Title

Finding the Gut Microbiome Connection to the Risk of Childhood Obesity Through an Examination of Gut Microbiota Among Children In Los. Angeles, California: A Cross-Sectional Pilot Study

Author

Basilio, Cecille Marie

Publication Date

2024

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Finding the Gut Microbiome Connection to the Risk of Childhood Obesity Through an Examination of Gut Microbiota Among Children in Los Angeles, California: A Cross-Sectional Pilot Study

A dissertation submitted in partial satisfaction of the

requirements for the degree

Doctor of Philosophy in Nursing

by

Cecille Marie Basilio

2024

ABSTRACT OF THE DISSERTATION

Finding the Gut Microbiome Connection to the Risk of Childhood Obesity Through an Examination of Gut Microbiota Among Children in Los Angeles, California: A Cross-Sectional Pilot Study

by

Cecille Marie Basilio

Doctor of Philosophy in Nursing

University of California, Los Angeles, 2024

Professor Felicia S. Hodge, Chair

Background: Childhood obesity has emerged as one of the most critical public health problems in the 21st century of national and global epidemic concern. Globally, in 2020, the number of children and adolescents between the ages of 5 and 19 who were classified as obese reached 150 million, and projections suggest this number could escalate to 254 million by 2030, according to the World Health Organization (WHO) 2021. In the United States (U.S.), obesity affects approximately 14.7 million children and adolescents aged 2-19 years (Centers for Disease Control and Prevention [CDC], 2022a). Considering the accelerating incidence of childhood obesity and its related comorbidities, environmental predispositions underlying childhood obesity and associated metabolic disorders need to be further explored. The cause of obesity is multifactorial. Genetic factors may determine an individual's susceptibility to obesity, and

environmental and lifestyle factors play a crucial role in the development of obesity. Thus, the gut microbial ecosystem has emerged as a significant environmental factor involved in the pathogenesis of childhood obesity. An altered gut microbiota has been linked to adult obesity. However, very little is known about the association of altered gut microbiota with childhood obesity.

Purpose: The purpose of this study was to examine the association between gut microbiota composition and child obesity. The specific aims were to: 1) evaluate the impact of child obesity status on three related outcomes to be assessed by extracting DNA from children's stool samples using 16S rRNA gene sequencing: alpha diversity of gut microbiota composition, Firmicutes: Bacteroidetes ratio, and abundance of SCFA-generating gut microbes between obese and non-obese school children; 2) compare the dietary habits and intakes between obese and non-obese school children; and 3) evaluate the association of body mass index differences in gut microbiota composition between obese and non-obese school children.

Methods: This pilot study used a cross-sectional design. Stool samples were collected and sequenced at the 16S rRNA gene in the V4 region. We recruited 27 participants, 13 in the obese and 14 in the lean groups. The outcomes are diversity of gut microbiota composition, Firmicutes: Bacteroidetes ratio, and abundance of short-chain fatty acid-generating gut microbes. We compared these outcomes between obese and non-obese school children using two sample T-tests. A logistic regression model was used to estimate the association between obese status and microbiota-related outcomes, adjusting for age, race/ethnicity, education of caregiver, and nutrition status.

Results: The alpha diversity was similar between the obese and non-obese groups. The F: B ratio in the o/o group was significantly increased (99.54 ±264.52) compared with non-o/o

children (31.25 ± 56.25) but not statistically significant at $p = 0.38$. There were no significant differences in the abundance of Firmicutes between non-o/o (0.71 ± 0.12) and o/o (0.69 ± 0.11) with $p = 0.59$. Overweight/obese children had an increased gut microbiota-producing SCFA (0.63 ± 0.25) compared with the non-o/o children (0.52 ± 022); however, it was not statistically significant ($p = 0.25$). Although o/o children consumed more calories (2021.46 ± 570.83) than non-o/o children (1744 ± (415.37), it was not significant at p = 0.16). The overweight/obese children also ate more foods rich in saturated fats (32.69 ± 21.01) than non-o/o children (24.07 ± 6.26), but it was insignificant at p = 0.18. Overweight/obese children had a higher diversity of fiber-rich foods (1.8 ± 1.77) than non-o/o children (1.39 ± 0.82). The differences were not significant ($p = 0.46$). There was no significant relationship between BMI z-score and Firmicutes, and BMI z-score was not a good predictor of the relative abundance of Firmicutes.

Discussion: There were no significant differences in alpha diversity at the phylum level between non-o/o and o/o groups. This suggests that obesity could not be determined as a causality of an alteration or dysbiosis in the gut microbiota among the o/o children in this study. The study's results provided limited evidence to support the F: B ratio as a biomarker for obesity. This study showed that SCFA-generating gut microbes were increased in o/o children compared with non-o/o children; however, it did not reach statistical significance. The daily gut microbiota friendliness score (DGMFS) was not statistically significant when comparing dietary intakes of high-energy, saturated fats, and fiber-rich foods. The study's results did not show an association between BMI z-score and relative abundance of Firmicutes.

The dissertation of Cecille Marie Basilio is approved.

Sarah E. Choi

Wendie A. Robbins

William J. McCarthy

Dena R. Herman

Felicia S. Hodge, Chair

University of California, Los Angeles

2024

DEDICATION

To my loving son Nikki, thank you for your patience, enduring love, and understanding when you thought I was done studying after receiving my MSN.

.

To Ewald, for your support, flexibility, and, most of all, for taking care of Nikki when I could not because I had to study, study, and study during my doctoral program.

To my family, friends, and coworkers for their love and support throughout this challenging and rewarding process.

To future doctoral students, perseverance is key. Know that there is always light at the end of the tunnel; just keep moving forward, and you will reach your goal.

To all the families and children who participated in this study and the people who helped with it, I could not have achieved this without your assistance.

To my committee, Dr. McCarthy, Dr. Robbins, Dr. Choi, and Dr. Herman for your immense support and guidance, and for sticking it out with me.

Thank you to Dr. Hodge for taking on the role of my Chair and advisor, for your mentorship, and for your commitment to my success.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

It has been a long journey, and I would like to acknowledge all who have made it possible to complete this dissertation. If I have forgotten anyone, which I am sure I have, I am truly sorry, it is unintentional.

Thank you to the UCLA School of Nursing team for your support and guidance. Dr. Mark Covin, Dr. Wiley, and Dr. Brauer, thank you for helping me with my readmission. To Dr. Farnaz Saadat, thank you for your kindness, support, and guidance. Thank you to Rachel Arbing, Angela Cortez, and Stephanie Varela for your support in coordinating the ordering of supplies I needed for my study and payment of invoices.

Thank you to Dr. Li and her team at Omega Bioservices for performing the DNA extraction and gene sequencing analysis. Thank you to Dr Yan Wang, UCLA School of Dentistry, for helping me with statistical analysis.

Thank you to all of the families and children who participated in the study. This dissertation could not have been completed without your involvement.

Lastly, I would like to thank my committee for all of their support and guidance. Dr. Hodge, thank you for your time, guidance, and mentorship. Dr. McCarthy, thank you for your expertise in gut microbiome research and methodology and for developing a proxy scoring scheme to analyze my dietary data. To Dr. Robbins and Dr. Choi, thank you for your support and guidance. To Dr. Herman, thank you for your expertise in diet and nutrition and for helping me understand dietary patterns and analyze dietary data.

BIOGRAPHICAL SKETCH

EDUCATION:

Dates	Institution and Location	Degree	Major
1980-1985	University of the Philippines Diliman, Quezon City	B.A.	Economics
2012-2014	Charles R. Drew University of Science and Medicine, Los Angeles, CA	M.S.N.	Nursing – entry level Masters

CAREER APPOINTMENTS:

Dates	Institution and Location	Appointment Title
2014 – 2017	University of California Los Angeles Los Angeles, CA	Research Nurse RN Coordinator,
2017 – present	Los Angeles Unified School District Los Angeles, CA	Credentialed School RN

ACADEMIC APPOINTMENTS:

Dates	Institution and Location	Appointment Title
2016 - 2017	UCLA School of Nursing Los Angeles, CA	Teaching Assistant Consultant; Teaching Associate

LICENSURE/CERTIFICATION:

Dates	Institution
Expires 12/31/25	CA RN License #95044403
Expires 12/31/25	CA PHN License #546779
Expires 02/2025	AHA Basic Life Support BLS Provider
Expires 08/12/25	American Red Cross First Aid
Issued 09/20/21	School Audiometrist Certification
Expires 07/28	School Nurse Services Credential

HONORS AND AWARDS:

Dates	Honor/Awards
2016-2018	University Fellowship Award, UCLA
2016-2018	Jonas Nurse Leader Scholarship Award
2016	Kaiser Permanente Deloras Jones RN Scholarship Award
2013-2014	NIG/NIGMS Pre-doctoral Training Grant for Enhancing Diversity of Nurses with Research Careers (5R25GM102777)
2012-2014	Kaiser Permanente Foundation Scholarship Award, Charles R. Drew University
2013-2014	Graduate Scholarship Award, Charles R. Drew University
2013-2014	Dr. Alfred M. Haynes Scholarship Award, Charles R. Drew University

PROFESSIONAL MEMBERSHIPS:
Activities:

Dates	Organizations
2015- present	Sigma Theta Tau International Honors Society of Nursing, Gamma Tau at Large Chapter, Los Angeles, CA

SCHOLARLY PRESENTATIONS:

1. **Basilio, C. M.** & Hodge, F. (2017). "Impact of spousal nutrition knowledge, attitudes and behaviors in Latino males receiving androgen deprivation therapy for late-stage prostate cancer." Poster presentation for 42nd Oncology Nursing Society Congress, Denver, Colorado.

2. **Basilio, C. M**., Fletes, S., Hicks, E. M., Maliski, S. (2016). "Staying Strong and Healthy: mHealth intervention in Latino men receiving androgen deprivation therapy for later stage prostate cancer." Poster presentation for Annual Research Day - University of California, Los Angeles School of Nursing.

3. **Basilio, C. M**., Fletes, S., Hicks, E. M. & Maliski, S. (2016). "Staying Strong and Healthy: mHealth intervention in Latino men receiving androgen deprivation therapy for later stage prostate cancer." Poster presentation for 41st Oncology Nursing Society Congress, San Antonio, Texas.

4. **Basilio, C. M**., Fletes, S. F., Hicks, E. & Maliski, S. M., PhD., RN. (2016). "A nursing-led Intervention for Latino Men with Later Stage Prostate Cancer. Poster Presentation." Poster presentation for Western Institute of Nursing 49th Annual Nursing Research Conference, Anaheim, California.

5. **Basilio, C. M**., Phillips, L. R., Mentes, J. C., Ume, E. P. & Evers-Manly, S. (2013). "Diabetes interventions in older Hispanics." Poster Presentation for Western Institute of Nursing 47th Annual Nursing Research Conference, Seattle, Washington.

6. **Basilio, C. M**., Phillips, L. R., Mentes, J. C., Ume, E. P. & Evers-Manly, S. (2014). "Diabetes interventions in older Hispanics." Poster Presentation for Annual Research Day - University of California, Los Angeles School of Nursing.

7. Kehinde, A., Phillips, L. R., Ume, E., Mentes, J., Evers-Manly, S., **Basilio, C. M.,** Ndukwe, M. & Chou, W. (2014). "Barriers to prostate cancer screening and prevention in older African-Americans." Poster presentation for Annual Research Day - University of California, Los Angeles School of Nursing.

INTRODUCTION

Childhood obesity has emerged as one of the most critical public health problems in the 21st century of national and global epidemic concern. Globally, in 2020, the number of children and adolescents between the ages of 5 and 19 who were classified as obese reached 150 million, and projections suggest this number could escalate to 254 million by 2030, according to the World Health Organization (WHO) 2021. In the United States (U.S.), obesity affects approximately 14.7 million children and adolescents aged 2-19 years (Centers for Disease Control and Prevention [CDC], 2022a).

There are direct and indirect consequences of high childhood obesity rates. The immediate consequences have health and social implications for children (CDC, 2022b; Ling et al., 2022). Compared with children at a healthy weight, those who are overweight or obese children and adolescents are at significant immediate and long-term risk for a range of health problems and obesity-related comorbidities, including metabolic syndrome, type 2 diabetes (T2D), certain cancers, joint and musculoskeletal problems (CDC, 2022b; Ling et al., 2022; Minghelli et al., 2015; Stohl, 2023; Taylor et al., 2006; Valerio et al., 2012), respiratory disorders (CDC, 2022b; Minghelli et al., 2015; Stohl, 2023), high blood pressure and high cholesterol, (CDC, 2022b; Magnussen et al., 2009; Minghelli et al., 2015), and orthopedic complications (CDC, 2022b; Valerio et al., 2012). Social implications of childhood obesity have been related to psychological and social problems that include anxiety, depression, low self-esteem, poor health-related quality of life, bullying, and social stigma (Beltrán-Garrayo, 2023; CDC, 2022b; Halfon et al., 2013; Ling et al., 2022; Morrison et al., 2015).

Childhood obesity accounts for $1.32 billion annually in medical care costs in the U.S. in 2019 dollars (Ward et al., 2021). Increases in childhood obesity can lead to rising healthcare

costs as it predicts additional increases in adult obesity and comorbidities (Ward et al., 2021; Ling et al., 2022). It is projected that by 2050, childhood obesity in the U.S. will cost nearly $14 billion annually in direct medical costs and $49 billion in indirect costs (Ling et al., 2022). The increase in total medical costs was found to be highest among adolescents aged 12 -18, attributable to increased health risks of obesity in developing chronic comorbidities such as type 2 diabetes and cardiovascular diseases in adolescence (Ling et al., 2022).

Metagenomics, first illustrated by Handelsman et al. (1998), involves the collective analysis of genomes from various microorganisms found in a particular environment (Zhang et al., 2021). Metagenomics represents an innovative approach for examining microorganisms in a specific habitat through techniques like functional gene screening or sequencing analysis. Metagenomic research primarily focuses on microbial diversity, community composition, genetic and evolutionary connections, functional roles, symbiotic interactions, and environmental interplay. The application of metagenomics is swiftly expanding across various domains, including medicine, agriculture, and environmental conservation (Zhang et al., 2021).

Through metagenomics, evidence is accumulating to implicate alterations in the composition and diversity of the gut microbiome as a heretofore underappreciated novel pathophysiological factor (Gill et al., 2006) in the development of obesity (Backhed et al., 2004; Cox & Blaser, 2013; Le Chatelier et al., 2013; Ley et al., 2005; Turnbaugh et al., 2008a; Turnbaugh et al. 2008b) and obesity-related metabolic disorders (Larsen et al., 2010; Le Chatelier et al., 2013; Qin et al., 2012). Studies have documented profound changes in the composition and metabolic function of the gut microbiota in obese subjects (Le Chatelier et al., 2013; Turnbaugh et al., 2008a; Turnbaugh et al., 2008b). In addition, some studies have demonstrated that the enormous capacity of the gut microbiota to harvest energy from the diet

influences host energy expenditure and storage within the host, thereby influencing /causing a chronic positive energy balance and systemic low-grade inflammation (Backhed et al., 2004; Ley et al., 2005; Turnbaugh et al., 2008a; Turnbaugh et al. 2008b). Other studies report that factors such as age, diet, geographical location (De Filippo et al., 2010; Mueller et al., 2010; Yatsunenko et al., 2012), mode of birth delivery (Azad et al., 2013; Dominguez-Bello et al., 2010; Jacobsson et al., 2014), breastfeeding (Notarbartolo et al., 2022) and the use of antibiotics (Aloisio et al., 2014; Greenwood et al., 2014) affect the composition of the gut microbiome with diet as the most significant influence of all (David et al., 2013; De Filippo et al., 2010; Singh et al., 2017). However, there is a lack of research on the impact of dietary habits on the gut microbiota in overweight/obese and normal-weight school-aged children.

Childhood Obesity

The COVID-19 pandemic has significantly intensified the existing epidemic of childhood obesity. Research involving 432,302 children in the U. S. revealed that the increase in Body Mass Index (BMI) has doubled during the pandemic, particularly affecting preschool and school-age children the most (Ling et al., 2022). During the COVID-19 pandemic, children and adolescents spent more time outside the traditional educational environment (Lange et al., 2021). This change particularly impacted households already grappling with factors that contribute to obesity. Many of these families faced further challenges, such as changes in income, food availability, and other critical health-related social factors (Lange et al., 2021). Consequently, children and adolescents were likely exposed to conditions that could promote rapid weight gain, including heightened stress, inconsistent eating schedules, limited availability of healthy food options, more time spent in front of screens, and reduced chances for physical exercise (Lange et al., 2021).

Obesity has increased over the past two decades throughout the U. S. (Trust for America's Health [TFAH], 2023). Data from the National Health and Nutrition Examination Survey indicate a 37% increase in obesity rates among adults aged 20 and above and a 42% increase among children and teenagers aged 2 to 19 between 1999-2000 and 2017-2020 (TFAH], 2023).

In March 2020, field operations for the National Health and Nutrition Examination Survey were suspended due to the outbreak of COVID-19 (Stierman et al., 2021). The incomplete 2019–2020 cycle data, denoted as 2019–March 2020, lacked national representation. To address this, the data were merged with the 2017–2018 data set to yield estimates representative of the national population. The National Health Statistics Report No. 158 documents the process of developing the 2017–March 2020 data files before the pandemic, outlines the recommended uses and potential limitations of these files, and provides calculated prevalence rates for specific health outcomes such as obesity among children and adolescents using these combined data sets (Stierman et al., 2021). The prevalence of obesity in 2017 – 2020 for children aged 2 – 19 years has more than tripled in the U.S. for more than four decades, from 5.5% to 19.7% (Fryar et al., 2020; Stierman et al., 2021). Obesity prevalence among children aged 2 – 5 years was 12.7%, 20.7% among children aged 6 – 11, and 22.2% among 12 – 19-year-olds (Stierman et al., 2021). Data from the National Survey of Children's Health (NSCH) indicate that in 2020-2021, an estimated 17.% of youth in the U.S. aged 10 – 17 were obese, while 16.3% were overweight (NSCH, 2021). In the 2022 NSCH, the incidence of obesity and overweight among children aged 10 to 17 showed minimal variation, holding at 16.6% for obese and 15.2% for overweight individuals in that age group (NSCH, 2022).

According to NSCH data, there are marked racial and ethnic disparities in childhood obesity among youth aged 10-17 in 2021-2022, with higher prevalence observed in non-Hispanic Black (22%), Hispanic (22.7%), and non-Hispanic American Indian/Alaska Native (21.4%) children compared to non-Hispanic Asian (9.6%) and non-Hispanic White (13.1%) children (Robert Wood Johnson Foundation [RWJF], 2023). Among youth aged 2 -19, the prevalence of obesity was observed at 26.2% among Hispanic children, 24.8% for non-Hispanic Black children, 16.6% for non-Hispanic White children, and 9.0% for non-Hispanic Asian children (Stierman et al., 2021).

Obesity has been addressed in multiple health initiatives (CDC, 2023; U. S. Department of Agriculture and National Institute of Food and Agriculture, n.d.), including Healthy People, with no U.S. State meeting the proposed goals of lower obesity prevalence in children and adolescents in 2000 (National Center for Health Statistics [NCHS], 2001) and 2010 (NCHS, 2012). The Office of Disease Prevention and Health Promotion (ODPHP, 2020) created Healthy People 2020 to reduce child obesity risk among children and adolescents aged 2 -19 by achieving the target of a 14.5% obesity rate, which was met with little or no change (NCHS, 2021). This objective was established without giving parents actionable advice on reducing their children's body fat percentage.

Once considered a problem exclusive to high-income countries, the prevalence of childhood obesity is now on the rise in low – and middle-income developing countries (WHO, 2020). In 1995, the global count of obese children under five was 18 million (WHO, 2020). By 2020, this figure had more than doubled, nearing 39 million and is expected to reach approximately 40 million by 2030 (WHO, 2021). In 2020, children 5 – 19 years old with obesity

were estimated at 150 million; if current trends continue unabated, the global number of obese children in this age range is predicted to increase to 254 million by 2030 (WHO, 2021).

The global challenge of childhood obesity is escalating at an alarming rate, marking a critical public health issue that transcends geographical and socio-economic boundaries. Initiatives like the WHO's Commission on Ending Childhood Obesity (ECHO) have outlined comprehensive strategies focusing on policy interventions, education during critical life-course periods, and specialized treatment for affected children, highlighting the need for a multifaceted and inclusive approach (Swinburn & Vandervijvere, 2016). Meanwhile, the Global Convention to Protect and Promote Healthy Diets by Consumers International and the World Obesity Federation underscores the urgency for high-level international commitments and actions akin to those in the battle against tobacco use (Vandevijvere, 2014). These efforts, combined with global initiatives like the United Nations' Decade of Action on Nutrition, which addresses obesity and undernutrition, signify a growing recognition of and response to this multifaceted crisis (Hanson et al., 2016).

In addition, school meal programs can play a crucial role in combating child obesity by providing nutritionally balanced meals and creating a supportive environment for healthy eating habits. Research highlights the significant impact of initiatives in the U.S., such as:

- The Healthy, Hunger-Free Kids Act (HHFKA) of 2010 enhanced nutrition standards for school meals, leading to a notable decline in obesity risk among children living in poverty. Obesity in this group would have been 47% higher in 2018 without the passage of the HHFKA (Kenney et al., 2020).

School nutrition programs, especially when part of a coordinated health intervention, have been shown to maintain healthy body mass index percentiles over time in low-income, multiethnic children (Natale et al., 2017).

- Implementations like Breakfast in the Classroom (BIC) increased meal program participation without leading to feared increases in obesity, pointing towards the potential of well-structured programs to promote health without adverse weight-related outcomes (Corcoran et al., 2014).

These findings suggest that school meal programs, when well-designed and effectively implemented, can be a valuable strategy in curbing child obesity and promoting a healthier future for children, particularly those from low-income families.

Obesity is a consequence of behaviors that affect excess weight gain. These behaviors include eating excess high-fat, high-calorie, and sugary foods, insufficient amounts of minimally processed fruits, vegetables, legumes, nuts, and seeds (Cohen et al., 2010; Zhang et al., 2022), low levels of physical activity (CDC, 2023) increase in sedentary behaviors (e.g., increased amounts of television screen time, video game playing) (Riddoch et al., 2009; Vinciguerra et al., 2019), and short sleep duration and unhealthy sleep patterns (Chaput et al., 2006; Kanellopoulou et al., 2021).

Elevated levels of technology utilization have been linked to an increase in childhood obesity, primarily due to heightened caloric intake and diminished physical activity (Murphy & Blackstock, 2018).

The exposure to technology begins at increasingly younger ages. In a 2021 U.S. survey, data revealed that children aged 8 to 18 with smartphones were at 94 percent, a slight increase from the 91 percent reported two years earlier. Additionally, there was a rise in the number of

children with access to streaming services, which climbed to 84 percent (Laricchia, 2023). Children spend a considerable amount of time online devoted to viewing television and movies. Recent statistics reveal that children between the ages of 8 and 12 spend two hours and 40 minutes daily watching TV or videos, compared to a mere nine minutes spent reading books (Guttmann, 2023). Children also spend a significant amount of time on social media platforms. In 2022, the United States found that children allocate approximately 113 minutes daily to TikTok and 90 minutes daily on Snapchat (Laricchia, 2024).

Research has demonstrated a significant relationship between screen time and childhood obesity. A study in Saudi Arabia found a correlation between increased screen time and higher BMI in children, suggesting that excessive screen time is linked with overweight and obesity in children aged less than one year to 5 years old (Alsaedi, Alasmari & Alzahrani, 2022). An analysis of overweight and obese students' screen time and physical activity levels found that screen time was a significant predictor of relative body mass index (RBMI) in children, with higher screen time associated with higher RBMI (Hosseinzadeh & Ahmadabad, 2015) and sedentary lifestyle which contributes to adiposity (Hingle & Kunkel, 2012).

In addition, research has shown that obese children and adolescents have lower educational engagement, more behavioral problems, and absences (An et al., 2017; Carey et al., 2015; Martin et al., 2018; Roberts et al., 2010). In addition, these studies have found that obese students are more likely to repeat a grade, have lower grade point averages and reading scores (Roberts et al., 2010), and demonstrate lower academic effort. Students with better academic grades have healthier behaviors. According to data from the 2019 National Youth Risk Behavior Surveillance System, students with higher grades are more likely to be physically active and less likely to watch television or play video games for three or more hours a day (CDC, 2020b). In

addition, students with higher grades are more likely to have healthy dietary behaviors, including eating breakfasts, fruits and vegetables, and avoiding soda (CDC, 2020b).

Obesity imposes immediate and long-term effects on health if left untreated during childhood. Health-related consequences of obesity include CVD, metabolic syndrome, T2D, orthopedic problems, obstructive sleep apnea (OSA), asthma, metabolic-associated fatty liver disease (MAFLD), polycystic ovarian syndrome (PCOS), psychiatric disease, and some types of cancer (Babey et al., 2012; Kelsey et al., 2014; Llewellyn et al., 2016; Pulgaron, 2013). Childhood obesity is specifically troubling because the early onset of such severe diseases increases the probability of early morbidity and premature mortality (Kelsey et al., 2014). In addition, the psychological, social, and behavioral consequences to the child can be devastating, as obesity can result in poor body image perception, low self-esteem, social isolation and discrimination, depression, and decreased quality of life (Pulgaron, 2013). Furthermore, the effects of childhood obesity during childhood have grave health consequences. Obesity-related disorders, such as hypertension, dyslipidemia, T2D, PCOS, MAFLD, and OSA, which were once the unhealthy domain exclusively of adults are increasingly observed in children and adolescents (Kelsey et al., 2014). Moreover, obese children have an increased likelihood of becoming obese as adults, as well as a higher risk of adult obesity-related complications (Kelsey et al., 2014; Llewellyn et al., 2016). The repercussion of accelerating pediatric obesity could exacerbate rates of adult obesity in the future, thus intensifying the risk for obesity-related diseases in obese adults (Kelsey et al., 2014; Llewellyn et al., 2016). Second only to tobacco, obesity is now considered a leading cause of preventable disease and mortality (Babey et al., 2012).

Body Mass Index and Definition of Terms

The CDC (2023a) defines overweight and obesity as terms to denote ranges of weight more than what is considered normal or healthy for a given age and height. The WHO (2021) defines overweight and obesity as excess body fat accumulation that increases the risk of certain diseases and other health issues. Body Mass Index (BMI) is widely used to define childhood overweight and obesity (CDC, 2023a). The BMI percentile is preferred for measuring the weight of children and young adults in the age bracket of 2-20 years, as it considers that children in this age group are in their growth phase and are growing at varying rates depending on their gender and age. Health professionals use growth charts to determine if a child's weight is within the healthy range for age, height, and gender. Children and teens of the same age and gender with a BMI of or above the 85th percentile and less than the 95th percentile are considered overweight. Children and teens of the same age and gender who are or above the 95th percentile are considered obese. Body Mass Index is age and gender-specific and is calculated by dividing the child's weight in kilograms by the square of height in meters (CDC, 2023a).

Childhood Obesity Rates in California

Although California has been a leader in advancing policies to prevent and reduce obesity, the state continues to battle an obesity epidemic among children. The latest findings from the 2021-2022 National Survey of Children's Health indicate that 16.3% of youth aged 10-17 in California are obese. This statistic positions California as the 28th state out of 51 in the national ranking for obesity rates within this age group (RWJF, 2023). The obesity rate among children aged 2 to 4 enrolled in the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) increased from 16% in 2016 to 17% in 2022 (RWJF, 2023). California ranks fifth among 51 states for the highest obesity rates among children in this age group. If left

unchecked, 17% of 2- to 4-year-olds could suffer the adverse health effects of obesity and sequelae later in adulthood (RWJF, 2023).

Childhood obesity rates among fifth--, seventh--, and ninth-graders in California public schools have shown little change since 2014 (California Department of Education [CDE], 2020; Population Reference Bureau [PRB], 2024). In 2019, 41% of fifth graders, 40% of seventh graders, and 38% of ninth graders in California were either overweight or obese (CDE, 2020; PRB, 2024). The obesity rate among California high school students increased from 13.9% in 2014 to 15.9% in 2019 and ranked 16th among the 51 states in the U.S. ((RWJF, 2023).

Certain racial and ethnic groups continue to experience higher obesity rates. For instance, in 2019, 53.2% of Native Hawaiian/Pacific Islander fifth graders were affected by obesity, which is one of the highest prevalence rates among all groups. Hispanic/Latino fifth graders also have a high rate at 49.7%, followed by American Indian/Alaska Native fifth graders at 44.3% and African American/Black fifth graders at 42%. In contrast, Asian-American and White fifth graders have obesity rates below 30% (PRB, 2024).

Childhood Obesity Rates in Los Angeles County

In 2019, childhood obesity rates among fifth graders in Los Angeles (LA) County showed no significant changes from previous years, but there were still notable differences among races and ethnicities. The highest rates of obesity were observed in Latino and Native Hawaiian/Pacific Islander fifth graders, at 52.5% and 53%, respectively. African American fifth graders had an obesity rate of 42.3%, while American Indian/Alaska Native fifth graders had a rate of 41%. Comparatively, the obesity rates for White and Asian fifth graders were lower, at 30% and 27%, respectively (PRB, 2024).

In LA County, racial and ethnic disparities in child obesity are influenced by various factors. A study by Kamali et al. (2017) found that although obesity prevalence among fifth graders in the Los Angeles Unified School District (LAUSD) declined from 2010 through 2013, higher prevalence persists, with notable demographic and socioeconomic disparities. The decline in obesity began earlier among whites and students attending schools in higher socioeconomic status (SES) groups, indicating inequalities by race/ethnicity and SES throughout the study period. Recent studies provide insights into the causes of racial and ethnic disparities in child obesity in LA County. A simulation model to study the life course incidence and trends of obesity and its effect on type 2 diabetes mellitus in LA County was developed by Nianogo and Arah (2022). This model provided insight into the future burden of obesity and type 2 diabetes and showed that the prevalence of obesity typically rose from 10% to 30% throughout a person's life, showing significant increases around the ages of 6-12 and 30-39 years (Nianogo & Arah, 2022). In contrast, the incidence of type 2 diabetes mellitus started at less than 2% in the 18-24 age group and progressively climbed, reaching a high of 25% in individuals aged 40-49. (Nianogo & Arah, 2022). Childhood obesity risk factors at both child and maternal levels contribute to racial/ethnic disparities in growth trajectories, with White, Black, and Latino children showing different growth trajectories (Pineros-Leano et al., 2022). More extended family WIC participation is associated with healthier beverage choices for infants and children but is not equally beneficial across racial/ethnic groups in Los Angeles County (Anderson et al., 2022).

The strong correlation between childhood obesity and economic hardship suggests that economic disadvantage is a dynamic force that drives the epidemic across the age spectrum (Los Angeles County Department of Public Health [LACDPH], 2011). For instance, the city of

Walnut Park has the highest prevalence rate of childhood obesity at 38.7%, which was found to be strongly correlated with economic hardship (LACDPH, 2011). It is in this regard that this study will be conducted in the county of Los Angeles to examine the effects of dietary habits of overweight or obese LAC children on gut microbiota composition that may predispose these children to develop chronic disease conditions at a later age.

The risk of child obesity has been proposed to begin during the maternal preconception period with significant influences on maternal birth weight, obesity status, and nutritional status (Haire-Joshu & Tabak, 2016), but early-in-life interventions can remedy suboptimal conditions during gestation and the neonatal period (Alderete et al., 2015; Lagstrom et al., 2020; Brown et al., 2019). Although efforts are made at state and national levels to bring obesity under control, multiple barriers, such as the lack of time, knowledge, and economic means, stand in the way of either clinicians or parents making any progress. At the same time, low-income families of overweight or obese children share cultural beliefs that are inconsistent with the prevention of obesity (Hughes et al., 2010). For instance, in Hispanic culture, there is a common belief that fatter children are healthier children; potential obesity is not a matter of parental concern when the child is young, that being overweight is a temporary condition that can be outgrown as the child approaches adulthood (Hughes et al., 2010). The pathogenesis of childhood obesity has been linked to several personal, behavioral, and environmental factors. For instance, the lack of knowledge about the importance of nutritious food and a healthy diet account for personal factors. The decreased amount of physical activity and reliance on calorie-rich, nutrient-poor foods are behavioral factors that contribute to child obesity risk. The cultural impact, economic resources, parenting styles, and family structures are other environmental causes of obesity. However, not a single or a combination of these factors fully explain the increasing prevalence

and growing epidemic of childhood obesity worldwide (Di Cesare et al., 2019; Khan et al., 2016). Recent scientific studies suggest that childhood obesity is closely linked to aberrant changes in the composition and metabolic function of the human gut microbiota (Bikel et al., 2021; Murga-Garrido et al., 2023). The association of gut microorganisms with metabolic disorders that include obesity and type 2 diabetes has attracted considerable recent attention (Thingholm et al., 2019; Valdes et al., 2018).

Gut Microbiota

Microbial cells in the human intestine are greater than the total number of human cells in the typical human and collectively harbor 150+ times more genes than the entire human genome (Tierney et al., 2019; Qin et al., 2010). The term gut microbiota refers to the extensive variety of microbes in the human intestine, including bacteria, viruses, fungi, archaea, and eukaryotic microbes (Asnicar et al., 2021; Bouter et al., 2017; Li & Wei, 2015). On the other hand, the gut microbiome is defined as the complete collection of all the genomic elements comprising a particular microbiota. The human gut microbiota plays a crucial role in maintaining health by protecting against pathogens and contributing to the integrity of the gut barrier. However, certain bacteria can pose risks by consuming the protective mucin layer when deprived of other food sources, potentially leading to "leaky gut" syndrome (Di Tommaso, Gasbarini & Ponziani,2021). Mucin, a vital component of the protective mucus layer, is consumed by specific gut bacteria, which are essential for barrier function and community stability. Alterations in mucin structure are associated with disorders like inflammatory bowel disease and colon cancer (Crouch et al., 2019). Commensal bacteria utilize mucins to promote their growth and colonization of the intestine. Mucus-derived components serve as nutrient and chemical cues for bacterial adaptation and pathogenesis (Sicard et al., 2017). The leaky gut may allow environmental factors to enter

the body, triggering autoimmune disease in genetically predisposed individuals. Probiotics have been shown to reverse the leaky gut by enhancing the production of tight junction proteins (Mu et al., 2017). Based on these studies, the balance of the gut microbiota is critical to maintaining the mucin layer and intestinal barrier integrity. Disruptions can lead to increased epithelial permeability, inflammation, and disease.

Furthermore, evidence suggests that the gut microbiota may affect food intake and satiety via gut peptide signaling (Cani et al., 2009; Musso et al., 2010). Gut hormones such as glucagon-like peptide-1 (GLP-1), peptide tyrosine tyrosine (PYY), cholecystokinin (CKK), and ghrelin play a critical role in transmitting signals of nutritional and energy status from the gut to the central nervous system to control food intake. Moreover, the study by Cani et al. (2009) showed increased gut microbiota fermentation, decreased appetite, improved postprandial glucose responses, and higher concentrations of GLP-1 and PYY after two weeks of prebiotic treatment, suggesting a role for prebiotics in the modulation of gut hormones.

The main bacterial phyla are the *Firmicutes* and the *Bacteroidetes*, which comprise more than 90% of the bacteria in the distal gut (Qin et al., 2010). The gut microbes can interact with the human host in multiple ways, including, but not limited to, an essential nutrient and vitamin metabolism upon dietary intake, fermenting of unused energy substrates, maintenance of gut epithelial integrity, educating the immune system, as well as protection against the invasion of intestinal pathogenic bacteria (Bouter et al., 2017; Bull & Plummer, 2014; Li & Wei, 2015). A commensal relationship commonly exists between the host and gut microbiota, ensuring a homeostatic balance of bacteria that helps keep the gastrointestinal tract healthy and protected from invasion by pathogenic bacteria (DeGrottola et al., 2016). However, an alteration in the composition of the gut microbiota may cause it to be highly vulnerable to pathogenic invasion

and colonization, resulting in dysbiosis. Dysbiosis is a disturbance to gut microbiota homeostasis caused by microbial imbalance, functional composition changes, metabolic activities, and changes in local microbial distribution (DeGrottola et al., 2016). Dysbiosis in the gut microbiota has been associated with a broad range of diseases, including inflammatory bowel disease, irritable bowel syndrome, celiac disease, allergic disorders, asthma, CVD, autism spectrum disorders, obesity, metabolic disorder, and colorectal cancer in both human and animal subjects (DeGrottola et al., 2016). In summary, the mechanisms in disease development of many of these conditions involve the crucial symbiotic relationship between the colonic bacteria, their metabolic products, and the host's immune system.

Gut Microbiota and Childhood Obesity

Because of the latest metagenomic developments, it has been primarily observed that a particular microbiota composition seems to be associated with the development of obesity. A predominant decrease in the diversity of the gut microbiota composition of obese subjects has been noted in the literature (Arslan, 2014; Crovey et al., 2020).

Firmicutes-Bacteroidetes Ratio

In several human and animal studies, alterations in gut microbiota composition have been observed between obese and lean subjects. These alterations are reflected at the phylum level of primarily *Firmicutes* and *Bacteroidete*s (Ley et al., 2006; Turnbaugh et al., 2006; Turnbaugh et al., 2008). An increased *Firmicutes-Bacteroidetes* (F/B) ratio has been associated with obesity in humans, which implies low intestinal concentrations of *Bacteroidetes* and high concentrations of *Firmicutes* (Bervoets et al., 2013; Riva et al., 2017; Tilg & Adolph, 2014; Turnbaugh et al., 2006). In European and North American populations, the human gut is predominated by two bacterial phyla: *Firmicutes* and *Bacteroidetes*. The ratio of these two phyla is associated with

microbial dysbiosis and the development of obesity. Obese individuals tend to have an elevated Firmicutes to Bacteroidetes ratio of these bacteria because excess body weight and body fat accumulation covary with this ratio. When obese individuals lose weight on a calorie-restricted diet, their concentration of *Bacteroidetes* increases in the gut microbiota (Tilg & Adolph, 2014; Turnbaugh et al., 2008). Experiments involving germ-free (GF) mice suggest that dysbiosis is probably a causal factor for obesity. GF and wild-type (WT) mice were fed the same high-fat diet (HFD) in these experiments. However, only the WT mice developed obesity.

When the obese microbiota was transplanted into the GF mice, obesity was then induced (Tilg & Adolph, 2014; Turnbaugh et al., 2008). In addition, when lean mice microbiota was transferred into obese mice through fecal microbial transplantation (FMT), symptoms of metabolic syndrome were alleviated. In a human study, FMT was used to transfer lean intestinal microbiota, which increased insulin sensitivity in subjects with metabolic syndrome (Vrieze et al., 2012). Numerous studies indicate that aberrant intestinal microbiota is closely associated with the development of obesity and that HFDs can change the microbiota composition. Disturbances in the gut microbiota could promote chronic inflammation-associated obesity. Differences in gut microbiota composition and energy metabolism between obese and lean subjects have been documented in the literature. While some study data suggest that an increased *Firmicutes-Bacteroidetes* (F: B) ratio plays an essential role in the etiology of obesity (Hollister et al., 2018; Hou et al., 2017; Pihl et al., 2016; Riva et al., 2017), the results of other studies contradict the F: B ratio (Ismail et al., 2011; Payne et al., 2011; Magne et al., 2020)). These inconsistencies could result from differences in sampling and gene sequencing. In addition, gut microbiota composition is significantly modified by diet, age, ethnicity, and geography (Bouter et al., 2017; Zoetendal et al., 2015). Nevertheless, recent data results show that genus members

of *Bacteroidetes* were more appropriate indicators of BMI *z*-score and obesity than *Firmicutes* due to conflicting responses of *Firmicutes* operational taxonomic units (Abdullah et al., 2023; Murga-Garrido et al., 2023; Riva et al., 2017).

Short-Chain Fatty Acids

One of the essential functions of the human gut microbiota is to ferment indigestible dietary fiber or resistant starch in the large intestine. The products of this fermentation process are the short-chain fatty acids (SCFA), with acetate, propionate, and butyrate as the most abundant SCFA, constituting 90% to 95% of the SCFA content (Canfora et al., 2015; Kim et al. 2019; Ríos-Covián et al., 2016) in a molar ratio of 60:20:20, respectively (Murugesan et al., 2018, Rahat-Rozenbloom et al., 2014). Members of both *Bacteroidetes* and *Firmicutes* produce SCFA that supplies the host with an additional source of energy (Chambers et al., 2015; Lu et al., 2016; Riva et al., 2017; Schwiertz et al., 2010). The *Bacteroidetes* phylum mainly produces acetate and propionate, while the *Firmicutes* phylum has butyrate as its most common metabolic product (Chambers et al., 2015; Den Besten et al., 2013b; Lu et al., 2016). Several studies have documented the significant physiological roles of SCFAs as mediators between the gut microbiota and host to regulate intestinal permeability, inflammation control, bile acid metabolism, immunological functions, and disease control (Conder et al., 2023; De la Cuesta-Zuluaga et al., 2018; Deleu et al., 2022; Goffredo et al., 2016; Hildebrand et al., 2023; Murugesan et al., 2015; Rahat-Rozenbloom et al., 2014; Ríos-Covián et al., 2016).

Acetate is utilized as a substrate for cholesterol synthesis and lipogenesis in the liver and enters the peripheral circulation (Murugesan et al., 2017; Rahat-Rozenbloom et al., 2014; Schwiertz et al., 2010), as well as a suppressor of appetite through a central hypothalamic mechanism (Frost et al., 2014). Butyrate serves as the primary energy source for colonocytes,

maintains colonic homeostasis, and functions as a histone deacetylase inhibitor (Holmes et al., 2020; Liu et al., 2018; Murugesan et al., 2017), thereby contributing to barrier integrity maintenance, and reduces levels of lipopolysaccharide, a potent inflammatory molecule which has been associated with metabolic endotoxemia, inflammation, insulin resistance, adiposity, and hepatic fat (Dela Cuesta-Zuluaga et al., 2018). Propionate is mainly taken by the liver and utilized as a harbinger for gluconeogenesis, liponeogenesis, and protein synthesis (Murugesan et al., 2017; Rahat-Rozenbloom et al., 2014; Schwiertz et al., 2010) and acts as a low-grade inflammation reducing agent (Murugesan et al., 2017). In addition, propionate stimulates the appetite hormones peptide tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1), which promotes reduced food intake (Chambers et al., 2015; Wilding et al., 2021). Propionate also stimulates immunosuppressive activities and insulin sensitivity via the incretin GLP-1 (Al-Lahham et al., 2012).

Emerging evidence from animal studies has shown that the gut microbiota and its SCFAs play an essential role in obesity (Den Besten et al., 2013a; Liou et al., 2013; Perry et al., 2016). The findings of these studies informed that micronutrients and SCFAs produced by the gut microbiota could regulate host energy metabolism in the development of diet-induced obesity, thereby increasing de novo lipogenesis in the liver and accumulation of lipids in all fat stores. Nevertheless, human studies have had inconsistent results regarding the relationship between SCFA and obesity. Numerous studies have observed significantly higher fecal concentrations of SCFA in obese adult subjects compared with adult normal-weight or lean subjects (De la Cuesta-Zuluaga et al., 2018; Fernandez et al., 2014; Rahat-Rozenbloom et al., 2014; Schwiertz et al., 2010). Several pediatric studies showed elevated levels of fecal concentration of SCFA among obese children compared with lean children (Kim et al., 2019; Payne et al., 2011; Pekmez et al.,

2018; Rahat-Rozenbloom et al., 2014; Riva et al., 2017). However, pediatric results are inconsistent as some studies reflected results of decreased fecal concentration of SCFA in obese compared with their lean counterparts (Barczyńska et al., 2018; Murugesan et al., 2015), and markers of obesity did not positively correlate with SCFA fecal concentration nor microbiota SCFA production capacity (Holmes at al., 2020). The inconsistent findings suggest that fecal SCFA concentration could be due to several factors, including increased or reduced colonic SCFA absorption, decreased or increased colonic transit time, increased SCFA production due to differences in dietary intake, or the obese microbiome may be associated with fewer microbial species that utilize SCFA as an energy source (Fernandes et al., 2014; Rahat-Rozenbloom et al., 2014).

It is hypothesized that the high levels of fecal SCFA observed in obese children may be the consequences of high colonic fermentation in obese children, decreased SCFA absorption due to low-grade inflammation, increased microbial production, alterations in microbial cross-feeding patterns, and rapid gut transit (Holmes et al., 2020; Kim et al., 2019; Payne et al., 2011; Pekmez et al., 2018; Riva et al., 2017; Schwiertz et al., 2010). The increased *Firmicutes*-*Bacteroidetes* ratio has been hypothesized to contribute to the pathophysiology of obesity and is linked with increased production of SCFA and energy harvest from colonic fermentation (Fernandez et al., 2014; Rahat-Rozenbloom et al., 2014; Riva et al., 2017).

