a reduction in the proportion of regulatory T cells and down regulation of the regulatory markers. Multiple researchers found the association between delivery mode and atopy (the genetic likelihood to develop an allergy). As well as those C-section delivered had a much higher risk of having asthma Kim et al. (2020). Also, C-section delivery is a risk factor for children's allergic outcomes, and the degree of risk varies by biological and environmental factors related to the infant and its parents. Kim et al. (2020) looked at Korean newborns receiving the same care services two weeks after birth using fecal samples collected at days 3, 7, and 14. Early gut microbiota development patterns were examined using 16S rRNA gene-based sequencing from the group of infants either born vaginally (VD= 64) or Cesarean section (CS= 68). Their findings supported earlier research that indicated an abundance of *Bifid bacterium*, *Bacteroides*, and *Lactobacillus* in infants born vaginally. Overall, there are still conflicting data, but with more recent studies, it is becoming more apparent that birth method significantly impacts the infant's microbiome.

Twins

There is not much data related to differences in microbiome in twins and single infants (Bao et al., 2021). looked at bacterial signatures that can influence diseases and food allergies in twins. Researchers compared healthy twins and twins with the allergies. Findings showed that twins have significant differences in their fecal microbiomes and metabolomes (the set of small molecules chemicals found within cell, organism) through adulthood. Meaning, the microbiota may play a protective role in patients with food allergies beyond infancy. This study does show how these early-life changes persist, despite years of separation and lifestyle changes between twins/siblings. Turnbaugh et al. (2009) focused on how genotype, environmental exposures, and host adiposity influence the microbiome, and looked at concordant fecal microbial communities of adult female monozygotic (MZ) and dizygotic (DZ) twin pairs for leanness or obesity. They found that identical MZ twin pairs gain weight more in response to overfeeding than unrelated people and are more parallel to body mass index (BMI) than DZ twin pairs. Overall, the gut microbiome is similar in family members, but each person's gut microbial community varies in the specific bacteria present. There are quite a few comparisons when it comes to co-variation between adult MZ and DZ twin pairs. However, there was no data on whether MZ and DZ twin pairs had different degrees of similarities at earlier stages of their lives. With minimal evidence, we hoped our data would indicate some relationship.

Obesity

Obesity is a growing problem in the US. One possible solution is to start as early as possible when it comes to breastfeeding. Obesity has been linked with decreased microbiome diversity. Infants with high *Streptococcus* at six months of age were more likely to be overweight at 18 months (about one and a half years) (Stiemsma & Michels, 2018). This may mean the chance of obesity can be predicted later in life by examining the fecal matter of the infant to look for differences in the microbiome. Also, it is possible that variations in gut microbiome in an infant were correlated with their cognitive abilities later in life, as well as their anxiety/ stress levels (Stiemsma & Michels, 2018). Scientists have narrowed the critical microbiome window to the time between conception and the first year of life. This is a particularly crucial time to ensure everything possible to allow the infant as much microbiota and diversity as possible. Obesity is also associated with phylum-level changes in the microbiota, reduced bacterial diversity, and altered representation of bacterial genes and metabolic pathways (Turnbaugh et al., 2009). In 2019, Mennella and colleagues examined whether maternal feeding modality had effects along with infant formula, type on early rapid weight gain. Using anthropometry, feeding patterns for formula feeds and maternal feeding, they concluded that the kind of infant formula directly impacted rapid weight gain early on. Findings did not pinpoint the difference in outcomes between formula feeding and breastfeeding. Forbes et al. (2018) gave further insight into the question at hand. Among 1087 infants from the prospective CHILD pregnancy cohort, revealed a feeding histories and fecal samples at 3-4 months and again at 12 months; 53.8% were exclusively breastfed at three months. Researchers found Microbiota profiles at 3 months were more associated with risk of obesity than the microbiota profiles at 12 months. Formula

supplementation was associated with higher diversity and enrichment of *Bacteroidaceae*. However, the breastfed infants showed lower variety and enrichment of *Bifidobacteriaceae* and *Veillonellaceae*. They found breastfeeding may be protective against obesity, and microbiota may contribute to this. Formula feeding did seem to create changes in microbiota that are associated with overweight. Also, slight microbiota differences emerge after brief exposure to formula in the hospital. These results identify important areas for future research more than anything else. (Forbes et al. 2018). Lemas and colleagues (2016) analyzed infant stool and fasting human milk samples from 18 normal-weight mothers, and their exclusively breastfed infants at two weeks postpartum. They found that human milk insulin and leptin are associated with beneficial microbiota and are predicted to increase barrier function, reducing intestinal inflammation (Lemas et al., 2016). They also found that babies born to obese mothers were exposed to 2-fold higher human milk insulin and leptin concentrations, lower *Gammaproteobacteria*, and elevated total SCFA content. The contribution of the gut microbiota to the development of overweight and obesity-related, low-grade inflammation is becoming clear as research goes on (Luoto, 2013). By influencing the nutritional and microbial ecology of the mother and the baby, the next generation's health may be modified.