However, some studies prove that SCFAs can influence metabolic functions associated with appetite control, calorie intake, and body weight management. A pilot study evaluated the impact of propionate on appetite control during a weight-loss diet and found that propionate may aid in weight loss through appetite modulation (Khatib et al., 2018). Acute increases in serum colonic short-chain fatty acids elicited by inulin do not increase GLP-1 or PYY responses but

may reduce ghrelin in lean and overweight humans. According to this study, inulin increased SCFA levels but did not affect GLP-1 or PYY responses, though it may reduce ghrelin levels, which could influence appetite (Rahat-Rozenbloom, 2016). A randomized clinical trial provided insight into the potential role of diet in modulating the gut microbiome to influence human energy balance, appetite, and body weight. The study found that a Microbiome Enhancer Diet led to significant shifts in microbial biomass, community structure, and fermentation, with parallel alterations to the host enteroendocrine system, without altering appetite or energy expenditure and resulting in additional calories lost in feces daily, thereby lowering metabolizable energy for the host by channeling more energy to the colon and microbes (Corbin et al., 2023).

The relationship between obesity and SCFAs is not yet fully understood but can be explained by the following hypotheses. An estimated 5% - 10% of daily calories come from the oxidation of SCFAs, add to the human energy balance, and contribute to lipogenesis and adipocyte accumulation, leading to energy harvest (Kim et al., 2018). In addition, higher fecal SCFA concentrations may be linked with gut dysbiosis, gut permeability, excess adiposity, and cardiometabolic risk factors (Dela Cuesta-Zuluaga et al., 2018). Some bacterial components associated with gut dysbiosis, such as flagellin, have been involved in the pathogenesis of obesity and various metabolic diseases by causing low-grade inflammation in adipose tissue and gut microbiota modifications (Chambers et al., 2015). In addition, the effects of SCFAs on body weight and food intake occur via G-protein coupled receptors (GPRs), GPR41 and GPR43, also known as free fatty acid receptors FFAR 3 and FFAR 2 (Chambers et al., 2015). Under conditions of high carbohydrate diets and obesity, the binding of SCFAs to GPRs as signal transduction molecules might be mitigated, leading to increased intestinal energy harvesting and

hepatic lipogenesis (Chambers et al., 2015; Den Besten et al., 2013). Short-chain fatty acids have also been shown to be involved in appetite regulation in human studies based on the finding that administrating propionate to patients with obesity led to enhanced gut hormone PYY and GLP–1 secretion with significantly reduced adiposity and overall weight (Chambers et al., 2015). The role of SCFAs in obese humans requires well-designed large-scale studies to elucidate the relationship between obesity and SCFAs further.

Intestinal Permeability or "Leaky" Gut

In studies involving both human and animal models, evidence suggests that obesity increases intestinal permeability, allowing the passing of bacterial products such as lipopolysaccharides (LPS) across the intestinal barrier (Arslan, 2014; Bleu et al., 2015). Lipopolysaccharides are commonly found in the outer membrane of gram-negative bacteria, usually of *Bacteroidetes* species, which comprise a lipid and a polysaccharide (Fathi & Wu, 2016; Manco et al., 2010). Lipopolysaccharides play a critical role in the chronic inflammatory state of obese individuals. Data from previous studies suggest that obesity increases LPS circulating levels, allowing it to cross the leaky epithelial barrier into the bloodstream, causing metabolic endotoxemia (Cani et al., 2007). One of the proposed mechanisms that link obesity and metabolic endotoxemia is the high-saturated fat diet. A high-saturated fat diet stimulates toll-like receptors, which promote an inflammatory response, contributing to increased intestinal permeability and, thus, the absorption of LPS (Cani et al., 2007; Erridge et al., 2007; Rocha et al., 2016). Immune detection of LPS in the host stimulates an inflammatory response. This results in chronically elevated low-grade inflammatory state among obese individuals (Cani et al., 2007; Erridge et al., 2007)

Increasing study data emphasize the link of an altered gut microbiota to adult obesity, but very little is known about the association with childhood obesity. There is a shortage of information on the role of the gut microbiome in provoking childhood obesity. Studies have yet to be conducted to explore the child's gut microbiota composition and its relation to gut homeostasis and various childhood diseases.

Gut Microbiome and Diet

Diet is one of the most critical determinants of the gut microbiota composition. (Asnicar et al., 2021). Dietary intake of carbohydrates, proteins, fiber, and fats provides nutrients for the proper maintenance of the gut microenvironment and significantly affects the composition of the gut microbiota, improving pH and transit time (De Filippo et al., 2010; Lee, 2013; Riva et al., 2017; Wu et al., 2011; Zhang et al., 2014). Study data show that low-calorie diets in obese subjects can effectively reverse the abnormal Firmicutes and Bacteroidetes ratio's abnormal balance to a level comparable with that of normal-weight individuals (Ley et al., 2006). Changes in dietary patterns have been shown to shift the gut microbiota composition detectably within 24 hours (Wu et al., 2011). In addition, long-term diets could influence and affect individuals' enterotypes such that diets rich in protein and animal fat favored the Bacteroides enterotype, and carbohydrate-enriched diets supported the Prevotella enterotype (Wu et al., 2011). Prevotella is taxonomically identified as a genus belonging to the Bacteroidetes phylum (Rinninella et al., 2019). Long-term dietary patterns were found to have distinct effects on gut microbiota composition and stability in children aged 4 – 8 years. In a longitudinal observational study of children aged 4 – 8 years, two dietary patterns were associated with distinct microbial profiles and variability of gut microbiota (Berding et al.,2018). Dietary Pattern 1, typified by an intake of fish, protein foods, refined carbohydrates, fruits, vegetables, juice and sweetened beverages,

kid's meals, snacks, and sweets, was associated with a higher relative abundance of Bacteroidetes, Bacteroides, and Ruminococcus, a genus in the phylum Firmicutes. Dietary Pattern 2, described by an intake of grains, dairy, legumes, nuts, and seeds, was associated with a higher and lower abundance of different genera belonging to the phylum Firmicutes, such as Phascolarctobacterium, Dorea, and Eubacterium. In addition, Berding et al. (2018) found that a dietary pattern defined by higher intakes of fruits, vegetables, and grains and reduced intakes of starchy foods, juice, and sweetened beverages was associated with more excellent microbiota stability over six months with an abundance of bacterial phyla, specifically Actinobacteria and Bacteroidetes. Lower intakes of vegetables and higher intakes of protein sources and dairy foods were associated with higher variability in gut microbiota composition over six months. These findings could be necessary as less variability in gut microbiota composition has been associated with significant resistance to pathogen invasion (Berding et al., 2018). No changes were observed in the alpha- or beta-diversity based on dietary patterns.

Long-term diets have been shown to shift gut microbiota composition (Wu et al., 2011). Interestingly, dietary intake affects the gut microbiota's metabolic function and activates the digestion of food components (Bouter et al. (2017). Many specific nutritional compounds have been linked to altered gut microbiota composition. Several food groups and nutrients were related to differences in gut microbiota composition. In addition, processed foods have been associated with altered microbial composition and the development of obesity and metabolic syndrome (Lane et al., 2020). Furthermore, red meat has been linked to altered gut microbiota composition and an adverse cardiometabolic state (Chassaing & Gewirtz, 2014; Koeth et al., 2013; Singh et al., 2017).

Diet is an essential influence on the homeostasis of gut microbiota. As demonstrated in several studies, it is vital from birth that breastfed infants have more Bifidobacteria in their gut microbiota than formula-fed infants. There is also evidence that the diversity of human milk oligosaccharides (a type of fiber) in breastmilk is inversely associated with the body adiposity of both mother and baby (Aldrete et al., 2015; Jantscher-Krenn et al., 2019; Lagstrom et al., 2020). Analysis of the microbiota of individuals who consume an ultra-processed Western diet showed an increase in Firmicutes, Proteobacteria phyla, and Bacteroides genus (Singh et al., 2017). In general, a Western diet, characterized by high proteins and fats but low in fiber, is associated with less diversity in microbiota composition. In a study by Herman et al., 2018, a Western diet, whether the protein source is animal or plant-based, was a significant factor in microbiome diversity. Results of a study on children's dietary patterns found that a higher dietary pattern in animal products was not associated with less substantial microbiota (Berding et al., 2018). Individuals who consume a minimally processed, culturally traditional diet display increased *Actinobacteria* and *Prevotella* (Lane et al., 2020; Singh et al., 2017).

Mounting evidence supports the significant effects of diet and dietary habits on the symbiotic relationship between gut microbiota and its host. Consumption of high saturated fat and ultra-processed foods, sugar-sweetened beverages, and low diversity of fiber-rich food sources are some of the factors that cause obesity and increased risk of metabolic syndrome in children. Several studies indicate that unhealthy alterations involving the gut microbiota are reversible with better adherence to the 2020-2025 *Dietary Guidelines for Americans* or to a Mediterranean dietary pattern (De Filippis et al., 2016; Singh et al., 2017)

Statement of the Study Purpose

This study aims to address the current gap in the literature by examining whether there are differences in the gut microbiota composition between obese and non-obese school children and if dietary habits affect the alpha-diversity of gut microbiota composition, the Firmicutes-Bacteroidetes (F: B) ratio and abundance of the primary short-chain fatty acids (SCFA -butyrate, propionate, and acetate) gut microbes. The following specific aims are:

1. To evaluate the impact of child obesity status on three related outcomes: alpha diversity of gut microbiota composition, F: B ratio, and abundance of SCFA-generating gut microbes between obese and non-obese children. All three outcomes would be assessed by extracting DNA from stool samples of children using 16S rRNA metagenomic sequencing.

Hypothesis 1: Obese children will have lower alpha-1 diversity (Shannon index) than non-obese children.

Hypothesis 2: Obese children will have a higher F/B ratio compared with non-obese school children.

Hypothesis 3: Obese children will have an increased relative abundance of SCFA-generating microbes compared with non-obese children.

2. To compare dietary habits and intakes (IV) between obese and non-obese school children measured by three 24-hour diet recalls.

Hypothesis 4: Obese children have higher energy and saturated fat dietary intakes compared with non-obese children.

Hypothesis 5. Obese children have a lower diversity of fiber-rich foods than non-obese school children (as measured by the daily gut microbiome friendliness score).

3. To evaluate the association of BMI (IV) with differences in gut microbiota composition (DV) obtained using 16S rRNA gene sequencing between obese and non-obese children.

Hypothesis 6: A higher BMI z-score is associated with a relative abundance of bacteria in the phylum *Firmicutes*

Nursing Implications

Knowledge gained from the study will provide new insights into nursing research and practice in the short term as a basis to explore the complex gut microbiota ecosystem of both non-obese and obese children in clinical intervention studies and, in the long term, contribute to the development of revolutionary strategies for the prevention and treatment of childhood obesity, and obesity-related diseases. When consistently followed throughout childhood, adolescence, and adulthood, childhood obesity prevention efforts that target the gut microbial ecosystem may improve health and help prevent tracking into adult obesity. It creates a window of opportunity for nurses to empower mothers of obese youth in the adoption of healthier lifestyle behaviors that may impact some of the childhood T2D risks associated with obesity. In addition, nurses can provide valuable pre– and post-natal dietary and lifestyle recommendations to mothers on improving their baby's gut health, such as encouraging breastfeeding and healthier eating and exercise during the breastfeeding period to increase the quality of the breastmilk (Harris et al., 2020; Lagstrom et al., 2020). Furthermore, the period of childhood may provide opportunities for pediatric microbiome interventions through research that could have broad implications to promote health or prevent diseases. Since childhood obesity has been associated with alterations in the composition and diversity of gut microbiota, the results of this study will help establish a baseline understanding of the pediatric gut microbiome that will inform future intervention

studies, targeting the gut microbiota for the prevention and treatment of childhood obesity and obesity-related diseases.

Chapter Summary

Although therapeutic lifestyle changes such as improving dietary habits and engaging in physical activity have been established as essential strategies to manage childhood obesity, they have been challenging to accomplish consistently. Low-income populations consume more ultra-processed, calorie-rich, fiber- and nutrient-poor foods than higher-income populations. This dietary pattern increases the risk of child obesity, decreasing motivation and interest in child physical activity. Considering the accelerating incidence of childhood obesity and its related comorbidities, environmental predispositions underlying childhood obesity and associated metabolic disorders need to be further explored. The cause of obesity is multifactorial. Genetic factors may determine an individual's susceptibility to obesity, and environmental and lifestyle factors play a crucial role in the development of obesity. Research studies have identified the gut microbiota as one such ecological factor (Bäckhed et al., 2004; Bäckhed et al., 2007; Ley et al., 2005; Turnbaugh et al., 2006; Turnbaugh et al., 2009). Thus, the gut microbial ecosystem has emerged as a significant environmental factor involved in the pathogenesis of childhood obesity. An altered gut microbiota has been linked to adult obesity. However, very little is known about childhood obesity. Diet, in particular, can have a substantial impact on the gut microbiome (Asnicar et al., 2021; David et al., 2013; De Filippo et al.,2010; Drasar et al., 1973; Ley et al., 2006; Rettger & Cheplin, 1921; Wu et al., 2011) and more accessible to modify in intervention studies to modulate the composition of the gut microbiota (Carmody et al., 2015; De Filippo et al., 2010; Lee, 2013; Riva et al., 2017; Singh et al., 2017; Wu et al., 2011; Zhang et al., 2014;) thus, it establishes a practical route for future therapeutic interventions.

Therefore, this study will examine how dietary habits affect gut microbiota composition and its association with childhood obesity. Understanding the underlying mechanisms between diet and gut microbiota diversity could pave the way to developing and implementing nutrition policies in the school setting that could help promote healthy dietary habits to prevent childhood obesity and related metabolic disorders.

REVIEW OF THE LITERATURE

Childhood obesity was once an obscure physiological phenomenon up until the late 20th century when the number of overweight or obese children far exceeded those suffering from malnutrition (Prentice, 2006). In recent years, specifically over the past two decades, there has been a notable increase in obesity prevalence throughout the United States. Data from the National Health and Nutrition Examination Survey indicates a significant uptick in obesity among both adults aged 20 and above, which climbed by 37%, and among young people aged 2 to 19, which increased by 42% from the 1999–2000 data collection to the 2017–2020 period. While the rise in obesity rates among adults was more gradual between 2003 and 2012, there was a more marked escalation in later years. In contrast, the obesity rates among youth remained relatively constant from 2003 until 2014, after which they began to escalate. (Fryar et al., 2020)

A paramount concern is that findings from large and small cohort studies consistently show that childhood obesity persists into adolescent and adult obesity (Engeland et al., 2004; Freedman et al., 2005; Magarey et al., 2003; Morrison et al., 2007; Reilly et al., 2011; Schmidt et al., 2010; Starc & Strel, 2011; Venn et al., 2007) with outcomes of increased BMI, waist circumference and skinfold thickness as significant indicators. In addition, the adverse health outcomes of childhood obesity are well documented, such as the increased risk for metabolic syndrome (Jacob & Reetha, 2017; Morrison et al., 2007; Schmidt et al., 2010), T2D (Juonala et al., 2011), CVD (Freedman et al., 2005; Magnussen et al., 2009), musculoskeletal problems (Valerio et al., 2012), as well as psychosocial issues (Halfon et al., 2013; Morrison et al., 2015), that may all become more prevalent with increasing age (Wu et al., 2023).

Research for intervention strategies to control the pervasiveness of childhood obesity is crucial. A growing body of evidence in gut microbiota research suggests that alterations in gut

microbiota composition may play a role in the development of childhood obesity (Backhed et al., 2004; Turnbaugh et al., 2006; Turnbaugh et al., 2008).

The literature review will focus on factors influencing the composition and development of infant gut microbiota, differences in gut microbiota between obese and non-obese children regarding the F: B ratio and presence of SCFA, and the impact of diet on gut microbiota diversity and composition.

Factors Affecting Early Colonization & Development of Infant Gut Microbiota

To date, a great deal of the investigation on the causes of childhood obesity revolved around dietary excesses, lack of physical activity, and the discovery of genes that promote excessive weight gain. The previously limited knowledge on human–microbe interactions as strictly pathogenic has undergone a remarkable development over the past two decades. While we now recognize the significant role of the microbiota as beneficial and vital to immune (Cebra, 1999) and metabolic health (Collado et al., 2016; Cox et al., 2014), we are just starting to grasp the mechanisms by which these microorganisms come together, and the factors in early life that disturb their natural ecological succession. The infancy period provides a unique opportunity for rapid colonization and development of infant gut microbiota that may impact subsequent health and predisposition to certain diseases, including childhood obesity (Smith et al., 2015; Wall et al., 2009). Childhood also provides a unique window of opportunity for modification of gut microbiota to achieve positive health outcomes in adulthood (Penders et al., 2006)). The difficult task of developing effective strategies to treat childhood obesity relies on an improved understanding of the factors that influence the initiation and persistence of this condition (Smith et al., 2015). Recognition of the determinants and progression of the initial gut microbiome

development will provide an understanding of how the microbiome can be influenced to improve metabolic health.

For this reason, research data suggest that mechanisms that lead to childhood obesity may already initiate during gestation and infancy (Ridaura et al., 2013; Turnbaugh et al., 2008). Fetal programming toward an obese phenotype may be inclined to develop due to intrauterine and extrauterine influences (Penders et al., 2006; Ridaura et al., 2013; Turnbaugh et al., 2008).

Intrauterine Factors

There is evidence of the importance of the prenatal period in determining the risk for obesity in childhood and its role in the establishment and development of the gut microbiome. The previous belief of a sterile fetus and intrauterine environment has been disputed in recent studies of healthy and pre-term pregnancies in both vaginal and caesarian-section (C-section) delivery. In a prospective cohort study by Satokari et al. (2008), commensal gut microbial DNA of Bifidobacterium and Lactobacillus rhamnosus were detected in all 39 placental samples without signs of tearing of membranes or maternal infection and independent of the mode of delivery. In addition, a randomized, double-blind, placebo-controlled trial study provided evidence of microbial DNA presence in placental samples of mothers who were given probiotic supplementation of Bifidobacterium lactis and Lactobacilli rhamnosus for 14 days before elective C-section delivery (Rautava et al., 2012). Furthermore, data from a population-based cohort of 320 placental specimens revealed a metabolically rich placental microbiome, highly similar to the human oral microbiome, that consists of beneficial and non-pathogenic bacteria from various phyla, including Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes and Fusobacteria phyla (Aagaard et al., 2014). This study also suggests that oral microbiota may be relevant in colonizing the infant's gut microbiota; however, the mechanism by which oral

microbes translocate to the placenta needs further exploration. The presence of bacterial DNA such as lactic acid bacteria, Bifidobacterium, Enterococcus, and Staphylococcus has been detected in meconium (Jimenez et al., 2008) and umbilical cord blood (Jimenez et al., 2005) of healthy neonates born by C-section delivery. Furthermore, Enterococcus faecium strains have been documented in the amniotic fluid and fetal intestine of pups delivered by pregnant mice that were orally administered with E. faecium (Jimenez et al., 2005; Jimenez et al., 2008).

The above studies provided evidence of the intrauterine presence of bacteria, demonstrating that infant gut colonization may start before birth by maternal microbiota transfer to the fetus through the placental barrier and bloodstream. Although bacterial presence has been associated in clinical practice as a marker for possible infection that may predispose to an increased risk of preterm delivery (DiGiulio et al., 2008), these studies did not associate bacterial presence with any signs of clinical infection or inflammation (Steel et al., 2005).

Maternal antibiotic use during pregnancy is considered a critical factor that has been reported to have a significant impact on the programming of an obesity-prone metabolic phenotype. Several studies report that antibiotic use before, during and after birth may alter the typical maternal-infant bacterial exchange, resulting in anomalistic microbial colonization of the infant's gut (Aloisio et al., 2014; Fouhy et al., 2012; Jaureguy et al., 2004; Keski-Nisula et al., 2013; Tanaka et al., 2009) with increased vulnerability to obesity in later life (Ajslev et al., 2011; Azad et al., 2014; Mueller et al., 2015; Trasande et al., 2013), as well as long-term implications on gut microbiota diversity and health.

A prospective study on infants found that infant gut colonization by gram-negative or gram-positive bacteria was delayed in antibiotic-exposed infants by 28% compared with 12% in non-antibiotic-exposed infants (Jaureguy et al., 2004). In addition, colonization by obligate

anaerobic bacteria, such as *Bifidobacterium and Clostridium,* was delayed by 36% in antibiotic-exposed infants compared with 24% in non-antibiotic-exposed infants, with colonization by Clostridium as significantly altered in antibiotic-exposed-infants, reflecting its susceptibility to antibiotic-resistant bacteria (Jaureguy et al., 2004). Similarly, Fouhy et al. (2012) found delayed intestinal colonization by *Bifidobacterium* and *Lactobacillus* in infants receiving parenteral antibiotics within 48 hours of birth. Lower counts of *Bifidobacterium* (Aloisio et al., 2014; Jaureguy et al., 2004; Tanaka et al., 2009) and *Lactobacillus* (Keski-Nisula et al., 2013) signify delayed colonization of infant gut by commensal bacteria due to prenatal antibiotic exposure that decreases microbial diversity (Aloisio et al., 2014; Jaureguy et al., 2004; Keski-Nisula et al., 2013).

In a longitudinal study of 436 mother-offspring dyads that were followed until seven years of age, Mueller et al. (2015) determined that prenatal use of antibiotics in the second or third trimester increased the risk of childhood obesity by 84% compared with children who were not exposed to antibiotics during pregnancy. In addition, antibiotic exposure during the second or third semester of pregnancy was positively correlated with higher BMI z-scores, waist circumference, and body fat percentage at significance $p \leq 0.05$. Results of the study suggest that maternal use of antibiotics altered the offspring's body composition. Furthermore, a large retrospective study of Danish school children aged between 7 – 16 years old, 33% of whom had prenatal exposure to systemic antimicrobials, reported a strong association with childhood obesity at a 26 - 29% increased prevalence of overweight and obesity at school age (Mor et al., 2015). Mor et al. (2015) observed that this strong association is likely attributed to a maternal gut microbiota altered by antibiotic use during pregnancy. Results of a longitudinal prospective study by Ajslev et al. (2011) of 28,354 mother-child dyads revealed an observed risk of

childhood overweight in infants treated with antibiotics at six months of age who are born of normal-weight mothers and reduced risk of overweight among infants of overweight mothers. Because early life is a crucial period for metabolic development (Dietz, 1994), microbiota disruption during this critical window due to antibiotic exposure could induce sustained effects on body composition, leading to an increased risk of overweight or obesity later in life (Ajslev et al., 2011; Murphy et al., 2013; Trasande et al., 2013).

Extrauterine Factors

Numerous studies have demonstrated the long-term influence of the mode of birth on the development of infant gut microbiota. Almost all studies, regardless of the method used, report profound differences in the fecal bacteria of infants born vaginally and infants delivered by caesarian section (CS) (Bouter et al., 2017). The infant's gut is rapidly increased with commensal bacteria during vaginal delivery (VD) from the mother's vaginal and fecal bacteria (den Besten et al., 2013; Edwards, 2017; Neu, 2015). Infants delivered by CS have delayed microbial colonization and lower gut microbial diversity (Gronlund et al., 1999; Jakobsson et al., 2014; Salminen et al., 2004). Colonization of commensal bacteria, such as *Bifidobacteria* and *Lactobacilli*, was observed to be delayed in infants delivered by CS by one month and ten days, respectively, compared to VD infants (Gronlund et al., 1999). In the meta-analysis by Li et al. (2013), after separate, pooled analyses, and without controlling for potential confounders, a 33% moderate increased risk of childhood obesity was reported that prevailed through to adulthood. In three birth-cohort studies conducted in 1982, 1993, and 2004 in Brazil, Mueller et al. (2015) observed an association between CS and a 46% higher risk of childhood obesity in a longitudinal study of 436 mother-child dyads that were followed from birth until seven years of age. In a longitudinal birth cohort study of children followed until 15 years of age, Blustein et al. (2013)

reported that at age 11 years, children born by CS were 1.83 times at higher odds of obesity (95% Cl: 1.24-2.70). In a prospective pre-birth cohort study of 1,255 children with body composition measured at three years of age, 15.7% of 284 children who were born by CS were obese compared to 7.5 children who were vaginally delivered (Huh et al., 2012).

The transfer of maternal microbes to newborns is also well established through breast-feeding. The relevant presence of *Bifidobacteria* in the microbiota of breast-fed infants is distinguishable from the gut microbiota of formula-fed infants that show a lower abundance of *Bifidobacteria* (Bouter et al., 2017; Edwards, 2017; Li & Wei, 2015). In breast-fed infants, *Bifidobacteria* begin to colonize 2 -5 days after birth, becoming the most predominant bacteria at 99% within one week, and making it the most significant bacterial component of the infant gut microbiota (Kurokawa et al., 2007; Yatsunenko et al., 2012). Breastfeeding modulates the infant's gut microbiota (Stark & Lee, 1982). It confers protective and immunomodulatory benefits to the infant against various infectious, allergic, and inflammatory diseases or disorders compared to formula-fed ones (Fernandez et al., 2013). The introduction to solid foods is deemed an essential determinant of gut microbiota composition and a significant influence in developing an adult-like microbiota (Bouter et al., 2017; Edwards, 2017). Thus, diet during infancy may affect the maturation of microbiota. In this regard, the *2020-25 Dietary Guidelines for Americans* now recommends that parents expose their infants to peanut butter as soon as they start solid foods because there is a maturational window during which early exposure of the infant to possible allergens prevents later-in-life allergies to those allergens (U. S. Department of Agriculture & U. S. Department of Health and Human Services, (2020)). The inclusion of prebiotics and probiotics in the infant's diet may increase *Bifidobacteria* and *Lactobacilli*

communities; however, there is a dearth of knowledge on whether these bacterial communities thrive long after the prebiotics and probiotics have been discontinued (Edwards, 2017).

Differences in Gut Microbiota Between Obese and Non-Obese Children: Firmicutes: Bacteroidetes Ratio, Short Chain Fatty Acids & Diet

The findings in the studies below demonstrate the power of combining human and gnotobiotic mouse models, specific dietary manipulations, and comparative metagenomics to define relationships between diet and gut microbial ecology with Firmicutes: Bacteroidetes (F: B) ratio and short-chain fatty acids (SCFA). In addition, the studies' results emphasized the importance of microbial and host contributions to regulating energy balance.

Gut Microbiota in Mouse Models of Obesity

A link between altered gut microbiota and obesity has been established in past studies using mouse models, in which the importance of diet in the gut microbiota profile was well exemplified. In 2005, Ley et al. hypothesized that gut microbial diversity affects host energy homeostasis. To test this hypothesis, Ley et al. (2005) performed an analysis of 5,088 bacterial 16s rRNA gene sequences extracted from the distal gut of genetically obese mice, lean mice, wild-type siblings, single-runted obese mice, and their mothers, who were all fed the same polysaccharide diet of Purina ad libitum. The study revealed that the two most abundant bacterial divisions in mice were the phylum *Bacteroidetes* and the phylum *Firmicutes.* The shifts were division-wide, with no specific loss or gain on *Bacteroidetes* or *Firmicutes* (Ley et al., 2005). Such shifts established a low abundance of *Bacteroidetes* in the distal gut microbiota of obese mice at 26.1%, a 50% reduction compared to lean mice, and a significantly more significant proportion of *Firmicutes* at 71.2%, independent of gender and family kinship (Ley et al., 2005). Ley et al. (2005) also discovered that the single-runted obese mice showed similar proportions of

Firmicutes and *Bacteroidetes* as the other obese mice despite significantly limited food consumption compared to lean mice. This suggests that limited food consumption and total body mass do not impact gut microbial diversity in the phylogenic division (Ley et al., 2005). The study by Ley et al. (2005) provided the very first evidence of the association between gut microbial ecology and obesity.

To add further to this knowledge, Turnbaugh et al. (2006) performed a study on a larger scale using shotgun sequencing and high-throughput 454 pyrosequencing techniques that underscored an increased ratio of *Firmicutes* to *Bacteroidetes* in obese mice compared to lean mice. In addition, the study reported an increased concentration of the SCFAs butyrate and acetate. This finding is consistent with numerous Firmicutes producing the SCFA butyrate (Barcenilla et al., 2000; Pryde et al., 2002). Moreover, the study revealed that the obese microbiome has an increased capacity for dietary energy harvest, resulting in significantly less energy remaining in feces than lean mice.

In 2009, Hildebrandt et al. investigated the relationships between diet, obesity, and microbiota composition by comparing the microbial communities of wild-type mice and mice with the resistin-like molecule beta (RELM) knockout (KO) phenotype. While both mice were lean and fed a standard chow (CHO) diet for 13 weeks, the wild-type mice became obese after switching to a high saturated fat diet for 21 days. Results of metagenomic 16S rDNA demonstrated considerable alterations in the gut microbiota composition associated with the high-fat diet change that included a decrease in *Bacteroidetes* and a remarkable increase in *Firmicutes* levels. Using the RELMβ KO genotype, the high-fat diet accounted for the altered microbial communities, not the obese state (Hildebrandt et al., 2009).

In a mouse model of diet-induced obesity (DIO), Turnbaugh et al. (2008) observed dramatic effects in the microbial ecology of male germ-free mice fed a high-fat/high-sugar prototypic Western diet for four weeks. Utilizing 16s rRNA gene sequencing, the Western diet-induced a gut microbial community significantly enriched in *Firmicutes* but depleted in *Bacteroidetes*. The overall diversity of the Western diet gut microbiota in germ-free mice was dramatically altered due to a bloom of a single class of *Firmicutes* called *Mollicutes* that accompanied a division-wide suppression of Bacteroidetes but not a division-wide shift in *Firmicutes* (Turnbaugh et al., 2008).

In a follow-up study, Turnbaugh et al. (2008) observed the same effects on conventionally raised mice fed either a Western or CHO diet. Those on the Western diet had a significant weight gain and greater adiposity than those on the CHO diet. Also observed was the presence of the *Mollicutes* lineage that was identified in the earlier study on germ-free mice.

In another experiment, Turnbaugh et al. (2008) demonstrated that dietary shifts could impact body weight, adiposity, and gut microbial ecology. In this study, Western-diet-fed conventionally raised mice with inherited microbial communities from mothers switched to a reduced carbohydrate (CARB-R) or reduced fat (FAT-R) diet. Mice on CARB-R and FAT-R consumed fewer calories and notably gained less weight [0.6±0.3g (FAT-R) and 0.0±0.3 g. (CARB-R) compared with the Western diet (2.0±0.3g)] (Turnbaugh et al., 2008). The epididymal fat gain was also reduced (1.90.3% of total body weight for FAT-R and 1.90.2% for CARB-R compared with 2.80.2% for the Western diet. Gene sequence analyses by 16s rRNA indicate that this diet-induced obesity animal model followed by weight and fat loss was accompanied by a remarkable decrease in the relative abundance of *Mollicutes* for FAT-R and a highly significant reduction for CARB-R compared with the Western diet (Turnbaugh et al.,

2008). A significant division-wide increase in the relative abundance of *Bacteroidetes* for FAT-R (2.8-fold) and CARB-R (2.2-fold) was also observed (Turnbaugh et al., 2008). In this regard, dietary interventions such as the FAT-R and CARB-R prove promising in inhibiting numerous consequences of Western diet-induced obesity, such as reduced adipose tissue mass, increase in abundance of Bacteroidetes, enhanced ability of the microbiota to suppress fat deposition and decreased bloom of *Mollicutes* lineage. Turnbaugh et al. (2008) performed a Unifrac analysis, which indicated that the gut communities in subjects consuming a Western diet shared a more remarkable resemblance than those in individuals. This pattern mirrors what is observed in the ob/ob obesity model. In the Western diet group, there was a notable increase in the proportion of Firmicutes and a decrease in Bacteroidetes in the gut microbiome.

The above mice studies demonstrated substantial evidence of causal associations between the gut microbiome and obesity and have highlighted the potential role of gut microbiota in regulating the development of obesity through diet. The above mice studies showed that dietary habits are the main contributors to the diversity and altered composition of mice gut microbiota, and nutritional interventions targeting the gut microbiota could be an excellent strategy to treat obesity. The mice models of obesity and gut microbiological ecology provided a platform for human studies.

***Firmicutes-Bacteroidetes* Ratio, Diet & Short Chain Fatty Acids**

Many studies have investigated and critically assessed the relationship between bacteria from Firmicutes and Bacteroidetes phyla and weight gain in the pediatric population. As gleaned from the mice studies, the relative abundance of the two predominant gut bacteria differs between lean and obese mice, with increased Firmicutes and fewer Bacteroidetes in genetically obese mice compared to their lean counterparts. To extend this observation to humans, a cutting-

edge initial human study by Ley et al. (2006) showed that 12 obese humans similarly exhibited a relative reduction in Bacteroidetes and elevated Firmicutes in their feces when randomly assigned to either a carbohydrate-restricted or fat-restricted diet over one year. Ley et al. (2006) observed a relative decrease in the F: B ratio with an increased abundance of Bacteroidetes and a decreased abundance of Firmicutes. The increased abundance of Bacteroidetes correlated with a percentage loss of body weight and no changes in dietary content. Ley et al. (2006) show that manipulating gut microbial communities could be a potential therapeutic approach in treating obesity and indicate a dynamic link between obesity and gut microbial ecology.

Subsequently, a much larger human study by Turnbaugh et al. (2009) demonstrated the association of obesity with the depletion of Bacteroidetes, reduced gut bacterial diversity, and enrichment in carbohydrate and lipid-utilizing genes in the entire microbiome. The study reported that 75% of obesity-enriched genes came from Actinobacteria, 25% from Firmicutes, and 42% of lean-enriched genes came from Bacteroidetes. The obesity-enriched genes provide the foundation for microbial biomarkers of the obese gut microbiome (Turnbaugh et al., 2009).

The initial investigation into the association between an altered gut microbiota composition and obesity development in children was conducted by Kalliomaki et al. (2008). Kalliomaki et al. (2008) examined obese and normal-weight children from birth, at the ages of 3, 6, 12, 18, and 24 months, and ages 4 and 7 years. Fecal gut microbiota composition was analyzed at ages 6 and 12 months. To avoid underestimating adiposity in children, weight status was determined using the International Obesity Task Force criteria for overweight and obesity in children (Cole et al., 2000). These criteria define BMI values for overweight and obesity at each age and gender in children associated with a predicted BMI of 25 and 30 at age 18 years (Cole et al., 2000). Kalliomaki et al. (2008) observed that children who remained lean at age seven years

showed higher levels of Bifidobacteria as infants compared with children who developed obesity and had higher levels of the pathobiont, Staphylococcus aureus, a species within the Firmicutes phylum, and lower levels of Bifidobacteria (Kalliomaki et al., 2008). *Bifidobacteria,* which falls under the *Actinobacteria* phylum, have been documented to characterize the gut microbiota composition of healthy breast-fed infants (Vaughan et al., 2002). It is suggested that breastfeeding protects against later onset of overweight or obesity (Armstrong & Reilly, 2002; Kramer, 1981) and that duration of breastfeeding is inversely associated with the risk of obesity (Poulton & Williams, 2001; von Kries et al., 1999).

Nonetheless, Kalliomaki et al. (2008) also emphasized the importance of *Staphylococcus spp.* in childhood obesity by demonstrating that a sizeable fecal concentration of *Staphylococcus spp.* in infancy predicted the development of overweight/obesity during childhood. Fecal gut microbiota composition was analyzed by fluorescent in-situ hybridization (FISH) with microscopic and flow cytometry detection and by quantitative real-time polymerase chain reaction (qRT-PCR).

In line with the findings of Kalliomaki et al. (2008), a relative abundance of *Bifidobacteria* was found in stool samples of children aged 1 – 6 years living in an African village of Burkina Faso (BF) who were breastfed for two years (De Filippo et al., 2010). Compared with BF children, European (EU) children of similar age in Florence, Italy, who were breastfed for only one year, had relatively low Bifidobacteria in their stool samples (De Filippo et al., 2010). In addition, the fecal microbiota of BF children showed a significant 58% enrichment in Bacteroidetes and 37.4% depletion of Firmicutes compared with 29.1% and 70.4%, respectively, in EU children. Firmicutes were twice as abundant in EU children, as evidenced by the different F: B ratios, indicating a markedly different gut bacterial colonization

in the two populations. According to De Filippo et al. (2010), diet plays a dominant role over other factors such as ethnicity, sanitation, hygiene, climate, and geography in shaping gut microbiota composition. The dietary habits of these two different populations may be responsible for this dramatic difference in gut microbial colonization (De Filippo et al., 2010). BF children subsisted on a diet of high-fiber and polysaccharide-rich foods that resemble Neolithic subsistence patterns of early African settlers; the diet of EU children is typified by a modern western diet, high in animal protein, fat, sugar, and starch, and low in fiber (De Filippo et al., 2010). In addition, fecal samples of BF children reflected an abundance of SCFAs, specifically propionate, and butyrate, compared with EU children (De Filippo et al., 2010). Specific bacterial genera in the Bacteroidetes phylum exclusive to BF children, such as Xylanibacter, Prevotella, Butyrivibrio, and Treponema, were responsible for producing the high levels of SCFA by gut microbial fermentation of dietary fibers (De Filippo et al., 2010). De Filippo et al. (2010) suggested a correlation between diet and SCFA production. The dietary implication of high consumption of sugar, animal fat, and calorie-dense foods in industrialized countries may reduce gut microbial richness and diversity, as well as limit the production of SCFA-producing bacteria that could afford a manifold of health-related effects, including prevention of gastrointestinal diseases (De Filippo et al., 2010). Most importantly, De Filippo et al. (2010) provided a model for assessing various environmental factors in two childhood populations and their impact on the gut microbiota. Gut microbial differences between these two groups were analyzed using high throughput 16s rDNA sequencing and biochemical analyses (De Filippo et al., 2010).

An increased F:B ratio has been proposed to contribute to the pathophysiology of obesity and is associated with increased production of SCFAs and energy harvest from colonic fermentation (Fernandes et al., 2014; Turnbaugh et al., 2006).

The results of an Egyptian pediatric study revealed a statistically significant increased F: B ratio within the obese group ($p = 0.003$) compared with the normal-weight children ($p < 0.001$) (Abdallah Ismail et al., 2011). The study also found an association between high fat intake and increased levels in Firmicutes and decreased levels in Bacteroidetes, and those with the highest carbohydrate intake were positive for both Bacteroidetes and Firmicutes. This finding highlights the potential role of dietary factors in modulating gut microbiota (Abdallah Ismail et al., 2011). A notable result was the presence of the biochemical marker high-sensitivity C-reactive protein (hs-CRP) in obese children and a positive trend for higher hs-CRP in children with positive Firmicutes ($p = 0.004$). C-reactive protein is a biochemical marker for systemic inflammation; it has been associated with several inflammation-related diseases such as CVD, diabetes mellitus, respiratory diseases, and rheumatoid arthritis (Ismail et al., 2011). The concentration of hs-CRP is elevated in obesity due to the expression of Interleukin-6 on adipose tissue and its release in the peripheral circulation (Cani et al., 2007). Ismail et al. (2011) concluded that their data support the role of gut microbiota in the pathophysiology of child obesity. Fecal microbiota samples were analyzed using the polymerase chain reaction method.

In addition, several studies have provided clear evidence to suggest that consumption of a high saturated fat diet is associated with metabolic endotoxemia and a two to three-fold increase in bacterial *Lipopolysaccharide* (LPS) in the blood (Cani et al., 2007; Khan et al., 2016; Sanchez-Tapia et al., 2021). Cani et al. (2007) found a dramatic change in gut microbiota composition (reduced *Lactobacillus* and *Bifidobacteria*), which was associated with an increase in gut permeability indicated by a reduction in the expression of Occludin and ZO-1 tight junction proteins (Cani et al., 2007, Sanchez-Tapia et al., 2021).

Research by Ferrer et al. (2013) indicates results of 94.6% abundance in *Firmicutes* compared to 3.2% of *Bacteroidetes* in the obese gut microbiota of a Spanish adolescent. Similarly, study data from Bervoets et al. (2013) reported higher concentrations of *Firmicutes* in the species level in overweight/obese (O/O) children compared with average weight (C) children. Additionally, an elevated F: B ratio was identified among overweight/obese (O/O) children compared with normal weight (C) children (Bervoets et al., 2013). It is important to note that *Bacteroides (B.) vulgatus*, a species in the *Bacteroidetes* phyla, appeared depleted in O/O children compared with (C) children. *B. vulgatus* was deemed a commensal gut bacterium and is generally considered an essential part of the core gut microbiota in healthy humans (Qin et al., 2010). *Lactobacillus* is the species-genus in the Firmicutes phyla that was found to be in high concentrations in the O/O gut microbiota, which is in line with findings of previous studies that suggest a capacity for *Lactobacillus spp*. to influence body weight and obesity (Million et al., 2012; Santacruz et al., 2009). In addition, the high concentrations of *Lactobacillus spp*. were positively correlated with hs-CRP in obese children and adolescents, per the findings of Abdallah Ismail et al. (2011). While some species of *Lactobacillus* promote weight gain, others may prevent weight gain, according to the comparative genomics analysis study by Drissi et al. (2014). Bervoets et al. (2013) also found *Staphylococcus* in stool samples of O/O children, which showed a greater efficiency in extracting energy from a given diet in obese children compared with the gut microbiota of lean children. The study results by Bervoets et al. (2013) indicate that *the Bacteroides group, Lactobacillus spp.*, and *Staphylococcus* impact the composition of gut microbiota and play a vital role in the pathophysiology of obesity. To identify and determine bacterial species in fecal samples for this study, qRT-PCR and matrix-assisted laser

desorption/ionization mass spectrometry (MALDI-TOF-MS) were utilized for in-depth analysis of species.

The results of an Italian pediatric study reported an elevated F: B ratio in significant variations within the obese group with significantly elevated levels of *Firmicutes* and diminished levels of *Bacteroidetes* (Riva et al., 2017). Higher concentrations of SCFA acetate, propionate, and butyrate were observed in the stools of obese children compared with normal-weight children. The overall concentration of SCFAs was highly associated with obesity and positively correlated with BMI *z*-score (Riva et al., 2017). Specifically, SCFAs acetate, propionate, and BMI *z*-score were significantly linked with microbiota composition at every taxonomic level (Riva et al., 2017). According to Riva et al. (2017), the higher fecal concentration of SCFAs in the obese group may indicate increased microbial fermentation, sluggish SCFA absorption due to low-grade inflammation, or greater colonic transit time. In addition, generalized linear regression models showed *Bacteroidetes* as a better predictor of BMI *z*-score and obesity than *Firmicutes.*

According to Riva et al. (2017), this could be attributed to the varying shifts of *Firmicutes* operational taxonomic units, which resulted in certain Firmicutes members being associated with obesity. Further studies would be needed to ascertain the role of certain members of *Firmicutes* on obesity. Nevertheless, the BMI *z*-score was positively correlated with significant levels of *Firmicutes* and negatively correlated with *Bacteroidetes*. However, in a meta-analysis of almost all of the studies, with one exception, a pattern emerges where the Firmicutes to Bacteroidetes ratio tends to be higher in obese individuals than in their lean counterparts. However, when evaluating the data with Wilcoxon rank-sum tests, the variations between the obese and lean groups did not reach statistical significance. Similarly, when

applying Fisher's Method to integrate various independent hypothesis tests, the Firmicutes to Bacteroidetes ratio disparity did not achieve statistical significance (Walters et al., 2014).

Obesity has been associated with an altered gut microbiota composition and dietary habits. Riva et al. (2017) reported high nutritional intakes of proteins, fats, sugars, and carbohydrates among obese children compared to normal-weight children. However, future studies are needed to establish if an altered gut microbiota is a causative factor in obesity or a consequence of diet (Riva et al., 2016). Overall, study data by Riva et al. (2017) present evidence of the association of obesity with an altered gut microbiota composition. Gut microbiota composition for this study was evaluated using 16s rRNA gene gene-targeted sequencing.

While globalization and urbanization afforded many advantages economically, politically, and socially, they also led to the urbanization of diets and, therefore, drastic changes in lifestyle and dietary habits (Solomons & Gross, 1995). Inevitably, a dietary trend toward consuming highly processed foods and foods high in unhealthy fats, sugar, and calories skyrocketed (Solomons & Gross, 1995; Shridhar et al., 2015; Lane et al., 2020). Processed plant foods have less fiber and lower amounts of other prebiotics than unprocessed food (Martinez Steele et al., 2017). Numerous data report the adverse effects of modern diets on human health, especially on the younger generation in developed and developing global areas (Imamura et al., 2015; Shridhar et al., 2015). The impact of the modernization of diet on gut microbiota between children living in rural and urban cities was consistent with previous studies on geographical variations in gut bacterial communities (De Filippo et al., 2010; Wu et al., 2011; Lee, 2013). Certainly, urbanization has altered the lifestyles of school-aged children living in some provincial towns and cities in Leyte, Philippines (Nakayama et al., 2017). Nakayama et al. (2017) reported that modern high-fat dietary habits of urban (UR) children aged 7 - 9 years

affected gut microbiota composition with a relatively high abundance of *Clostridia and Erysipelotrichia* classes belonging to the *Firmicutes* phyla and depleted levels of the genus *Prevotella* belonging to the *Bacteroidetes* phyla, compared with the gut microbiota composition of rural (RU) children of similar age. The depleted levels of *Prevotella* may be explained by the fact that this class of species is typically found in high levels in a vegetarian diet (David et al., 2013).

High fat intake positively correlated with F: B ratio and BMI. High-fat intake increases the intestinal level of bile acids, thus limiting bile-sensitive bacteria and promoting more bile-tolerant bacteria (Islam et al., 2011). This may illustrate the reduced prevalence of the *Bacteroidetes genus Prevotella* in UR children. Nakayama et al. (2017) described the diet of UR children as high in fat, protein, and sugar intake, and they ate fast food 4.0 times a week on average, reflecting the modernization of dietary habits in the UR group. The diet of RU children consisted of high daily consumption of regional fruits, vegetables, and grains, low meat, fat, and sugar intake, and eating fast food 0.90 times a week on average. Total energy intake did not statistically differ between the two groups. According to the CDC BMI criteria, urban children in this study were described as overweight and obese, while rural children were described as normal and underweight. Nakayama et al. (2017) demonstrated that *Prevotella* might be vital in the healthful response to less caloric, sugary, and low animal protein and fat diets. The investigators also suggest that the Prevotella-depleted and high F: B gut microbiota is the outcome of the evolutionary adaptation of the gut microbial community to a high-fat and high-calorie modern diet. This study suggests that the long-term reduced intake of diverse fiber-rich and minimally processed foods such as fruits and vegetables, whole grains, legumes, seeds, and nuts may eventually lead to obesity as a consequence of the enhanced gut permeability of plasma

lipopolysaccharide (LPS) into the host circulation that triggers chronic inflammation (Chen, He & Huang, 2014), and as a consequence of insufficient propionate to stimulate GLP-1 satiety signaling (Bodnaruc et al., 2016).

However, it remains unclear whether the altered gut microbiota is a result of obesity or an aftermath of an altered dietary habit. Nevertheless, the study signified an association between an altered gut microbiota and obesity among children in developing areas due to a modern diet. High-throughput 16s rRNA gene sequence analysis was conducted to analyze gut microbiota composition.

Chapter Summary

Strong evidence from numerous studies suggests the association between childhood obesity and an altered gut microbiota composition. While modern dietary habits (especially fiber-poor, processed foods), increasingly sedentary lifestyles, c-section delivery, antibiotic use, and formula feeding are major contributory factors to an obesogenic condition, many researchers are gaining a valuable appreciation of an altered gut microbiota composition as an essential risk factor (Clarke et al., 2012). Diet strongly influences the composition and diversity of gut microbiota and is best portrayed in human and animal studies with shaping the gut microbial community. The link between diet and gut microbiota composition has been extensively studied, ranging from increased F: B ratio, elevated SCFA level, and higher BMI in obese microbiota with short- and long-term dietary outcomes and weight gain and loss. However, it is unclear whether an altered gut microbiota composition is causal for obesity or the impact of dietary habits. Evidence from the study by Ridaura et al. (2013) suggests that an altered gut microbiota composition is probably causal for obesity as fecal microbiota transplants from obese adult human twins have been shown to transmit the obesity risk to genetically identical, germ-free

mice fed a low-fat mouse food and different levels of saturated fat, and fruits and vegetable intake typically in the United States. Therefore, this study will compare the dietary intake of overweight/obese and normal-weight school-aged children to investigate the effect on gut microbiota composition and its SCFA metabolites in terms of F: B ratio, SCFA concentrations, and alpha-1 diversity.

THEORETICAL FRAMEWORK

There is a consensus that the increase in childhood obesity is partly due to environmental changes (Hill et al., 2003) that can lead to increased food intake and an overall decline in total energy expenditure attributable to a decrease in basal metabolic rate rather than a decrease in physical activity, which has important implications for understanding obesity trends (Speakman et al., 2023). For instance, in the family environment, there has been an increase in the percentage of children with television in their bedrooms (Dennison et al., 2002). Eating has been linked with television watching (Matheson et al., 2004), often shifting time away from physical activity (Durant et al., 1994), thus reducing energy expenditure. In addition, changes in the environment also influence dietary intake (French et al., 2001). With the growing number of single-parent families and families in which both parents work (Bowers, 2003), there has been an increase in eating out at restaurants (National Restaurant Association, 2019) where larger portion sizes are served (Rolls, 2003), and with more fats and oils added to the food supply, resulting in increased food and calorie consumption (Costa et al., 2019; Lin et al., 1999). The more significant issue with restaurant meals is that they tend to be highly processed, high in sugar, sodium, and saturated fat, and lacking in fiber, thus less satiating and needing to consume more to achieve satiety (Costa et al., 2019).

During the past decade, groundbreaking research has provided strong evidence of an association between obesity and an altered gut microbiota composition that triggers a decrease in satiety-signaling, an increase in energy harvest, chronically elevated low-grade inflammation and the alteration of host gene expression (Backhed et al., 2004; Fernandes et al., 2014; Ley et al., 2005; Ley et al., 2006; Turnbaugh et al., 2006; Turnbaugh et al., 2009). The gut microbiota is considered an environmental factor that plays a significant role in the pathogenesis of obesity

(Backhed et al., 2004; Backhed et al., 2005; Ley et al., 2005; Turnbaugh et al., 2006, 2009). The two significant phyla – *Bacteroidetes* and *Firmicutes* – have been found to differ in proportions between obese and lean children, and these proportions have been shown to change during weight loss and highly correlate with the percentage of body weight lost (Ley et al., 2006).

Several studies have strongly suggested that diet is the most profound determinant of gut microbial functioning throughout life (Moschen et al., 2012; Tilg & Moschen, 2015), as it can have a significant impact on the structure and composition of the gut microbiota in both the short- (David et al., 2013; Wu et al., 2011) and long-term (Ley et al., 2006; Wu et al., 2011). Most importantly, diet can, with regular exercise, establish an environment that can promote the long-term stability of a healthy microbiota (Moschen et al., 2012). The interactions between gut microbiota and diet in mammals are complex. Any significant lifestyle or dietary changes will likely affect microbial stability (David et al., 2013; Wu et al., 2011). Diet is the primary determinant in the microbiota colonization patterns from the first stages of human life and is also a risk factor in childhood obesity (Stanislawski et al., 2018). Thus, the impact of diet and dietary behaviors on gut microbiota composition has received much attention in research to help mitigate obesity risk and reduce obesity prevalence.

The prevailing number of obese children and adolescents is still dramatically higher than it was historically across the nation (Hales et al., 2018; Ogden et al., 2015; Ogden et al., 2016) and in the world (Olds et al., 2011), There are 150 million children worldwide (WHO, 2021), and an estimated 14.7 million children in the U. S. who are obese. The increasing trend in rates of obesity in children is alarming given the significant short- and long-term physical health (Juonala et al., 2011; Kelsey et al., 2014; Llewellyn et al., 2016) and psychological consequences (Beck, 2016; CDC, 2017b; Halfon et al., 2013; Llewellyn et al., 2015; Morrison et al., 2015),

economic implications owing to increased medical costs (Finkelstein et al., 2014; Hammond & Levine, 2010), and the robust tracking of childhood obesity into adulthood (Freedman et al., 2005; Freedman et al., 2005a; Simmonds et al. 2015; Whitaker et al., 1997) which could adversely impact future adult obesity rates (Kelsey et al., 2014; Llewellyn et al., 2016). Intervention in childhood is critical to combat the obesity epidemic and its related complications. Programs combining dietary and physical activity elements have been shown to lower obesity risk (measured by zBMI and BMI) in children between 0 and 5 years old. The study by Brown et al. (2011) suggests potential benefits of diet-only programs for this age group; however, physical activity seems ineffective for this younger age group. On the other hand, for children between 6 and 12 years and adolescents between 13 and 18, programs focused solely on increasing physical activity have decreased obesity risk, measured by BMI. However, more evidence is needed for these age groups that diet-only programs are effective. Still, there is some indication that combining dietary efforts with physical activity may yield positive outcomes (Brown et al., 2011).

To address childhood obesity, it is essential to consider the contexts or ecological niche in which the child is situated to understand the emergence of risk factors implicated in the development of childhood obesity. The family environment is an environmental context shown in studies to contribute to the development of obesity, specifically by shaping the child's dietary behaviors and the ability to self-regulate dietary intake (Epstein, 1996; Johnson & Birch, 1994). In addition, it is essential to understand how the gut microbiota, an environmental obesity risk factor, is influenced by dietary behavior that puts children at risk for obesity. These contexts or ecological niches will be given primary consideration in this research. Other ecological contexts,

such as school and community, will be given secondary consideration to show how these contexts may play a role in producing an obesogenic environment for the child.

This study explores whether childhood obesity can be understood through ecological principles, considering the interplay between individuals and their environments. It will evaluate the diet, dietary behaviors, and specific child characteristics such as gut microbiota composition, age, and gender of overweight/obese and normal healthy weight school-aged children. Additionally, it will consider the various ecological settings or environments that influence the development of child obesity. To offer insight and understanding of the process by which childhood obesity develops, the ecological systems theory (EST) will be utilized to provide a meaningful guide for this research. Ecological systems theory will help evaluate how the child is affected and the degree of influence by an environment with unhealthy triggers.

Ecological Systems Theory

As indicated in previous research, the development of childhood obesity is complex and multifaceted. A set of factors from multiple contexts interact with each other and place a child at risk of obesity. This multidimensional system can be conceptualized using the ecological systems theory (EST) developed by Urie Bronfenbrenner (1979), a psychologist and cofounder of the Head Start program.

Description of Ecological Systems Theory

Ecological systems theory conceptualizes human development from a contextual orientation with evolving interactions between the developing child and the environment (Bronfenbrenner, 1975, 1979, 1986a, 1986b; Bronfenbrenner & Morris, 1998). To be precise, EST is described as the ecology of human development, with the developing child consistently viewed as influencing and being influenced by a dynamic environment. According to EST, the

development or change in individual characteristics can be effectively defined only when the person's ecological niche or context is considered. An ecological niche includes the immediate context in which an individual is nested and the contexts in which that context is situated. The ecological niche where development occurs for the child consists of the home and school, rooted in larger social contexts of community and society in general. In addition to these larger contexts, physical and biological characteristics specific to the child, such as genetics, gender, and age, interact with familial and societal characteristics to affect development.

In EST, the concept of environment in terms of development is reflected in five nested and interconnected systems, progressively interacting with the child. In developing his theory, Bronfenbrenner (1975) qualified the environment as ecological, intrinsically connected to the individuals within it. In other words, EST focuses not on the environment or context but on the ecological system where the developing child is included (Bronfenbrenner, 1975).

Ecological Systems - Defined

Ecological systems theory examines individual development within nested settings or environments. These five interconnected ecological systems influence and interact with a child's development, directly and indirectly, and include the microsystem, mesosystem, exosystem, macrosystem (Bronfenbrenner, 1979; Bronfenbrenner, 1988b), and chronosystem, which was added later, the (Bronfenbrenner, 1986a, 1986b; Bronfenbrenner & Morris, 1998).

Microsystem. This ecological environment is defined as the most proximal setting of the child, such as home and school. It involves structures and processes and face-to-face interactions between the child, immediate family or caregivers, and their school (Bronfenbrenner, 1979; Bronfenbrenner, 1988b). The constitutive element of the setting is one in which activities and interpersonal roles and relations are engaged over time (Bronfenbrenner, 1975, 1986a). The

family plays a key role. In the ecological approach to combat childhood obesity, the family must be involved (Epstein, 1996; Epstein et al., 2007), and it does so in the microsystem context (Bronfenbrenner, 1979).

Mesosystem. This ecological system refers to the interrelations between two or more microsystems in which the child actively participates and the processes involved (e.g., the relations between home and school, school and work, etc.). In other words, the mesosystem is a system of microsystems. This system is developed or enlarged when the child enters a new setting (Bronfenbrenner, 1979; Bronfenbrenner, 1988b) and reduced when the opposite happens. The developmental characteristics are similar to those of the microsystem, with activities, interpersonal roles, and relations occurring across settings (Bronfenbrenner, 1979; Bronfenbrenner, 1988b).

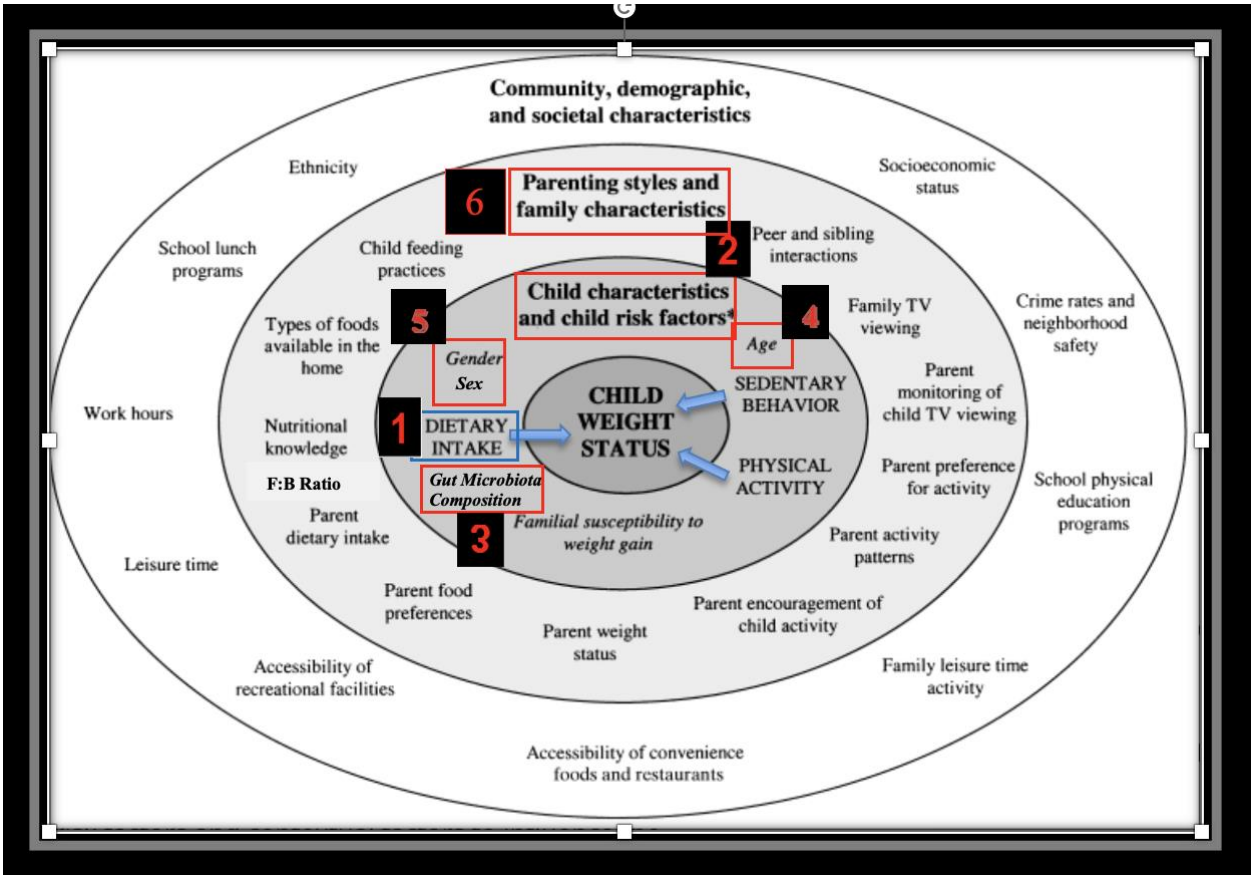
Exosystem. This is the third circle of the ecological model. Exosystem embodies the relations and processes that are taking place in two or more settings, one of which the developing child is not situated and does not actively participate in but in which events occur that influence the processes of the immediate setting that does include the child (Bronfenbrenner, 1979; Bronfenbrenner, 1988b). Although the child may not be in the same setting or context, the child can sometimes influence it (Bronfenbrenner, 1979; Bronfenbrenner, 1988b), whether formally or informally (Bronfenbrenner, 1978). The effect on the child's development is indirect; for instance, events at the parent's workplace have an ensuing impact on the home (Bronfenbrenner, 1978). In addition, the exosystem has an essential societal role in social policy making (Bronfenbrenner, 1974), such as programs that facilitate child-care centers and policy decisions on the type of care and the type of education young children will receive.

Macrosystem. This system encompasses the institutional systems of a culture or sub-culture, such as the social, economic, educational, legal, and political systems (Bronfenbrenner, 1978). The efficiency of how the lower systems function is indicative of the influence of the macrosystem on the other ecological systems (e.g., family, school) (Bronfenbrenner, 1988b). A remarkable feature of the macrosystem is its overarching ideology or belief system pattern common to a particular culture or sub-culture (Bronfenbrenner, 1979). Consequently, children's daily experiences may be similar in a given ethnic, socioeconomic, societal, or religious group (Bronfenbrenner, 1979; 1988b).

Chronosystem. This system was added to highlight the influence of changes and continuity on child development over time in the environments in which the child lives (Bronfenbrenner, 1986a, 1986b). Bronfenbrenner (1975, 1978, 1979) stressed that person characteristics change progressively over time and space, which indicates continuity both in the person and in the environment, as well as changes due to the dynamic relations of person, environment, and other people within that environment all engaged in reciprocal activities that become progressively more complex over time. In the chronosystem, time is considered as important as the environment for human development (Bronfenbrenner, 1986a, 1986b, 1988). Changes caused by events or experiences throughout the individual's life are considered. These experiences may originate from the external environment (e.g., going to school, parents divorcing) or within the developing child's body (e.g., overweight/obesity, puberty, illness). Changes can either be normative, that is, change is expected, such as school entry, or non-normative, when change is unexpected, such as sudden death or severe illness of a family member (Bronfenbrenner, 1986b, 1988).

In summary, EST posits that development occurs as an outcome of the interactions within and among these contexts in which characteristics of the child interact with processes in the family and school, which are impacted by the characteristics of the community and society. Ecological systems theory has been utilized to model predictors of childhood obesity (Figure 3.1) (Davison & Birch, 2001). The application of EST to this research will provide a framework to evaluate and integrate research in assessing influences of dietary intake and behavior and their impact on gut microbiota composition while incorporating the child's characteristics such as age, gender, and gut microbiota composition, and considering the familial and societal factors that influence the emergence of child risk behaviors. The ecological systems most relevant to this research will be microsystem and mesosystem, as family and social and environmental pressures on nutrition primarily influence obesity. An improved understanding of the micro- and mesosystems influencing dietary behaviors might elucidate the persistence of childhood obesity.

Figure 0.1. The ecological systems theory model of childhood obesity predictors (Davison & Burch, 2001) was modified to show gut microbiota as a child's characteristic. Childhood obesity risk factors (shown in the upper case) pertain to child behaviors that have been linked with the development of childhood obesity. Shown in italics are characteristics of the child that interact with child risk factors and contextual factors that influence the development of obesity. This ecological model conveys the influence of child, family, and community on child risk factors in the micro - and mesosystems. This study focuses on 1) the child's dietary intake as a risk factor for obesity, 2) the child's characteristics such as 3) gut microbiota composition, 4) age, 5) sex and gender, and 6) Parenting styles, genetic influence, and family characteristics.



Application of Ecological Systems Theory to Research

The application of EST to this research is presented in a model by Davison and Burch (2001) (Figure 3.1). This ecological theory model of childhood obesity illustrates the development of childhood obesity based on numerous research studies evaluating predictors of childhood obesity (David & Burch, 2001). Following this model, dietary intake is a child risk factor (shown in upper case in Figure 3.1) that can place a child at risk of obesity. The impact of dietary intake on the development of childhood obesity is moderated by child characteristics that include age and sex and, for this research, gut microbiota composition. In addition, dietary intake is shaped by family processes in the microsystem, such as parenting styles, parents' dietary eating patterns, food preferences, nutritional knowledge, and child feeding practices. Besides family characteristics, the model shows school characteristics that influence child weight status based on the dietary quality of school lunches. This research will not explore the dietary quality of school meals, but data on whether the subject consumed school meals will be considered. As this ecological model further illustrates, community, demographic, and more prominent societal factors influence parenting practices and child dietary habits. Moreover, this EST model depicts bi-directional relationships among the various levels of influence, which means that the impact of factors from another level moderates factors at one level. Therefore, the EST model of the development of childhood obesity stresses the necessity of addressing the child's characteristics and the familial and societal contexts in which the child is embedded to understand the process by which childhood obesity develops.

Children's Dietary Behavior

According to study results, children's dietary patterns and behaviors may be influenced by factors that contribute to childhood obesity and include high energy intake compared to

needs, low responsiveness to satiety signals, high receptiveness to external food cues, and high preference for energy-dense foods (Blundell et al., 1993; Corbin. Et al., 2023; Klesges et al., 1995; Lawton et al., 1993; Rolls et al., 1994; Rolls & Hammer, 1995;). In addition, the study by Montano et al. (2015) revealed that dietary habits are molded at a young age and retained for years (Montano et al., 2015). Furthermore, these deep-rooted nutritional habits in childhood continue with indications of fuzziness, poor dietary diversification, high responsiveness to food cues, and increased obesity risk (Montano et al., 2015). Early experiences with food and food acceptance are influenced by children's food preferences and previous exposure/lack of exposure to new plant foods and food supply (Klesges et al., 1983; Klesges et al., 1991; Wardle et al., 2003). The phenomenon of food neophobia, where both children and adults exhibit an inherent resistance to new plant flavors, presents a significant obstacle to dietary diversification. An initially disliked food typically requires 8 to 14 exposures to become palatable. This learning process often involves initial rejection and consequent waste, which wealthier families may accommodate but can pose a substantial challenge for those with limited resources. Consequently, lower-income families tend to have a more limited variety of vegetables deemed acceptable, exacerbating nutritional disparities (Appleton et al., 2018; Wardle et al., 2003)

Food preferences. Children learn food preferences based on associations with taste, flavors, and textures of high energy-dense foods and quickly learn to choose foods high in fat (Birch et al., 1991; Birch & Fisher, 1995; Nestle et al., 1998). Studies have found that children, in general, eat a high level of dietary fat (Salz et al., 1983; Wardle & Beales, 1986), which may be attributed to the increased palatability and energy of foods rich in dietary fats, as well the low satiating effect of fats (Klesges et al., 1995; Rolls et al., 1994; Rolls & Hammer, 1995). A study by Fisher and Birch (1995) observed that children's food choices consisted of a wide range of

dietary fat intake, ranging from 25% to 42% of energy, despite an array of healthy foods. In addition, Fisher and Burn (1995) reported that children's preference for high-fat foods positively correlated with the overall level of dietary fat, which was consistent with the findings of other studies (Ricketts, 1997; Tucker et al., 1997).

Research data indicate that children have an innate preference for sweet and salty tastes and reject sour and bitter flavors (Birch et al., 1987; Steiner, 1971). Thus, acceptance of new food in children does not occur instantly and may require between five and 15 repeated exposures to develop preferences and the increased intake of fresh foods (Birch & Marlin, 1982; Birch et al., 1987; Maxwell et al., 2018; Sullivan & Birch, 1990, 1994; Wardle et al., 2003). Evidence from these studies emphasizes the significance of early experiences with food and food acceptance in that children tend to like and eat what is familiar. What is expected is what exists in the environment. Parents provide the food environment to their children through early exposure to fruits and vegetables (Baranowski et al., 1993) and to foods high in energy, sugar, and fat (Fisher & Birch, 1995; Nguyen et al., 1996; Olivier et al., 1992). The food environment that parents provide affects children's food preferences and acceptance, which is directly associated with children's adiposity (Fisher & Birch, 1995; Nguyen et al., 1996; Olivier et al., 1992). Children repeatedly exposed to high-energy dense foods learn to prefer those foods (Birch & Fisher, 1995; Fisher & Birch, 1995). According to Wang et al. (2021), the estimated proportion of total energy intake from ultra-processed foods has significantly increased among U.S. youths over the past two decades. Specifically, the consumption of ultra-processed foods rose from 61.4% to 67.0%, while the intake of unprocessed or minimally processed foods decreased from 28.8% to 23.5%. The study also observed an increase in energy from ready-to-heat and eat-mixed-dish, sweet snacks, and sweets but a decrease in energy from sugar-

sweetened beverages and processed fats and oils. Notably, there was a more significant increase in ultra-processed food consumption among non-Hispanic Black and Mexican American youths compared to non-Hispanic White youths. This trend underscores the growing dominance of ultra-processed foods in the diets of young Americans. It highlights potential areas for public health intervention to address nutritional quality and reduce health disparities.

Some studies supported that school meal programs can effectively expose children to healthier food options, a critical step in addressing childhood obesity. Grainger et al. (2007) analyzed the impact of reforms on promoting healthier eating in a high school lunch program. The study showed that the new lunch program was associated with improving the nutritional quality of students' food choices, indicating that school meal programs can encourage students to choose healthier foods. Implementing the Healthy, Hunger-Free Kids Act of 2010 was associated with a significant decrease in BMI among school-aged youths. The Act, which strengthened nutritional standards of school-based meals, showed a reduction in annual BMI trends after its full implementation, particularly among youths aged 12 to 18 and living in households with lower annual income. The findings suggest that school meal programs are a crucial opportunity for interventions to combat childhood obesity (Chandran et al., 2023).

Furthermore, other studies found that children’s higher percentage of fat intake is positively correlated with a higher percentage of body fat, BMI, fat mass, and increased triceps skinfold measurements (Blundell et al., 1993; Fisher & Birch, 1995; Gazzaniga & Burns, 1993; Ricketts et al., 1997; Tucker et al., 1997). Moreover, the higher percentage of fat intake has been prospectively linked with immense gains in children’s skinfold thickness over one year (Robertson et al., 1997) and more significant increases in BMI over two years (Klesges et al., 1995). Consuming high levels of fat through diet may promote fat storage, as ingested fat is

preferentially stored compared with other macronutrients such as carbohydrates or proteins (Flatt, 1988; Schutz et al., 1989).

Dietary fat intake has been related to body fatness in childhood (McGloin et al., 2002), and diets high in fat have been associated with childhood obesity after controlling for possible confounding factors (Blundell et al., 1993; Fisher & Birch, 1995; Lawton et al., 1993; Ricketts, 1997; Rolls & Hammer, 1995; Tucker et al., 1997). The evidence suggests that only high energy-dense/high-fat diets, and not high-fat diets per se, may lead to passive overconsumption of energy (Ramirez & Friedman, 1990; Stubbs et al., 1995), potentially resulting in increased fat storage and subsequent obesity in children (Gazzaniga & Burns, 1993; Klesges et al., 1995). Nuts are high-fat foods, but eating more nuts is associated with lower body weight (Sugizaki & Naves, 2018). Several studies have also shown that long-term adherence to a low-fat diet results in the gradual loss of body fat in obese children (De Filippo et al., 2010; Woo et al., 2004; Yackobovitch-Gavan et al., 2008), and preference for fruits and vegetables may function as a protective factor for the development of obesity (Epstein et al., 2001; Rosettie et al., 2018).

In summary, cross-sectional and longitudinal research illustrates an association between children's dietary behavior and weight status, although findings are more consistent for children's fat intake and preference for fat. Children's saturated fat intake promotes increased food intake and fat gain due to the saturated fat intrinsic properties of high palatability, high energy density, and low satiety, stimulating an inflammatory response, high palatability, high energy density, and low satiety. Saturated fats and omega-3 fatty acids are equally tasty, yet they have contrasting impacts on feelings of fullness due to their differing effects on inflammation (Buckley &. Howe, 2010; Milanski et al., 2009). Inconsistencies in identifying associations may be elucidated not only by the challenges of assessing children's dietary intake but also by the

under-reporting of food intake by the parents (Maffeis et al., 1998) and obese children (Maffeis et al., 1994). In addition, inconsistencies in research may be elucidated by the fact that research has seldom considered child characteristics that modulate the relationship between dietary behavior/patterns and weight status, the contexts in which children's eating patterns develop, and the processes by which such eating patterns produce (Davison & Birch, 2001).

Child Characteristics

Dietary risk factors' impact on the development of childhood obesity is moderated by child characteristics such as age, sex, and gut microbiota composition.

Gut microbiota composition. The most crucial child characteristic for this study is gut microbiota composition. According to studies, childhood obesity is associated with an altered gut microbiota composition characterized by elevated levels of *Firmicutes* and reduced levels of *Bacteroidetes* (Bervoets et al., 2013; De Filippo et al., 2010; Ferrer et al., 2013; Riva et al., 2016). Differences in gut microbiota composition have been observed between obese and lean children in pediatric studies. Gut microbiota composition of obese children has been characterized by elevated levels of *Firmicutes* and low levels of *Bacteroidetes* compared with normal-weight children (Abdallah Ismail et al., 2011; Bervoets et al., 2013; Ferrer et al., 2013; Riva et al., 2016). What children eat is the most critical environmental factor that affects gut microbiota composition. Pediatric studies have revealed impactful results on the interaction of different types of diet with the gut microbiota of rural and urban children. A Western-type diet consisting of high-saturated fat, high-energy dens, and low-fiber foods was positively correlated with elevated levels of *Firmicutes* and decreased levels of *Bacteroidetes* in gut microbiota composition of urban children (De Filippo et al., 2010; Nakayama et al., 2017). The gut microbiota composition of rural children subsisting on a diet rich in fiber, starch, and plant

polysaccharides was characterized by increased levels of *Bacteroidetes* and low levels of *Firmicutes* in their gut microbiota (De Filippo et al., 2010; Nakayama et al., 2017). Daily food choices can influence gut microbiota composition and play a vital role in the long-term regulation of energy balance and the development of obesity.

Age and gender. Age and gender differences in fat mass have been shown in several studies. Results of studies indicate a higher fat mass in girls than boys between ages 4 – 7 years, but this could not be attributed to a higher fat intake due to maternal bias in reporting of daughter's intake (Gazzaniga & Burns, 1993; Ku et al., 1981; Nguyen et al., 1996). In addition, substantial evidence in studies analyzing body composition using anthropometric measures, dual-energy X-ray absorptiometry (DEXA), and bio-electrical impedance analysis (BIA) of children and adolescents aged 3 – 20 years reveals that the percentage of body fat was higher in girls than boys at all ages (Boot et al., 1997; Mast et al., 1998 Nagy et al., 1997; Taylor et al., 1997). Nevertheless, studies have shown that fat intake was positively and significantly correlated with body fat in boys after adjusting for physical activity energy expenditure (Maffeis et al., 1994; Nguyen et al., 1996).

In energy regulation, the extent to which parents exert control over what and how much their children eat was found to have a positive correlation with low self-control in energy regulation with children (Johnson & Birch, 1994). Boys were better than girls at adjusting their energy intakes, and this difference may be attributable to how boys and girls were parented concerning food and eating in that mothers of boys may not impose dietary restraints on their sons (Johnson & Birch, 1994). For girls, poor energy regulation was associated with their adiposity (McLaughlin et al., 2015; Handford et al., 2018). In addition, parental control was linked to girls' adiposity, with mothers exerting influence on their daughters' body image and

eating habits by modeling their own negative body image-related beliefs and dieting behaviors (Handford et al., 2018; McLaughlin et al., 2015). Cultural pressures produce increases in dieting and compulsion toward thinness for girls and increasing muscles for boys (McCabe & Ricciardelli, 2005) such that the onset of dieting has been reported in girls as young as six years of age (McLaughlin et al., 2015).

The energy needs of children vary between girls and boys depending on their growth rate and age, and the timing of growth spurts differs for girls and boys, particularly during adolescence. Therefore, gender and growth rate are likely to interact with energy intake, possibly percent fat intake, affecting children's weight status (Davison & Birch, 2001). Age and gender characteristics have been used as variables in research, but their effects have been controlled for in analysis rather than being directly examined.

Familial susceptibility to weight gain. Although familial patterns of obesity will not be assessed in this study, it is essential to mention this child characteristic here to shed light on the role of parents in childhood obesity as they provide the genetic predisposition and the environment where this predisposition is expressed.

According to study findings, a genetic predisposition to obesity strongly correlates with children's weight status (Garn & Clark, 1976; Maffeis et al., 1998; McGloin et al., 2002; Price et al., 1998; Whitaker et al., 1997), and highly correlates with obesogenic home environments (Shrempft et al., 2018). A growing body of evidence suggests parental obesity is not only a highly significant predictor of obesity development in children but may also be an influencing factor (Freeman et al., 2012; Keane et al., 2012). Keane et al. (2012) established that the obesity status of parents is a predominant risk factor for childhood obesity, with children of obese parents having a much higher likelihood of being overweight or obese themselves. The research

underscored the importance of considering parental weight when assessing the risk of childhood obesity. The weight status of fathers is an influential factor in the development of obesity in their children, suggesting that interventions to treat or prevent childhood obesity should also focus on the weight status of the father (Freeman et al., 2012). The implications are significant, as they suggest that family-based interventions may be necessary to tackle the issue of childhood obesity effectively.

A child with non-obese parents has a 7% estimated chance of becoming obese, 40% if one parent is obese, and 80% if both parents are obese (Garn & Clark, 1976). A study by Galley et al. (2014 examined whether maternal obesity was linked to differences in the composition of the gut microbiome in children in early life. It discovered significant effects of maternal obesity on the gut microbiome composition of offspring, particularly among those of higher socioeconomic status. Children of obese mothers exhibited greater homogeneity in their gut microbiomes and differences in abundances of specific bacterial groups linked to weight and diet, providing novel evidence that maternal obesity is associated with early-life gut microbiome differences (Galley et al., 2014). In addition, studies have shown that maternal obesity has the power to affect the dietary fat intake of their children in that children with obese mothers and normal-weight fathers consumed higher dietary fat than children with no overweight or obese parents (Eck et al., 1992; Nguyen et al., 1996; Oliviera et al., 1993). Furthermore, children who are at higher risk of obesity because of one or two obese parents tend to consume a higher proportion of energy from dietary fat, resulting in significant weight gain over one year than children with no obese parents and are therefore at low risk of obesity (Eck et al., 1992). Thus, dietary fat intake may have a more potent effect on the body composition of children with a genetic predisposition to obesity (Nguyen et al., 1996).

Familial adiposity patterns suggest that variation in body fatness is partly explained by a genetic or heritable component (Hughes et al., 2016). For instance, the heritability of obesity from the parents may influence the body's metabolism by altering the body's fat content and the balance of energy intake and expenditure in children. In addition, the genetic cause of obesity is partly attributed to appetitive phenotypes, with individuals who inherit lower sensitivity to satiety or greater avid appetite having a greater tendency to overeat in response to the food environment (Llewellyn & Wardle, 2015). The study by Llewellyn and Wardle (2015) used various research approaches and epidemiological studies to show that appetite plays a vital role in weight development and demonstrated that appetite is highly heritable. The study by Galley et al., 2014 underscores the potential role of the maternal gut microbiome in the transmission of obesity risk to children, highlighting the need for interventions targeting maternal health and microbiome composition to mitigate obesity in future generations.

Various studies have demonstrated the genetic component of childhood obesity; however, genes alone may not exclusively demonstrate the rapid increase in the prevalence of childhood obesity. The genes responsible for weight gain indirectly cause obesity by increasing the susceptibility to fat gain in children exposed to a specific high-risk environment (Hughes et al., 2016). The genetic predisposition to obesity demonstrates that parental obesity is a significant risk factor in the development of childhood obesity.

Parenting Styles and Family Characteristics - Family Processes

Evidence indicates that parents provide the genetic predisposition to obesity (Hughes et al., 2016) as well as the environment (Bassul et al., 2021; Finnae et al., 2016; Frankel et al., 2012; Hughes & Frazier-Wood, 2016; Tan & Holub, 2010) where this predisposition may be expressed. The home environment is a crucial context for the development of children's dietary

behaviors that eventually may affect their weight outcome (Bassul et al., 2020; Finnane et al., 2016; Frankel et al., 2012; Hughes & Frazier-Wood, 2016; Tan & Holub, 2010). Most importantly, parents influence the development of their children's food preferences and dietary intake through the foods they make available to their children. Similarities have been consistently observed in child and parent patterns of dietary intake, and positive associations have been found between the food preferences of children and parents (Finnane et al., 2016; Frankel et al., 2012; Hughes & Frazier-Wood, 2016; Tan & Holub, 2010). For example, when parents constantly expose their children to high-energy-dense foods, they learn to prefer them. Parents can influence the development of a healthy child's dietary intake by creating an environment in which children are consistently exposed to a variety of nutritious foods and limiting exposure to high-energy and high-fat foods (Bassul et al., 2020; Savage et al., 2016). However, this child-feeding strategy of restricting access to high-energy and high-fat foods may result in the child's over-consumption of those foods in reaction to parental restrictions.

A variety of child-feeding strategies are utilized by well-meaning parents that are intended to ensure adequate and well-balanced food intake, but these strategies have been demonstrated to be counter-productive, especially when they involve the parents acting controlling and coercive (Costanzo & Woody, 1994; Rollins et al., 2014). Previous research reports that parental use of bribes, threats, and food rewards to control their children's food intake may have adverse and unintended effects on children's food preferences and may undermine the child's self-regulation of energy intake (Costanzo & Woody, 1994; Fisher & Birch, 1999a; Fisher & Birch, 1999b; Johnson & Birch, 1994). However, it is up to the parents to ensure their infants are fed minimally processed, fiber-rich foods that will provide the infants' microbes with all the substrates they need. Well-fed gut microbes will increase satiety-signaling

and decrease blood glucose levels without the infant having to "self-regulate" energy intake. Infant obesity is not a problem with infant self-regulation of energy intake but rather evidence that the infants' parents are feeding their infants an insufficient amount of minimally processed, fiber-rich foods.

Children's self-regulation of energy is defined as the children's inborn and socialized ability to eat and not eat in response to cues of hunger and satiety (Frankel et al., 2012; Hughes & Frazier-Wood, 2016; Tan & Holub, 2010). Child eating self-regulation is established across the lifespan from early childhood, with individual differences developing and continuing to develop associated with weight status (Frankel et al., 2012; Hughes & Frazier-Wood, 2016; Tan & Holub, 2010). Research shows that overweight children tend to have inadequate self-regulation of energy intake compared with normal-weight children (Frankel et al., 2012; Hughes & Frazier-Wood, 2016; Tan & Holub, 2010). This difference may be due to overweight children having less sensitivity to internal signals of hunger (Frankel et al., 2012) or poor inhibitory control (Tan & Holub, 2010) and being less able to self-regulate their energy intake in the presence of external food cues. Parent feeding styles and practices, specifically with regards to restricting or controlling children's food intake and the use of rewards and punishments, might influence children's development of self-regulation of energy intake and have been linked with children's overweight status (Frankel et al., 2012; Hughes & Frazier-Wood, 2016; Tan & Holub, 2010). The type and degree of parental control utilized over their children's eating may foster rather than prevent the development of childhood obesity and eating problems (Finnane et al., 2017; Costanzo & Woody, 1994; Fisher & Birch, 1999a; Fisher & Birch, 1999b; Johnson & Birch, 1994; Savage et al., 2016). Authoritarian and controlling styles of child-feeding may negatively influence children's liking of these foods by teaching them to dislike the very foods

that parents want their children to consume and to prefer foods that should be consumed in relatively limited quantities (Costanzo & Woody, 1994; Fisher & Birch, 1999) (a); Fisher & Birch, 1999(b); Johnson & Birch, 1994. Parents must provide their children with a greater variety of minimally processed, fiber-rich foods and leave it to them to choose which minimally processed, fiber-rich foods to eat. The study's results by Tan and Holub (2010) found that self-regulation in eating was positively correlated with inhibitory control and predicted the parent's restrictive feeding style. Parents who believed their children could self-regulate utilized less restrictive feeding styles and practices (Frankel et al., 2012; Hughes & Frazier-Wood, 2016; Tan & Holub, 2010).

Parents tend to encourage their children to eat more fruits and vegetables and discourage or limit their children's intake of foods high in energy, sugar, and fat. Research shows that when the family eats together, parents can likely model healthy eating behaviors (Bassul et al., 2020; Finnane et al., 2017; Scaglioni et al., 2018). Parental role modeling of the consumption of fruits and vegetables may help increase their children's daily consumption of healthful foods (Bassul et al., 2020; Draxten et al., 2014; Finnane et al., 2017; Scaglioni et al., 2018). Children who regularly eat meals with their parents eat more fruits and vegetables than children who eat without parents in front of the TV. This is especially true if parents model the importance of eating fruits and vegetables. Nevertheless, the home food environment also matters. Providing 24/7 access to fresh fruits and vegetables and limiting access to sodas and chips can help children maintain a healthy weight (Bassul et al., 2021). In addition, differences in child feeding practices of successful and unsuccessful dieting parents, especially dieting mothers, may have an impact on the energy regulation of their children, specifically for girls (Costanzo & Woody, 1994; Cutting et al., 1999; Johnson & Birch, 1994; Rollins et al., 2014; Zocca et al., 2011). When

mothers reported higher levels of coercion and control in their child-feeding practices, their children demonstrated less evidence of controlling their food intake, which was especially clear for girls (Costanzo & Woody, 1994; Johnson & Birch, 1994; Zocca et al., 2011). In addition to child-feeding approaches that promote the development of obesity, evidence suggests that intergenerational transfer of *Firmicutes* gut microbiota and unhealthy feeding behaviors also contribute to familial patterns of obesity (Tun et al., 2018).

Healthfully feeding young children requires some nutritional knowledge by the parent or caregiver to ascertain that feeding practices, foods, and amounts of calories offered are appropriate. Among low-income immigrant women, telling them to eat more like how their grandmother ate is more effective than teaching them about nutrition. Traditional diets were richer in minimally processed, fiber-rich foods than today's highly processed foods. Parental nutritional knowledge has been shown to have a positive association with children's healthy dietary behaviors (Gibson et al., 1998) and negatively associated with children's total energy and fat intake (Romanos-Nanclares et al., 2018). Parents with excellent nutrition knowledge and healthy attitudes can interpret nutrition information and labels evident in their purchases of more nutritious foods and make them available and readily accessible in the home environment (Romanos-Nanclares et al., 2018). Food availability and accessibility at home have been found to positively correlate with eating fruits and vegetables (Baranowski et al., 1993; Bassul et al., 2020). These are significant causal elements in children's food preference for and intake of such foods (Birch & Marlin, 1982). It is likely that poor parental nutrition accounts for the greater availability of high energy-dense foods at home, which encourages the consumption of such foods, thereby increasing the risk of obesity.

There is plenty of evidence to implicate parenting practices and family environment in the development of childhood obesity. The environment of watching television in the home also influences children's obesity risk (Bassul et al., 2021). The findings reveal that the home environment is the primary setting for children's early experiences with food and eating shapes their food intake and may influence the promotion of obesity (Johnson & Birch, 1994; Haire-Joshu & Nanney, 2002). Parents have a challenging role as they are lifelong examples and should model good dietary habits to positively influence their children's dietary intake and behaviors.

Societal Context - School Environment

Parent-feeding practices and other ecological niches, such as the school environment, shape children's dietary habits. Since children typically consume a quarter of their daily intake at school, the school environment becomes a significant social context in developing children's lifelong dietary patterns. The school environment has been shown to influence children's food preferences and consumption as peers' dietary behaviors in that environment serve as a model for the developing child (Birch, 1980). Food choices and dietary behaviors of peer models have been shown to shape children's food preferences and dietary patterns (Birch, 1980). One of the few studies that examined this possibility found that children aged 2-5 years in a daycare center changed their food preferences and increased consumption of disliked foods after repeated exposures to other children with different food preferences (Birch, 1980). The influence of peers on children's food preferences was evident 1- 8 weeks after the research procedure, suggesting long-term effects on preferences (Birch, 1980).