The mother's diet is essential because obese mothers with a maternal high-fat diet during pregnancy and breastfeeding may cause dysbiosis in the offspring's microbiome (Stiemsma et al.,2018). Several studies found, during pregnancy, the Western diet (which provides high intakes of animal protein, saturated fat, and sodium but is low in monounsaturated fat and n–3 fatty acids) was associated with decreased short chain fatty acid (SCFAs) production this is believed to cause the same effect in human babies. The Mediterranean diet, however, has greater amounts of monounsaturated fatty acids (MUFAs), is mainly plant-derived protein, with some

protein from fish and dairy, and is low in refined sugars. These are the two diets that have been primarily looked at concerning this research topic. The fat content in the breast milk, particularly the long-chain polyunsaturated fatty acids (LCFAs), is also mainly affected by the mother's diet. "*Staphylococcus* seemed to be negatively related with MUFA percentages in milk triglycerides, and the genus *Streptococcus* was negatively correlated with the abundance of SFAs in milk triglycerides. *Bifid bacterium* and *Lactobacillus* genera showed negative associations with MUFAs and n–3 PUFAs in milk phospholipids" (Demmelmair et al., 2020, p 7). In *Maternal and Perinatal Factors Associated with the Human Milk Microbiome, Demmelmair et al.* found that the probiotic bacteria in the diet might reach the mammary gland and be integrated into milk. In addition, micro-and macronutrient intake could influence the composition of bacteria living in the maternal GI tract (2020). These may reach the mammary gland via the intramammary pathway. Alternatively, different bacteria may live in the mammary gland via altered micronutrient content in that microenvironment (Demmelmair et al., 2020). Also, it is believed that chronic nutrient intake is more important than acute nutrient intake in shaping the GI microbiome. Even though this is gaining evidence, a lot more research is still needed to understand how the mother's diet could affect the milk and, therefore, the baby's microbiome.

Conclusion

The literature includes evidence that breastfeeding can have a positive impact on an infant’s life when looking in terms of microbiome and fecal fermentation profile. It is still a growing research area, and it will be interesting to see how this research continues.

Chapter 3: Materials and Methods

Approval for the study was obtained from ETSU's Institutional Review Board. Participants were recruited during well child pediatric appointments at ETSU's Pediatric primary care clinic. Researchers explained the requirements of the study, and after informed consent was obtained, participants were provided with a food frequency questionnaire (FFQ) for children ages 2-7, and the procedures for how to collect and return the stool samples. The child's history was obtained, including current age, birth length and weight, delivery type (C-section or vaginal), and feeding method (breast, bottle fed, or both) and for how long. The child's weight and height were obtained from the medical chart. Participants were given a $10 gas card.

Protocol for sample collection and drop off:

Stool samples (either in diapers or sealed containers) were placed in freezer bags and kept frozen. Participants then transported stool samples and the FFQ to the ETSU Pediatric facility within 24 hours, where they were collected by researchers. Participants were then provided with a $20 gift card upon drop off of materials. The stool samples were then stored in a temperature-controlled biohazard cooler and transported to the Human Nutrition and Dietetics Research Laboratory on ETSU's Valleybrook campus, where the samples were stored at -80° Celsius for analysis.

Block Questionnaire for Ages 2-7- Kids 2-7 FFQ

This questionnaire included about 90 questions and asks about a child's typical intake over the previous 6-month period. The food list was developed from NHANES III dietary recall data. The nutrient database was developed from the USDA Nutrient Database for Standard Reference. Individual portion size is asked for beverages but not other foods. Completed

questionnaires were mailed to Berkeley Analytics, Inc (dba NutritionQuest) in Berkley, California, for processing, where they were nutrient estimates were calculated and provided electronically for statistical analysis.

SCFA Extraction and Determination

2.6.3 SCFA Extraction and Analysis SCFA extractions were performed using a procedure developed by Schwiertz et al. that was modified [137]. One mL of the SCFA extraction solution, containing Oxalic acid (0.1 mol/L), Sodium Azide (40 mmol/L), and Caproic acid (0.1 mmol/L)(internal standard) was added to 80 mg of a freeze-dried stool sample in a 16 x 100 mm disposable culture tube. The tube was capped and vortexed for 30 seconds. The tube was placed on a horizontal shaker for 1 hour. The tube was centrifuged at 4000 rpm for 20 minutes. After centrifuging, the supernatant was removed and placed in a 1.5mL polypropylene Fisherbrand micro-centrifuge tube. The solution was re-centrifuged at 12,000 rpm for 15 minutes. Again, the supernatant was removed and placed in a new 1.5 mL micro-centrifuge tube. The solution was re-centrifuged at 12,000 rpm for 15 minutes. Finally, the supernatant was removed, placed in a 2 mL amber vial, and was stored at -80◦C until being analyzed using a Shimadzu GC2010 gas chromatograph with SigmaAldrich ZB-Wax Plus capillary column. Samples were run using a method adapted from Schaefer et al [138]. The method included injecting 1 μL of solution with an SPL1 temperature of 250◦C. The initial column temperature was 50◦C, held for 2 minutes, which rose at a rate of 15 degrees/minute until reaching 140◦C with a hold of 5 minutes, followed by a rise at rate of 10 degrees/minute until reaching 160◦C with a hold of 3 minutes and a rise of 10 degrees/minute until reaching 175◦C with a hold of 3 minutes. The ame ionization detector temperature was 180◦C, and the end time of the run was 24 minutes. Samples were run in duplicate, and values for each participant were averaged.