Schools are appealing settings for promoting healthy behaviors in children, with several population-based studies that have successfully improved diet or physical activity to reduce the

prevalence of obesity. The implementation of the Healthy Hunger-Free Kids Act (HHKA) of 2010 paved the way for improving the nutritional quality of school foods and beverages across the country (U. S. Department of Agriculture [USDA], 2013), which is consistent with the current Dietary Guidelines for Americans (U. S. Department of Health and Human Services [DHHS] & USDA, 2015) through federally sponsored programs such as the National School Lunch Program (NSLP) and School Breakfast Program (SBP). The core policies for NSLP and SBP, aligned with provisions of HHKA of 2010, are designed to improve eating patterns in the school environment by serving nutritionally balanced school meals. School meals contribute approximately half of the daily caloric intake of participating children, with school lunches accounting for an estimated 31% of daily calories and school breakfasts accounting for up to 22% (Au et al., 2016). Because of the HHKA of 2010, studies have found that children who ate school lunches had overall higher diet quality and limited solid fats and added sugar than children who ate lunch from home (Romo-Palafox et al., 2015; Au et al., 2016). In addition, children who ate school breakfast consumed more fruits and dairy than those who ate breakfast at home (Romo-Palafox et al., 2015; Au et al., 2016).

Furthermore, several studies have documented that the nutritional quality of packed meals from home is lower than that of school meals (Bhattacharya et al., 2006; Farris et al., 2014; Hur et al., 2011; Johnston et al., 2012). These findings also suggest that parents may need more nutritional knowledge (or financial wherewithal) to pack healthier lunches to meet the Healthy Eating Index 2010 that include vegetables, plant proteins, and whole grains (Au et al., 2016; Romo-Palafox et al., 2015 Overall, findings show that school meals have improved dietary outcomes of students participating in the NSLP and SBP across a wide array of different measures.

Chapter Summary

In summary, the ecological systems theory presented in a model of childhood obesity predictors is practical, helpful, and applicable to this cross-sectional study. The usefulness is pertinent to researchers and practitioners who are involved in the treatment of childhood obesity and the development of prevention and treatment programs. It applies to the nurse's role in preventing and treating childhood obesity by taking an ecological approach and considering the contextual complexity and dynamic systems within which childhood obesity risk factors emerge. Nurses and other health care providers working with children should always take an ecological approach in the prevention and treatment of obesity as the child is embedded in the family and social systems, and it would be difficult for obese children to alter their dietary habits if not supported by their families and school. Obese and overweight children would benefit from an ecological approach to treatment that considers child and parent characteristics, parent practices, and family and school environment, all of which influence children's dietary habits.

Since the incidence of childhood obesity is mainly attributed to diet and lack of physical activity, efforts to combat obesity and related metabolic diseases have focused on whether the gut microbiota has a mediating role that can predispose the host to diet-induced obesity. Evidence from animal studies has identified obese microbiota with the capacity for increased harvest energy from diet and the capacity to induce adiposity in the host by transplantation (Turnbaugh et al., 2006; Turnbaugh et al., 2008). Two dominant gut microbiota divisions in the gut have been revealed to associate obesity with changes in the relative abundance of these two key gut microbiota divisions – *Bacteroidetes* and *Firmicutes* (Ley et al., 2005; Turnbaugh et al., 2006; Turnbaugh et al., 2008) thus, identifying the gut microbiota as a contributing factor in the pathophysiology of obesity. An improved understanding informed by ecological theory of how

gut microbiota composition may be altered through diet may bring a new perspective to clinical obesity interventions by using the gut microbiota as new targets for obesity control.

METHODS

The purpose of this pilot study is to examine whether there are differences in the gut microbiota composition between obese and lean school children and if dietary intakes affect the diversity of gut microbiota composition in terms of the Firmicutes-Bacteroidetes (F: B) ratio, alpha-1 microbial diversity and increased abundance of short-chain fatty acids (SCFA)-producing gut microbes. First, the study will compare the gut microbiota composition (dependent variable [DV]) of obese and lean school children [obesity status is the IV], using 16S rRNA gene sequencing on DNA extracted from fecal samples (dependent variable [DV]) obtained from obese and lean school children. This pilot study will test the hypotheses that obese children will have lower alpha diversity in gut microbiota composition, a high F: B ratio, and an increased relative abundance of SCFA-generating microbes in stools. Second, the study will examine associations between dietary intakes (IV) and an altered gut microbiota composition (DV), characterized by a high F: B ratio, when controlling for children with or without obesity. The study will test the hypothesis that obese children with high energy and fat dietary intakes have an altered gut microbiota characterized by a high F: B ratio. Third, the study will assess the association of body mass index (BMI) (IV) with differences in gut microbiota composition (DV), as obtained by 16S rRNA gene sequencing, of obese and lean children. The study will test the hypothesis that high BMI z-scores are associated with an abundance of bacteria in the phylum *Firmicutes.*

Research results may provide new knowledge to pediatric nurses in identifying an altered gut microbiota composition as a risk factor for childhood obesity. Thus, the results could provide new insights into nursing research and practice as a foundation for exploring the gut microbiota system of obese and non-obese children in clinical intervention studies and contribute to

developing strategies for preventing and treating obesity and obesity-related complications. Moreover, the outcome of this research could provide a window of opportunity for pediatric nurses to adopt an ecological approach to treating obesity by including parents in obesity prevention efforts. Obesity prevention efforts should be firmly established in schools. School nurses have the knowledge, skills, and expertise to promote the prevention and reduction of overweight and obese children and adolescents in schools. Relevant obesity information from this study can empower school nurses to promote and implement obesity school-based strategies such as identifying, assessing, referral, and follow-up with children and adolescents who are at risk of health problems associated with overweight or obesity, promoting healthy messages to encourage consumption of nutritious foods and daily physical activity, and educating students, parents and school communities (especially school Food Services) about healthy lifestyle behaviors, daily physical requirements, and preventable health risks associated with obesity.

Design

This pilot study used a correlational, cross-sectional design to compare and characterize the gut microbiota composition in obese vs. lean children. Stool samples were collected by parents and stored in the refrigerator. The collection took place in the same week as the dietary record. Subsequently, it was transported to the laboratory with dry ice and stored until further analysis was performed. Height and weight measurements were taken at enrollment, and BMI and BMI Z-scores were calculated using the Children's Hospital of Philadelphia Research Institute's pediatric Z-score calculator. This tool is based on the Centers for Disease Control and Prevention (CDC) cut-off points BMI-for-age growth charts and calculates the BMI, BMI percentile, and z-score of young children and adolescents between the ages of 2 and 20 years (Children's Hospital of Philadelphia [CHP], 2023). Children's dietary information was collected

using the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool, version ASA24-2020 (National Cancer Institute, 2020), with the assistance of parents. The ASA24-2020 is a web-based tool that collects and analyses dietary data. The PI contacted participants' parents to obtain dietary information by administering three non-consecutive 24-hour dietary recalls, including two weekdays and one weekend. Parents are considered the best authority on their children's diet and dietary behaviors as they are the ones who prepare their packed lunch, feed them at home, and observe their dietary habits. Data from the 24-hour dietary recalls were manually entered into the Automated Self-Administered 24-hour Dietary Assessment Tool (ASA24), version ASA24-2020 (National Cancer Institute, 2020).

Study Population and Setting

The obese and lean sample for this study were recruited from the University Village student family apartments owned by the University of California Los Angeles (UCLA), where there is access to fellow students with children. The recruitment site provides an adequate number and ethnically diverse sample. Some of the highest prevalence rates of obesity among children were among public school fifth graders in 2018 of Latino, Native Hawaiian/Pacific, African American/Black, and American Indian/Alaska Native racial and ethnic backgrounds (CDE PFTRF, 2018). Considering the high prevalence rates among fifth graders, the target population selected were children enrolled in Kindergarten through fourth grades in a public school in West Los Angeles (LA). Identifying students in these grade levels who are obese and are potentially at high risk of obesity-related health problems such as hypertension, type 2 diabetes, respiratory, and joint and muscular problems (Minghelli et al., 2015) may help alleviate the obesity prevalence burden in fifth grade. In addition to obesity-related health problems, obesity is associated with poorer educational outcomes (An et al., 2017; Carey et al., 2015;

Martin et al., 2018; Roberts et al., 2010). Furthermore, obesity and low aerobic fitness were associated with poorer scores on standardized tests in children (Roberts et al., 2010).

Sample Selection

The inclusion criteria were: 1) school children aged 5 – 10 years; 2) English or Hispanic speaking; 3) no history or current diagnosis of type 1 or type 2 diabetes; 4) children with BMI ≥ 85^{th} percentile for same age and gender; 5) children with BMI ≥ 95^{th} percentile for same age and gender; and 6) children with BMI = 5^{th} percentile or < 85^{th} percentile for same age and gender. The exclusion criteria include 1) corticosteroid and antibiotic use in the last month before the study and during the study; 2) hospitalization in the last month before the study and during the study; and 3) significant comorbidities such as acute infection or chronic disease.

Sample

Based on power analysis run by G*Power (version 3.1), using independent samples *t*-test, a sample size of 42 subjects – 21 in the obese group and 21 in the lean group allows for the detection of a large effect size (0.8) at an alpha of 0.05 and power of 0.80 (Faul et al., 2009). A 95% confidence interval will be considered in all tests.

Based on the power analysis and using Pearson's Correlation to detect a correlation between obesity and the F: B ratio, increased gut microbiota producing SCFA in stool, and between BMI and F: B ratio, a sample size of 46 – 23 in the obese group and 23 in the lean group are needed. Pearson's correlation ® will measure the strength of the association between quantitative continuous variables.

The Healthy Eating Index, which is reasonable based on previous studies, was used to compare dietary intakes between groups (Asnicar et al., 2021; Romo-Palafox et al., 2015). Based on the power analysis and using ANOVA to detect differences in dietary intakes between obese

and lean groups, a sample size of 12 is needed. To compare dietary intakes and patterns between obese and lean groups, the Chi-square test ($\chi 2$) was used.

Due to time constraints (limited time to recruit), lack of resources, and the impact of the COVID-19 pandemic on schools, the pilot sample needed to be decreased to 24 subjects (12 obese, 12 lean). However, all attempts were made to recruit as many subjects as possible during the period. Data collection occurred over a 3–6-month period +/—2 months.

Sampling procedure. School children who met the inclusion criteria were eligible for participation in the study during the 3–6 month (+/—months) data collection period.

Sample size. Due to constraints on time, resources, and the limited availability of school children amid the COVID-19 pandemic, it became necessary to reduce the pilot sample size. Efforts were focused on enlisting as many participants as feasible throughout the data gathering.

Variables

Independent variable. The independent variables include dietary intakes from 24-hour diet recall, BMI z-scores of obese and lean school children, and 16s rRNA gene sequencing. The PI did not manipulate these variables. BMI was calculated using CDC guidelines.

Dependent variable. The outcome variables related to obesity are Firmicutes, Bacteroidetes, and Firmicutes to Bacteroidetes ratio (F:B ratio) extracted from DNA in fecal samples of obese and lean school children and analyzed by 16s rRNA gene sequencing. Other outcome variables include alpha diversity of gut microbiota composition and abundance of SCFA-producing gut microbes.

Dependent	Description	Type
Firmicutes	Phyla	Continuous
Bacteroidetes	Phyla	Continuous
F:B ratio	Firmicutes: Bacteroidetes	Continuous

Independent	Description	Type
24-hour dietary recall	Food frequency questionnaire	Categorical
BMI-z score	Standard deviation of BMI	Continuous
16s rRNA gene sequencing	Metagenomics analysis	Categorical

Planned Statistical Analysis

The primary data analysis will be performed on all subjects enrolled in the study. It will include outcomes from 16s rRNA gene sequencing, three non-consecutive 24-hour dietary recalls, and height and weight. Statistical analysis was performed with IBM SPSS Statistics Version 27 (SPSS Inc. Chicago, IL, USA).

Descriptive statistics were used to report subject characteristics (age, gender, height, weight, BMI, and dietary data). This analysis will include mean and standard deviations for continuous variables and frequencies and percentages for categorical variables.

All categorical values were analyzed with the Chi-square test ($\chi 2$) for the trend of ordinal variables with three or more categories. Continuous variable comparisons were performed using independent samples t-tests if there was no significant deviation from normality. Otherwise, the Wilcoxon test was used.

Comparison of F:B ratio, gut microbiota producing SCFA, and dietary intakes between obese and lean groups were analyzed by Chi-square test ($\chi 2$). Multiple linear regression was applied to quantify associations between gut microbiota composition (alpha-1 diversity; F:B ratio) and gut microbiota producing SCFA concentration, diet, and BMI z-scores, with gender and age of child included as covariates. To compare the gut microbial richness between obese

and lean groups, the nonparametric method, Chao1 index, was used (Chao, 1984; Hughes, Hellmann, Ricketts, & Bohannan, 2001). The nonparametric Shannon diversity index was applied to assess the gut microbial diversity between the two groups. Differences in *Firmicutes* and *Bacteroidetes* levels between obese and lean groups were evaluated by a parametric test (ANCOVA) (with age and gender as covariates) or a nonparametric test (Kruskal-Wallis) if outcomes were not multivariate normally distributed.

Study Procedures

Approval for the study was obtained from the University of California, Los Angeles (UCLA) institutional review board. The PI thoroughly reviewed the application process and adhered to the proposal submission procedures. There was minimal or no risk to subjects, and this study did not involve an intervention or manipulation of variables. However, UCLA consent for parents and parental permission for minors to participate in research was provided to all parents. All participating children were provided with their separate consent. Parents of subjects were provided with additional information regarding their children's participation in the study.

Recruitment, Screening, and Enrollment

Screening. A one-page study summary was developed, and copies of study flyers were distributed at the University Village (Appendix F). The PI approached parents of potentially eligible children by telephone or electronic email during University-sponsored events and at playgrounds and provided the study flyer. A phone (Appendix C) and electronic email screening script (Appendix B) were developed.

Recruitment. At a mutually agreed upon time and place (in a private and quiet location), the PI met the participants' parents, explained detailed study information, and allowed parents to ask questions and express concerns. Time was given for parents to discuss with their eligible

child/student as needed. However, parent(s)/legal guardian were allowed to decide to participate within a week after receiving the study information. If the parent(s)/ legal guardian agreed to participate, all study procedures were explained, and the UCLA consent for parent and parental permission for the minor to participate in the research were provided to the parent.

Enrollment. Enrollment occurred after informed consent information was provided to the parent(s)/legal guardian. Study participation was one-time (cross-sectional), measuring height and weight, calculating BMI, collecting fecal samples, extracting DNA from fecal samples, and obtaining demographic information from electronic school health records.

Data Collection Procedures

Anthropometric measurements. After enrollment, the PI coordinated a convenient time for the parent to bring the participant to the University Village community center. The PI took height and weight measurements, following standard procedure, using the Salter Brecknell Weight and Height Physician mechanical scale purchased for specific use in the study. Measurements of height, weight, and BMI calculations were recorded in the Confidential Subject Information Report form (Appendix A). The scale was calibrated regularly using standardized Troemner calibration weights.

Fecal sample collection. The parents of all subjects were instructed to collect stool samples at home. They were provided with a collection kit, including a toilet basin to eliminate cross-contamination, a sterile vial with a built-in spoon, and gloves. All collected samples were immediately stored in the participant's freezer until transported to the laboratory. The stool sample collection occurred in the same week as the 2-day 24-hour food recording.

DNA extraction and sequencing. Following the manufacturer's instructions, total DNA will be obtained from the collected stools using a QIAamp DNA Stool Mini Kit (Qiagen, Inc.,

Hilden, Germany). DNA concentrations will be determined by spectrophotometer (Nano-Drop 2000, Thermo Scientific, Wilmington, USA), and ten μL(microliter) of each DNA sample will be checked for integrity on 1% agarose gel. All samples received at the research facility will be stored at -80 ^{0} C until further analysis.

Microbial communities will be profiled using V1-V3[24] amplicons of the 16s rRNA gene, Illumina's (Illumina, Inc., San Diego, CA) 16S Metagenomic Sequencing Library Preparation protocol (Illumina, 2021), and using Illumina's MiSeq600 cycle V3 kit with Nextera XT dual-index barcodes. Libraries will be normalized and pooled before sequencing in paired-end mode on the MiSeq platform by Illumina (Illumina, Inc., San Diego, CA).

DNA extraction and preparation of 16s rRNA gene amplicon libraries will be performed in collaboration with Dr. Susanne Henning at the UCLA Center for Human Nutrition.

Collection of Data and Recording

The PI collected the participants' socio-demographic and parent information, including the participants' height, weight, and BMI measurements. All subject demographic and assessment data were compiled on a confidential subject information report form (Appendix A).

Dietary data. Participants' parents completed three 24-hour diet recalls administered by the PI within seven days using the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool, version ASA24-2020 (National Cancer Institute, 2020).

Protection of Human Subjects in Research

The protection of human subjects was ensured. Subjects of all racial and ethnic groups were eligible for inclusion in the study. Human subject protection approval was obtained from UCLA IRB. The PI completed and complied with the requirements of the Collaborative

Institutional Training Initiative (CITI Program) for UCLA. The study commenced only after IRB approval.

Informed Consent

This cross-sectional pilot study did not include an intervention or manipulation of variables. Therefore, a waiver of signed informed consent was requested from the IRB because the research involved no more than minimal risk to the subjects. However, the information contained within the informed consent was provided to the subjects' parents.

Potential Risks

The human subjects in this study were children in good general health attending a public school in West LA.

Potential risks. There was a minimal risk to subjects in this study. A potential risk facing participants in this study was a breach of confidentiality. The collection of stools may be inconvenient for some parents. Therefore, convenience measures in stool collection, such as a convenient sterile collection kit, were provided to parents. Overall, no additional risks were associated with participation in the study.

Privacy and confidentiality. The PI obtained a waiver of HIPAA authorization for eligibility screening but obtained HIPAA permission to participate in the study. The private health information (PHI) collected followed the data security plan outlined in the IRB application to protect identifiers from improper use or disclosure.

Minimizing risks to confidentiality. No student identifiers were collected; the PI assigned each subject a number that cannot be traced back to the specific subject. The subject numbering system started with 100. Each subsequent subject was given more than the previous patient number. For instance, subject one was assigned the number 200, and subject two was

given the number 201. These numbers did not correlate with any student identifier, such as name, school health record, or birth date. A concurrent review of student school health records and data collection of subject information included students' demographics (e.g., age, gender, home address, height, weight, BMI, and parent information).

Immediately following data collection, the completed confidential subject information report form was placed in an envelope by the PI, and the results were not disclosed. The PI maintained the envelope containing the confidential subject information form in a secure, locked cabinet until reviewed and entered into the statistical analysis program (IBM, SPSS Statistics Version 27) SPSS Inc., Chicago, IL, USA) by the PI. The password-protected ASA24 Dietary Assessment Tool stored participants' dietary data. The ASA24 system captured no personally identifiable information as a code number will identify each participant. Industry-standard security controls protected participants' dietary data. All data entered into the ASA24 system at the PI's password-protected computer were encrypted by the internet browser and transmitted to ASA24 servers using Secure Socket Layer technology. Only the PI accessed the participant's dietary data using a username and a strong password.

Potential Benefits

Subject. There was no direct benefit to school children participating in the research study. However, increased knowledge of childhood obesity and its associations with gut microbiota composition could potentially benefit society.

Generation of knowledge. This study and original research will contribute to nursing knowledge about assessing family and environmental factors in planning strategies for treating and preventing childhood obesity.

A plethora of research exists linking an aberrant gut microbiota composition to obesity in adulthood. However, there is a lack of research on childhood obesity and its association with an altered gut microbiota composition. This research highlighted the complex interaction of factors influencing childhood obesity and the importance of considering the contexts in which the child is embedded and all the contexts in which the child interacts.

Risk and benefit analysis. The benefits outweighed the risks in the proposed study. The potential benefit to society was increased knowledge, possibly leading to the development of a. treatment or therapy or school nutrition policies to control or minimize the risk of childhood obesity, with the gut microbiota as the intervention target. In addition, although individual subjects did not benefit from participation, this study's results will significantly contribute to understanding potential environmental causes of childhood obesity. The risk/benefit ratio was favorable for this study, and adverse events were not anticipated.

Payment to Participants

Participants' parents received two $25 Visa gift cards for $50 upon completing all study measures. At enrollment of participants and after completion of height and weight measurements, the parent received a $25 Visa gift card. After completing the three non-consecutive 24-hour dietary recalls and providing the stool sample, the parent received a second $25 Visa gift card. The amount was minimal to reduce the possibility of monetary coercion related to recruitment, which was a small token of appreciation for participation in the study.

Costs of Participation

There were no other costs associated with participation in the study. All procedures were performed at no financial cost to the family.

Chapter Summary

The methods chapter presented the design, sample, settings, data collection procedures, and planned data analyses. The data analysis plan described the proposed statistical tests to achieve study aims. Findings from this study will increase knowledge on the association of an altered gut microbiota composition with childhood obesity and how diet, as influenced by family factors, can dramatically shift the composition of gut microbiota composition to improve health, thereby helping prevent the tracking of childhood obesity into adulthood. In addition, this research is foundational to future studies investigating the gut microbial system and its impact on childhood obesity and health during childhood and later in life.

RESULTS

This study examined the possible differences in gut microbiota composition between overweight/obese (o/o) and non-overweight/obese (non-o/o) school children on three related outcomes: 1) alpha-diversity of gut microbiota (GM) composition; 2) Firmicutes-Bacteroidetes (F: B) ratio; and 3) the abundance of short-chain fatty acid (SCFA) -generating gut microbes. The study also compared the dietary intakes between non-o/o and o/o groups. Data on dietary intake and GM composition were collected and analyzed. This chapter presents the study findings. Section one describes the study participants, study site, recruitment, and retention, and section two describes 16s metagenomic sequencing, alpha diversity of GM, F: B ratio, GM producing SCFA, BMI z-scores, and dietary intakes. Section three addresses the research aims:

1) comparing alpha diversity of gut microbiota composition, F: B ratio, and abundance of SCFA-generating gut microbes between overweight/obese (o/o) and non-overweight/obese children (non-o/o),
2) comparing dietary intakes between o/o and non-o/o children and the association to gut microbiota composition and obesity status, and
3) higher BMI z-score and the association to the relative abundance of gut microbe belonging to the phylum *Firmicutes*.

Section four covers the secondary analyses of potential confounding factors.

Study Site

The study site selected was the University of California University Village (UCUV), located at two sites - South Sawtelle and South Sepulveda - in the Los Angeles Palms/Mar Vista area. Resident Services Coordinator Leslie Wright says the site has more than 1,120 units. The units are designed for married students with children, students with same-sex domestic partners,

and single parents (UCLA Housing, 2023). At the time of recruitment, approximately 952 families were residing at UCUV. However, data on children from UCLA Housing were unavailable to determine how many children lived at the two sites by age range at the time of recruitment. From observation, it could be easily assumed that a large majority of UCUV residents have children because, at any given moment, many children were running around and playing on site. The UCLA community as a whole is ethnically and racially diverse, and this diversity was reflected in the UCUV community.

Recruitment and Retention

Recruitment for study participants began in May 2022 following IRB approval of two requested changes: the study setting and the extension of the study timeframe through November 2022. Recruitment consisted of posting and distributing study flyers in common areas such as the two sites' entrance gates and laundry rooms. Parents of potentially eligible participants were approached by the principal investigator (PI) by telephone or email and were provided study flyers. Recruitment was also accomplished via direct contact with parents, approaching them at site playgrounds, which facilitated the opportunity to explain the study and hand out flyers to interested individuals.

Retention Strategies

Since participation in the study required considerable time and effort, strategies were used to retain study participants. Retention strategies included calling/emailing parents to remind them to complete the three non-consecutive 24-hour dietary records and to collect the stool sample on or after the third day. In addition, a $50 Visa gift card was awarded to study participants once the three non-consecutive 24-hour dietary records were completed and stool

samples were given to the PI. Participants' parents referred other enrolled participants once the criteria were met.

Sample Characteristics

A total of 27 children were enrolled in the study. Stool samples were collected from 14 non-o/o children (average BMI z-score: -0.43) and 13 o/o children (average BMI z-score: 1.63; $p < 0.0001$). The non-o/o group was comprised of n=10 males and n=4 females. The o/o group was comprised of n=8 males and n=5 females. Sample characteristics, including sex, F: B ratio, BMI z-score, ethnicity/race, mode of delivery in childbirth, history of breastfeeding or formula feeding as an infant, education of parent, and annual household income, were considered. Table 5.1 illustrates the sample's characteristics separated by non-o/o and o/o groups. There was no significant relationship between the history of breastfeeding or formula feeding as an infant with non-o/o and o/o children (Fisher exact test; $p = 0.0628$). Children born either by cesarian section or vaginal delivery did not reach statistical significance at the $p = 0.05$ level (Fisher exact test; $p = 0.2281$). Although sex is considered one of the critical variables affecting the gut microbiota, sex was not statistically significant and did not show sex differences in gut microbiota (Fisher exact; $p = 0.2749$). However, the two groups had significant differences in ethnicity/race. There were significantly more Hispanics in the o/o group (61.54%) and more Whites in the non-o/o group (42.86%) ($p = 0.0006$). There were significantly more parents who were high school graduates in the o/o group and more parents who were college graduates in the non-o/o group ($p = 0.0007$). Annual household income reached statistical significance at $p = < 0.0001$, with more families earning an annual household income between $20,000 - $30,000 in the o/o group and more families earning an annual household income between $31,000 - $60,000 in the non-o/o group. There was a significant relationship in BMI z – score between non-o/o and o/o groups at

p = < 0.0001. There was no statistical difference in F: B ratio between the non-o/o and o/o groups.

Table 0.1. Participant characteristics by group. Fisher exact test was used to generate the difference in p values.

	Overweight/obese	**Non-overweight/non-obese**	***p*-value**
Sex			0.2749
Male - 0	8 (61.54%)	10 (71.43%)	
Female - 1	5 (38.46%)	4 (28.57%)	
Ethnicity/race			0.0006
White - 0	2 (15.38%)	6 (42.86%)	
Hispanic - 1	8 (61.54%)	2 (14.29%)	
Asian - 2	1 (7.69%)	2 (14.29%)	
Middle Eastern - 4		3 (21.43%)	
Other Race - 5	2 (15.38%)	1 (7.14%)	
Mode of delivery			0.2281
Vaginal - 0	10 (76.92%)	13 (92.86%)	
Caesarian Section - 1	3 (23.08%)	1 (7.14%)	
History of Feeding			0.0628
Breast fed - 0	8 (61.54%)	12 (85.71%)	
Mixed feeding – 0, 1	3 (23.08%)	2 (14.29%)	
Formula Feeding - 1	2 (15.38%)	0	
Education of parent			0.0007
High school graduate - 0	4 (30.77%)	1 (7.14%)	
Some College - 1	3 (23.08%)	2 (14.29%)	
College Graduate - 2	3 (23.08%)	11 (78.57%)	
Advanced Degree - 3	3 (23.08%)		
Annual Household Income (thousands)			<.0001
<$20k - 0	4 (30.77%)	0	
$21k-$30k - 1	3 (23.08%)	0	
$31k-$60k - 2	3 (23.08%)	6 (42.86%)	
$61k-$75k - 3		5 (35.71%)	
>$75K - 4	3 (23.08%)	3 (21.43%)	
Age (years)	7.85 (1.72, 6-10)	6.64 (1.39, 5-10)	0.0563
Z-score of *BMI-for-age	1.63 (0.58, 1.04-2.59)	-0.43 (0.69, -1.65-0.66)	<.0001
*F reads	11010.54 (2901.35, 5688-15553)	10538.57 (3788.2, 5859-16803)	0.7209
*B reads	1730 (1439, 13-4802)	1607.29 (1446.42, 0-4254)	0.827
*FB ratio	99.54 (264.52, 2.08-970.08)	31.25 (56.25, 1.88-209.21)	0.379

*Abbreviations: F = Firmicutes; B = Bacteroidetes; FB ratio = Firmicutes/Bacteroidetes ratio; BMI = body mass index; k = thousand. *BMI-for-age z-score based on sex-specific, age-adjusted CDC growth chart-derived norms for BMI values.

16s Metagenomic Sequencing

Metagenomics 16S sequencing was performed by Omega Bioservices (Norcross, GA, USA). DNA was isolated using Omega Biotek Mag-Bind® Universal Pathogen DNA Kit following the manufacturer`s protocol. DNA concentration was measured using the QuantiFluor dsDNA System on a Quantus Fluorometer (Promega, Madison, WI, USA).

The V3-V4 region of the bacterial 16S rRNA gene sequences was amplified using the primer pair containing the gene-specific sequences and Illumina adapter overhang nucleotide sequences. The full-length primer sequences are 16S Amplicon PCR Forward Primer (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 16S Amplicon PCR Reverse Primer(5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC).

Amplicon PCR was performed to amplify the template out of input DNA samples. Briefly, each 25 μL of polymerase chain reaction (PCR) reaction contains 12.5 ng of sample DNA as input, 12.5 μL 2x KAPA HiFi HotStart ReadyMix (Kapa Biosystems <https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwiT28rzztXPAhUDQSYKHVmoB2gQFggdMAA&url=https%3A%2F%2Fwww.kapabiosystems.com%2F&usg=AFQjCNH2ckStYKfNu_G3Rp82oDoDyfcUvQ&sig2=na72hk3Ut1ekBIuNHRZFOA>, Wilmington, MA) and five μL of 1 μM of each primer. PCR reactions were carried out using the following protocol: 1) an initial denaturation step performed at 95°C for three minutes followed by 25 cycles of denaturation (95°C, 30 s), 2) annealing (55°C, 30 s) and extension (72°C, 30 sec), and 3) a final elongation of 5 min at 72°C. The PCR product was cleaned from the reaction mix with Mag-Bind RxnPure Plus magnetic beads (Omega Bio-tek, Norcross, GA).

A second index PCR amplification, used to incorporate barcodes and sequencing adapters into the final PCR product, was performed in 25 μL reactions using the same master mix conditions described above. Cycling conditions were as follows: 95°C for 3 minutes, followed by eight cycles of 95°C for 30", 55°C for 30" and 72°C for 30". A final 5-minute elongation step was performed at 72°C.

The library, ~600 bases in size, was checked using an Agilent 2200 Tapestation and quantified using QuantiFluor dsDNA System (Promega). Libraries were normalized, pooled, and sequenced (2 x 300 bp paired-end read setting) on the MiSeq (Illumina, San Diego, CA).

Phylogenetic Sequencing

Phylogenetic sequencing (PGS) of the samples' gut microbiota was done to reach the small subunit ribosomal RNA (16s rRNA) gene. A typical method of PGS is barcoded amplicon sequencing. QIIME 2 was utilized to analyze data such as decoding, similarity clustering, and defining operational taxonomic units (OTUs).

Phyloseq

Research data was imported and transferred into phyloseq to analyze data and seamless exploratory transformation and plotting. Phyloseq is a Bioconductor package that integrates "abundance data, phylogenetic information, and covariates to perform exploratory transformations, plots and confirmatory testing and diagnostic plots" (McMurdie & Holmes, 2012, p. 1)

Alpha Diversity of Gut Microbiota Composition

Alpha diversity refers to the within-sample diversity in a single ecosystem or sample while considering the number of species observed. Many researchers are interested in estimating

alpha diversity since differences between groups have been associated with several health-related outcomes.

The most straightforward measure of alpha diversity is richness, or the number of operational taxonomic units (OTUs) observed in the sample. Alpha diversity also takes into account how OTUs are evenly distributed. Other alpha diversity metrics are often weighted by the abundances at which OTUs are observed. Using the plug-in estimates for observed richness, Shannon diversity, and phylogenetic diversity on the sub-sampled data (Figure 5.3), differences according to obese status were estimated and tested. Figure 5.1 shows that the observed OTUs are correlated with the total reads. Using the Wilcoxon Rank Sum Test to test for differences in alpha diversity between non-o/o and o/o groups, the boxplot in Figure 5.2 shows the variation in alpha diversity between non-o/o and o/o children groups and indicates no significant differences between them.

Table 5.2 illustrates the means and standard deviations of various outcome variables separated by non-o/o and o/o children. The alpha diversity between the two groups was not statistically significant using observed gut microbiota richness ($p = 0.68$). As estimated by the Shannon index, the alpha diversity between non-o/o and o/o children indicated no significant differences ($p = 0.45$). As measured by the Chao1 index, the alpha diversity suggested that the gut microbiota richness between samples from non-o/o and o/o children was not statistically significant, with a $p = 0.70$.

Figure 0.1. Observed OTUs vs. Total Reads

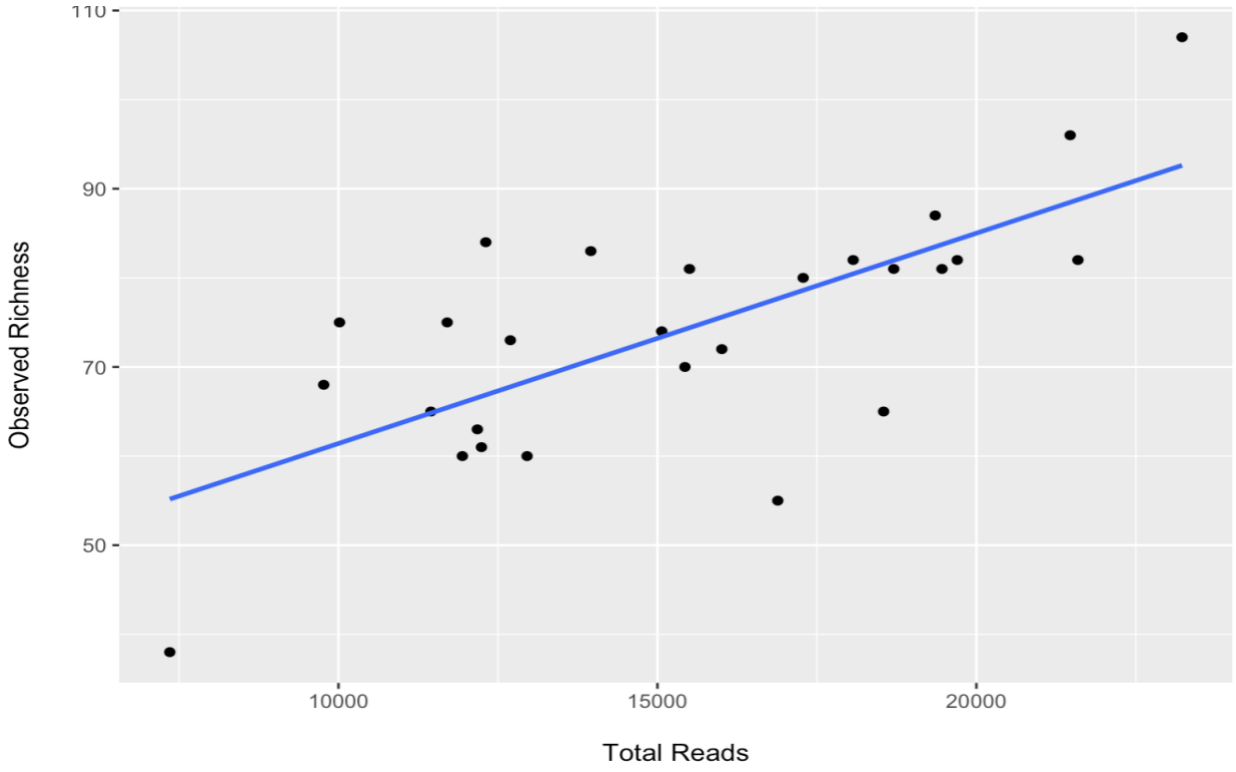


Figure 0.2. Alpha Diversity Boxplot

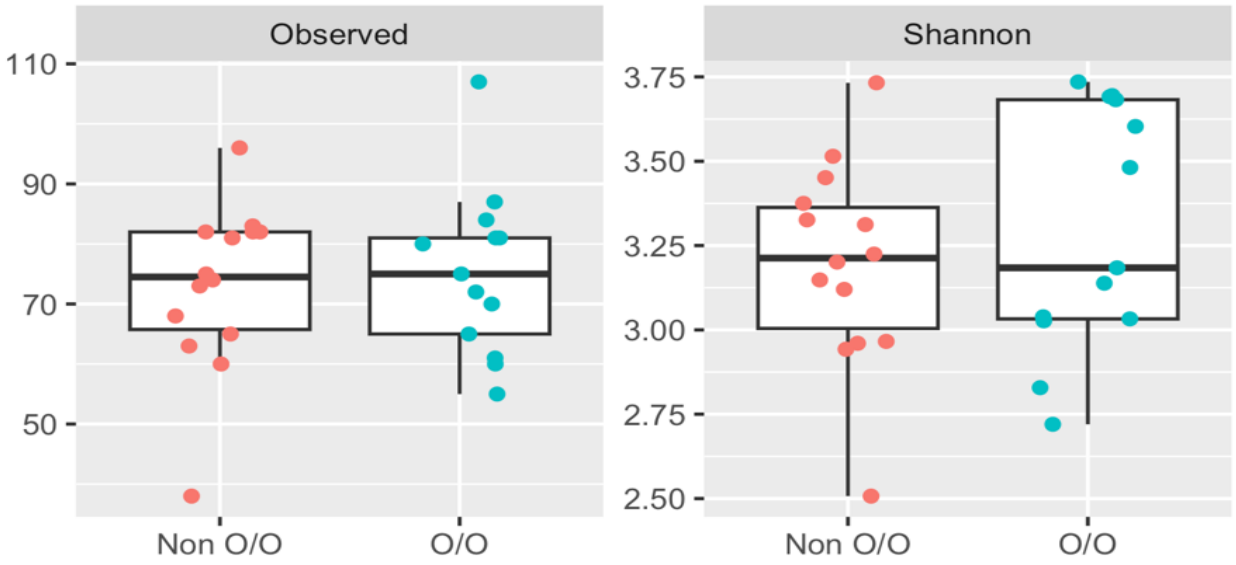


Figure 0.3. Observed Richness and Alpha Diversity using Chao1 and Shannon Indices

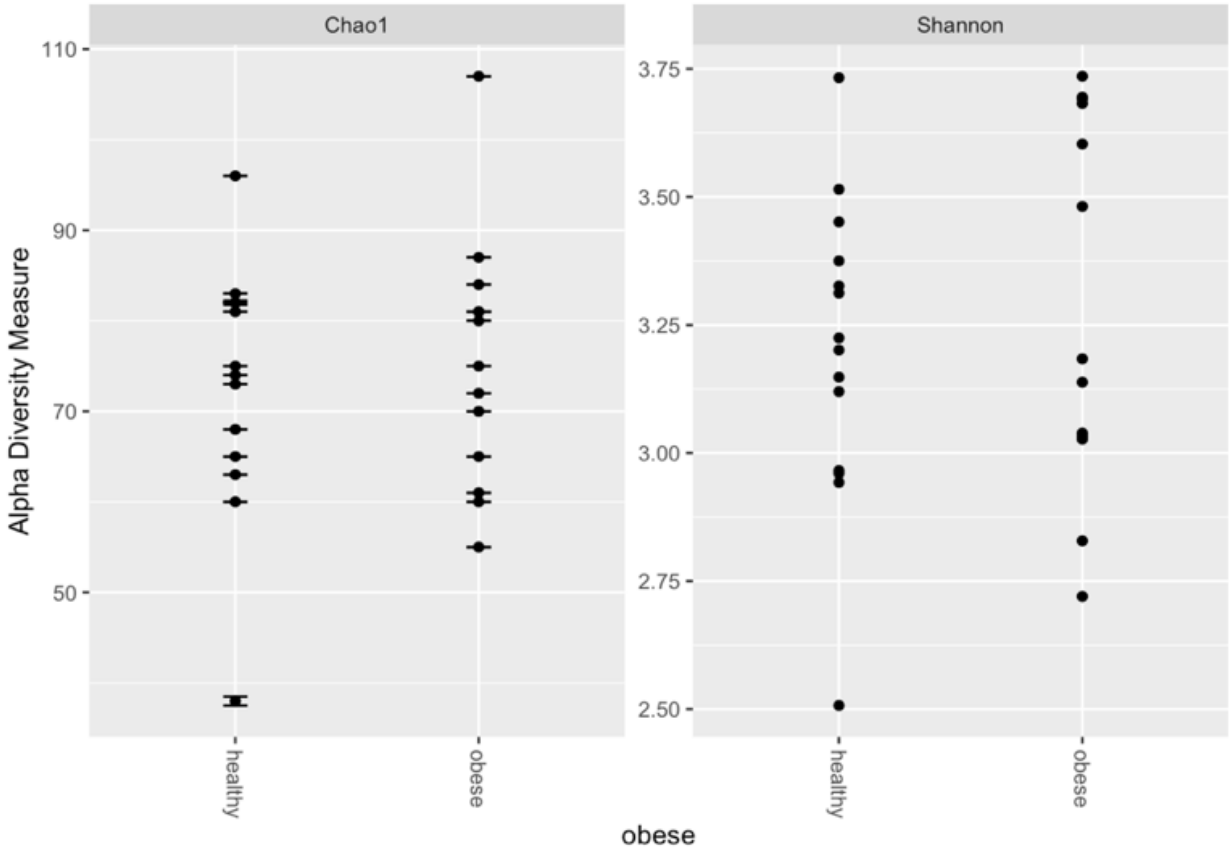


Table 0.2. Outcome variables by group: Alpha diversity, F: B ratio, Firmicutes relative abundance, and SCFA-generating gut microbes Using Pooled Variance Method

	NON-O/O	**O/O**	
Outcome variable	**N=14**	**N=13**	**p-value**
α-diversity: Observed	73 (13.93), 38-96	75.23 (13.85), 55-107	0.6803
α-diversity: Chao 1	73.24 (14.35), 38-99	75.35 (14.14), 55-108.5	0.7041
α-diversity: Shannon	3.2 (0.3), 2.51-3.73	3.3 (0.36), 2.72-3.74	0.4481
α-diversity: InvSimpson	14.67 (5.78), 6.4-27.11	17.24 (8.22), 7.07-29.15	0.3526
F:B ratio	31.25 (56.25), 1.88-209.21	99.54 (264.52), 2.08-970.08	**0.379
F relative abundance	0.71 (0.12), 0.45-0.86	0.69 (0.11), 0.47-0.85	0.5932
SCFA 10K	0.52 (0.22), 0.18-0.86	0.63 (0.25), 0.16-1.16	0.246
All use pooled variance except ** using Satterthwaite method			

Relative Abundance of Gut Microbiota

The overall composition of gut microbiota at the OTU and taxonomic levels from genus to phylum were not significantly impacted by obesity status, as illustrated by the bar/boxplots at the phylum level in Figure 5.4 and genus level (Figure 5.6). There are 11 phyla, and their relative abundance looks similar between non-o/o and o/o children.

Figure 0.4. Relative Abundance at Phylum Level

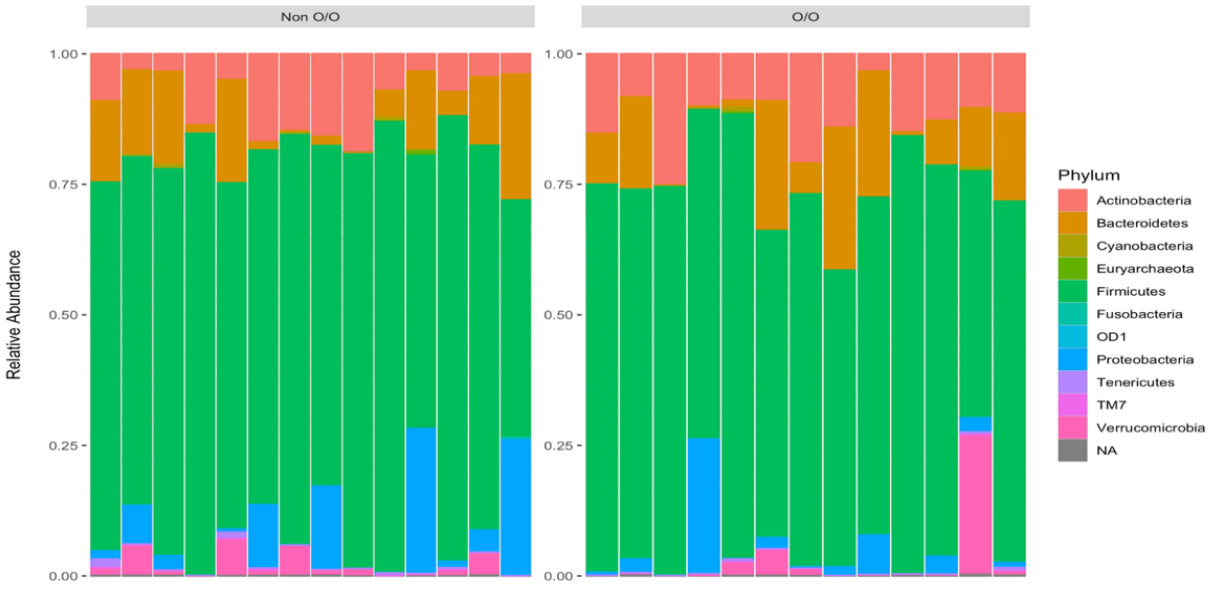


Figure 0.5. Boxplot of Relative Abundance at Phylum Level

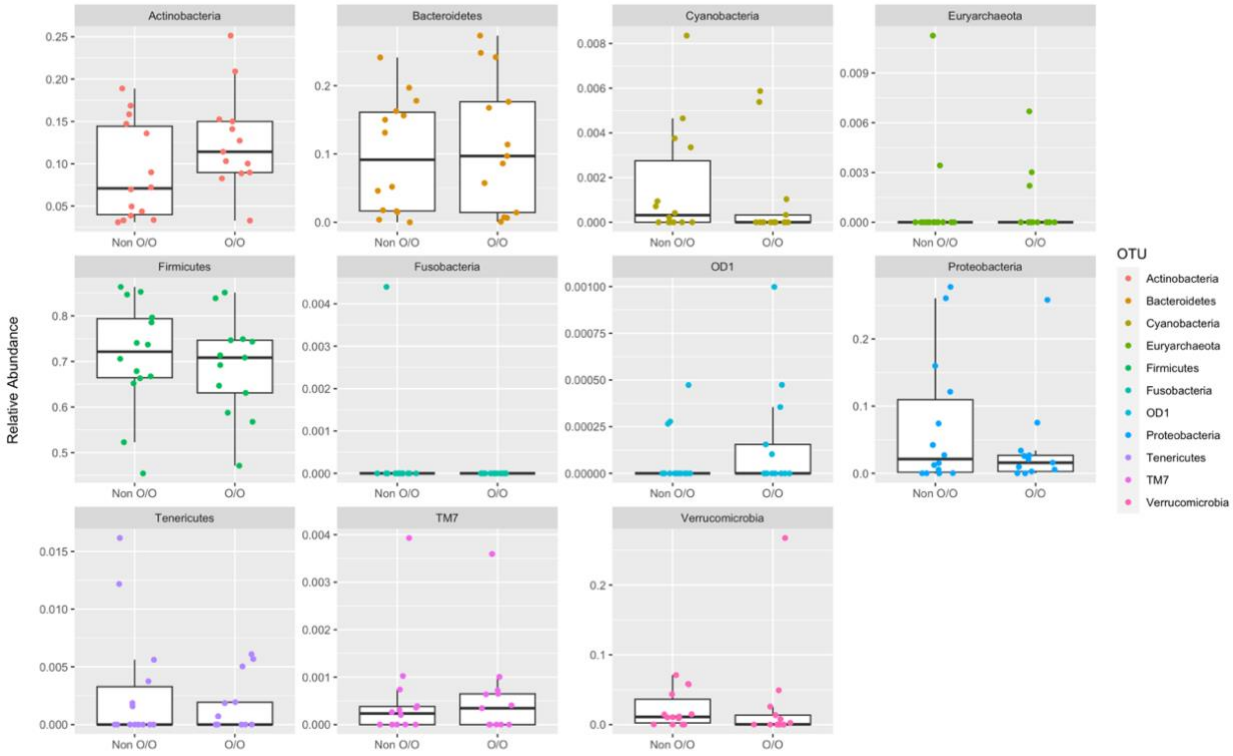
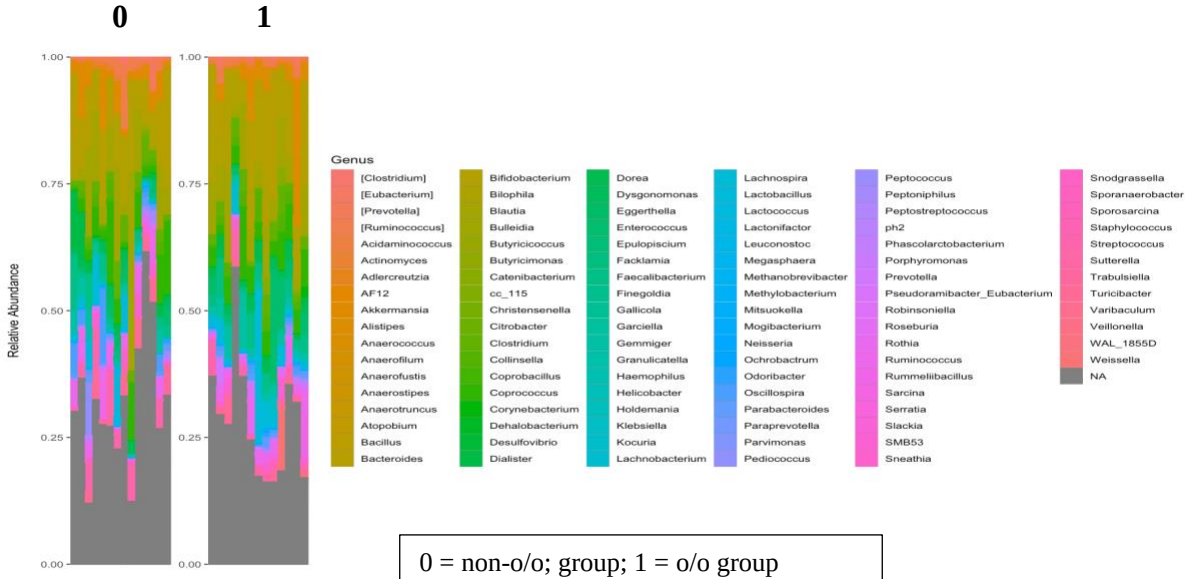


Figure 0.6. Relative Abundance at Genus Level



0 = non-o/o; group; 1 = o/o group

Firmicutes/Bacteroidetes Ratio

The metagenomic analysis of fecal bacteria from non-o/o and o/o children at the phylum level showed that F: B ratio in the o/o group is higher (99.54 ± 264.52) compared with non-o/o (31.25 ± 56.25) but it is not significant at $p = 0.379$ (Table 5.2). The conclusion with p-value indicated that there was no difference in the taxa frequency for Firmicutes and Bacteroidetes between non-o/o and o/o groups, as shown in Table 5.3. There were no significant differences in the Firmicutes level between the non-o/o (10500 ± 3790) and o/o (11000 ± 2900) children and in the Bacteroidetes level of non-o/o (1610 ± 1450) and o/o (1730 ± 1430). Table 5.3 shows slight differences in mean values for Firmicutes, Bacteroidetes, and F: B ratio between the two groups. PROC CORR was used to calculate the Pearson correlation coefficient to assess the linear relationship between F: B ratio and alpha diversity. Table 5.4 shows a significant negative relationship between the F: B ratio and alpha diversity at p =.05. When controlling for age and gender, associations between Firmicutes, Bacteroidetes, and alpha diversity were tested using multiple linear regression. There is a strong negative association between Firmicutes and alpha diversity and a strong positive association between Bacteroidetes and alpha diversity. When controlling for age and gender: 1) alpha diversity decreases by 0.3 for every 10000 increase in Firmicutes, which was significant at $p = .0026$, and 2) alpha diversity increases by 1 for every 10000 increase in Bacteroidetes, which was highly significant at $p = .0000$. (Table 5.5). Table 5.5 also shows that when considering a model that includes Firmicutes and Bacteroidetes as predictors while controlling for age and gender, the study found that Firmicutes and Bacteroidetes are good predictors of alpha diversity (Shannon Index). The scatter plots in Figure 5.7 depict the correlations between alpha diversity, Firmicutes, and Bacteroidetes without controlling for gender and age. It shows a significant negative correlation between alpha

diversity and Firmicutes (-0.3853; $p = 0.0472$) and a significant positive correlation between alpha diversity and Bacteroidetes (0.6941; $p = 0.0000$). Although significant, neither Firmicutes nor Bacteroidetes are good predictors of alpha diversity (Shannon Index).

Table 0.3. Mean values of outcome variables for non-o/o and o/o children

Variable	Non-O/O Children (N=14)	O/O Children (N=13)	Total (N=27)	P-value
F				
Mean (SD)	10500 (3790)	11000 (2900)	10800 (3330)	0.721
Median [Min, Max]	9860 [5860, 16800]	11400 [5690, 15600]	11000 [5690, 16800]	
B				
Mean (SD)	1610 (1450)	1730 (1440)	1670 (1420)	0.827
Median [Min, Max]	1470 [0, 4250]	1670 [13.0, 4800]	1670 [0, 4800]	
FB Ratio				
Mean (SD)	29.0 (54.7)	99.5 (265)	63.0 (187)	0.338
Median [Min, Max]	5.06 [0, 209]	7.66 [2.08, 970]	5.61 [0, 970]	
SCFA				
Mean (SD)	5250 (2220)	6320 (2460)	5760 (2360)	0.246
Median [Min, Max]	5320 [1760, 8550]	5880 [1550, 11600]	5790 [1550, 11600]	
SCFA10K				
Mean (SD)	0.525 (0.222)	0.632 (0.246)	0.576 (0.236)	0.246
Median [Min, Max]	0.532 [0.176, 0.855]	0.588 [0.155, 1.16]	0.579 [0.155, 1.16]	
Observed				
Mean (SD)	73.0 (13.9)	75.2 (13.8)	74.1 (13.7)	0.68
Median [Min, Max]	74.5 [38.0, 96.0]	75.0 [55.0, 107]	75.0 [38.0, 107]	
Chao1				
Mean (SD)	73.2 (14.3)	75.3 (14.1)	74.3 (14.0)	0.704
Median [Min, Max]	74.5 [38.0, 99.0]	75.0 [55.0, 109]	75.0 [38.0, 109]	
Shannon				
Mean (SD)	3.20 (0.299)	3.30 (0.363)	3.25 (0.329)	0.448
Median [Min, Max]	3.22 [2.51, 3.73]	3.18 [2.72, 3.74]	3.20 [2.51, 3.74]	
InvSimpson				
Mean (SD)	14.7 (5.78)	17.2 (8.22)	15.9 (7.04)	0.353
Median [Min, Max]	14.1 [6.40, 27.1]	12.4 [7.07, 29.2]	13.8 [6.40, 29.2]	

Table 0.4. Alpha Diversity Correlations with BMI Z-score, FB Ratio, and SCFA-Producing Gut Microbiota Variables Using CORR Procedure

3 With Variables:	**BMI z-score** FB ratio **SCFA-producing Gut Microbiota**
4 Variables:	**Observed Chao1 Shannon InvSimpson**

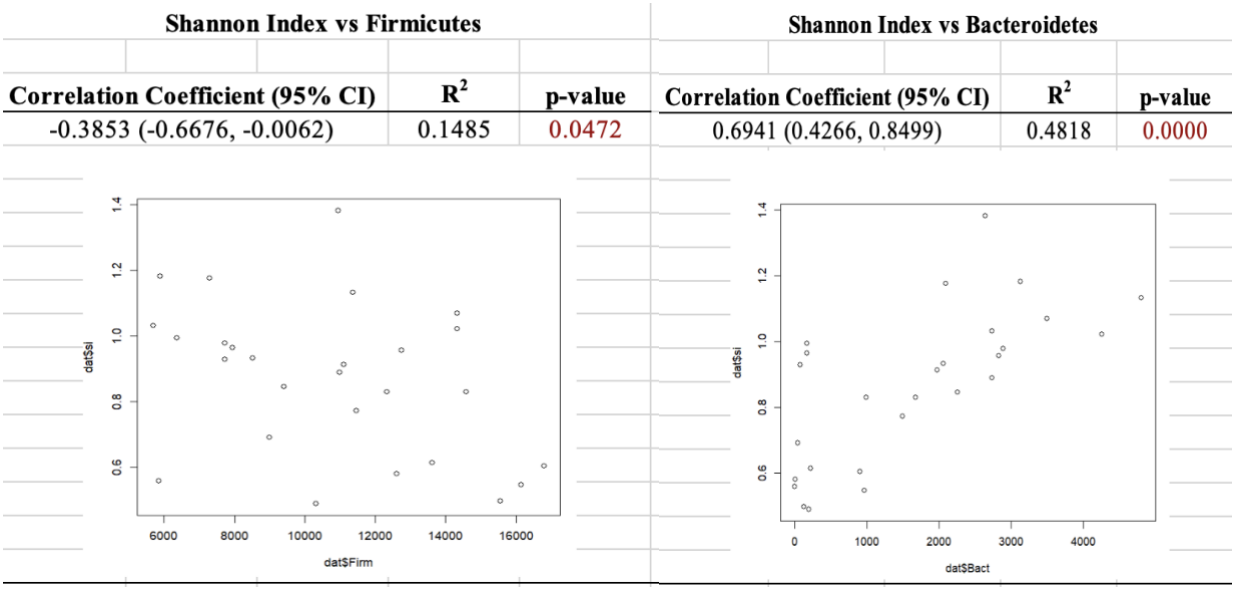
Simple Statistics						
Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**
z-score	27	0.56259	1.21792	15.19000	-1.65000	2.59000
FB ratio	26	65.39510	190.57187	1700	1.88235	970.07692
SCFA- producing Gut Microbiota	27	5764	2358	155641	1550	11568
Observed	27	74.07407	13.66896	2000	38.00000	107.00000
Chao1	27	74.25296	14.01086	2005	38.00000	108.50000
Shannon	27	3.24593	0.32873	87.64000	2.51000	3.74000
InvSimpson	27	15.90704	7.04087	429.49000	6.40000	29.15000

Pearson Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations				
	Observed	**Chao1**	**Shannon**	**InvSimpson**
BMI z-score	0.07759 0.7005 27	0.06509 0.7470 27	0.08025 0.6907 27	0.10022 0.6189 27
FB ratio	-0.44818 0.0217 26	-0.43910 0.0248 26	-0.39532 0.0456 26	-0.22463 0.2699 26
SCFA- producing Gut Microbe	0.46131 0.0154 27	0.46817 0.0138 27	0.45071 0.0183 27	0.41162 0.0329 27

Table 0.5. Predicting Alpha Diversity From Firmicutes, Bacteroidetes, and BMI Z-Score (with age and gender as covariates)

Factor/Covariate	Coefficient	Standard Error	p-value	Model Summary
Firmicutes	-0.00003	0.0000	0.0026	R^2 = 0.6758 p- value = 0.0001
Bacteroidetes	0.0001	0.0000	0.0000	
BMI	0.0158	0.0284	0.5829	
Age	0.0010	0.0221	0.9630	
Gender	-0.0805	0.0703	0.2649	

Figure 0.7. Scatter Plots To Show Correlations between Alpha Diversity, Firmicutes, and Bacteroidetes

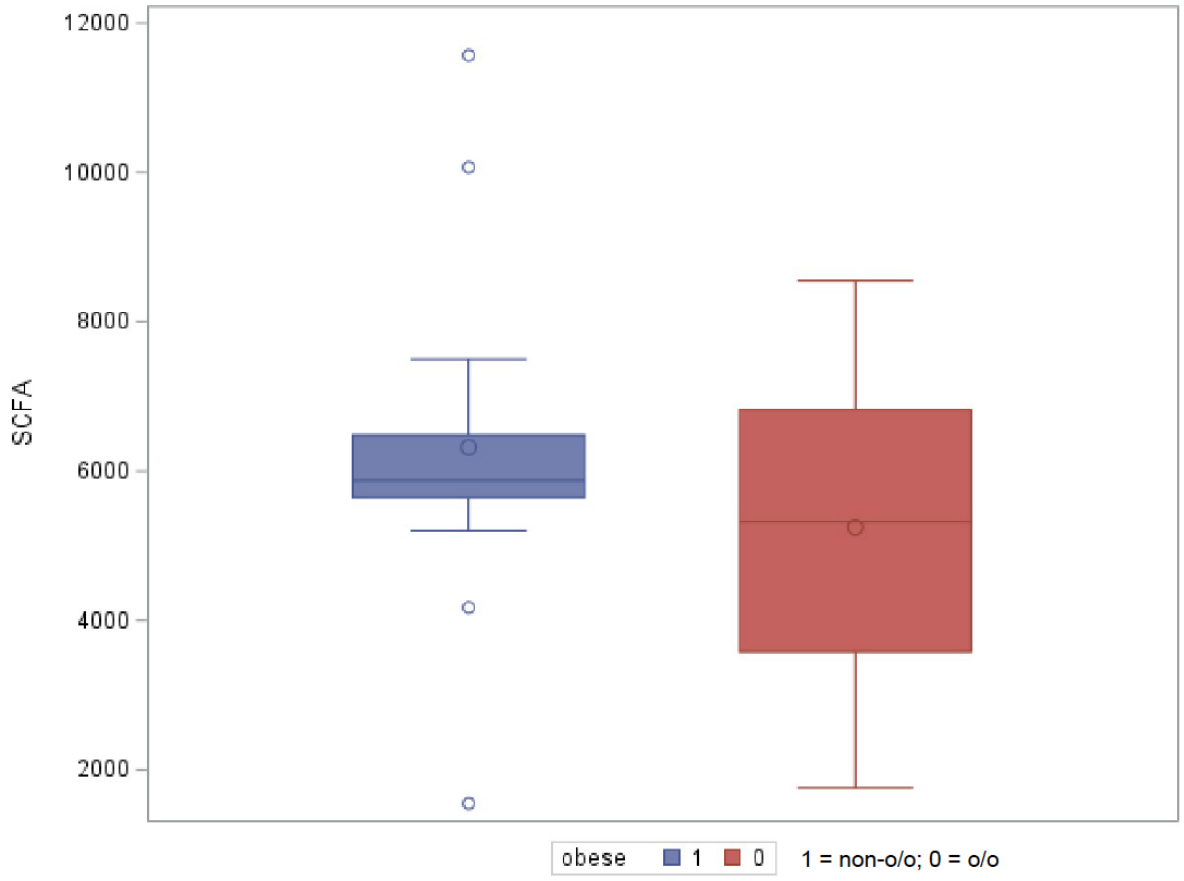


Gut Microbiota Producing Short Chain Fatty Acids (SCFA)

This study observed significantly higher concentrations of gut microbiota producing SCFA in the stools of o/o children (0.632 ± 0.246) compared to non/o/o children (0.525 ± 0.222). The boxplot (Figure 5.8) shows that overweight/obese children have an increased relative abundance of gut microbiota producing SCFA compared with non-o/o children. However, it was

not significant (p = 0.246). Pearson correlation coefficient was computed using the CORR procedure to assess the correlation between gut microbiota producing SCFA and alpha diversity. The two variables had a significant positive correlation with r = .45 and p=0.02 (Table 5.4).

Figure 0.8. Gut Microbiota producing SCFA between Non-O/O and O/O Groups



Body Mass Index Z-score

Body Mass Index (BMI) is a widely used tool to help classify children into healthy, overweight, and obese weight categories for both research purposes and clinical assessments. This study used BMI z-score to divide participants into non-o/o and o/o groups. Table 5.1 shows a significance (p = <.0001) in BMI z-score related to weight status. Table 5.6 describes the variability in the BMI z-score mean for study participants, showing that data was clustered

around the mean. The Pearson correlation coefficient was computed using the CORR procedure to assess whether the BMI z-score is correlated with alpha diversity. A weak positive correlation exists between the two variables with r = 0.08 and $p = 0.69$. In addition, the scatter plot (Figure 5.9) shows no significant relationship between gut microbiota producing SCFA and BMI z-score. In addition, when predicting alpha diversity from BMI z-score while controlling for age and gender, the study found no significant association between BMI z-score and alpha diversity (r =0.0158; $p = 0.5829$) (Table 5.5).

Table 0.6. BMI z-scores mean for participants (n = 27)

Variable	N	Mean	Std Dev	Minimum	Maximum
BMI z-score	27	0.56	1.22	-1.65	2.59
DGMFS	27	1.59	1.35	0.33	6.57

Figure 0.9. Scatter plot to show the relationship between SCFA-producing

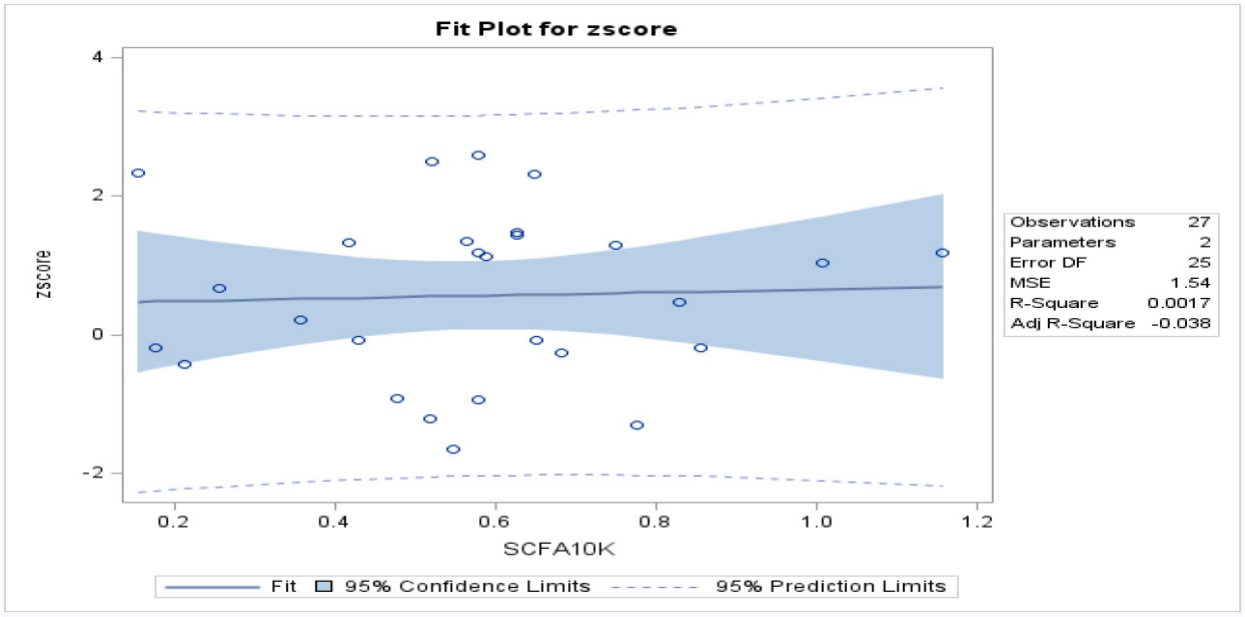
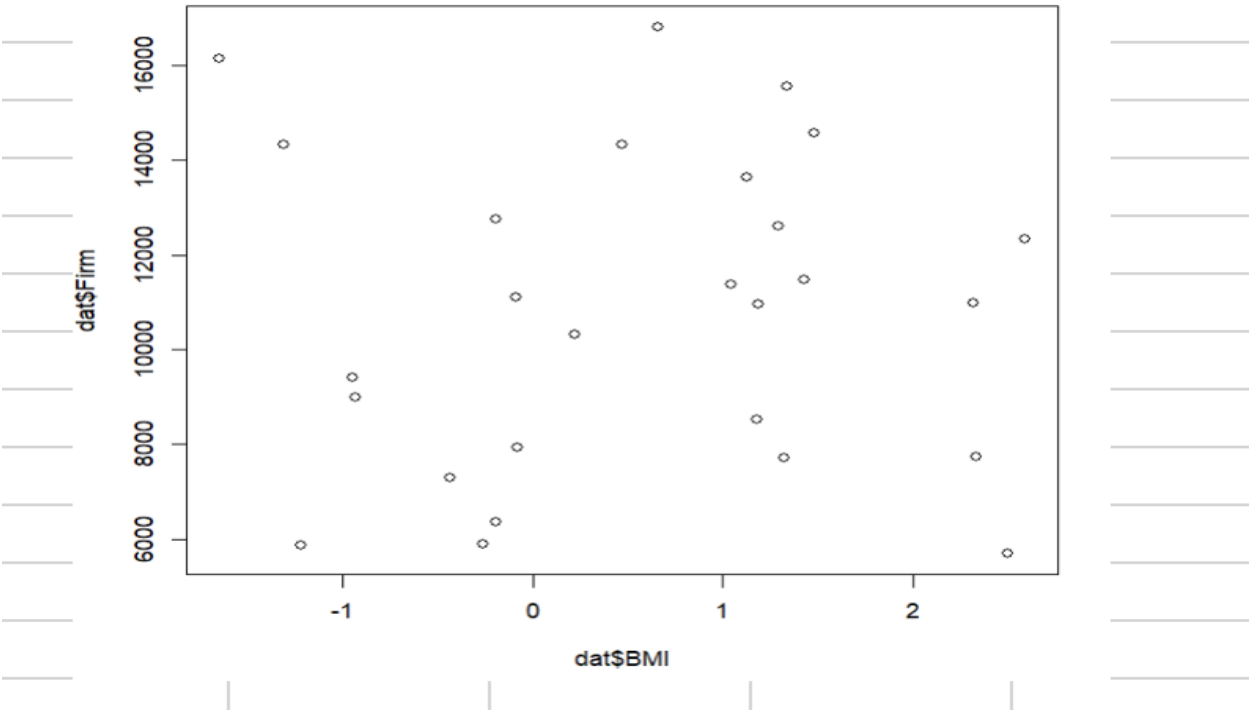


Table 0.7. Regression Analysis of BMI Z-Score and Relative Abundance of Firmicutes

Regression analysis of BMI Z-score and relative abundance of Firmicutes Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.76990	1.52041	1.16	0.2554
Firmicutes	1	-1.72329	2.14391	-0.80	0.4291
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-1.64286	1.70717	-0.96	0.3459
age	1	0.40961	0.12886	3.18	0.0042
dgmfs	1	0.12355	0.15717	0.79	0.4399
Firmicutes	1	-1.35440	1.86516	-0.73	0.4751

Table 0.8. Correlation Between BMI Z-score and Firmicutes Using R2 Coefficient of Determination

Correlation Coefficient (95% CI)	R^2	p-value
0.0188 (-0.3639, 0.3959)	0.0004	0.926



Dietary Intakes

Table 5.9 describes the dietary intakes between non-o/o and o/o children.

Table 0.9. Means and Standard Deviations of Dietary Intakes Using T-test

Variable	Non-O/O Children (N=14)	O/O Children (N=13)	Total (N=27)	P-value
Calories				
Mean (SD)	1740 (415)	2020 (571)	1880 (507)	0.159
Median [Min, Max]	1790 [1130, 2570]	1980 [1290, 2880]	1920 [1130, 2880]	
Carbohydrate (Carb_g)				
Mean (SD)	223 (52.7)	242 (74.9)	232 (63.7)	0.471
Median [Min, Max]	226 [134, 304]	220 [124, 394]	224 [124, 394]	
Total Carbohydrates (Tot_carbs)				
Mean (SD)	0.511 (0.0430)	0.480 (0.0720)	0.496 (0.0597)	0.187
Mean (SD)	65.3 (18.2)	79.6 (20.6)	72.2 (20.4)	0.0665
Median [Min, Max]	60.5 [43.0, 101]	75.0 [56.0, 132]	71.0 [43.0, 132]	
Saturated Fats (Satfats)				
Mean (SD)	24.1 (6.26)	32.7 (21.0)	28.2 (15.6)	0.154
Median [Min, Max]	23.5 [13.0, 35.0]	28.0 [11.0, 92.0]	25.0 [11.0, 92.0]	
Total Fat (Tot fat)				
Mean (SD)	0.341 (0.0425)	0.366 (0.0699)	0.353 (0.0577)	0.26
Median [Min, Max]	0.345 [0.280, 0.430]	0.370 [0.260, 0.520]	0.350 [0.260, 0.520]	
Protein				
Mean (SD)	65.3 (18.2)	79.6 (20.6)	72.2 (20.4)	0.0665
Median [Min, Max]	60.5 [43.0, 101]	75.0 [56.0, 132]	71.0 [43.0, 132]	
Total Protein (tot_prot)				
Mean (SD)	0.151 (0.0234)	0.176 (0.0287)	0.163 (0.0287)	0.018
Median [Min, Max]	0.140 [0.120, 0.190]	0.170 [0.140, 0.230]	0.160 [0.120, 0.230]	
Whole Grain (whole_g)				
Mean (SD)	1.89 (1.20)	1.46 (1.24)	1.68 (1.22)	0.363
Median [Min, Max]	1.50 [0.500, 4.50]	1.60 [0, 4.15]	1.60 [0, 4.50]	

Variable	Non-O/O Children (N=14)	O/O Children (N=13)	Total (N=27)	P-value
Grains				
Mean (SD)	4.56 (1.61)	5.95 (2.43)	5.23 (2.13)	0.0899
Median [Min, Max]	4.00 [2.00, 7.75]	5.50 [2.00, 9.25]	4.75 [2.00, 9.25]	
Dairy				
Mean (SD)	1.55 (1.17)	1.56 (0.867)	1.55 (1.02)	0.978
Median [Min, Max]	1.13 [0.250, 4.25]	1.50 [0, 3.50]	1.25 [0, 4.25]	
Vegetable				
Mean (SD)	0.729 (0.663)	1.00 (0.540)	0.859 (0.611)	0.257
Median [Min, Max]	0.500 [0.250, 2.25]	1.00 [0.250, 2.00]	0.750 [0.250, 2.25]	
Plant				
Mean (SD)	3.41 (4.39)	1.77 (1.85)	2.62 (3.45)	0.223
Median [Min, Max]	1.88 [0, 14.3]	1.10 [0, 5.25]	1.75 [0, 14.3]	
Added Sugar (add_sugar)				
Mean (SD)	35.5 (16.0)	51.2 (30.3)	43.1 (24.8)	0.103
Median [Min, Max]	29.0 [17.0, 75.0]	50.0 [20.0, 115]	34.0 [17.0, 115]	
Sodium				
Mean (SD)	2740 (991)	3370 (1080)	3040 (1070)	0.129
Median [Min, Max]	2530 [1770, 5380]	3170 [1870, 5460]	2920 [1770, 5460]	
Total Fiber (tot_fib)				
Mean (SD)	19.6 (6.44)	19.7 (7.58)	19.7 (6.87)	0.986
Median [Min, Max]	18.8 [11.0, 36.0]	20.0 [9.00, 32.0]	19.0 [9.00, 36.0]	
Daily Gut Microbiota Friendliness (dgmfs)				
Mean (SD)	1.39 (0.821)	1.80 (1.77)	1.59 (1.35)	0.444
Median [Min, Max]	1.20 [0.330, 3.73]	0.980 [0.340, 6.57]	1.00 [0.330, 6.57]	

Matching Code:

Total Daily Avg. Calories	calories
Daily Avg Carbs Eaten (g)	carb_g
Total Carb Range %	tot_carb
Daily Avg Saturated Fat Eaten (g)	satfats
Total Fat Range %	tot_fat
Daily Avg Protein Eaten (meat, poultry, eggs) (g)	protein
Total Protein Range %	tot_prot
Daily Avg Whole Grains Eaten (oz).	whole_g
Daily Avg Refined Grains Eaten (oz)	grains
Daily Avg Fruits Eaten; 100% juice (c.)	
Daily Avg Soymilk/ Dairy/Dairy Products Eaten (c.)	
Daily Avg Vegetables Eaten (dark green, red, orange, other) (c.)	veg
Daily Avg Plant Proteins (Legumes (beans, peas), nuts, seed, soy) (oz.)	plant
Daily Avg Added Sugars Avg Eaten (g)	add_sugar
Daily Avg Sodium Eaten (mg.)	
Daily Avg Total Fiber (g)	tot_fib
Daily GM Friendliness Score	dgmfs

On average, compared with the non-o/o group, the o/o group consumed more calories, higher energy, and saturated fat foods, fruits, vegetables, and foods high in sugar and sodium. In addition, the o/o group has increased protein consumption from meat, poultry, and eggs. The non-o/o group had increased consumption of plant proteins from legumes, beans, peas, nuts, and seeds. Using the *t*-test, the DGMFS in non-o/o is lower (1.39 ± 0.821) than the o/o children (1.80 ± 1.77), but it was insignificant at $p = 0.444$. The *t*-test Satterthwaite method showed DGMFS at $p = 0.46$, which was also negligible.

Table 0.10. Outcome Variables for Calories, Saturated Fats, and Daily Gut Microbiota Friendliness Score (DGMFS) Using t-test Pooled Variance and Satterthwaite Methods

	NON-O/O	O/O	
Outcome variable	**N=14**	**N=13**	**p-value**
Total Daily Avg. Calories	1744 (415.37), 1126-2572	2021.46 (570.83), 1292-2876	0.1589
Daily Avg Saturated Fats Eaten (g)	24.07 (6.26), 13-35	32.69 (21.01), 11-92	**0.1770
Daily GM Friendliness Score	1.39 (0.82), 0.33-3.73	1.8 (1.77), 0.34-6.57	**0.4589
All use pooled variance except ** using Satterthwaite method			

Daily Gut Microbiota Friendliness Score

Because a Healthy Eating Index could not be derived from the limited ASA 24 output, a proxy scoring scheme was developed by Dr. William McCarthy, UCLA Fielding School of Public Health Professor, who calculated the gut microbiota friendliness of daily food intake. See Appendix H for the calculation of gut microbiota friendliness of daily food intake using ASA 24 output. This scoring scheme gives a zero to a maximum weight of 1.0 to each food group: whole grains, whole fruit, dark green and red-orange vegetables, other vegetables, and plant proteins. Consumption of these foods provides substrates to the gut microbiota, yielding more SCFAs. Food components that undermine gut microbiota are saturated fat and sugar. The total weights are added as a denominator for the overall daily food gut microbiota friendliness score. A score of 1.0 or greater is healthy, and a score less than 1.0 is unhealthy (McCarthy, 2023).

Research Aims

Specific Aim 1: Compare all outcomes: alpha diversity and richness of gut microbiota, F: B ratio, relative abundance of F, and abundance of SCFA-generating gut microbes between overweight/obese (o/o) and non-overweight/obese children (non-o/o). Table 5.11 presents the

variables using the t-test to compare the means and detect any differences between the two groups. Table 5.2 shows the means, standard deviation, and *p-values* using the Pooled Variance and Satterthwaite Method.

Table 0.11. Means and Standard Deviations For Each Outcome Variable

OBESE_ STATUS	N Obs	Variable	N	Mean	Std Dev	Minimum	Maximum
NON-O/O	14	Observed	14	73.00	13.93	38.00	96.00
		Chao1	14	73.24	14.35	38.00	99.00
		Shannon	14	3.20	0.30	2.51	3.73
		InvSimpson	14	14.67	5.78	6.40	27.11
		FB ratio	13	31.25	56.25	1.88	209.21
		Firmicutes	14	0.71	0.12	0.45	0.86
		SCFA10K	14	0.52	0.22	0.18	0.86
O/O	13	Observed	13	75.23	13.85	55.00	107.00
		Chao1	13	75.35	14.14	55.00	108.50
		Shannon	13	3.30	0.36	2.72	3.74
		InvSimpson	13	17.24	8.22	7.07	29.15
		FB ratio	13	99.54	264.52	2.08	970.08
		Firmicutes	13	0.69	0.11	0.47	0.85
		SCFA10K	13	0.63	0.25	0.16	1.16

Hypothesis 1: **Overweight/obese (o/o) children will have lower alpha-1 diversity (Shannon index) than non-overweight/obese children.** No significant differences in alpha-1 diversity exist between non-o/o (3.20 ± 0.30) and o/o children (3.30 ± 0.36) with $p = 0.45$.

Hypothesis 2: **Overweight/obese children will have a higher F: B ratio compared with non-o/o school children.** The F: B ratio in the o/o group was significantly increased (99.54 ±264.52) compared with non-o/o children (31.25 ± 56.25) but not statistically significant at $p = 0.38$.

Hypothesis 3: **Overweight/obese children will have a higher relative abundance of Firmicutes compared with non-overweight/obese children.** Overweight/obese children did not

have a higher relative abundance of Firmicutes than non-o/o children. There were no significant differences between non-o/o (0.71 ± 0.12) and o/o (0.69 ± 0.11) with $p = 0.59$.

Hypothesis 4: Overweight/obese children will have an increased relative abundance of gut microbiota producing SCFA compared with non-overweight/obese children. There were differences in gut microbiota-producing SCFA between non-o/o and o/o children ($p = 0.25$). Overweight/obese children had an increased gut microbiota-producing SCFA (0.63 ± 0.25) compared with the non-o/o children (0.52 ± 022); however, it was not significant ($p = 0.25$).

Specific Aim 2: To compare three 24-hour diet recalls of higher energy and saturated fat, dietary intakes, and diversity of fiber-rich foods, measured by daily gut microbiota friendliness score (DGMFS) between overweight/obese and non-overweight/obese children.

Hypothesis 4*:* Obese children have higher energy and saturated fat dietary intakes compared with non-obese children. However, o/o children consumed more calories (2021.46 ± 570.83) than non-o/o children (1744 ± (415.37); it was not significant at p = 0.16). The overweight/obese children also ate more foods rich in saturated fats (32.69 ± 21.01) than non-o/o children (24.07 ± 6.26), but it was insignificant at p = 0.18.

Hypothesis 5. Overweight/obese children have a lower diversity of fiber-rich foods than non-obese school children as measured by the Daily Gut Microbiota Friendliness Score (DGMFS). Overweight/obese children had a higher diversity of fiber-rich foods (1.8 ± 1.77) than non-o/o children (1.39 ± 0.82). The differences were not significant ($p = 0.46$).

Specific Aim 3: To evaluate the association of BMI with differences in gut microbiota composition obtained using 16S rRNA gene sequencing between obese and non-obese children.

Hypothesis 6*:* A higher BMI z-score is associated with a relative abundance of bacteria in the phylum Firmicutes. Table 5.7 shows the regression analysis of BMI z-score and relative abundance of Firmicutes. When controlling for age and DGMFS, a higher BMI z-score was not associated with a relative abundance of Firmicutes ($p = 0.47$). The correlation of determination (r^2), Figure 5.8, shows no significant relationship between BMI z-score and Firmicutes, and BMI z-score was not a good predictor of the relative abundance of Firmicutes.

DISCUSSION

Childhood obesity rates have continued to climb and could be attributed to the COVID-19 pandemic. As of 2023, 1 in 5 children in the U. S. is obese (CDC, 2023). From 2017 to 2020, the childhood prevalence rate was 19.7% (CDC, 2022). Obesity prevalence among 2 – 5-year-olds was 12.7%, 20.7% among 6 – 11-year-olds, and 22.2% among 12 – 19-year-olds (CDC, 2022). Among ethnic/racial populations, Hispanic children have the highest obesity prevalence at 25.2%, followed by non-Hispanic Black children at 24.8%, 16.9% among non-Hispanic White children, and 9% among non-Hispanic Asian children (CDC, 2022). The increasing rates of childhood obesity put children and adolescents at high risk for adverse health conditions. Childhood obesity is a severe problem in the United States and worldwide.

A growing body of evidence in gut microbiota research suggests that alterations in gut microbiota composition may play a role in the development of childhood obesity. Gut microbiota manipulation should be considered an intervention strategy to control the pervasiveness of childhood obesity.

Overview of Findings

There were no significant differences in alpha diversity at the phylum level between non-o/o and o/o groups. This suggests that obesity could not be determined as a causality of an alteration or dysbiosis in the gut microbiota among the o/o children in this study. This result aligns with previous research that showed no differences in alpha diversity between lean and obese children (Hollister et al., 2018; Houtman et al., 2022; Shin & Cho, 2020; Squillario et al., 2023). In addition, a significant inverse relationship existed between the F: B ratio and alpha diversity among the non-o/o and o/o groups.

The study's results provided limited evidence to support the F:B ratio as a biomarker for obesity. Numerous earlier studies (Bervoets et al., 2013; Ley et al., 2006; Riva et al., 2017; Tilg & Adolph, 2014; Turnbaugh et al., 2006 & 2008) and current studies (Alcazar et al., 2022; Cho, 2023; DaSilva et al., 2020; Shin & Cho, 2020) have implicated an increased F:B ratio as a biomarker for obesity and BMI. The findings of this study are in agreement with previous studies regarding the lack of association between the F: B ratio and obesity and, therefore, BMI (Chen et al.,2020; Hollister et al.,2018; Houtman et al., 2022; López-Contreras et al.,2018; Squillario et al., 2023).

Several observational studies suggest a positive association with obesity and SCFA. Randomized controlled trials of microbe-accessible foods versus the Western dietary pattern have shown increased intake of microbe-accessible foods to stimulate increases in SCFA levels while reducing excess body fat (Corbin et al., 2023; Seethaler et al., 2022; Zhao et al., 2018). Previous pediatric studies showed elevated levels of fecal concentration of SCFA among obese children compared with lean children (Kim et al., 2019; Payne et al., 2011; Pekmez et al., 2018). Since this study did not assess SCFA concentrations in stools or plasma, we could not replicate those results. However, in theory, our innovative SCFA-generating gut microbe variable, which represents the sum of relative abundances of SCFA-generating phyla, should be correlated to SCFA concentrations in stool and plasma. This study showed that SCFA-generating gut microbes were increased in o/o children compared with non-o/o children; however, it did not reach statistical significance. The study's results agree with previous research regarding higher fecal concentrations of SCFA in obese children. The study's results also contradict previous pediatric research showing decreased fecal concentrations of SCFA in o/o children (Barczyńska

et al., 2018; Holmes et al., 2020). Significantly, the Pearson correlation showed a positive correlation between SCFA-generating gut microbes and alpha diversity.

The study showed that the daily gut microbiota friendliness score (DGMFS) was not statistically significant when comparing dietary intakes of high-energy, saturated fats, and fiber-rich foods. There was a statistically non-significant trend for o/o children to consume more total grams of fiber-rich foods than non-o/o children. When adjusted for total daily calorie intake, compared with non-o/o children, the o/o group tended to adhere better to the Federal 14g fiber per 1000 kilocalories per day recommendation. This may account for the increased relative abundance of Prevotella in the o/o group, which, according to some studies, is enriched in vegetarian diets (Nakayama et al.,2017; Tomova et al., 2019). However, their findings are inconsistent with a previous study by Dong et al. (2022), which showed an abundance of Prevotella and Bacteroides in obese participants with a distinct brain-gut microbiome connection to the brain's reward center. Nakayama et al. (2017) demonstrated that Prevotella might be vital in the healthful response to less caloric, sugary, and low animal protein and fat diets. Further research needs to be established to clarify the role of Prevotella in determining its association with obesity and healthy weight status. Although the o/o participants in this study consumed slightly more foods rich in saturated fats than the non-o/o participants, it was insignificant.

The study's results did not show an association between BMI z-score and relative abundance of Firmicutes. In addition, BMI z-scores did not correlate with the alpha diversity of participants' gut microbiota composition. In addition, we did not find a significant relationship between BMI z-score and SCFA-generating gut microbes.

Study Limitations

There were significant differences in age, gender, and racial/ethnicity between groups, and a homogenous sampling was not achieved. Due to our small sample size, we could not generate significant associations among the different variables between the two groups. A larger sample may have shown significant differences in gut microbiota results. The target number of participants to recruit was not reached due to difficulties in recruitment. Factors that could have affected recruitment include: 1) the time and effort required to participate in the study, which others may have found inconvenient; 2) the recruitment sites did not have a big pool of o/o children to recruit from; more non-o/o children wanted to participate in the study, 3) direct recruitment of potential study participants was highly challenging due to privacy concerns and cautionary measures needed to take so that potential participants do not feel pressured to participate, and 4) lack of funding.

The diet collection from the three 24-hour dietary recalls may not be accurate due to issues such as overreporting of healthy foods eaten, underreporting of unhealthy foods eaten, memory recall leading to forgotten items, items not consumed, and the unavailability of foods eaten on the diet survey.

Implications to Nursing Practice

The increasing incidence of obesity is staggering and seems unstoppable. As obesity continues to be a significant national and global health threat, so does the increased risk for a variety of diseases, including cardiovascular diseases, diabetes, and cancer, as well as increasing evidence of an association between obesity and gut microbiota. Managing gut microbiota has been proposed as a new method for treating obesity. Agonists targeting the GLP-1 receptor have successfully decreased excess weight in patients. These efficacious treatments offer optimism for

youth and a potential decrease in obesity rates. Studies support the idea that school meal programs can effectively expose children to healthier food options, which is a critical step in addressing childhood obesity, potentially helping to lower obesity rates.

Knowledge gained from the study will provide insights into nursing practice by exploring the complex gut microbiota ecosystem of non-obese and obese children for the long-term prevention and treatment of obesity. In addition, knowledge gained from this study could be used to develop childhood obesity prevention programs that target the gut microbial ecosystem to improve health and prevent the tracking of childhood obesity into adult obesity.