Data Analysis:

This one-way ANOVA analysis was generated using SAS software, Version 9.4 of the SAS System, Copyright © 2013 SAS Institute Inc. One-way ANOVA is a statistical test that compares the variance in the group means within a sample while also considering only one independent variable.

Chapter 6: Results

Sample 1

Forty participants were recruited; of these, 30 completed the study and were included in analysis. The sample consisted of 53% (n=16) males and 47% (n=14) females, with an age range of 24-58 months. 43% (n=13) reported birth via cesarean-section, 53% (n=16) reported vaginal births. 76.7% (n=23) receives some breastmilk; 30% (n=9) were exclusively breastfed, 23.3% (n=7) formula fed, and 46.7% (n=14) fed a combination. Length of breastfeeding varied from 0.5 months to 24+ months, with a mean length of 8.92 months. Lastly, 20% (n=6) of the toddlers in the study were obese.

There were a few surprises in the initial findings. Statistical analysis did not show significant differences in the SCFA composition of obese vs non-obese toddlers in the sample, indicating that the SCFAs present were not associated with weight status. However, there were significant differences in the quantity of specific SCFAs (isobutyrate, isovaleric acid, and octanoic acid) in toddlers who were formula fed as infants versus toddlers who were breastfed, and those fed a combination of breastmilk, and formula ($p < 0.05$). This is important because feeding modality is a key factor being explored as impacting long term health. The presence of

these branched SCFAs may be a possible marker of protein fermentation and may increase with age. These factors should be considered in future analysis. There is a chance this is influenced by insoluble dietary fiber intake, depending on the toddler's diet.

SCFA	Breast	Formula	Combination	P-value	Comments
n	9	7	14		
acetate	34.5 ± 6.0	28.6 ± 3.2	33.1 ± 6.3	0.1161	One-Way ANOVA
butyrate	36.1 ± 11.7	29.3 ± 2.1	32.8 ± 6.2	0.2444	One-Way ANOVA
caproic	0.1 ± 0.1	0.3 ± 0.4	0.2 ± 0.2	0.4732	One-Way ANOVA
isobutyrate	2.5 ± 1.2	4.6 ± 1.1	3.2 ± 1.4	0.0096	One-Way ANOVA
isocaproic	0.3 ± 0.3	0.1 ± 0.1	0.2 ± 0.2	0.1469	One-Way ANOVA
isovaleric	4.4 ± 2.2	9.1 ± 2.7	5.9 ± 2.9	0.006	One-Way ANOVA
octanoic	1.3 ± 0.6	2.6 ± 1.2	1.5 ± 1.0	0.0245	One-Way ANOVA
propionic	18.1 ± 7.9	19.7 ± 5.0	19.0 ± 3.9	0.844	One-Way ANOVA
valeric	2.7 ± 1.4	5.6 ± 0.8	4.2 ± 1.8	0.0036	One-Way ANOVA

Chapter 5: Discussion

The findings included some interesting results regarding SCFAs and feeding modality, though important to remember this is all preliminary data. The literature suggests that the presence of increased branched short chain fatty acids may lead to the production of other fermentation products that can be harmful for the colon epithelium (Rios-Covian et al., 2020). Branched chain fatty acids (BCFA) are mostly saturated fatty acids (SFA) with one or more methyl branches on the carbon chain. The main BCFAs are isovaleric and isobutyric acids. Which are produced in less amounts and their fecal levels in different human groups, and influence on health are not widely known at this point (Rios-Covian et al., 2020). If this is connected to specific feeding modalities, possible interventions to offset these outcomes may become evident. Analysis of the dietary intake may help determine if these initial results may be contributed to diet and fiber intake.

Limitations included having a small sample size. Due to recruiting limitations, we enrolled fewer obese toddlers than we would have expected. Due to this being the south we figure a few more of the participants would be obese. Due to the low number is was more difficult to draw associations. Further, the mothers and toddlers in the study were from rural East Tennessee, and shared similar backgrounds, so results may not be generalizable to the greater population. Even though this is a good representation of the Appalachians, more data and participants are needed to see how this effects other populations. Also, while the available data allows researchers to draw some predictions about the health of the participants, further analysis of the microbiome and quality of the dietary intake will add a more complete picture. The

microbiome data is being looked over in Colorado which should be concluded soon. This will allow more associations to be made.

Chapter 6: Conclusion

In conclusion the findings were promising though didn't completely answer the hypotheses. Hopefully with the further diet information and microbiome data more connections will present themselves. In general, for both the literature review and this research process that consistent conclusion is this is a start, but further research is needed. This is, in some cases, needing a larger and more diverse sample size. In other it is they found promising data but needed to follow the participants later in life to see the long-term effects. Though a healthy gut is critical and once more research is conducted these hypotheses plus others can be better understood.

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