It creates a window of opportunity for nurses and healthcare providers to empower mothers of youth with obesity in the adoption of healthier lifestyle behaviors that may impact some of the childhood type-2 diabetes risks associated with obesity. In addition, nurses can provide valuable pre– and post-natal dietary and lifestyle recommendations to mothers on improving their baby's gut health, such as encouraging breastfeeding and healthier eating and exercise during the breastfeeding period to increase the quality of the breastmilk (Harris et al., 2020; Lagstrom et al., 2020). Furthermore, the period of childhood may provide opportunities for pediatric microbiome interventions through research that could have broad implications to promote health or prevent diseases. Since childhood obesity has been associated with alterations in the composition and diversity of gut microbiota, the results of this study will help establish a baseline understanding of the pediatric gut microbiome that will inform future intervention studies, targeting the gut microbiota for the prevention and treatment of childhood obesity and obesity-related diseases.

The outcomes of this study provide a window of opportunity for pediatric nurses to adopt an ecological approach to treating obesity by including parents of children with obesity in

obesity prevention efforts. Obesity prevention efforts should be firmly established in schools. School nurses recognize the positive impact of healthy eating and physical activity on academic success and are essential in promoting a culture of health and well-being for all students. In addition, school nurses play an indispensable role in affecting policy changes to improve the health of students and the communities in which they live (National Association of School Nurses, 2015). The American Academy of Pediatrics Council on School Health (2016) recognizes the vital role of school nurses in the continuum of care of children and adolescents. It states that a school nurse's daily presence may contribute to reducing childhood obesity.

School nurses have the knowledge, skills, and expertise to promote the prevention and reduction of overweight and obesity in school children and adolescents. Relevant obesity information from this study can empower school nurses to promote and implement obesity school-based strategies such as identifying, assessing, referral, and follow-up with children and adolescents who are at risk of health problems associated with overweight or obesity, promoting healthy messages to encourage consumption of microbe-friendly healthy foods, engage in daily physical activity, as well as educating students, parents, and school communities (especially school Food Services) about healthy lifestyle behaviors, daily physical requirements, and preventable health risks associated with obesity.

Future Research

Childhood provides a unique opportunity for obesity interventions to prevent tracking obesity into adulthood. The results of this study suggest no significant associations between obesity and alpha-diversity, F: B ratio, SCFA-generating gut microbes, and BMI z-score. Due to the scarcity of pediatric studies regarding gut microbiota and obesity, more studies are needed to

elucidate gut microbiota as a novel method for the prevention and treatment of not just obesity but other health conditions as well, such as diabetes, Crohn's Disease, asthma, and even autism.

Future pediatric studies will involve larger samples to determine whether an altered gut microbiota is caused by obesity or vice versa. A cohort or longitudinal study will be ideal for conducting in the future to improve understanding of the dynamic processes involved in human gut microbiota, identify changes over time, and gather insights into the cause-and-effect relationships between gut microbiota and obesity and other health conditions such as diabetes.

Future pediatric studies are needed to understand the various structures and functions of gut microbiota in children to design dietary intervention studies that may eventually translate into nutritional recommendations for children and adults.

Since children spend most of their time at school, this is an opportunity for school nurses to adopt relevant obesity information from this study to promote and implement obesity school-based strategies. Such school-based strategies include: 1) identifying, assessing, referral, and follow-up with children and adolescents who are at risk of health problems associated with overweight or obesity, 2) promoting healthy messages through health class teaching, nutritional flyers, and brochures to encourage consumption of healthy foods, daily physical activity, and 3) educate students, parents, and school communities about healthy lifestyle behaviors, daily physical requirements, and preventable health risks associated with obesity.

Conclusion

Although this study was not able to determine significant associations between obesity and gut microbiota composition, specifically Firmicutes and Bacteroidetes, BMI z-score, SCFA-generating gut phyla, and impacts of higher energy, saturated fats, and fiber-rich dietary intakes, it supports the need for novel therapies for the treatment and prevention of obesity and other

obesity-related health conditions. Obesity rates have tremendously skyrocketed since the COVID-19 pandemic, and it is a grave health concern to reckon with in the national and global arena. Gut microbiota intervention studies have successfully shown beneficial effects in weight loss. Gut microbiota interventions via dietary, physical activity, or transplantation manipulations present promising pathways by which obesity and obesity-related health problems can be treated and prevented.

Appendix A. Confidential Subject Data Abstraction Form

1. Date: MM/DD/YY _____
2. Group:
    a. Obese = 0 - _____
    b. Lean =1 - _____
3. Demographic Information
    a. DOB: MM/DD/YY _____
    b. Gender:
        i. Male = 0 - _____
        ii. F = 1- _____
    c. Ethnicity:
        i. Caucasian (non-Hispanic) = 0 - _____
        ii. Hispanic/Latino = 1 - _____
        iii. Asian/Pacific Island = 2 - _____
        iv. African American = 3 - _____
        v. Middle Eastern = 4 - _____
        vi. Other = 5 _____
4. Mode of delivery in childbirth:
    a. Vaginal = 0 _____
    b. Caesarian = 1_____
5. History of early feeding:
    a. Breastfed = 0 _____
    b. Formula-fed = 1 _____
6. Anthropometric Measures:
    a. Weight (lbs.): _____
    b. Height (in.): _____
    c. BMI percentile (%): _____
    d. BMI z-score: _____
7. Parent Educational Status:
    a. High School Graduate = 0 - _____
    b. Some college = 1- _____
    c. College Graduate = 2 - _____
    d. Advanced degrees = 3 - _____
8. Annual Household income:
    a. < $20,000 = 0 - _____
    b. $20,000 - $30,000 = 1 - _____
    c. $31,000 - $60, 000 = 2 - _____
    d. $61,000 - $75,000 = 3 - _____
    e. < $75,000 = 4 - _____

Appendix B: Email Recruitment Script

Association of Gut Microbiota Composition with Childhood Obesity

WRITTEN SCRIPT TO SEND BY MAIL OR EMAIL TO PARENTS OF POTENTIAL PARTICIPANTS

Dear [Parent of Participant],

My name is Cecille Marie Basilio, and I am a doctoral candidate in the School of Nursing at the University of California Los Angeles. I am conducting a research study examining the association of gut microbiota composition with childhood obesity, and I am the Principal Investigator. Your child is invited to participate in the study.

Participating in the study will involve the data collection of socio-demographic information (i.e., age, gender, race/ethnicity), birth and infant feeding history, height and weight for BMI calculation, dietary information, and a one-time collection of a stool specimen. A sterile stool collection kit and gloves will be provided to study participants for convenience. The collection of socio-demographic information is anticipated to take at most 15 minutes to complete. Measuring your child's height and weight at the school will take at most 10 minutes. Dietary information will be collected by three non-consecutive 24-hour dietary recalls (two weekdays and one weekend) that I will administer by telephone interview. Each 24-hour dietary recall will take approximately less than 30 minutes. The study will take about one hour and 45 minutes of your time. Appreciating your time and willingness, you will receive a $50.00 Visa gift card after all study measures. One $25 Visa gift card will be shown after socio-demographic, height, and weight information are taken; a second $25 Visa gift card will be given after stool collection and collection of the three 24-hour dietary recalls.

Your answers will be confidential. No one will know the answers except for me. If your child is eligible and wants to participate in the study, your answers will be kept with the research records. Your answers will be destroyed if your child does not qualify for the study. Participation in this study is voluntary. Your child's identity as a participant will remain confidential during and after the study. No student identifiers will be collected; I will assign each study participant a number that cannot be traced back to the participant. For instance, Participant One will be given the number 200, and Participant Two will be assigned the number 201. These numbers will not be correlated with any student identifier, such as name, school health record, or birth date.

If you have questions or want your child to participate, please email me at cbasilio1114@g.ucla.edu or call me at (310) 770 – 3653.

Thank you for your participation,

Cecille Marie Basilio, MSN, RN, PHN
Doctoral Candidate
School of Nursing, UCLA

Appendix C: Telephone Recruitment Script

Finding the Gut Microbiome Connection to the Risk of Childhood Obesity

---

SCRIPT FOR INTRODUCING THE STUDY TO POTENTIAL PARTICIPANTS OR PARENTS OF POTENTIAL PARTICIPANTS AND INITIAL SCREENING THROUGH THE TELEPHONE

The following script would respond to the participant's interest in participating in the study via telephone. They will be speaking with the Principal Investigator from the school of nursing.

Thank you for calling about the study "Finding the Gut Microbiome Connection to the Risk of Childhood Obesity." My name is Cecille Basilio, and I am the Principal Investigator. I need to ask you a few questions to determine whether your child can participate in the research. Before I begin, let me tell you about the study.

The study's purpose is to learn about the association of gut microbiome composition with childhood obesity, which is considered one of the significant public health concerns in the nation. The gut microbiome plays a critical role in our health by helping control digestion, benefiting our immune system, and affecting many other aspects of health. An imbalance of unhealthy and healthy microbes in the intestines may contribute to weight gain, high blood sugar, high cholesterol, and other disorders.

Would you like to continue with the screening to determine if your child is eligible? The screening will take about 5 minutes or less. Your participation in the screening is voluntary. A decision on whether or not to participate in the screening will not affect your relationship with LAUSD. You will not benefit from the screening.

Your answers will be confidential. No one will know the answers except for the research team.

If your child is eligible and wants to participate in the study, your answers will be kept confidential, along with the research and other records. Your answers will be destroyed if you or your child do not qualify for the study.

Would you like to continue with the screening?

[*If no, thank the person and hang up*].

[*If yes, continue with the screening*

Does your child have a diagnosis of Type 1 or Type 2 diabetes? (Yes/No)

Has your child been treated with antibiotics or steroids in the past month? (Yes/No)

Has your child been hospitalized in the past month? (Yes/No)

Does your child have any chronic disorders? (Yes/No)

Thank you for answering the screening questions.

[Indicate whether the child is eligible or is not suitable and explain why.]

If the potential subject meets the study criteria

I have a copy of the consent form that I can mail/email you or give at the school when you drop off/pick up your child. I can also read the consent form for you now over the telephone. Please feel free to let me know if you have any questions that I can answer for you.

If you agree to participate, please mail the signed consent form in the pre-stamped envelope. You can also give me consent if you decide to participate in the study today (the day of the school visit). After I have received your signed informed consent, your child's participation in the study will begin.

Participation in the study will involve you completing a socio-demographic questionnaire (i.e., age, gender, race/ethnicity, birth, and early feeding history), height and weight measurement for BMI calculation), collection of three non-consecutive 24-hour dietary recalls administered by phone and a one-time collection of a stool specimen that will be done in the privacy of your home. You will be provided with a sterile stool collection kit for your convenience. The study will require approximately 1 hour and 45 minutes of your time. Appreciating your time and willingness, you will receive a $50.00 Visa gift card after all study measures. One $25 Visa gift card will be given after socio-demographic, height, and weight information are taken; a second $25 Visa gift card will be provided after the stool collection and collection of the three 24-hour dietary recalls.

Do you have any questions about the screening or research? I will give you a couple of telephone numbers to call if you have any questions later. Do you have a pen? If you have questions about the research screening, contact me at 213-640-6190, and I will answer them.

If you have questions regarding the rights of research subjects or if you have complaints or concerns about the research and cannot reach the Principal Investigator or want to talk to someone other than the Investigator, you may call the UCLA Office of the Human Research Protection Program at (310) 825-7122.

Appendix D: Consent

University of California, Los Angeles

CONSENT TO PARTICIPATE IN RESEARCH

Finding the Gut Microbiome Connection to the Risk of Childhood Obesity through an Examination of the Gut Microbiota among School Children in Los Angeles, California

INTRODUCTION

Cecille Marie Basilio, MSN, RN, PHN, and Felicia S. Hodge, DrPH, from the School of Nursing at the University of California, Los Angeles (UCLA) are conducting a research study. You and your child were selected as possible participants in this study because your child is between the ages of 5 - 10 years, has a BMI percentile = 5^{th}, $\geq 85^{th}$ or $\geq 95\%$, has no type 1 or type 2 diabetes and has not been hospitalized or been treated with antibiotics or corticosteroids in the past month before the study. Your participation in this research study is voluntary. Please read the information below and ask questions about anything you do not understand before deciding whether to participate in the study.

WHAT SHOULD I KNOW ABOUT A RESEARCH STUDY?

- Someone will explain this research study to you.
- Whether or not you take part is up to you.
- You can choose not to take part.
- You can agree to take part and later change your mind.
- Your decision will not be held against you.
- You can ask all the questions you want before you decide.

WHY IS THIS RESEARCH BEING DONE?

The study aims to examine whether there are differences in the gut microbiota composition of obese and non-obese schoolchildren and whether diet and dietary habits affect the gut microbiota composition of participants.

HOW LONG WILL THE RESEARCH LAST, AND WHAT WILL I NEED TO DO?

Participation will take about one hour and forty minutes. Measuring height and weight at school will take about 10 minutes. The collection of dietary information will take about 90 minutes, with each 24-hour dietary recall taking approximately 30 minutes or less.

If you volunteer to participate in this study, the researcher will ask you to do the following:

- Information will be collected about your child's birth date, gender, race/ethnicity, mode of delivery (vaginal or C-section), infant's early feeding history (breast milk or formula), educational status, and annual income.

- Your child's height and weight will be measured at the school to calculate their body mass index. This procedure will take approximately ≤ 10 minutes.
- The Principal Investigator will collect three non-consecutive 24-hour dietary recalls (two weekdays and one weekend). Each 24-hour dietary recall will take about 30 minutes or less.

- Within the weekend after the third and last 24-hour dietary recall, the parent will collect a stool sample from your child in the convenience of your home. You will be provided with a stool collection kit to collect a stool specimen. This will take about 15 minutes.

ARE THERE ANY RISKS IF I PARTICIPATE?
There is a minimal risk to participants in this study. The potential risk to this study will be a breach of confidentiality. Overall, no additional risks are associated with participation in the study.

ARE THERE ANY BENEFITS IF I PARTICIPATE?
There is no direct benefit to school children participating in the study. The potential benefit to society is that we will learn about the association of gut microbiome composition with childhood obesity and how diet and dietary patterns affect gut microbiome composition. The increased knowledge gained from this study may lead to the development of a new treatment or therapy or school nutrition policies to control or minimize the risk of childhood obesity, with the gut microbiota as the target of intervention. The risk/benefit ratio is favorable for this study, and adverse events are not anticipated.

ALTERNATIVES TO PARTICIPATION
This is not a treatment study. You may choose not to be part of the study.

HOW WILL INFORMATION ABOUT ME AND MY PARTICIPATION BE KEPT CONFIDENTIAL?
Members of the research team and, if appropriate, your school nurses will know you are participating in the research study. All results will be kept confidential but may be available if you wish. No information about you or provided by you during the research will be disclosed to others without your written permission, except:
- if necessary, to protect your rights or
- if required by law (i.e., child or elder abuse, harm to self or others, reports of certain infectious diseases).

No information revealing your identity will be included when the research results are published or discussed at conferences. Authorized representatives of the UCLA Institutional Review Board (IRB) may need to review records of individual subjects. As a result, they may see your name, but they are bound by confidentiality rules not to reveal your identity to others.

The researchers will do their best to keep your private information confidential. Information about you will be handled as confidentially as possible, but participating in research may involve

a loss of privacy and the potential for a breach of confidentiality. Study data will be physically and electronically secured. Data security breaches are risky when using electronic means to store data.

Use of personal information that can identify you:
There will be no personal information that can identify subjects. No student identifiers will be collected; the PI will assign each subject a number that cannot be traced back to the specific subject. The subject numbering system will start with 100. Each subsequent subject will be given more than the previous patient number. For instance, subject one will be assigned the number 200, and subject two will be given the number 201. These numbers will not be correlated with any student identifier, such as name, school health record, or birth date.

How information about you will be stored:
Immediately following data collection, the PI will place the completed confidential subject information report form in an envelope, and the results will not be disclosed. The PI will maintain the envelope containing the confidential subject information form in a secure, locked cabinet until it is reviewed and entered into the statistical analysis program.

People and agencies that will have access to your information:
The research team and authorized UCLA personnel may have access to study data and records to monitor the study. Research records provided to allowed non-UCLA personnel will not contain identifiable information about you. Publications and presentations from this study will not identify you by name.

Employees of the University may have access to identifiable information as part of routine processing of your data, such as lab work or processing payment. However, university employees are bound by strict confidentiality rules.

USE OF DATA FOR FUTURE RESEARCH:
Your data, including de-identified data, may be kept for use in future research

WILL I BE PAID FOR MY PARTICIPATION?

- In appreciation for your time and willingness to allow your child to participate in the study, you will receive a $50 Visa gift card at the end of the study.
- If your child cannot complete the study, you will receive a $25 Visa gift card if half of the study is finished.
- You will be asked to sign a receipt for the payment form. If you choose to withdraw from the study and not complete all required observations, you will be paid for the extent to which your child could be part of the study.

FINANCIAL OBLIGATION:

You are not responsible for any of the costs involved in the study.

WHO CAN I CONTACT IF I HAVE QUESTIONS ABOUT THIS STUDY?
The research team:

You can talk to the researchers if you have any questions, comments, or concerns about the research. Please contact:
Principal Investigator: Cecille Marie Basilio, MSN, RN, PHN
Phone: 310-770-3653
Email: cbasilio1114@g.ucla.edu
Faculty Sponsor: Felicia S. Hodge, DrPH
Phone: 310-267-2265
Email: fhodge@sonnet.ucla.edu

UCLA Office of the Human Research Protection Program (OHRPP):
If you have questions about your rights as a research subject, or you have concerns or suggestions, and you want to talk to someone other than the researchers, you may contact the UCLA OHRPP by phone: (310) 206-2040; by email: participants@research.ucla.edu or by mail: Box 951406, Los Angeles, CA 90095-1406.

WHAT ARE MY RIGHTS IF I TAKE PART IN THIS STUDY?

- You can choose whether or not to participate in this study, and you may withdraw your consent and discontinue participation at any time.
- Whatever decision you make, you will not be penalized and will not lose any benefits to which you were otherwise entitled.
- You may refuse to answer any questions you do not want to answer and remain in the study.

You will be given a copy of this information for your records.

HOW DO I INDICATE MY AGREEMENT TO PARTICIPATE?

You should sign and provide the date below to participate in this study.

SIGNATURE OF THE PARTICIPANT

_____
Name of Participant

_____ _____
Signature of Participant Date

SIGNATURE OF PERSON OBTAINING CONSENT

_____ _____
Name of Person Obtaining Consent Contact Number

_____ _____
Signature of Person Obtaining Consent Date

Appendix E. Screening Form

Date Screening Started _____

Date Screening Completed _____

Inclusion / Exclusion	**Inclusion Criteria Met (Yes or No)**	**Exclusion Criteria Met (Yes or No)**	**Information obtained from Parent / health record**	**Initials of Screener**
Child is between 5 -10 years old				
BMI = 5th percentile or < 85th percentile				
BMI ≥ 85th percentile				
BMI ≥ 95th percentile				
Has no history or current diagnosis of type 1 or type 2 diabetes				
No antibiotics or corticosteroid treatment one month prior to the study and during the study				
No significant comorbidities such as acute infection or chronic disease				
No hospitalization in the last month prior to the study				
No significant comorbidities such as acute infection or chronic disease				

_____ _____

IRB Approved Screener (print name) IRB Approved Screener (signature)

Appendix F. Study Information Sheet

Finding the Gut Microbiome Connection to the Risk of Childhood Obesity Through the Examination of Microbiota Composition Among School Children in Los Angeles

Project Summary:
The overall goal is to determine whether an altered gut microbiota composition is associated with childhood obesity and whether dietary factors influence an altered gut microbiota composition.

Specific Aims:
1) Evaluate the impact of child obesity status on three related outcomes: gut microbiota composition, Firmicutes: Bacteroidetes (F:B) ratio, and abundance of primary short-chain fatty acids producing gut microbes between obese and non-obese school children.
2) Compare the dietary habits and intakes between obese and non-obese school children; and
3) Evaluate the association of Body Mass Index differences with gut microbiota composition in obese and non-obese schoolchildren.

Study Design:
This pilot study will use a correlational, cross-sectional design.

Subjects:
21 Overweight or obese children
21 Lean children

Inclusion/Exclusion Criteria:
1) children aged 5 – 10 years
2) English speaking
3) no history or current diagnosis of type 1 or type 2 diabetes
4) children with BMI ≥ 85th percentile for same age and gender
5) children with BMI ≥ 95th percentile for same age and gender
6) children with BMI = 5^{th} percentile or < 85^{th} percentile for same age and gender
7) No corticosteroid and antibiotic use in the last month before the study
8) No hospitalization in the last month before study and during the study
9) No significant comorbidities such as acute infection or chronic disease

Subject Payment: $50 Visa gift card upon completion of all study measures

If you have questions or potential subjects to refer to, please contact:
Cecille Marie Basilio, MSN, RN, PHN
Doctoral Candidate, UCLA, and School RN, LAUSD
Phone: 310-770-3653 Email: cbasilio1114@g.ucla.edu

Appendix G. Child Assent

UNIVERSITY OF CALIFORNIA LOS ANGELES

ASSENT TO PARTICIPATE IN RESEARCH

Finding the Gut Microbiome Connection to the Risk of Childhood Obesity through an Examination of the Gut Microbiota among School Children in Los Angeles, California

1. My name is Cecille Marie Basilio.

2. We are asking you to participate in a research study because we are trying to learn more about how the bacteria in your stomach affect your weight and health and how the foods you eat affect these bacteria in your gut.

3. Three things will happen if you agree to participate in this study. First, I will take your height and weight at school. Second, I will call your parents on three different days to tell them the foods you ate on those days. The last thing will be for your mom or dad to get a bit of your stool after you do number two because that is how we can study the bacteria in your stomach.

4. You have a small risk if you participate in the study. The small risk to you will be a breach of confidentiality, which means telling someone information we have collected about you. When you participate in the study, we will try not to break that confidentiality. So, we have security plans in place to protect your privacy. For example, your identity will not be directly connected to the data we collect about you because we will not use your name or other information to identify you. Instead, we will assign you an identification number as a study participant. All the information we collect about you will remain confidential and kept in a locked file cabinet.

5. The anticipated benefits of participating in the study include increasing knowledge about how foods affect one's body and health and improving support for obesity screening and prevention.

6. Please discuss this with your parents before deciding whether to participate. We will also ask your parents to permit you to participate in this study. But even if your parents say “yes,” you can still decide not to do this.

7. If you don’t want to be in this study, you don’t have to participate. Remember, being in this study is up to you, and no one will be upset if you don’t want to participate or even if you change your mind later and want to stop. Being in this study will not affect your grades, relationship with the school, or other benefits to which you may otherwise be entitled.

8. You can ask any questions that you have about the study. If you have a question later that you didn’t think of now, call me at 310-770-3653 or ask me next time.

9. Signing your name at the bottom means agreeing to be in this study. You and your parents will be given a copy of this form after you have signed it.

_____ _____

Name of Subject Dat**e**

Appendix H. Calculation of the Daily Gut Microbiota

Friendliness Score (DGMFS) (Dr. William McCarthy)

To calculate gut microbiota-friendliness of daily food intake using ASA24 output, do the following:

Grain-friendliness = 0 to target(5 oz. max) / (5 oz) [score range from 0 to 1.0]

fruit-friendliness = 0 to target (1.5 cups max) / (1.5 cups) [score range from 0 to 1.0]

dark green vegetable friendliness = 0 to target (0.5 cups max) / (0.5 cups) [score range fr 0 to 1.0]

red-orange vegetable friendliness = 0 to target (0.5 cups max) / (0.5 cups) [score range fr 0 to 1.0]

other vegetable friendliness = 0 to target (0.5 cups max) / (0.5 cups) [score range from 0 to 1.0]

plant protein-friendliness = 0 to target (4 oz. max of plant protein only*) / (4 oz.) [score range from 0 to 1.0]

*plant protein = vegetable legumes + protein legumes + seeds + nuts

<u>Pro-inflammatory components that undermine gut microbiota friendliness of the above:</u>
Sugar-unfriendliness = eaten (g) / limit (g) [value is under 1.0 when healthy, over 1.0 when unhealthy]
Saturated fat-unfriendliness = eaten (g) /limit (g) [value is under 1.0 when healthy, over 1.0 when unhealthy]
Overall gut microbiota friendliness of food score:
(grain-friendliness + fruit-friendliness + dark green vegetable friendliness + red-orange vegetable friendliness + other vegetable friendliness + plant protein friendliness) / (sugar-unfriendliness + saturated fat-unfriendliness)

This example: ((0.8/5.0 + 0.9/1.5 + 0.0/0.5 + 0.2/0.5 + 0.0/0.5 + (0.5 + 7.6 = max 4.0)/4.0)) / ((29/35) + (39/16)) = (0.16 + 0.6 + 0.0 + 0.4 + 0.0 + 1.0) / (0.83 + 2.44) = 2.16 / 3.27 = 0.66.

Note. A daily food gut microbiota friendliness score of 0.66 is not impressive and is likely to be associated with obesity.

REFERENCES

Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., & Versalovic, J. (2014). The placenta harbors a unique microbiome. *Science Translational Medicine, 6*(237), 237ra265-237ra265. doi:10.1126/scitranslmed.3008599

Aagaard, K., Riehle, K., Ma, J., Segata, N., Mistretta, T-A., Coarfa, C.,...Versalovic, J. (2012). A metagenomic approach to the characterization of the vaginal microbiome signature during pregnancy. *Plos One, 7*(6), 1-15. doi:10.1371/journal.pone.0036466

Abdallah Ismail, N., Ragab, S. H., Abd Elbaky, A., Shoeib, A. R., Alhosary, Y., & Fekry, D. (2011). Frequency of firmicutes and bacteroidetes in gut microbiota in obese and normal weight Egyptian children and adults. *Arch Med Sci, 7*(3), 501-507. doi:10.5114/aoms.2011.23418

Abdullah, B., Idorus, M., Daud, S., Aazmi, S., Pillai, T., & Zain, Z. (2023). Gut Microbiota Composition in the First and Third Trimester of Pregnancy among Malay Women is Associated with Body Mass Index: A Pilot Study. *The Malaysian Journal of Medical Sciences: MJMS*, 30, 116 - 128. https://doi.org/10.21315/mjms2023.30.1.10.

Alcazar, M., Escribano, J., Ferré, N., Closa-Monasterolo, R., Selma-Royo, M., Feliu, A., . . . Balcells, E. (2022). Gut microbiota is associated with metabolic health in children with obesity. *Clinical Nutrition, 41*(8), 1680-1688. doi:https://doi.org/10.1016/j.clnu.2022.06.007

Al-Lahham, S., Roelofsen, H., Rezaee, F., Weening, D., Hoek, A., Vonk, R., & Venema, K. (2012). Propionic acid affects immune status and metabolism in adipose tissue from overweight subjects. *Eur J Clin Invest, 42*(4), 357-364. doi:10.1111/j.1365-2362.2011.02590

Aloisio, I., Mazzola, G., Corvaglia, L. T., Tonti, G., Faldella, G., Biavati, B., & Di Gioia, D. (2014). Influence of intrapartum antibiotic prophylaxis against group b streptococcus on the early newborn gut composition and evaluation of the anti-streptococcus activity of bifidobacterium strains. *Appl Microbiol Biotechnol, 98*(13), 6051-6060. doi:10.1007/s00253-014-5712-9

Aguirre, M., Jonkers, D. M., Troost, F. J., Roeselers, G., & Venema, K. (2014). In vitro characterization of the impact of different substrates on metabolite production, energy extraction, and gut microbiota composition from lean and obese subjects. *Plos One, 9*(11), e113864. doi:10.1371/journal.pone.0113864

Ajslev, T. A., Andersen, C. S., Gamborg, M., Sorensen, T. I., & Jess, T. (2011). Childhood overweight after establishment of the gut microbiota: The role of delivery mode, pre-pregnancy weight and early administration of antibiotics. *Int J Obes (Lond), 35*(4), 522-529. doi:10.1038/ijo.2011.27

Alderete, T. L., Autran, C., Brekke, B. E., Knight, R., Bode, L., Goran, M. I., & Fields, D. A. (2015). Associations between human milk oligosaccharides and infant body composition in the first 6 mo of life. Am J Clin Nutr, 102(6), 1381-1388. doi:10.3945/ajcn.115.115451.

Alsaedi, A., Alasmari, A., & Alzahrani, S. (2022). Screen time use and its health effects among children attending well baby clinic at primary health care centers, Makkah, Saudi Arabia, 2020. *International Journal of Advanced Research*. https://doi.org/10.21474/ijar01/14774.

Aloisio, I., Mazzola, G., Corvaglia, L. T., Tonti, G., Faldella, G., Biavati, B., & Di Gioia, D. (2014). Influence of intrapartum antibiotic prophylaxis against group b streptococcus on

the early newborn gut composition and evaluation of the anti-streptococcus activity of bifidobacterium strains. *Appl Microbiol Biotechnol, 98*(13), 6051-6060. doi:10.1007/s00253-014-5712-9

American Academy of Pediatrics, Council on School. (2016). Role of the school nurse in providing school health services. *Pediatrics, 137*(6). doi:10.1542/peds.2016-0852

Anderson, P. ., & Butcher, K. E. (2006). Childhood obesity: trends and potential causes. *The Future of children, 16*(1), 19–45. https://doi.org/10.1353/foc.2006.0001

An, R., Yan, H., Shi, X., & Yang, Y. (2017). Childhood obesity and school absenteeism: A systematic review and meta-analysis. *Obes Rev, 18*(12), 1412-1424. doi:10.1111/obr.12599.

Anderson, C., Martinez, C., O'Malley, K., Ritchie, L., & Whaley, S. (2022). Longer Participation in the Special Supplemental Nutrition Program for Women, Infants, and Children Is Not Associated with Reduced Sugar-Sweetened Beverage Intake among Black Participants. *Nutrients*, 14. https://doi.org/10.3390/nu14051048.

Appleton, K. M., Hemingway, A., Rajska, J., & Hartwell, H. (2018). Repeated exposure and conditioning strategies for increasing vegetable liking and intake: Systematic review and meta-analyses of the published literature. *Am J Clin Nutr, 108*(4), 842-856. doi:10.1093/ajcn/nqy143

Armstrong, J., & Reilly, J. J. (2002). Breastfeeding and lowering the risk of childhood obesity. *The Lancet, 359*(9322), 2003-2004. doi:10.1016/s0140-6736(02)08837-2

Arslan, N. (2014). Obesity, fatty liver disease and intestinal microbiota. *World J Gastroenterol, 20*(44), 16452-16463. doi:10.3748/wjg.v20.i44.16452

Asnicar, F., Berry, S. E., Valdes, A. M., Nguyen, L. H., Piccinno, G., Drew, D. A., . . . Segata, N. (2021). Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. Nat Med. doi:10.1038/s41591-020-01183-8

Au, L. E., Rosen, N. J., Fenton, K., Hecht, K., & Ritchie, L. D. (2016). Eating school lunch is associated with higher diet quality among elementary school students. *J Acad Nutr Diet, 116*(11), 1817-1824. doi:10.1016/j.jand.2016.04.010

Azad, M. B., Bridgman, S. L., Becker, A. B., & Kozyrskyj, A. L. (2014). Infant antibiotic exposure and the development of childhood overweight and central adiposity. *Int J Obes (Lond), 38*(10), 1290-1298. doi:10.1038/ijo.2014.119

Azad, M. B., Konya, T., Maughan, H., Guttman, D. S., Field, C. J., Chari, R. S., . . . Kozyrskyj, A. L. (2013). Gut microbiota of healthy Canadian infants: Profiles by mode of delivery and infant diet at four months. *Cmaj, 185*(5), 385-394. doi:10.1503/cmaj.121189

Babey, S. H., Wolstein, J., Diamant, A. L., Bloom, A. & Goldstein, H. (2012). Overweight and obesity among children by California cities - 2010. *UCLA Center for Health Policy Research and California Center for Public Health Advocacy*, 1-8.

Backhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., . . . Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A, 101*(44), 15718-15723. doi:10.1073/pnas.0407076101

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., & Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science, 307*(5717), 1915-1920. doi:10.1126/science.1104816

Baranowski, T., Domel, S., Gould, R., Baranowski, J., Leonard, S., Treiber, F., & Mullis, R. (1993). Increasing fruit and vegetable consumption among 4th and 5th grade students:

Results from focus groups using reciprocal determinism. *Journal of Nutrition Education, 25*(3), 114-120. doi:10.1016/s0022-3182(12)80567-x

Barcenilla, A., Pryde, S. E., Martin, J. C., Duncan, S. H., Stewart, C. S., Henderson, C. & Flint, H. J. (2000). Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microbiol, 66*, 1654-1661.

Barczyńska, R., Litwin, M., Sliżewska, K., Szalecki, M., Berdowska, A., Bandurska, K., . . . Kapuśniak, J. (2018). Bacterial microbiota and fatty acids in the feces of overweight and obese children. *Pol J Microbiol, 67*(3), 339-345. doi:10.21307/pjm-2018-041

Bassul, C., Corish, C. A., & Kearney, J. M. (2021). Associations between home environment, children's and parents' characteristics and children's tv screen time behavior. *Int J Environ Res Public Health, 18*(4). doi:10.3390/ijerph18041589

Beales, J., & Kulick, R. (2013). Does advertising on television cause childhood obesity? A longitudinal analysis. *Journal of Public Policy & Marketing*, *32*(2), 185-194. http://dx.doi.org/10.1509/jppm.11.051

Beck, A. R. (2016). Psychosocial Aspects of Obesity. *NASN School Nurse, 31*(1), 23-27. doi:10.1177/1942602X15619756

Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell, 157*(1), 121-41.

Beltrán-Garrayo, L., Solar, M., Blanco, M., Graell, M., & Sepúlveda, A. R. (2023). Examining associations between obesity and mental health disorders from childhood to adolescence: A case-control prospective study. *Psychiatry Research, 326*, 115296. doi:https://doi.org/10.1016/j.psychres.2023.115296

Berding, K., Holscher, H. D., Arthur, A. E., & Donovan, S. M. (2018). Fecal microbiome composition and stability in 4- to 8-year-old children is associated with dietary patterns and nutrient intake. *J Nutr Biochem, 56*, 165-174. doi:10.1016/j.jnutbio.2018.01.002

Bervoets, L., Van Hoorenbeeck, K., Kortleven, I., Van Noten, C., Hens, N., Vael, C., Goosens, H., Desager, K. N. & Vankerckhoven, V. (2013). Differences in gut microbiota composition between obese and lean children: a cross-sectional study. *Gut Pathogens*, *5*(1), 10. http://dx.doi.org/10.1186/1757-4749-5-10

Bhattacharya, J., Currie, J., & Haider, S. J. (2006). Breakfast of champions? The school breakfast program and the nutrition of children and families. *The Journal of Human Resources, 41*(3), 445-=466.

Bikel, S., López-Leal, G., Cornejo-Granados, F., Gallardo-Becerra, L., García-López, R., Sánchez, F., Equihua-Medina, E., Ochoa-Romo, J., López-Contreras, B., Canizales-Quinteros, S., Hernández-Reyna, A., Mendoza-Vargas, A., & Ochoa-Leyva, A. (2021). Gut dsDNA virome shows diversity and richness alterations associated with childhood obesity and metabolic syndrome. *iScience*, 24. https://doi.org/10.1016/j.isci.2021.102900.

Birch, L. L. (1980). Effects of peer models' food choices and eating behaviors on preschoolers' food preferences. *Child Development, 51*(2), 489-496.

Birch, L. L., & Fisher, J. A. (1995). Appetite and eating behavior in children. *Pediatric Clinics of North America, 42*(4), 931-953. doi:10.1016/s0031-3955(16)40023-4

Birch, L. L., Johnson, S. L., Andresen, G., Peters, J. C., & Schulte, M. C. (1991). The variability of young children's energy intake. *N Engl J Med, 324*(4), 232-235.

Birch, L. L., & Marlin, D. W. (1982). I don't like it; I never tried it: Effects of exposure on two-year-old children's food preferences. *Appetite, 3*(4), 353-360. doi:10.1016/s0195-6663(82)80053-6

Birch, L. L., McPhee, L., Shoba, B. C., Pirok, E., & Steinberg, L. (1987). What kind of exposure reduces children's food neophobia?: Looking vs. Tasting. *Appetite, 9*(3), 171-178. doi:https://doi.org/10.1016/S0195-6663(87)80011-9

Bleau, C., Karelis, A. D., St-Pierre, D. H., & Lamontagne, L. (2015). Crosstalk between intestinal microbiota, adipose tissue, and skeletal muscle as an early event in systemic low-grade inflammation and the development of obesity and diabetes. Diabetes Metab Res Rev, 31(6), 545-561. doi:10.1002/dmrr.2617

Block, G., Hartman, A. M., Dresser, C. M., Carroll, M. D., Gannon, J., & Gardner, L. (1986). A data-based approach to diet questionnaire design and testing. *Am J Epidemiol, 124*(3), 453-469. doi:10.1093/oxfordjournals.aje.a114416

Blundell, J. E., Burley, V. J., Cotton, J. R., & Lawton, C. L. (1993). Dietary fat and the control of energy intake: Evaluating the effects of fat on meal size and postmeal satiety. *Am J Clin Nutr, 3*(57), 772S-778S.

Blustein, J., Attina, T., Liu, M., Ryan, A. M., Cox, L. M., Blaser, M. J., & Trasande, L. (2013). Association of caesarean delivery with child adiposity from age 6 weeks to 15 years. *Int J Obes (Lond), 37*(7), 900-906. doi:10.1038/ijo.2013.49

Bodnaruc, A. M., Prud'homme, D., Blanchet, R., & Giroux, I. (2016). Nutritional modulation of endogenous glucagon-like peptide-1 secretion: A review. *Nutrition & Metabolism, 13*(1), 92. doi:10.1186/s12986-016-0153-3

Boot, A. M., Bouquet, J., de Ridder, M. J. A., Krenning, E. P., & de Muinck Keitzer-Schrama. (1997). Determinants of body composition measured by dual-energy x-ray absorptiometry in Dutch children and adolescents. *Am J Clin Nutr, 66,* 232-238.

Bouchard, C., Pérusse, L., Leblanc, C., Tremblay, A., & Thériault, G. (1988). Inheritance of the amount and distribution of human body fat. *International journal of obesity*, *12*(3), 205–215.

Bouchard, C. (1997). Genetic determinants of regional fat distribution. *Human Reproduction, 12*, 1-5.

Braet, C., O'Malley, G., Weghuber, D., Vania, A., Erhardt, É., & Nowicka, P. et al. (2014). The assessment of eating behaviour in children who are obese: A psychological approach. A position paper from the European childhood obesity group. *Obesity Facts*, *7*(3), 153-164. http://dx.doi.org/10.1159/000362391

Börnhorst, C., Siani, A., Tornaritis, M., Molnár, D., Lissner, L., Regber, S., . . . Pigeot, I. (2016). Potential selection effects when estimating associations between the infancy peak or adiposity rebound and later body mass index in children. *International Journal of Obesity, 41*(4), 518-526. doi:10.1038/ijo.2016.218

Bouter, K. E., van Raalte, D. H., Groen, A. K., & Nieuwdorp, M. (2017). Role of the gut microbiome in the pathogenesis of obesity and obesity-related metabolic dysfunction. *Gastroenterology, 152*(7), 1671-1678. doi:10.1053/j.gastro.2016.12.048

Bowers, D. E. (2000). Cooking trends echo changing roles of women. *Food Review, 23*(1-4), 23-29.

Bronfenbrenner, U. (1974). Developmental research, public policy, and the ecology of

childhood. *Child Development, 45*(1), 1-5. Retrieved from https://www.jstor.org/stable/1127743?seq=1#metadata_info_tab_contents

Bronfenbrenner, U. (1975). Reality and research in the ecology of human development. *Proceedings of the American Philosophical Society, 119*, 439–469.

Bronfenbrenner, U. (1978). The social role of the child in ecological perspective. *Zeitschrift fur Soziologie, 7*(1), 4-20.

Bronfenbrenner, U. (1979). The ecology of human development: Experiments in nature and design. Cambridge, MA: Harvard University Press.

Bronfenbrenner, U. (1986a). Ecology of the family as a context for human development: Research perspectives. *Developmental Psychology, 22*(6), 723-742.

Bronfenbrenner, U. (1986b). Recent advances in research on the ecology of human development. In R. K. Silbereisen, K. Eyferth, & G. Rudinger (Eds.), Development as action in context: Problem behavior and normal youth development (pp. 287-309). Heidelberg and New York: Springer-Verlag.

Bronfenbrenner, U. (1988). Interacting systems in human development. Research paradigms: Present and future. In Bolger, N., Caspi, A., Downey, G., & Moorehouse, M. (Eds.), *Persons in contexts: Developmental processes* (pp. 25-49). Cambridge, UK: Cambridge University Press.

Bronfenbrenner, U. & Morris, P. A. (1998). The ecology of developmental processes. In Damon, W., & Eisenberg, N. (Eds.), *The handbook of child psychology* (pp. 993-1027). New York, NY: John Wiley & Sons. Retrieved from http://www5.galib.uga.edu/reserves/docs/scanner%20pc%20shelter/ill%20scans/2_14_2019/bronfenbrenner_the_ecology_of_developmental_processes.p

Brown, T., Moore, T. H., Hooper, L., Gao, Y., Zayegh, A., Ijaz, S., . . . Summerbell, C. D. (2019). Interventions for preventing obesity in children. *Cochrane Database Syst Rev, 7*(7), Cd001871. doi:10.1002/14651858.CD001871.pub4

Browne, N., Hodges, E., Small, L., Snethen, J., Frenn, M., Irving, S., Gance-Cleveland, B., & Greenberg, C. (2021). Childhood obesity within the lens of racism. *Pediatric Obesity*, 17. https://doi.org/10.1111/ijpo.12878.

Buckley, J. D., & Howe, P. R. C. (2010). Long-chain omega-3 polyunsaturated fatty acids may be beneficial for reducing obesity-a review. *Nutrients, 2*(12), 1212-1230. doi:10.3390/nu2121212

Bull, M. J., & Plummer, N. T. (2014). Part 1: The human gut microbiome in health and disease. *Integr Med (Encinitas), 13*(6), 17-22.

California Department of Education (CDE), (2019). Largest & smallest public-school districts – CalEdFacts. Retrieved from https://www.cde.ca.gov/ds/sd/cb/ceflargesmalldist.asp

California Department of Education (CDE). (2020). PFT statewide research files.. Retrieved from https://www.cde.ca.gov/ta/tg/pf/pftresearch.asp

Canfora, E. E., Jocken, J. W., & Blaak, E. E. (2015). Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol, 11*(10), 577-591. doi:10.1038/nrendo.2015.128

Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., . . . Burcelin, R. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes, 56*(7), 1761-1772. doi:10.2337/db06

Cani, P. D., Lecourt, E., Dewulf, E. M., Sohet, F. M., Pachikian, B. D., Naslain, D., . . . Delzenne, N. M. (2009). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *The American Journal of Clinical Nutrition, 90*(5), 1236-1243. doi:10.3945/ajcn.2009.28095

Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M., & Owen, L. J. (2015). Dysbiosis of the gut microbiota in disease. *Microbial Ecology in Health & Disease, 26*(0). doi:10.3402/mehd.v26.26191

Carmody, R. N., Gerber, G. K., Luevano, J. M., Jr., Gatti, D. M., Somes, L., Svenson, K. L., & Turnbaugh, P. J. (2015). Diet dominates host genotype in shaping the murine gut microbiota. *Cell Host Microbe, 17*(1), 72-84. doi:10.1016/j.chom.2014.11.010

Carey, F. R., Singh, G. K., Brown, H. S., 3rd, & Wilkinson, A. V. (2015). Educational outcomes associated with childhood obesity in the United States: Cross-sectional results from the 2011-2012 national survey of children's health. *Int J Behav Nutr Phys Act, 12 Suppl 1*(Suppl 1), S3. doi:10.1186/1479-5868-12-s1-s3

Centers for Disease Control and Prevention (CDC). (2018, October 15). *Whole school, whole community, whole child, a collaborative approach to learning and health*. Retrieved from https://www.cdc.gov/healthyyouth/wscc/

Centers for Disease Control and Prevention (CDC). (2022a). *Childhood obesity facts: Prevalence of childhood obesity in the United States, R*etrieved from https://www.cdc.gov/obesity/data/childhood.html - print

Centers for Disease Control and Prevention (CDC). (2022b). *Childhood obesity causes and consequences*. https://www.cdc.gov/obesity/childhood/causes.html

Centers for Disease Control and Prevention (CDC). (2023a, March 31). *Defining childhood obesity: BMI for children and teens.* https://www.cdc.gov/obesity/childhood/defining.html

Centers for Disease Control and Prevention (CDC). (2023b, December 20). *State and local programs.* Retrieved from https://www.cdc.gov/nccdphp/dnpao/state-local-programs/index.html

Centers for Disease Control and Prevention (CDC). (2023c, April 27). *Adolescent and school* health: *2019 Youth risk behavior surveillance system*. Retrieved from https://www.cdc.gov/healthyyouth/data/yrbs/index.htm

Centers for Disease Control and Prevention (CDC). (2023d, November 21). *Preventing childhood obesity: 5 things families can do.* Retrieved from https://www.cdc.gov/nccdphp/dnpao/features/childhood-obesity/index.html#:~:text=Children%20aged%203–5%20years,making%20it%20a%20family%20affair

Chambers, E. S., Morrison, D. J., & Frost, G. (2015). Control of appetite and energy intake by SCFA: What are the potential underlying mechanisms? *Proc Nutr Soc, 74*(3), 328-336. doi:10.1017/s0029665114001657

Chandran, A., Burjak, M., Petimar, J., Hamra, G., Melough, M. M., Dunlop, A. L., . . . Knapp, E. (2023). Changes in body mass index among school-aged youths following implementation of the healthy, hunger-free kids act of 2010. *JAMA Pediatr, 177*(4), 401-409. doi:10.1001/jamapediatrics.2022.5828Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics, 11*(4), 265-270.

Chaput, J. P., Brunet, M., & Tremblay, A. (2006). Relationship between short sleeping hours and childhood overweight/obesity: Results from the 'Quebec en forme' project. *Int J Obes (Lond), 30*(7), 1080-1085. doi:10.1038/sj.ijo.0803291

Chassaing, B., & Gewirtz, A. T. (2014). Gut microbiota, low-grade inflammation, and metabolic syndrome. Toxicol Pathol, 42(1), 49-53. doi:10.1177/0192623313508481 Collado, M. C., Rautava, S., Aakko, J., Isolauri, E., & Salminen, S. (2016). Human gut colonization may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Sci Rep, 6*, 23129. doi:10.1038/srep23129

Chen, J., He, X., & Huang, J. (2014). Diet effects in gut microbiome and obesity. J Food Sci, 79(4), R442-451. doi:10.1111/1750-3841.12397

Chen, X., Sun, H., Jiang, F., Shen, Y., Li, X., Hu, X., . . . Wei, P. (2020). Alteration of the gut microbiota associated with childhood obesity by 16s rRNA gene sequencing. *PeerJ, 8*, e8317. doi:10.7717/peerj.8317

Cho, K. Y. (2023). Association of gut microbiota with obesity in children and adolescents. *Clin Exp Pediatr, 66*(4), 148–154. doi:10.3345/cep.2021.01837

Clarke, S. F., Murphy, E. F., Nilaweera, K., Ross, P. R., Shanahan, F., O'Toole, P. W., & Cotter, P. D. (2012). The gut microbiota and its relationship to diet and obesity: New insights. *Gut Microbes, 3*(3), 186-202. doi:10.4161/gmic.20168

Cohen, D., Sturm, R., Scott, M., Farley, T., & Bluthenthal, R. (2010). Not Enough Fruit and Vegetables or Too Many Cookies, Candies, Salty Snacks, and Soft Drinks?. *Public Health Reports*, 125, 88 - 95. https://doi.org/10.1177/003335491012500112

Collado, M. C., Rautava, S., Aakko, J., Isolauri, E., & Salminen, S. (2016). Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Sci Rep, 6*, 23129. doi:10.1038/srep23129

Cole, T. J., Bellizzi, M. C., Flegal, K. M. & Dietz, W. H. (2000). Establishing a standard definition for child overweight and obesity worldwide: International survey. *British Medical Journal*, *320*(7244), 1240-1243.

Conder, E., Shay, H., Vekaria, H., Erinkitola, I., Bhogoju, S., Goretsky, T., Sullivan, P., Barrett, T., & Kapur, N. (2023). B1919utyrate-induced mitochondrial function improves barrier function in inflammatory bowel disease (IBD). *Inflammatory Bowel Diseases*. https://doi.org/10.1093/ibd/izac247.135

Coppola, S., Avagliano, C., Calignano, A., & Canani, . (2021). The protective role of butyrate against obesity and obesity-related diseases. Molecules, 26(682), 1-19. doi:10.3390/molecules

Corbin, K. D., Carnero, E. A., Dirks, B., Igudesman, D., Yi, F., Marcus, A., . . . Smith, S. R. (2023). Host-diet-gut microbiome interactions influence human energy balance: A randomized clinical trial. *Nat Commun, 14*(1), 3161. doi:10.1038/s41467-023-38778-x

Corcoran, S., Elbel, B., & Schwartz, A. (2014). The Effect of Breakfast in the Classroom on Obesity and Academic Performance: Evidence from New York City.. *Journal of policy analysis and management : [the journal of the Association for Public Policy Analysis and Management]*, 35 3, 509-32. https://doi.org/10.1002/PAM.21909.

Costa, C. S., Rauber, F., Leffa, P. S., Sangalli, C. N., Campagnolo, P. D. B., & Vitolo, M. R. (2019). Ultra-processed food consumption and its effects on anthropometric and glucose

profile: A longitudinal study during childhood. *Nutr Metab Cardiovasc Dis, 29*(2), 177-184. doi:10.1016/j.numecd.2018.11.003

Costanzo, P. R., & Woody, E. Z. (1984). Parental perspectives on obesity in children: The importance of sex differences. *Journal of Social and Clinical Psychology, 2*(4), 305-313.

Cox, L. M., & Blaser, M. J. (2013). Pathways in microbe-induced obesity. *Cell Metabolism, 17*(6), 883-894. doi:10.1016/j.cmet.2013.05.004

Cox, Laura M., Yamanishi, S., Sohn, J., Alekseyenko, Alexander V., Leung, Jacqueline M., Cho, I., . . . Blaser, Martin J. (2014). Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell, 158*(4), 705-721. doi:10.1016/j.cell.2014.05.052

Crovesy, L., Masterson, D., & Rosado, E. L. (2020). Profile of the gut microbiota of adults with obesity: A systematic review. Eur J Clin Nutr, 74(9), 1251-1262. doi:10.1038/s41430-020-0607-6

Crouch, L., Liberato, M., Urbanowicz, P., Baslé, A., Lamb, C., Stewart, C., Cooke, K., Doona, M., Needham, S., Brady, R., Berrington, J., Madunić, K., Wuhrer, M., Chater, P., Pearson, J., Glowacki, R., Martens, E., Zhang, F., Linhardt, R., Spencer, D., & Bolam, D. (2019). Prominent members of the human gut microbiota express endo-acting O-glycanases to initiate mucin breakdown. *Nature Communications*, 11. https://doi.org/10.1038/s41467-020-17847-5.

Cutting, T., M., Fisher, J. O., Grimm-Thomas, K., & Birch, L. L. (1999). Like mother, like daughter: Familial patterns of overweight are mediated by mothers' dietary disinhibition. *Am J Clin Nutr, 69*, 608-613.

Daniels, S. R., Arnett, D. K., Eckel, R. H., Gidding, S. S., Hayman, L. L., Kumanyika, S., . . . Williams, C. L. (2005). Overweight in children and adolescents: Pathophysiology, consequences, prevention, and treatment. *Circulation, 111*(15), 1999-2012. doi:10.1161/01.CIR.0000161369.71722.10

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., . . . Turnbaugh, P. J. (2013). Diet rapidly and reproducibly alters the human gut microbiome. *Nature, 505*(7484), 559-563. doi:10.1038/nature1282

Davis, C., & Carter, J. (2014). If certain foods are addictive, how might this change the treatment of compulsive overeating and obesity? *Current Addiction Reports*, *1*(2), 89-95. http://dx.doi.org/10.1007/s40429-014-0013-z

Dao, M., Everard, A., Aron-Wisnewsky, J., Sokolovska, N., Prifti, E., & Verger, E. et al. (2015). Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: Relationship with gut microbiome richness and ecology. *Gut, 65*(3), 426-436. http://dx.doi.org/10.1136/gutjnl-2014-308778

D'Argenio, V., & Salvatore, F. (2015). The role of the gut microbiome in the healthy adult status. *Clinica Chimica Acta, 451,* 97-102. http://dx.doi.org/10.1016/j.cca.2015.01.003

Da Silva, C. C., Monteil, M. A., & Davis, E. M. (2020). Overweight and obesity in children are associated with an abundance of firmicutes and a reduction of bifidobacterium in their gastrointestinal microbiota. *Child Obes, 16*(3), 204-210. doi:10.1089/chi.2019.02

Data Points. (2016). *Kidsdata.org*. Retrieved 5 August 2017, from http://www.kidsdata.org/blog/?p=7516

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., . . . Turnbaugh, P. J. (2013). Diet rapidly and reproducibly alters the human gut microbiome. *Nature, 505*(7484), 559-563. doi:10.1038/nature12820

Davison, K. K., & Birch, L. L. (2001). Childhood overweight: A contextual model and recommendations for future research. *Obes Rev, 2*(3), 159-171. doi:10.1046/j.1467-789x.2001.00036.x

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J., & Massart, S. et al. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of The National Academy of Sciences, 107*(33), 14691-14696. http://dx.doi.org/10.1073/pnas.1005963107

De Filippis, F., Pellegrini, N., Vannini, L., Jeffery, I. B., La Storia, A., Laghi, L., . . . Ercolini, D. (2016). High-level adherence to a mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut, 65*(11), 1812-1821. doi:10.1136/gutjnl-2015-309957

DeGruttola, A. K., Low, D., Mizoguchi, A., & Mizoguchi, E. (2016). Current understanding of dysbiosis in disease in human and animal models. *Inflammatory bowel diseases, 22*(5), 1137-1150. doi:10.109

Deleu, S., Arnauts, K., Deprez, L., Machiels, K., Ferrante, M., Huys, G., Thevelein, J., Raes, J., & Vermeire, S. (2023). High Acetate Concentration Protects Intestinal Barrier and Exerts Anti-Inflammatory Effects in Organoid-Derived Epithelial Monolayer Cultures from Patients with Ulcerative Colitis. *International Journal of Molecular Sciences*, 24. https://doi.org/10.3390/ijms24010768.

De la Cuesta-Zuluaga, J., Mueller, N. T., Álvarez-Quintero, R., Velásquez-Mejía, E. P., Sierra, J. A., Corrales-Agudelo, V., . . . Escobar, J. S. (2018). Higher fecal short-chain fatty acid levels are associated with gut microbiome dysbiosis, obesity, hypertension and cardiometabolic disease risk factors. *Nutrients, 11*(1). doi:10.3390/nu11010051

Dennison, B. A., Erb, T. A., & Jenkins, P. L. (2002). Television viewing and television in bedroom associated with overweight risk among low-income preschool children. *Pediatrics, 109*(6), 1028–1035. https://doi.org/10.1542/peds.109.6.1028

De Silva-Sanigorski, A. M., Bell, A. C., Kremer, P., Nichols, M., Crellin, M., Smith, M., . . . Swinburn, B. A. (2010). Reducing obesity in early childhood: Results from romp & chomp, an Australian community-wide intervention program. *Am J Clin Nutr, 91*(4), 831-840. doi:10.3945/ajcn.2009.28826

Den Besten, G., Lange, K., Havinga, R., van Dijk, T. H., Gerding, A., van Eunen, K., . . . Reijngoud, D. J. (2013a). Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am J Physiol Gastrointest Liver Physiol, 305*(12), G900-910. doi:10.1152/ajpgi.00265.2013

Den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013b). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res, 54*(9), 2325-2340. doi:10.1194/jlr.R03601

Dietz, W. H. (2004). Overweight in childhood and adolescence. *N Engl J Med, 350*(9), 856-857.

DiGiulio, D. B., Romero, R., Amogan, H. P., Kusanovic, J. P., Bik, E. M., Gotsch, F., . . . Relman, D. A. (2008). Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: A molecular and culture-based investigation. *Plos One, 3*(8), e3056. doi:10.1371/journal.pone.0003056

Drasar, B. S., Crowther, J. S., Goddard, P., Hawksworth, G., Hill, M. J., Peach, S., . . . Renwich, A. (1973). The relation between diet and the gut microflora in man. *Proceedings of the Nutrition Society, 32*(2), 49-52. doi:10.1079/pns19730014

Draxten, M., Fulkerson, J. A., Friend, S., Flattum, C. F., & Schow, R. (2014). Parental role modeling of fruits and vegetables at meals and snacks is associated with children's adequate consumption. Appetite, 78, 1-7. doi:10.1016/j.appet.2014.02.017

Drissi, F., Merhej, V., Angelakis, E., El Kaoutari, A., Carrière, F., Henrissat, B., & Raoult, D. (2014). Comparative genomics analysis of Lactobacillus species associated with weight gain or weight protection. Nutrition & diabetes, 4(2), e109. https://doi.org/10.1038/nutd.2014.6

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *PNAS.org, 107*(26), 11971-11975.

Dong, T. S., Guan, M., Mayer, E. A., Stains, J., Liu, C., Vora, P., . . . Gupta, A. (2022). Obesity is associated with a distinct brain-gut microbiome signature that connects prevotella and bacteroides to the brain's reward center. *Gut Microbes, 14*(1), 2051999. doi:10.1080/19490976.2022.2051999

DuRant, R. H., Baranowski, T., Johnson, M., & Thompson, W. O. (1994). The relationship among television watching, physical activity, and body composition of young children. *Pediatrics, 94*(4 Pt 1), 449–455.

Eck, L. H., Klesges, R. C., Hanson, C. L., & Slawson, D. (1992). Children at familial risk for obesity: an examination of dietary intake, physical activity, and weight

status. *International journal of obesity and related metabolic disorders : Journal of the International Association for the Study of Obesity, 16*(2), 71–78.

Edgar, R. C. (2018). Updating the 97% identity threshold for 16s ribosomal rna otus. *Bioinformatics, 34*(14), 2371-2375. doi:10.1093/bioinformatics/bty113

Edwards, C. A. (2017). Determinants and duration of impact of early gut bacterial colonization. *Annals of Nutrition and Metabolism, 70*(3), 246-250. doi:10.1159/000466711

Epstein, L. H. (1996). Family-based behavioural intervention for obese children. *Int J Obes Relat Metab Disord, 20 Suppl 1,* S14-21.

Epstein, L. H., Paluch, R. A., Roemmich, J. N., & Beecher, M. D. (2007). Family-based obesity treatment, then and now: Twenty-five years of pediatric obesity treatment. *Health Psychol, 26*(4), 381-391. doi:10.1037/0278-6133.26.4.381

Engeland, A., Bjørge, T., Tverdal, A., & Søgaard, A. J. (2004). Obesity in adolescence and adulthood and the risk of adult mortality. *Epidemiology, 15*(1), 79-85. doi:10.1097/01.ede.0000100148.40711.59

Erridge, C., Attina, T., Spickett, C. M., & Webb, D. J. (2007). A high-fat meal induces low-grade endotoxemia: Evidence of a novel mechanism of postprandial inflammation. *Am J Clin Nutr, 86*(5), 1286-1292.

Fallani, M., Amarri, S., Uusijarvi, A., Adam, R., Khanna, S., Aguilera, M., . . . team, I. (2011). Determinants of the human infant intestinal microbiota after the introduction of first complementary foods in infant samples from five European centres. *Microbiology, 157*(Pt 5), 1385-1392. doi:10.1099/mic.0.042143-0

Farajian, P., Panagiotakos, D., Risvas, G., Malisova, O., & Zampelas, A. (2014). Hierarchical analysis of dietary, lifestyle and family environment risk factors for childhood obesity:

the GRECO study. *European Journal of Clinical Nutrition, 68*(10), 1107-1112. http://dx.doi.org/10.1038/ejcn.2014.89

Faith, M. S., & Krall, T. V. E. (2006). Social environmental and genetic influences on obesity and obesity-promoting behaviors: Fostering research and integration. In Fernandez, L. M. & Blazer, D. G. (Eds.), *Institute of Medicine committee on assessing interactions among social, behavioral, and genetic factors in health; genes, behavior, and the social environment: Moving beyond the nature/nurture debate* (pp. 236-280). Retrieved from http:// https://www.ncbi.nlm.nih.gov/books/NBK19929/pdf/Bookshelf_NBK19929.pdf

Farris, A. R., Misyak, S., Duffey, K. J., Davis, G. C., Hosig, K., Atzaba-Poria, N., . . . Serrano, E. L. (2014). Nutritional comparison of packed and school lunches in pre-kindergarten and kindergarten children following the implementation of the 2012-2013 national school lunch program standards. *J Nutr Educ Behav, 46*(6), 621-626. doi:10.1016/j.jneb.2014.07.007

Fathi, P., & Wu, S. (2016). Isolation, detection, and characterization of enterotoxigenic bacteroides fragilis in clinical samples. *Open Microbiol J, 10*, 57-63.

Faul, F., Erdfelder, E., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using g*power 3.1: Tests for correlation and regression analyses. *Behav Res Methods, 41*(4), 1149-1160. doi:10.3758/BRM.41.4.1149

Ferchaud-Roucher, V., Pouteau, E., Piquet, H., Zair, Y., & Krempf, M. (2005). Colonic fermentation from lactulose inhibits lipolysis in overweight subjects. *Am J Physiol Endocrinol Metab, 289*(4), E716-720. doi:10.1152/ajpendo.00430.2004

Fernandez, L., Langa, S., Martin, V., Maldonado, A., Jimenez, E., Martin, R., & Rodriguez, J. M. (2013). The human milk microbiota: Origin and potential roles in health and disease. *Pharmacol Res, 69*(1), 1-10. doi:10.1016/j.phrs.2012.09.001

Fernandes, J., Su, W., Rahat-Rozenbloom, S., Wolever, T. M., & Comelli, E. M. (2014). Adiposity, gut microbiota, and fecal short chain fatty acids are linked in adult humans. *Nutr Diabetes, 4*, e121. doi:10.1038/nutd.2014.23

Ferrer, M., Ruiz, A., Lanza, F., Haange, S.-B., Oberbach, A., Till, H., . . . Suarez, A. (2013). Microbiota from the distal guts of lean and obese adolescents exhibit partial functional redundancy besides clear differences in community structure. *Environmental Microbiology, 15*(1), 211-226. doi:10.1111/j.1462-2920.2012.02845.x

Finnane, J. M., Jansen, E., Mallan, K. M., & Daniels, L. A. (2017). Mealtime structure and responsive feeding practices are associated with less food fussiness and more food enjoyment in children. *J Nutr Educ Behav, 49*(1), 11-18.e11. doi:10.1016/j.jneb.2016.08.007

Fisher, J. O., & Birch, L. L. (1995). Fat preferences and fat consumption of 3- to 5-year-old children are related to parental adiposity. *Journal of the American Dietetic Association, 95*(7), 759-764. doi:10.1016/s0002-8223(95)00212-x

Fisher, J. O., & Birch, L. L. (1999a). Restricting access to foods and children's eating. *Appetite, 32*, 405-419.

Fisher, J. O., & Birch, L. L. (1999b). Restricting access to palatable foods affects children's behavioral response, food selection and intake. *Am J Clin Nutr, 69*(6), 1264-1272.

Fllatt, J. P. (1988). Importance of nutrient balance in body weight regulation. *Diabetes/Metabolism Reviews, 4*(6), 571-581.

Fouhy, F., Guinane, C. M., Hussey, S., Wall, R., Ryan, C. A., Dempsey, E. M., . . . Cotter, P. D. (2012). High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin. *Antimicrob Agents Chemother, 56*(11), 5811-5820. doi:10.1128/aac.00789-12

Frankel, L. A., Hughes, S. O., O'Connor, T. M., Power, T. G., Fisher, J. O., & Hazen, N. L. (2012). Parental influences on children's self-regulation of energy intake: Insights from developmental literature on emotion regulation. *Journal of obesity, 2012*, 327259-327259. doi:10.1155/2012/327259

Freedman, D. S., Khan, L. K., Serdula, M. K., Dietz, W. H., Srinivasan, S. R., & Berenson, G. S. (2005). The relation of childhood BMI to adult adiposity: The Bogalusa heart study. *Pediatrics, 115*(1), 22-27. doi:10.1542/peds.2004-0220

Freedman, D. S., Khan, L. K., Serdula, M. K., Dietz, W. H., Srinivasan, S. R. & Berenson, G. S. (2005a). Racial differences in the tracking of childhood BMI to adulthood. *Obesity Research, 13*(5), 928-932.

Freeman, E., Fletcher, R., Collins, C., Morgan, P., Burrows, T., & Callister, R. (2012). Preventing and treating childhood obesity: time to target fathers. *International Journal of Obesity*, 36, 12-15. https://doi.org/10.1038/ijo.2011.198. French, S. A., Story, M., & Jeffrey, R. W. (2001). Environmental influences on eating and physical activity. *Annual Review of Public Health, 22*, 309-335.

Frost, G., Sleeth, M. L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., . . . Bell, J. D. (2014). The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun, 5*, 3611. doi:10.1038/ncomms4611

Fryar, C. D., Carroll, M. D., & Afful, J. (2020). Prevalence of overweight, obesity and severe obesity among children and adolescents aged 2-19 years: United States, 1963-1965 through 2017-2018. National Center for Health Statistics Health E-Stats, December 2020. Retrieved from https://www.cdc.gov/nchs/data/hestat/obesity-child-17-18/overweight-obesity-child-H.pdf

Galley, J., Bailey, M., Dush, C., Schoppe-Sullivan, S., & Christian, L. (2014). Maternal obesity is associated with alterations in the gut microbiome in toddlers. *PLoS ONE*, 9. https://doi.org/10.1371/journal.pone.0113026.

Garn, S. M., & Clark, D. C. (1976). Trends in fatness and the origins of obesity. *Pediatrics*, *57*(443-456).

Gazzaniga, J. M., & Burns, T. L. (1993). Relationship between diet composition and body fatness, with adjustment for resting energy expenditure and physical activity, in pre-adolescent children *Am J Clin Nutr*, *58*, 21-28.

Gebel, E. (2012). *More Kids Than Ever Have Type 2 Diabetes*. *Diabetes Forecast*. Retrieved 3 August 2017, from http://www.diabetesforecast.org/2012/nov/more-kids-than-ever-have-type-2-diabetes.html

Gibson, P. R., Moeller, I., Kagelari, O., Folino, M., & Young, G. P. (1992). Contrasting effects of butyrate on the expression of phenotypic markers of differentiation in neoplastic and non-neoplastic colonic epithelial cells in vitro. *J Gastroenterol Hepatol*, *7*(2), 165-172.

Gibson, E. L., Wardle, J., & Watts, C. J. (1998). Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children. *Appetite, 31*, 205-228.

Gill, S. R., Pop, M., Deboy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., . . . Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. *Science, 312*(5778), 1355-1359. doi:10.1126/science.1124234

Goffredo, M., Mass, K., Parks, E. J., Wagner, D. A., McClure, E. A., Graf, J., . . . Santoro, N. (2016). Role of gut microbiota and short-chain fatty acids in modulating energy harvest and fat partitioning in youth. *The Journal of Clinical Endocrinology & Metabolism, 101*(11), 4367-4376. doi:10.1210/jc.2016-1797

Greenwood, C., Morrow, A. L., Lagomarcino, A. J., Altaye, M., Taft, D. H., Yu, Z., . . . Schibler, K. R. (2014). Early empiric antibiotic use in preterm infants is associated with lower bacterial diversity and higher relative abundance of enterobacter. *J Pediatr, 165*(1), 23-29. doi:10.1016/j.jpeds.2014.01.010

Gronlund, M. M., Lehtonen, O. P., Eerola, E., & Kero, P. (1999). Fecal microflora in healthy infants born by different methods of delivery: Permanent changes in intestinal flora after cesarean delivery. *J Pediatr Gastroenterol Nutr, 28*(1), 19-25.

Goldstein, H. (2016). *Reining in California's Skyrocketing Diabetes Epidemic*. *Chcf.org*. Retrieved 3 August 2017, from http://www.chcf.org/articles/2016/08/reining-skyrocketing-diabetes-epidemic

Gomez, M. (2015). *Survey emphasizes childhood obesity epidemic in LA County*. *ABC7 Los Angeles*. Retrieved 5 August 2017, from http://abc7.com/news/survey-emphasizes-childhood-obesity-epidemic-in-la-county/1023534/

Guinane, C., & Cotter, P. (2013). Role of the gut microbiota in health and chronic gastrointestinal disease: Understanding a hidden metabolic organ. *Therapeutic Advances in Gastroenterology*, *6*(4), 295-308. http://dx.doi.org/10.1177/1756283x13482996

Gurnani, M., Birken, C., & Hamilton, J. (2015). Childhood Obesity. *Pediatric Clinics of North America, 62*(4), 821-840. http://dx.doi.org/10.1016/j.pcl.2015.04.001

Gustaw-Rothenberg, K. (2009). Dietary patterns associated with Alzheimer's disease: Population based study. *Int J Environ Res Public Health, 6*(4), 1335-1340. doi:10.3390/ijerph6041335

Guttmann, A. (2023). Daily entertainment screen time among children in the U.S., by activity. Retrieved from https://www.statista.com/statistics/1312623/average-daily-entertainment-screen-time-children-us-activity/

Hales, C. M., Fryar, C. D., Carroll, M. D., Freedman, D. S. & Ogden, C. L. (2018). Trends in obesity and severe obesity prevalence in U.S. youth and adults by sex and age, 2007-2008 to 2015-2016. *Jama, 319*(16), 1723-1725. doi:10.1001/jama.2018.3060

Hales, C. M., Carroll, M. D., Fryar, C. D., & Ogden, C.L. (2017). Prevalence of obesity among adults and youth: United States, 2015–2016. NCHS data brief, no 288. Hyattsville, MD: National Center for Health Statistics.

Haire-Joshu, D. N., & Nanney, M. S. (2002). Prevention of overweight and obesity in children: Influences on the food environment. *The Diabetes Educator, 28*(3), 415-423.

Haire-Joshu, D., & Tabak, R. (2016). Preventing obesity across generations: Evidence for early life intervention. *Annu Rev Public Health, 37*, 253-271. doi:10.1146/annurev-publhealth-032315-021859

Halfon, N., Larson, K., & Slusser, W. (2013). Associations between obesity and comorbid mental health, developmental, and physical health conditions in a nationally representative sample of US children aged 10 to 17. *Acad Pediatr, 13*(1), 6-13. doi:10.1016/j.acap.2012.10.007

Hammond, R. A., & Levine, R. (2010). The economic impact of obesity in the United States. *Diabetes, metabolic syndrome, and obesity : targets and therapy, 3*, 285-295. doi:10.2147/DMSOTT.S7384

Handford, C. M., Rapee, R. M., & Fardouly, J. (2018). The influence of maternal modeling on body image concerns and eating disturbances in preadolescent girls. *Behav Res Ther, 100*, 17-23. doi:10.1016/j.brat.2017.11.001

Hanson, M., Gluckman, P., & Bustreo, F. (2016). Obesity and the health of future generations.. *The lancet. Diabetes & endocrinology*, 4 12, 966-967 . https://doi.org/10.1016/S2213-8587(16)30098-5.

Health Communication | Social Cognitive Theory. (2016). *Universiteit Twente*. Retrieved 4 August 2017, from https://www.utwente.nl/en/bms/communication-theories/sorted-by-cluster/Health%20Communication/Social_cognitive_theory/

Herman, D. R., Rhoades, N., Mercado, J., Argueta, P., Lopez, U., & Flores, G. E. (2020). Dietary habits of 2- to 9-year-old American children are associated with gut microbiome composition. *J Acad Nutr Diet, 120*(4), 517-534. doi:10.1016/j.jand.2019.07.024

Hildebrandt, M. A., Hoffmann, C., Sherrill–Mix, S. A., Keilbaugh, S. A., Hamady, M., Chen, Y. Y., . . . Wu, G. D. (2009). High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology, 137*(5), 1716-1724.e1712. doi:10.1053/j.gastro.2009.08.042

Hildebrand, C. B., Lichatz, R., Pich, A., Mühlfeld, C., Woltemate, S., Vital, M., & Brandenberger, C. (2023). Short-chain fatty acids improve inflamm-aging and acute lung injury in old mice. *American journal of physiology. Lung cellular and molecular physiology*, *324*(4), L480–L492. https://doi.org/10.1152/ajplung.00296.2022

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., . . . Sanders, M. E. (2014). The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*(8), 506-514. doi:10.1038/nrgastro.2014.66

Hill, J. O., Wyatt, H. R., Reed, G. W. & Peters, J. C. (2003). Obesity and the environment: Where do we go from here? *Science, 299*(5608), 853-855.

Hingle, M., & Kunkel, D. (2012). Childhood obesity and the media.. *Pediatric clinics of North America*, 59 3, 677-92, ix . https://doi.org/10.1016/j.pcl.2012.03.021.

Hoelscher, D., Kirk, S., Ritchie, L., & Cunningham-Sabo, L. (2013). Position of the Academy of Nutrition and Dietetics: Interventions for the Prevention and Treatment of Pediatric Overweight and Obesity. *Journal of The Academy of Nutrition and Dietetics*, *113*(10), 1375-1394. http://dx.doi.org/10.1016/j.jand.2013.08.004

Hollister, E. B., Foster, B. A., Dahdouli, M., Ramirez, J., & Lai, Z. (2018). Characterization of the stool microbiome in Hispanic preschool children by weight status and time. *Childhood obesity (Print)*, *14*(2), 122–130. https://doi.org/10.1089/chi.2017.0122

Holmes, Z. C., Silverman, J. D., Dressman, H. K., Wei, Z., Dallow, E. P., Armstrong, S. C., . . . David, L. A. (2020). Short-chain fatty acid production by gut microbiota from children with obesity differs according to prebiotic choice and bacterial community composition. *MBio, 11*(4), e00914-00920. doi:10.1128/mBio.00914-20

Hosseinzadeh, K., & Ahmadabad, M. (2015). Screen Time and Physical Activity in Overweight and Obese Students. *Biotechnology and Health Sciences*, 2. https://doi.org/10.17795/BHS-31211.

Hou, Y. P., He, Q. Q., Ouyang, H. M., Peng, H. S., Wang, Q., Li, J., . . . Yin, A. H. (2017). Human gut microbiota associated with obesity in Chinese children and adolescents. *Biomed Res Int, 2017*, 7585989. doi:10.1155/2017/7585989

Houtman, T. A., Eckermann, H. A., Smidt, H., & de Weerth, C. (2022). Gut microbiota and bmi throughout childhood: The role of firmicutes, bacteroidetes, and short-chain fatty acid producers. *Sci Rep, 12*(1), 3140. doi:10.1038/s41598-022-07176-6

Hughes, S. O., & Frazier-Wood, A. C. (2016). Satiety and the self-regulation of food take in children: A potential role for gene-environment interplay. *Current obesity reports, 5*(1), 81-87. doi:10.1007/s13679-016-0194-y

Hughes, C., Gooze, R., Finkelstein, D., & Whitaker, R. (2010). Barriers to Obesity Prevention in Head Start. *Health Affairs*, *29*(3), 454-462. http://dx.doi.org/10.1377/hlthaff.2009.0499

Hughes, J. B., Hellmann, J. J., Ricketts, T. H., & Bohannan, B. J. M. (2001). Counting the uncountable: Statistical approaches to estimating microbial diversity. *Appl Environ Microbiol, 67*(10), 4399-4406. doi:10.1128/aem.67.10.4399-4406.2001

Huh, S. Y., Rifas-Shiman, S. L., Zera, C. A., Edwards, J. W. R., Oken, E., Weiss, S. T., & Gillman, M. W. (2012). Delivery by caesarean section and risk of obesity in preschool age children: A prospective cohort study. *Archives of Disease in Childhood, 97*(7), 610-616. doi:10.1136/archdischild-2011-301141

Hunt, K. M., Foster, J. A., Forney, L. J., Schutte, U. M., Beck, D. L., Abdo, Z., . . . McGuire, M. A. (2011). Characterization of the diversity and temporal stability of bacterial communities in human milk. *Plos One, 6*(6), e21313. doi:10.1371/journal.pone.0021313

Hur, I., Burgess-Champoux, T., & Reicks, M. (2011). Higher quality intake from school lunch meals compared with bagged lunches. *ICAN: Infant, Child, & Adolescent Nutrition, 3*(2), 70-75. doi:10.1177/1941406411399124

Illumina. (2021). 16s metagenomic sequencing library preparation. Retrieved from https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

Imamura, F., Micha, R., Khatibzadeh, S., Fahimi, S., Shi, P., Powles, J., & Mozaffarian, D. (2015). Dietary quality among men and women in 187 countries in 1990 and 2010: A systematic assessment. *The Lancet Global Health, 3*(3), e132-e142. doi:10.1016/s2214-109x(14)70381-x

Islam, K. B., Fukiya, S., Hagio, M., Fujii, N., Ishizuka, S., Ooka, T., . . . Yokota, A. (2011). Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. *Gastroenterology, 141*(5), 1773-1781. doi:10.1053/j.gastro.2011.07.046

Jackson, M., Walker, S., Simpson, C., McFarlane-Anderson, N., & Bennett, F. (2009). Are food patterns associated with prostate cancer in Jamaican men: A preliminary report. *Infect Agent Cancer, 4 Suppl 1*(Suppl 1), S5. doi:10.1186/1750-9378-4-s1-s5

Jacob, A. & Reetha, S. G. (2017). Prevalence of metabolic comorbidities in obese children. *International Journal of Contemporary Pediatrics, 4*(4), 1450. doi:10.18203/2349-3291.ijcp20172684

Jakobsson, H. E., Abrahamsson, T. R., Jenmalm, M. C., Harris, K., Quince, C., Jernberg, C., . . . Andersson, A. F. (2014). Decreased gut microbiota diversity, delayed bacteroidetes colonization and reduced th1 responses in infants delivered by caesarean section. *Gut, 63*(4), 559-566. doi:10.1136/gutjnl-2012-303249

Jandhyala, S. (2015). Role of the normal gut microbiota. *World Journal of Gastroenterology*, *21*(29), 8787. http://dx.doi.org/10.3748/wjg.v21.i29.8787

Jantscher-Krenn, E., Treichler, C., Brandl, W., Schonbacher, L., Kofeler, H., & van Poppel, M. N. M. (2019). The association of human milk oligosaccharides with glucose metabolism in overweight and obese pregnant women. Am J Clin Nutr, 110(6), 1335-1343. doi:10.1093/ajcn/nqz202

Jaureguy, F., Carton, M., Panel, P., Foucaud, P., Butel, M. J., & Doucet-Populaire, F. (2004). Effects of intrapartum penicillin prophylaxis on intestinal bacterial colonization in infants. *J Clin Microbiol, 42*(11), 5184-5188. doi:10.1128/jcm.42.11.5184-5188.2004

Jernberg, C., Lofmark, S., Edlund, C., & Jansson, J. K. (2010). Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology, 156*(Pt 11), 3216-3223. doi:10.1099/mic.0.040618-0

Jerrett, M., McConnell, R., Wolch, J., Chang, R., Lam, C., & Dunton, G. et al. (2014). Traffic-related air pollution and obesity formation in children: a longitudinal, multilevel analysis. *Environmental Health*, *13*(1). http://dx.doi.org/10.1186/1476-069x-13-49

Ji, Y., Ma, N., Zhang, J., Wang, H., Tao, T., Pei, F., & Hu, Q. (2021). Dietary intake of mixture coarse cereals prevents obesity by altering the gut microbiota in high-fat diet fed mice. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association, 147, 111901. doi:10.1016/j.fct.2020.111901

Jimenez, E., Fernandez, L., Marin, M. L., Martin, R., Odriozola, J. M., Nueno-Palop, C., . . . Rodriguez, J. M. (2005). Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Curr Microbiol, 51*(4), 270-274. doi:10.1007/s00284-005-0020-3

Jimenez, E., Marin, M. L., Martin, R., Odriozola, J. M., Olivares, M., Xaus, J., Fernandez, L., Rodriguez, J. M., . (2008). Is meconium from healthy newborns actually sterile? *Research in Microbiology, 159*, 187-193. doi:10.1016/j.resmic.2007.12.007

Johnson, S. L., & Birch, L. L. (1994). Parents' and children's adiposity and eating style. *Pediatrics, 94*(5), 653.

Johnston, C. A., Moreno, J. P., El-Mubasher, A., & Woehler, D. (2012). School lunches and lunches brought from home: A comparative analysis. *Child Obes, 8*(4), 364-368. doi:10.1089/chi.2012.0012

Juonala, M., Magnussen, C. G., Berenson, G. S., Venn, A., Burns, T. L., Sabin, M. A., . . . Raitakari, O. T. (2011). Childhood adiposity, adult adiposity, and cardiovascular risk factors. *N Engl J Med, 365*(20), 1876-1885. doi:10.1056/NEJMoa1010112

Kalliomaki, M., Collado, Maria Carmen, Salminen, Seppo & Isolauri, Erika. (2008). Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr, 87*, 534-538.

Kallus, S. J., & Brandt, L. J. (2012). The intestinal microbiota and obesity. *J Clin Gastroenterol, 46*(1), 16-24. doi:10.1097/MCG.0b013e31823711fd

Kamali, A., Hameed, H., Shih, M., & Simon, P. (2017). Turning the curve on obesity prevalence among fifth graders in the Los Angeles Unified School District, 2001–2013. *Preventing Chronic Disease, 14*. doi:10.5888/pcd14.160377

Kanellopoulou, A., Notara, V., Magriplis, E., Antonogeorgos, G., Rojas-Gil, A. P., Kornilaki, E. N., . . . Panagiotakos, D. B. (2021). Sleeping patterns and childhood obesity: An epidemiological study in 1,728 children in Greece. *Journal of Clinical Sleep Medicine, 17*(5), 1093-1101. doi:doi:10.5664/jcsm.9160

Kantor, L. S. (1999). A comparison of the U. S. food supply with the food guide pyramid recommendations. In Frazao, E. (Ed.), *America's eating habits: Changes and consequences* (pp. 71-95). Retrieved from https://ageconsearch.umn.edu/record/33604?ln=en.

Karlamangla, S. (2015). *L.A. County launches campaign against childhood obesity*. *latimes.com*. Retrieved 5 August 2017, from http://www.latimes.com/local/california/la-me-adv-water-campaign-20151011-story.html

Keane, E., Layte, R., Harrington, J., Kearney, P., & Perry, I. (2012). Measured Parental Weight Status and Familial Socio-Economic Status Correlates with Childhood Overweight and Obesity at Age 9. *PLoS ONE*, 7. https://doi.org/10.1371/journal.pone.0043503. Kelsey, M., Zaepfel, A., Bjornstad, P., & Nadeau, K. (2014). Age-Related Consequences of Childhood Obesity. *Gerontology, 60*(3), 222-228. http://dx.doi.org/10.1159/000356023

Kenney, E. L., Barrett, J. L., Bleich, S. N., Ward, Z. J., Cradock, A. L., & Gortmaker, S. L. (2020). Impact of the healthy, hunger-free kids act on obesity trends. *Health Aff (Millwood), 39*(7), 1122-1129. doi:10.1377/hlthaff.2020.00133

Keski-Nisula, L., Kyynarainen, H. R., Karkkainen, U., Karhukorpi, J., Heinonen, S., & Pekkanen, J. (2013). Maternal intrapartum antibiotics and decreased vertical transmission of lactobacillus to neonates during birth. *Acta Paediatr, 102*(5), 480-485. doi:10.1111/apa.12186

Key, J., Burnett, D., Babu, J., & Geetha, T. (2023). The Effects of Food Environment on Obesity in Children: A Systematic Review. *Children*, 10. https://doi.org/10.3390/children10010098.

Khan, M., Gerasimidis, K., Edwards, C., & Shaikh, M. (2016). Role of Gut Microbiota in the Aetiology of Obesity: Proposed Mechanisms and Review of the Literature. *Journal of Obesity, 2016*, 1-27. http://dx.doi.org/10.1155/2016/7353642

Khodayar-Pardo, P., Mira-Pascual, L., Collado, M. C., & Martinez-Costa, C. (2014). Impact of lactation stage, gestational age, and mode of delivery on breast milk microbiota. *J Perinatol, 34*(8), 599-605. doi:10.1038/jp.2014.47

Kim, B. R., Shin, J., Guevarra, R., Lee, J. H., Kim, D. W., Seol, K. H., . . . Isaacson, R. (2017). Deciphering diversity indices for a better understanding of microbial communities. *J Microbiol Biotechnol, 27*(12), 2089-2093. doi:10.4014/jmb.1709.09027

Kim, K. N., Yao, Y., & Ju, S. Y. (2019). Short chain fatty acids and fecal microbiota abundance in humans with obesity: A systematic review and meta-analysis. *Nutrients, 11*(10). doi:10.3390/nu11102512

Kimura, I., Ozawa, K., Inoue, D., Imamura, T., Kimura, K., Maeda, T., . . . Tsujimoto, G. (2013). The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor gpr43. *Nat Commun, 4*, 1829. doi:10.1038/ncomms2852

Klesges, R. C., Coates, T. J., Brown, G., Sturgeon-Tillisch, J., Moldenhauer-Klesges, L. M., Holzer, B., . . . Vollmer, J. (1983). Parental influences on children's eating behavior and relative weight. *J Appl Behav Anal, 16*(4), 371-378. doi:10.1901/jaba.1983.16-371

Klesges, R. C., Stein, R. J., Eck, L. H., Isbell, T. R., & Klesges, L. M. (1991). Parental influence on food selection in young children and its relationships to childhood obesity. *Am J Clin Nutr, 53*(4), 859-864. doi:10.1093/ajcn/53.4.859

Klesges, R. C., Klesges, L. M., Eck, L. H., & Shelton, M. L. (1995). A longitudinal analysis of accelerated weight gain in preschool children. *Pediatrics, 95*(1), 126–130.

Koeth, R. A., Wang, Z., Levison, B. S., Buffa, J. A., Org, E., Sheehy, B. T., . . . Hazen, S. L. (2013). Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med, 19*(5), 576-585. doi:10.1038/nm.3145

Koren, O., Goodrich, J. K., Cullender, T. C., Spor, A., Laitinen, K., Backhed, H. K., . . . Ley, R. E. (2012). Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell, 150*(3), 470-480. doi:10.1016/j.cell.2012.07.008

Kovatcheva-Datchary, P., & Arora, T. (2013). Nutrition, the gut microbiome, and the metabolic syndrome. *Best Practice & Research Clinical Gastroenterology*, *27*(1), 59-72. http://dx.doi.org/10.1016/j.bpg.2013.03.017

Kramer, M. S. (1981). Do breastfeeding and delayed introduction of solid foods protect against subsequent obesity. *J Pediatr, 98*(6), 883-887.

Ku, L. C., Shapiro, L. R., Crawford, P. B., & Huenemann, R. L. (1981). Body composition and physical activity in 8-year-old children. *The American Journal of Clinical Nutrition, 34*, 2770-2775.

Kurokawa, K., Itoh, T., Kuwahara, T., Oshima, K., Toh, H., Toyoda, A., . . . Hattori, M. (2007). Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. *DNA Research, 14*(4), 169-181. doi:10.1093/dnares/dsm018

Lagstrom, H., Rautava, S., Ollila, H., Kaljonen, A., Turta, O., Makela, J., . . . Bode, L. (2020). Associations between human milk oligosaccharides and growth in infancy and early childhood. Am J Clin Nutr, 111(4), 769-778. doi:10.1093/ajcn/nqaa010

Lancaster, G. A., Dodd, S., & Williamson, P. R. (2004). Design and analysis of pilot studies: Recommendations for good practice. *Journal of Evaluation in Clinical Practice, 10*(2), 307-312.

Lane, M., Howland, G., West, M., Hockey, M., Marx, W., Loughman, A., . . . Rocks, T. (2020). The effect of ultra-processed very low-energy diets on gut microbiota and metabolic outcomes in individuals with obesity: A systematic literature review. *Obes Res Clin Pract, 14*(3), 197-204. doi:10.1016/j.orcp.2020.04.006

Larsen, N., Vogensen, F., van den Berg, F., Nielsen, D., Andreasen, A., & Pedersen, B. et al. (2010). Gut Microbiota in Human Adults with Type 2 Diabetes Differs from Non-Diabetic Adults. *Plos ONE, 5*(2), e9085. http://dx.doi.org/10.1371/journal.pone.0009085

Lawton, C. L., Burley, V. J., Wales, J. K., & Blundell, J. E. (1993). Dietary fat and appetite control in obese subjects: weak effects on satiation and satiety. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity, 17*(7), 409–416.

Laricchia, F. (2024). Children and technology - Statistics and facts. *Statista.* Retrieved from https://www.statista.com/topics/9840/children-and-technology/#topicOverview

Laricchia, F. (2023). Share of U.S. children with an electric device in their home 2019-2021, by type. *Statista.* Retrieved from https://www.statista.com/statistics/1324245/children-owning-an-electronic-device-at-home-by-type-us/

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., . . . Pedersen, O. (2013). Richness of human gut microbiome correlates with metabolic markers. *Nature, 500*(7464), 541-546. doi:10.1038/nature12506

Lee, Y.-K. (2013). Effects of diet on gut microbiota profile and the implications for health and disease. *Bioscience of microbiota, food, and health, 32*(1), 1-12. doi:10.12938/bmfh.32.1

Ley, R. E., Backhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A, 102*(31), 11070-11075. doi:10.1073/pnas.0504978102

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Microbial ecology: Human gut microbes associated with obesity. *Nature, 444*(7122), 1022-1023. doi:10.1038/4441022a

Liu, H., Wang, J., He, T., Becker, S., Zhang, G., Li, D., & Ma, X. (2018). Butyrate: A double-edged sword for health? Adv Nutr, 9(1), 21-29. doi:10.1093/advances/nmx00 Llewellyn, C., & Wardle, J. (2015). Behavioral susceptibility to obesity: Gene–environment interplay in the development of weight. *Physiology & Behavior*, *152*, 494-501. http://dx.doi.org/10.1016/j.physbeh.2015.07.006

Llewellyn, A., Simmonds, M., Owen, C. G., & Woolacott, N. (2016). Childhood obesity as a predictor of morbidity in adulthood: A systematic review and meta-analysis. *Obesity Reviews, 17*(1), 56-67. doi:10.1111/obr.12316

Li, H., & Wei, C. (2015). Diet, Gut Microbiota and Obesity. *Journal of Nutritional Health & Food Science*, *3*(4), 01-06. http://dx.doi.org/10.15226/jnhfs.2015.00150

Lin, B. H., Guthrie, J., & Frazao, E. (1999). Nutrient contribution of food away from home. In Frazao, E. (Ed.), *America's eating habits: Changes and consequences* (pp. 213-242). Retrieved from http:// https://ageconsearch.umn.edu/record/33604?ln=en.

Ling, J., Chen, S., Zahry, N. R., & Kao, T. A. (2023). Economic burden of childhood overweight and obesity: A systematic review and meta-analysis. *Obes Rev*, *24*(2), e13535. doi:10.1111/obr.13535

Liou, A. P., Paziuk, M., Luevano, J. M., Jr., Machineni, S., Turnbaugh, P. J., & Kaplan, L. M. (2013). Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Sci Transl Med, 5*(178), 178ra141. doi:10.1126/scitranslmed.3005687

Lobstein, T., Jackson-Leach, R., Moodie, M., Hall, K., Gortmaker, S., & Swinburn, B. et al. (2015). Child and adolescent obesity: part of a bigger picture. *The Lancet, 385*(9986), 2510-2520. http://dx.doi.org/10.1016/s0140-6736(14)61746-3

López-Contreras, B. E., Morán-Ramos, S., Villarruel-Vázquez, R., Macías-Kauffer, L., Villamil-Ramírez, H., León-Mimila, P., . . . Canizales-Quinteros, S. (2018). Composition of gut microbiota in obese and normal-weight Mexican school-age children and its association with metabolic traits. *Pediatr Obes, 13*(6), 381-388. doi:10.1111/ijpo.12262

Los Angeles County Department of Public Health, Office of Health Assessment and Epidemiology, (2011). Obesity and related mortality Los Angeles County: A cities and communities health report, September 2011.

Lu, Y., Fan, C., Li, P., Lu, Y., Chang, X., & Qi, K. (2016). Short-chain fatty acids prevent high-fat-diet-induced obesity in mice by regulating g protein-coupled receptors and gut microbiota. *Sci Rep, 6*, 37589. doi:10.1038/srep37589

Maffeis, C., Schutz, Y., Zaffanello, M., Piccoli, R., & Pinelli, L. (1994). Elevated energy expenditure and reduced energy intake in obese prepubertal children: Paradox of poor dietary reliability in obesity. *The Journal of Pediatrics, 124*(3), 348-354.

Maffeis, C. (1999). Childhood obesity: The genetic-environmental interface. *Bailliere's Clinical Endocrinology and Metabolism, 13*(1), 31-46.

Maffeis, C., Talamini, G., & Tato, L. (1998). Influence of diet, physical activity, and parents' obesity on children's adiposity: A four-year longitudinal study. *International Journal of Obesity, 22*, 758-764.

Magnussen, C. G., Venn, A., Thomson, R., Juonala, M., Srinivasan, S. R., Viikari, J. S., . . . Raitakari, O. T. (2009). The association of pediatric low- and high-density lipoprotein cholesterol dyslipidemia classifications and change in dyslipidemia status with carotid intima-media thickness in adulthood evidence from the cardiovascular risk in young Finns study, the Bogalusa heart study, and the CDAH (childhood determinants of adult health) study. *J Am Coll Cardiol, 53*(10), 860-869. doi:10.1016/j.jacc.2008.09.061

Manco, M., Putignani, L., & Bottazzo, G. F. (2010). Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. *Endocr Rev, 31*(6), 817-844. doi:10.1210/er.2009-0030

Magarey, A. M., Daniels, L. A., Boulton, T. J., & Cockington, R. A. (2003). Predicting obesity in early adulthood from childhood and parental obesity. *Int J Obes Relat Metab Disord, 27*(4), 505-513. doi:10.1038/sj.ijo.0802251

Marchioni, D. M. L., Latorre, M. d. R. D. d. O., Eluf-Neto, J., Wünsch-Filho, V., & Fisberg, R. M. (2005). Identification of dietary patterns using factor analysis in an epidemiological study in São Paulo. *Sao Paulo Medical Journal, 123*, 124-127.

Margulies, M., Egholm, M., Altman, W. E., Attiya, S., Bader, J. S., Bemben, L. A., . . . Rothberg, J. M. (2005). Genome sequencing in microfabricated high-density picolitre reactors. *Nature, 437*(7057), 376-380. doi:10.1038/nature03959

Martin, A., Booth, J. N., Laird, Y., Sproule, J., Reilly, J. J., & Saunders, D. H. (2018). Physical activity, diet, and other behavioural interventions for improving cognition and school

achievement in children and adolescents with obesity or overweight. *Cochrane Database Syst Rev, 3*, CD009728. doi:10.1002/14651858.CD009728.pub4

Martínez Steele, E., Popkin, B. M., Swinburn, B., & Monteiro, C. A. (2017). The share of ultra-processed foods and the overall nutritional quality of diets in the U.S: Evidence from a nationally representative cross-sectional study. *Popul Health Metr, 15*(1), 6. doi:10.1186/s12963-017-0119-3

Mast, M., Kortzinger, I., Konig, E. & Muller, M. J. . (1998). Gender differences in fat mass of 5 - 7-year-old children. *International Journal of Obesity*, *22*, 878-884.

Matheson, D. M., Killen, J. D., Wang, Y., Varady, A., & Robinson, T. N. (2004). Children's food consumption during television viewing. *The American journal of clinical nutrition*, *79*(6), 1088–1094. https://doi.org/10.1093/ajcn/79.6.1088

Maxwell, A. E., Castillo, L., Arce, A. A., De Anda, T., Martins, D., & McCarthy, W. J. (2018). Eating veggies is fun! An implementation pilot study in partnership with a YMCA in south Los Angeles. Prev Chronic Dis, 15, E132. doi:10.5888/pcd15.180150

McCabe, M. P., & Ricciardelli, L. A. (2005). A prospective study of pressures from parents, peers, and the media on extreme weight change behaviors among adolescent boys and girls. *Behav Res Ther, 43*(5), 653-668. doi:10.1016/j.brat.2004.05.004

McConnell, R., Shen, E., Gilliland, F., Jerrett, M., Wolch, J., & Chang, C. et al. (2014). A Longitudinal Cohort Study of Body Mass Index and Childhood Exposure to Secondhand Tobacco Smoke and Air Pollution: The Southern California Children's Health Study. *Environmental Health Perspectives*. http://dx.doi.org/10.1289/ehp.1307031

McGloin, A. F., Livingstone, M. B. E., Greene, L. C., Webb, S. E., Gibson, J. M. A., Jebb, S.A., Cole…Prentice, A. M. (2002). Energy and fat intake in obese and lean children at varying risk of obesity. *International Journal of Obesity, 26*, 200-207.

McLaughlin, E. A., Belon, K. E., Smith, J. E., & Erickson, S. J. (2015). Mothers' and daughters' beliefs about factors affecting preadolescent girls' body satisfaction. *Body Image, 13*, 9-17. doi:10.1016/j.bodyim.2014.11.002

McMurdie, P. J., & Holmes, S. (2012). Phyloseq is a bioconductor package for handling and analyzing high-throughput phylogenetic sequence data. *Pacific Symposium on Biocomputing. Pacific Symposium on Biocomputing*, 235–246.

Memish, Z., El Bcheraoui, C., Tuffaha, M., Robinson, M., Daoud, F., & Jaber, S. et al. (2014). Obesity and Associated Factors — Kingdom of Saudi Arabia, 2013. *Preventing Chronic Disease*, *11*. http://dx.doi.org/10.5888/pcd11.140236

Michaelides, M., Miller, M., Subrize, M., Kim, R., Robison, L., & Hurd, Y. et al. (2013). Limbic activation to novel versus familiar food cues predicts food preference and alcohol intake. *Brain Research*, *1512*, 37-44. http://dx.doi.org/10.1016/j.brainres.2013.03.006

Milanski, M., Degasperi, G., Coope, A., Morari, J., Denis, R., Cintra, D. E., . . . Velloso, L. A. (2009). Saturated fatty acids produce an inflammatory response predominantly through the activation of tlr4 signaling in hypothalamus: Implications for the pathogenesis of obesity. *J Neurosci, 29*(2), 359-370. doi:10.1523/jneurosci.2760-08.2009

Million, M., Maraninchi, M., Henry, M., Armougom, F., Richet, H., Carrieri, P., . . . Raoult, D. (2011). Obesity-associated gut microbiota is enriched in lactobacillus reuteri and depleted in bifidobacterium animalis and methanobrevibacter smithii. *International Journal of Obesity, 36*(6), 817-825. doi:10.1038/ijo.2011.153

Minghelli, B., Oliveira, R., & Nunes, C. (2015). Association of obesity with chronic disease and musculoskeletal factors. *Rev Assoc Med Bras (1992), 61*(4), 347-354. doi:10.1590/1806-9282.61.04.347

Montano, Z., Smith, J. D., Dishion, T. J., Shaw, D. S., & Wilson, M. N. (2015). Longitudinal relations between observed parenting behaviors and dietary quality of meals from ages 2 to 5. *Appetite, 87*, 324–329. doi:10.1016/j.appet.2014.12.219

Mor, A., Antonsen, S., Kahlert, J., Holsteen, V., Jorgensen, S., Holm-Pedersen, J., . . . Ehrenstein, V. (2015). Prenatal exposure to systemic antibacterials and overweight and obesity in Danish schoolchildren: A prevalence study. *Int J Obes (Lond), 39*(10), 1450-1455. doi:10.1038/ijo.2015.129

Moreno-Indias, I., Cardona, F., Tinahones, F., & Queipo-OrtuÃ±o, M. (2014). Impact of the gut microbiota on the development of obesity and type 2 diabetes mellitus. *Frontiers in Microbiology, 5*. http://dx.doi.org/10.3389/fmicb.2014.00190

Morris, M., Beilharz, J., Maniam, J., Reichelt, A., & Westbrook, R. (2015). Why is obesity such a problem in the 21st century? The intersection of palatable food, cues and reward pathways, stress, and cognition. *Neuroscience & Biobehavioral Reviews, 58*, 36-45. http://dx.doi.org/10.1016/j.neubiorev.2014.12.002

Morrison, J. A., Friedman, L. A., & Gray-McGuire, C. (2007). Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: The Princeton lipid research clinics follow-up study. *Pediatrics, 120*(2), 340–345. doi:10.1542/peds.2006-1699

Morrison, K. M., Shin, S., Tarnopolsky, M., & Taylor, V. H. (2015). Association of depression & health related quality of life with body composition in children and youth with obesity. *J Affect Disord, 172*, 18-23. doi:10.1016/j.jad.2014.09.014

Moschen, A. R., Wieser, V., & Tilg, H. (2012). Dietary factors: Major regulators of the gut's microbiota. *Gut Liver, 6*(4), 411-416. doi:10.5009/gnl.2012.6.4.411

Mshvildadze, M., Neu, J., Shuster, J., Theriaque, D., Li, N., & Mai, V. (2010). Intestinal microbial ecology in premature infants assessed with non-culture-based techniques. *J Pediatr, 156*(1), 20-25. doi:10.1016/j.jpeds.2009.06.063

Mueller, N. T., Whyatt, R., Hoepner, L., Oberfield, S., Dominguez-Bello, M. G., Widen, E. M., . . . Rundle, A. (2015). Prenatal exposure to antibiotics, cesarean section, and risk of childhood obesity. *Int J Obes (Lond), 39*(4), 665–670. doi:10.1038/ijo.2014.180

Mueller, S., Saunier, K., Hanisch, C., Norin, E., Alm, L., Midtvedt, T., . . . Blaut, M. (2006). Differences in fecal microbiota in different European study populations in relation to age, gender, and country: A cross-sectional study. *Appl Environ Microbiol, 72*(2), 1027-1033. doi:10.1128/aem.72.2.1027-1033.2006

Murga-Garrido, S., Ulloa-Pérez, E., Díaz-Benítez, C., Orbe-Orihuela, Y., Cornejo-Granados, F., Ochoa-Leyva, A., Sánchez-Flores, A., Cruz, M., Castañeda-Márquez, A., Plett-Torres, T., García, A., & Lagunas-Martínez, A. (2023). Virulence Factors of the Gut Microbiome Are Associated with BMI and Metabolic Blood Parameters in Children with Obesity. *Microbiology Spectrum*. https://doi.org/10.1128/spectrum.03382-22.

Murphy, L., & Blackstock, S. (2018). The influence of technology on obesity in children and adolescents threats to, and opportunities for, health in our digital world. *Archives of Disease in Childhood*, 103, A174 - A174. https://doi.org/10.1136/archdischild-2018-rcpch.417

Murphy, E. F., Cotter, P. D., Healy, S., Marques, T. M., O'Sullivan, O., Fouhy, F., . . . Shanahan, F. (2010). Composition and energy harvesting capacity of the gut microbiota:

Relationship to diet, obesity, and time in mouse models. *Gut, 59*(12), 1635-1642. doi:10.1136/gut.2010.215665

Munyaka, P. M., Khafipour, E., & Ghia, J. E. (2014). External influence of early childhood establishment of gut microbiota and subsequent health implications. *Front Pediatr, 2*, 109. doi:10.3389/fped.2014.00109

Murugesan, S., Nirmalkar, K., Hoyo-Vadillo, C., García-Espitia, M., Ramírez-Sánchez, D., & García-Mena, J. (2018). Gut microbiome production of short-chain fatty acids and obesity in children. *Eur J Clin Microbiol Infect Dis, 37*(4), 621-625. doi:10.1007/s10096-017-3143-0

Murugesan, S., Ulloa-Martínez, M., Martínez-Rojano, H., Galván-Rodríguez, F. M., Miranda-Brito, C., Romano, M. C., . . . García-Mena, J. (2015). Study of the diversity and short-chain fatty acids production by the bacterial community in overweight and obese Mexican children. *European Journal of Clinical Microbiology & Infectious Diseases, 34*(7), 1337-1346. doi:10.1007/s10096-015-2355-4

Must, A., Jacques, P. F., Dallal, G. E., Bajema, C. J., & Dietz, W. H. (1992). Long-term morbidity and mortality of overweight adolescents. A follow-up of the Harvard growth study of 1922 to 1935. *N Engl J Med, 327*(19), 1350-1355. doi:10.1056/nejm199211053271904

Nagy, T. R., Gower, B. A., Trowbridge, C. A., Dezenberg, C., Shewchuk, R. M., & Goran, M. I. (1997). Effects of gender, ethnicity, body composition and fat distribution on serum leptin concentrations in children. *Journal of Clinical Endocrinology and Metabolism, 82*(7), 2148-2152.

Nakayama, J., Yamamoto, A., Palermo-Conde, L. A., Higashi, K., Sonomoto, K., Tan, J., & Lee, Y. K. (2017). Impact of westernized diet on gut microbiota in children on Leyte Island. *Front Microbiol, 8*, 197. doi:10.3389/fmicb.2017.00197

Natale, R., Messiah, S., Asfour, L., Uhlhorn, S., Englebert, N., & Arheart, K. (2017). Obesity Prevention Program in Childcare Centers: Two-Year Follow-Up. *American Journal of Health Promotion*, 31, 502 - 510. https://doi.org/10.1177/0890117116661156.

National Association of School Nurses (NASN). (2015). Framework for 21st century school nursing practice. *NASN School Nurse, 130*(4), 219-231. doi: 10.1177/1942602X15589559

National Cancer Institute (NCI). (2020). Automated self-administered 24-hour dietary assessment tool (2020, October 5). https://epi.grants.cancer.gov/asa24/

National Center for Health Statistics NCHS). (2001). Healthy People 2000 Final Review, 76. Hyattsville. MD. https://www.cdc.gov/nchs/data/hp2000/hp2k01.pdf

National Center for Health Statistics (NCHS). (2012). Healthy People 2010 Final Review, 19-3. Hyattsville. MD. Retrieved from https://www.cdc.gov/nchs/data/hpdata2010/hp2010_final_review.pdf

National Center for Health Statistics (NCHS). (2021). Healthy People 2020 Final Review. Hyattsville. MD. Retrieved from https://stacks.cdc.gov/view/cdc/11117

National Survey of Children's Health (NSCH). (2021). Child and adolescent health measurement 2020-2021 initiative. *Data Resource Center for Child & Adolescent Health.* Retrieved from https://www.childhealthdata.org/browse/survey/results?q=9246&r=1

National Survey of Children's Health (NSCH). (2022). Child and adolescent health measurement initiative. *Data Resource Center for Child & Adolescent Health.* Retrieved from https://www.childhealthdata.org/browse/survey/results?q=10043&r=1

National Restaurant Association, (2019). Restaurant industry factbook. Washington, DC:

Neu, J. (2015). Developmental aspects of maternal-fetal, and infant gut microbiota and implications for long-term health. *Maternal Health, Neonatology and Perinatology, 1*(1). doi:10.1186/s40748-015-0007-4

Nestle, M., Wing, R., Birch, L., DiSogra, L., Drewnoski, A., Middleton, S, M.,… Economos, C. (1998). Behavioral and social influences on food choice. *Nutr Rev, 56*(5), S50-S74.

Nguyen, V. T., Larson, D. E., Johnson, R. K., & Goran, M. I. (1996). Fat intake and adiposity in children of lean and obese parents. *Am J Clin Nutr, 63*, 507-513.

Nianogo, R., & Arah, O. (2022). Forecasting Obesity and Type 2 Diabetes Incidence and Burden: The ViLA-Obesity Simulation Model. *Frontiers in Public Health*, 10. https://doi.org/10.3389/fpubh.2022.818816

Nieto, F. J., Szklo, M., & Comstock, G. W. (1992). Childhood weight and growth rate as predictors of adult mortality. *Am J Epidemiol, 136*(2), 201-213.

Nogal, A., Valdes, A. M., & Menni, C. (2021). The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. *Gut Microbes, 13*(1), 1-24. doi:10.1080/19490976.2021.1897212

Notarbartolo, V., Giuffrè, M., Montante, C., Corsello, G., & Carta, M. (2022). Composition of human breast milk microbiota and its role in children's health. *Pediatr Gastroenterol Hepatol Nutr, 25*(3), 194-210. doi:10.5223/pghn.2022.25.3.194

Office of Disease Prevention and Health Promotion (ODPHP). (2020). Nutrition and weight status: Weight status, nws-10.4. *Healthy People 2020.* U.S. Department of Health and Human Services. Retrieved from https://www.healthypeople.gov/2020/topics-objectives/topic/nutrition-and-weight-status/objectives

Office of Disease Prevention and Health Promotion (ODPHP). (2020a). Nutrition and weight status: Weight status, nws-10.4. *Healthy People 2020.* U.S. Department of Health and Human Services. Retrieved from https://www.healthypeople.gov/2020/data-search/Search-the-Data#objid=4928

Office of Disease Prevention and Health Promotion (ODPHP). (2020b). Nutrition and weight status: Weight status, nws-10.2. *Healthy People 2020.* U.S. Department of Health and Human Services. Retrieved from https://www.healthypeople.gov/2020/data-search/Search-the-Data#objid=4926

Office of Disease Prevention and Health Promotion (ODPHP). (2020c). Nutrition and weight status: Weight status, nws-10.3. *Healthy People 2020.* U.S. Department of Health and Human Services. Retrieved from https://www.healthypeople.gov/2020/data-search/Search-the-Data#objid=4927

Ogden, C. L., Carroll, M. D., Fryar, C. D., & Flegal, K. M. (2015). Prevalence of obesity among adults and youth: United States, 2011–2014. NCHS data brief, no 219. Hyattsville, MD: National Center for Health Statistics. Retrieved from https://www.cdc.gov/nchs/data/databriefs/db219.pdf

Ogden, C., Carroll, M., Lawman, H., Fryar, C., Kruszon-Moran, D., Kit, B., & Flegal, K. (2016). Trends in obesity prevalence among children and adolescents in the United States, 1988-1994 through 2013-2014. *JAMA*, *315*(21), 2292. http://dx.doi.org/10.1001/jama.2016.6361

Ogden C. L, & Flegal, K. M. (2010). Changes in terminology for childhood overweight and obesity. National health statistics reports; no 25. Hyattsville, MD.

Olds, T., Maher, C., Zumin, S., Peneau, S., Lioret, S., Castetbon, K., . . . Summerbell, C. (2011). Evidence that the prevalence of childhood overweight is plateauing: Data from nine countries. *Int J Pediatr Obes, 6*(5-6), 342-360. doi:10.3109/17477166.2011.605895

Oliveria, S. A., Ellison, R. C., Moore L. L. Gillman, M. W., Garrahie, E. J., & Singer, M. R. (1992). Parent-child relationships in nutrient intake: The Framingham children's study. *Am J Clin Nutr, 56*, 593–598.

Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A., & Brown, P. O. (2007). Development of the human infant intestinal microbiota. *PLoS Biol*, *5*(7), e177. doi:10.1371/journal.pbio.0050177

Pan, L., Freedman, D. S., Park, S., Galuska, D. A., Potter, A., & Blanck, H. M. (2019). Changes in obesity among US children aged 2 through 4 years enrolled in WIC during 2010-2016. *Jama, 321*(23), 2364-2366. doi:10.1001/jama.2019.5051

Payne, A. N., Chassard, C., Zimmermann, M., Muller, P., Stinca, S., & Lacroix, C. (2011). The metabolic activity of gut microbiota in obese children is increased compared with normal-weight children and exhibits more exhaustive substrate utilization. *Nutr Diabetes, 1*, e12. doi:10.1038/nutd.2011.8

Pearl, R., & Lebowitz, M. (2014). Beyond personal responsibility: Effects of causal attributions for overweight and obesity on weight-related beliefs, stigma, and policy support. *Psychology & Health*, *29*(10), 1176-1191. http://dx.doi.org/10.1080/08870446.2014.916807

Pekmez, C. T., Dragsted, L. O., & Brahe, L. K. (2019). Gut microbiota alterations and dietary modulation in childhood malnutrition - the role of short chain fatty acids. *Clin Nutr, 38*(2), 615-630. doi:10.1016/j.clnu.2018.02.014

Penders, J., Thijs, C., Vink, C., Stelma, F. F., Snijders, B., Kummeling, I., . . . Stobberingh, E. E. (2006). Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics, 118*(2), 511-521. doi:10.1542/peds.2005-2824

Perry, R. J., Peng, L., Barry, N. A., Cline, G. W., Zhang, D., Cardone, R. L., . . . Shulman, G. I. (2016). Acetate mediates a microbiome-brain-β-cell axis to promote metabolic syndrome. *Nature, 534*(7606), 213–217. doi:10.1038/nature18309

Perusse, L., Tremblay, A., Leblanc, C. Cloninger, C. R., Reich, T., Rice, & Bouchard, C. (1988). Familial resemblance in energy intake: Contribution of genetic and environmental factors. *Am J Clin Nutr, 47*, 629-635.

Pihl, A. F., Fonvig, C. E., Stjernholm, T., Hansen, T., Pedersen, O., & Holm, J.-C. (2016). The role of the gut microbiota in childhood obesity. *Childhood Obesity, 12*(4), 292-299. doi:10.1089/chi.2015.0220

Piirainen, T., Isolauri, E., Lagström, H., & Laitinen, K. (2007). Impact of dietary counselling on nutrient intake during pregnancy: A prospective cohort study. *British Journal of Nutrition, 96*(06), 1095. doi:10.1017/bjn20061952

Pike, K. M., & Rodin, J. (1991). Mothers, daughters, and disordered eating. *Journal of abnormal psychology*, *100*(2), 198–204. https://doi.org/10.1037//0021-843x.100.2.198

Pineros-Leano, M., Grafft, N., & Aguayo, L. (2022). Childhood obesity risk factors by race and ethnicity. *Obesity*, 30, 1670 - 1680. https://doi.org/10.1002/oby.23500.

Potts, N. L. & Mandleco, B. (2012). *Pediatric Nursing: Caring for children and families, third edition.* Clifton Park, NY: Delmar Cengage Learning.

Poulton, R., & Williams, S. (2001). Breastfeeding and risk of overweight. *Jama, 286*(12), 1448-1450. doi:10.1001/jama.286.12.1447Pryde, S. E., Duncan, S. H., Hold, G. L., Stewart, C.

S. & Flint, H. J. (2002). The microbiology of butyrate formation in the human colon. *FEMS Microbilology Letters, 217*, 133-139

Population Reference Bureau (PRB). (2024). Students who are overweight or obese by race/ethnicity and grade level. *Kidsdata,org.* Retrieved from https://www.kidsdata.org/topic/727/overweight-race/table#fmt=1028&loc=2,364&tf=124&ch=623,7,11,70,10,72,9&sortColumnId=0&sortType=asc

Prentice, A. M. (2006). The emerging epidemic of obesity in developing countries. *Int J Epidemiol, 35*(1), 93-99. doi:10.1093/ije/dyi272

Price, R. A., Reed, D. R., & Lee, J. H. (1998). Obesity related phenotypes in families selected for extreme obesity and leanness. *International Journal of Obesity, 22*, 406-413.

Pulgaron, E., & Delamater, A. (2014). Obesity and Type 2 Diabetes in Children: Epidemiology and Treatment. *Current Diabetes Reports*, *14*(8). http://dx.doi.org/10.1007/s11892-014-0508-y

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., . . . Wang, J. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature, 464*(7285), 59-65. doi:10.1038/nature08821

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., & Zhang, F. et al. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*, *490*(7418), 55–60. http://dx.doi.org/10.1038/nature11450

Rahat-Rozenbloom, S., Fernandes, J., Gloor, G. B., & Wolever, T. M. (2014). Evidence for greater production of colonic short-chain fatty acids in overweight than lean humans. *Int J Obes (Lond), 38*(12), 1525-1531. doi:10.1038/ijo.2014.46

Ramirez, I., & Friedman, M. I. (1990). Dietary hyperphagia in rats: Role of fat, carbohydrate, and energy content. *Physiology & Behavior, 4*, 1157–1163.

Rautava, S., Collado, M. C., Salminen, S., Isolauri, E. (2012). Probiotics modulate host-microbe interaction in the placenta and fetal gut: A randomized double-blind placebo-controlled trial. *Neonatology, 102*, 178-184. doi:10.1159/000339182

Reilly, J. J., Bonataki, M., Leary, S. D., Wells, J. C., Davey-Smith, G., Emmett, P., . . . Sherriff, A. (2011). Progression from childhood overweight to adolescent obesity in a large contemporary cohort. *International Journal of Pediatric Obesity, 6*(2-2), e138-e143. doi:10.3109/17477166.2010.497538

Rettger, L. F. & Cheplin, H. A. (1921). *A treatise on the transformation of the intestinal flora with special reference to the implantation of bacillus acidophilus*. New Haven, Connecticut: Yale University Press. doi: https://doi.org/10.5962/bhl.title.24080

Ricketts, C. D. (1997). Fat preferences, dietary fat intake and body composition in children. *European Journal of Clinical Nutrition, 51*, 778-781.

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., … Gordon, J. I. (2013). Cultured gut microbiota from twins discordant for obesity modulate adiposity and metabolic phenotypes in mice. *Science (New York, N.Y.), 341*(6150), 10.1126/science.1241214. http://doi.org/10.1126/science.1241214

Riddoch, C. J., Leary, S. D., Ness, A. R., Blair, S. N., Deere, K., Mattocks, C., . . . Tilling, K. (2009). Prospective associations between objective measures of physical activity and fat mass in 12–14-year-old children: The avon longitudinal study of parents and children (alspac). *Bmj, 339*, b4544. doi:10.1136/bmj.b4544

Ringel-Kulka, T., Cheng, J., Ringel, Y., Salojarvi, J., Carroll, I., Palva, A., . . . Satokari, R. (2013). Intestinal microbiota in healthy U.S. Young children and adults--a high throughput microarray analysis. *Plos One, 8*(5), e64315. doi:10.1371/journal.pone.0064315

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G., Gasbarrini, A., & Mele, M. (2019). What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms, 7*(1). doi:10.3390/microorganisms7010014

Ríos-Covián, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., de Los Reyes-Gavilán, C. G., & Salazar, N. (2016). Intestinal short chain fatty acids and their link with diet and human health. *Front Microbiol, 7*, 185. doi:10.3389/fmicb.2016.00185

Riva, A., Borgo, F., Lassandro, C., Verduci, E., Morace, G., Borghi, E., & Berry, D. (2017). Pediatric obesity is associated with an altered gut microbiota and discordant shifts in firmicutes populations. Environmental Microbiology, 19(1), 95-105. doi:10.1111/1462-2920.13463

Roberts, C. K., Freed, B., & McCarthy, W. J. (2010). Low aerobic fitness and obesity are associated with lower standardized test scores in children. *J Pediatr, 156*(5), 711-718, 718.e711. doi:10.1016/j.jpeds.2009.11.039

Robert Wood Johnson Foundation (RWJF). (2023). *State of childhood obesity: Ages 10 -17.* Retrieved from https://stateofchildhoodobesity.org/demographic-data/ages-10-17/#:~:text=Roughly%20one%20in%20six%20youth,ethnicity%20at%20the%20national%20level

Roberto, C., Swinburn, B., Hawkes, C., Huang, T., Costa, S., & Ashe, M. et al. (2015). Patchy progress on obesity prevention: emerging examples, entrenched barriers, and new thinking. *The Lancet*, *385*(9985), 2400-2409. http://dx.doi.org/10.1016/s0140-6736(14)61744-x

Robertson, S. M., Cullen, K. W., Baranowski, J., Baranowski, T. O. M., Hu, S., & de Moor, C. (1999). Factors related to adiposity among children aged 3 to 7 years. *Journal of the American Dietetic Association, 99*(8), 938-943. doi:10.1016/s0002-8223(99)00224-2

Rocha, D. M., Caldas, A. P., Oliveira, L. L., Bressan, J., & Hermsdorff, H. H. (2016). Saturated fatty acids trigger tlr4-mediated inflammatory response. *Atherosclerosis, 244*, 211-215. doi:10.1016/j.atherosclerosis.2015.11.015

Rokholm, B., Baker, J. L., & Sorensen, T. I. (2010). The levelling off of the obesity epidemic since the year 1999--a review of evidence and perspectives. *Obes Rev, 11*(12), 835-846. doi:10.1111/j.1467-789X.2010.00810.x

Rollins, B. Y., Loken, E., Savage, J. S., & Birch, L. L. (2014). Maternal controlling feeding practices and girls' inhibitory control interact to predict changes in BMI and eating in the absence of hunger from 5 to 7 y. *Am J Clin Nutr, 99*(2), 249–257. doi:10.3945/ajcn.113.063545

Rolls, B. J., Kim-Harris, S., Fishman, M. W., Foltin, R. W., Moran, T. H., & Stoner, S. A. (1994). Satiety after preloads with different amounts of fat and carbohydrate: Implications of obesity. *Am J Clin Nutr, 60*, 476-487.

Rolls, B., J., & Hammer, V. A. (1995). Fat, carbohydrate, and the regulation of energy intake. *Am J Clin Nutr, 62*, 1086S-1095S.

Rolls B. J. (2003). The Supersizing of America: Portion Size and the Obesity Epidemic. *Nutrition today*, *38*(2), 42–53. https://doi.org/10.1097/00017285-200303000-00004

Rolls, B. J., Morris, E. L., & Roe, L. S. (2002). Portion size of food affects energy intake in normal-weight and overweight men and women. *The American journal of clinical nutrition*, *76*(6), 1207–1213. https://doi.org/10.1093/ajcn/76.6.1207

Romanos-Nanclares, A., Zazpe, I., Santiago, S., Marin, L., Rico-Campa, A., & Martin-Calvo, N. (2018). Influence of parental healthy-eating attitudes and nutritional knowledge on nutritional adequacy and diet quality among preschoolers: The SENDO project. *Nutrients*, *10*(12). doi:10.3390/nu10121875

Romero, R., Hassan, S. S., Gajer, P., Tarca, A. L., Fadrosh, D. W., Nikita, L.,...Ravel, J. (2014). The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome,* (4), 1–19.

Romo-Palafox, M. J., Ranjit, N., Sweitzer, S. J., Roberts-Gray, C., Hoelscher, D. M., Byrd-Williams, C. E., & Briley, M. E. (2015). Dietary quality of preschoolers' sack lunches as measured by the healthy eating index. *J Acad Nutr Diet, 115*(11), 1779-1788. doi:10.1016/j.jand.2015.05.017

Rosenbaum, M., & Leibel, R. L. (1998). The physiology of body weight regulation: Relevance to the etiology of obesity in children. *Pediatrics, 101*, 525-539.

Rosettie, K. L., Micha, R., Cudhea, F., Penalvo, J. L., O'Flaherty, M., Pearson-Stuttard, J., . . . Mozaffarian, D. (2018). Comparative risk assessment of school food environment policies and childhood diets, childhood obesity, and future cardiometabolic mortality in the United States. *Plos One, 13*(7), e0200378. doi:10.1371/journal.pone.0200378

Rook, G. A., Lowry, C. A., & Raison, C. L. (2015). Hygiene and other early childhood influences on the subsequent function of the immune system. *Brain Res, 16*, 47–62. doi:10.1016/j.brainres.2014.04.004

Russell, W., Gratz, S., Duncan, S., Holtrop, G., Ince, J., & Scobbie, L. et al. (2011). High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *American Journal of Clinical Nutrition*, *9*(5), 1062–1072. http://dx.doi.org/10.3945/ajcn.110.002188

Sabin, M., Kao, K., Juonala, M., Baur, L., & Wake, M. (2015). Viewpoint article: Childhood obesity - looking back over 50 years to begin to look forward. *Journal of Paediatrics and Child Health, 5*(1), 82–86. http://dx.doi.org/10.1111/jpc.12819

Safavi, M., Farajian, S., Kelishadi, R., Mirlohi, M., & Hashemipour, M. (2013). The effects of symbiotic supplementation on some cardio-metabolic risk factors in overweight and obese children: A randomized triple-masked controlled trial. *Int J Food Sci Nutr, 64*(6), 687–693. doi:10.3109/09637486.2013.775224

Salminen, S., Gibson, G. R., McCartney, A. L., & Isolauri, E. (2004). Influence of mode of delivery on gut microbiota composition in seven-year-old children. *Gut, 53*(9), 1388-1389. doi:10.1136/gut.2004.041640

Sanchez-Tapia, M., Miller, A. W., Granados-Portillo, O., Tovar, A. R., & Torres, N. (2020). The development of metabolic endotoxemia is dependent on the type of sweetener and the presence of saturated fat in the diet. Gut Microbes, 12(1), 1801301. doi:10.1080/19490976.2020.1801301

Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A, 74*(12), 5463-5467.

Sanmiguel, C., Gupta, A., & Mayer, E. (2015). Gut Microbiome and Obesity: A Plausible Explanation for Obesity. *Current Obesity Reports, 4*(2), 250-261. http://dx.doi.org/10.1007/s13679-015-0152-0

Salz, K. M., Tamir, I., Ernst, N., Kwiterovich, P., Glueck, C., Christensen, B.,…Scott, L. W. (1983). Selected nutrient intakes of free-living white children ages 6-19 years: The lipid research clinics program prevalence study. *Pediatric Research, 17*, 124–130.

Satokari, R., Gronroos, T., Laitinen, K., Salminen, S., & Isolauri, E. (2009). Bifidobacterium and lactobacillus DNA in the human placenta. *Lett Appl Microbiol, 48*(1), 8-12. doi:10.1111/j.1472-765X.2008.02475.x

Savage, J. S., Birch, L. L., Marini, M., Anzman-Frasca, S., & Paul, I. M. (2016). Effect of the insight responsive parenting intervention on rapid infant weight gain and overweight status at age 1 year: A randomized clinical trial. *JAMA Pediatrics, 170*(8), 742-749. doi:10.1001/jamapediatrics.2016.0445

Scaglioni, S., De Cosmi, V., Ciappolino, V., Parazzini, F., Brambilla, P., & Agostoni, C. (2018). Factors influencing children's eating behaviours. *Nutrients, 10*(6). doi:10.3390/nu10060706

Scheppach, W. (1994). Effects of short chain fatty acids on gut morphology and function. *Gut, 35*(1 Suppl), S35-38.

Schmidt, M. D., Dwyer, T., Magnussen, C. G., & Venn, A. J. (2010). Predictive associations between alternative measures of childhood adiposity and adult cardio-metabolic health. *International Journal of Obesity, 35*(1), 38-45. doi:10.1038/ijo.2010.205

Schwiertz, A., Taras, D., Schafer, K., Beijer, S., Bos, N. A., Donus, C., & Hardt, P. D. (2010). Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring), 18*(1), 190-195. doi:10.1038/oby.2009.167

Schulze, M. B., Fung, T. T., Manson, J. E., Willett, W. C., & Hu, F. B. (2006). Dietary patterns and changes in body weight in women. *Obesity, 14*(8), 1444-1453. doi:https://doi.org/10.1038/oby.2006.164

Schutz, Y., Flatt, J. P., & Jequier, E. (1989). Failure of dietary fat intake to promote fat oxidation: A factor favoring the development of obesity. *Am J Clin Nutr, 50*, 307-314.

Seethaler, B., Nguyen, N. K., Basrai, M., Kiechle, M., Walter, J., Delzenne, N. M., & Bischoff, S. C. (2022). Short-chain fatty acids are key mediators of the favorable effects of the Mediterranean diet on intestinal barrier integrity: Data from the randomized controlled libre trial. *Am J Clin Nutr, 116*(4), 928-942. doi:10.1093/ajcn/nqac175

Shaping behavior and attitude in childhood obesity – Physiopedia (2016). *Physio-pedia.com*. Retrieved 4 August 2017, from http://www.physio-pedia.com/Shaping_behaviour_and_attitude_in_childhood_obesity

Shen, J., Obin, M., & Zhao, L. (2012). The gut microbiota, obesity, and insulin resistance. *Molecular Aspects of Medicine, 34*(1), 39-58. http://dx.doi.org/10.1016/j.mam.2012.11.001

Shin, S., & Cho, K. Y. (2020). Altered gut microbiota and shift in Bacteroidetes between young obese and normal-weight Korean children: A cross-sectional observational study. *Biomed Res Int, 2020*, 6587136. doi:10.1155/2020/6587136

Shreiner, A., Kao, J., & Young, V. (2015). The gut microbiome in health and in disease. *Current Opinion in Gastroenterology, 31*(1), 69-75. http://dx.doi.org/10.1097/mog.0000000000000139

Shridhar, G., Rajendra, N., Murigendra, H., Shridevi, P. . (2015). Modern diet and its impact on human health. *Journal of Nutrition & Food Sciences, 05*(06). doi:10.4172/2155-9600.1000430

Shubrook, J. (2011). *Childhood obesity and the risk of diabetes in minority populations* (p. 20). DOs Against DIABETES. Retrieved from http://www.cecity.com/aoa/healthwatch/jan_11/print5.pdf

Simmonds, M., Llewellyn, A., Owen, C. G., & Woolacott, N. (2015). Predicting adult obesity from childhood obesity: A systematic review and meta-analysis. *Obes Rev, 17*(2), 95-107. doi:10.1111/obr.12334

Singh, R. K., Chang, H. W., Yan, D., Lee, K. M., Ucmak, D., Wong, K., . . . Liao, W. (2017*).* Influence of diet on the gut microbiome and implications for human health. *J Transl Med, 15*(1), 73. doi:10.1186/s12967-017-1175-y

Smith, J. D., Montano, Z., Dishion, T. J., Shaw, D. S., & Wilson, M. N. (2015). Preventing weight gain and obesity: Indirect effects of the family check-up in early childhood. *Prev Sci, 16*(3), 408-419. doi:10.1007/s11121-014-0505-z

Schrempft, S., van Jaarsveld, C. H. M., Fisher, A., Herle, M., Smith, A. D., Fildes, A., & Llewellyn, C. H. (2018). Variation in the heritability of child body mass index by obesogenic home environment. JAMA Pediatrics, 172(12), 1153-1160. doi:10.1001/jamapediatrics.2018.1508

Shridhar, G., Rajendra, N., Murigendra, H., Shridevi, P. . (2015). Modern diet and its impact on human health. *Journal of Nutrition & Food Sciences, 05*(06). doi:10.4172/2155-9600.1000430.

Sicard, J., Bihan, G., Vogeleer, P., Jacques, M., & Harel, J. (2017). Interactions of Intestinal Bacteria with Components of the Intestinal Mucus. *Frontiers in Cellular and Infection Microbiology*, 7. https://doi.org/10.3389/fcimb.2017.00387

Solomons, N. W. G., R. (1995). Urban nutriiton in developing countries. *Nutr Rev, 53*(4), 90 - 95.

Speakman, J. R., de Jong, J. M. A., Sinha, S., Westerterp, K. R., Yamada, Y., Sagayama, H., . . . Wong, W. W. (2023). Total daily energy expenditure has declined over the past three decades due to declining basal expenditure, not reduced activity expenditure. *Nat Metab, 5*(4), 579-588. doi:10.1038/s42255-023-00782-2

Squillario, M., Bonaretti, C., La Valle, A., Di Marco, E., Piccolo, G., Minuto, N., . . . Biassoni, R. (2023). Gut-microbiota in children and adolescents with obesity: Inferred functional analysis and machine-learning algorithms to classify microorganisms. *Sci Rep, 13*(1), 11294. doi:10.1038/s41598-023-36533-

Stanislawski, M. A., Dabelea, D., Wagner, B. D., Iszatt, N., Dahl, C., Sontag, M. K., . . . Eggesbo, M. (2018). Gut microbiota in the first 2 years of life and the association with body mass index at age 12 in a Norwegian birth cohort. *MBio, 9*(5). doi:10.1128/mBio.01751-18

Starc, G., & Strel, J. (2011). Tracking excess weight and obesity from childhood to young adulthood: A 12-year prospective cohort study in Slovenia. *Public Health Nutrition, 14*(1), 49-55. doi:10.1017/S1368980010000741

Stark, P. L., & Lee, A. (1982). The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. *J Med Microbiol, 15*(2), 189-203. doi:10.1099/00222615-15-2-189

Statistics - Childhood Obesity Foundation. (2016). *Childhood Obesity Foundation*. Retrieved 5 August 2017, from http://childhoodobesityfoundation.ca/what-is-childhood-obesity/statistics/

Steel, J. H., Malatos, S., Kennea, N., Edwards, A. D., Miles, L., Duggan, P., . . . Sullivan, M. H. F. (2005). Bacteria and inflammatory cells in fetal membranes do not always cause preterm labor. *Pediatric Research, 57*(3), 404-411. doi:10.1203/01.pdr.0000153869.96337.90

Steiner, J. E. (1977). Facial expressions of the neonate infant indicating the hedonics of food-related chemical stimuli. In J. M. Weiffenbach (Ed.), *The Genesis of Sweet Preference* (pp. 173-189). Bethesda, Maryland: NIH.

Stierman, Bryan et al. (2021). National Health and Nutrition Examination Survey 2017–March 2020 Prepandemic Data Files Development of Files and Prevalence Estimates for Selected Health Outcomes. (158).

Stunkard, A. J., Sørensen, T. I., Hanis, C., Teasdale, T. W., Chakraborty, R., Schull, W. J., & Schulsinger, F. (1986). An adoption study of human obesity. *The New England journal of medicine*, *314*(4), 193–198. https://doi.org/10.1056/NEJM198601233140401

Stubbs, R. J., Ritz, P., Coward, W. A., & Prentice, A. M. (1995). Covert manipulation of the ratio of dietary fat to carbohydrate and energy density: Effect on food intake and energy balance in free-living men eating ad libitum. *Am J Clin Nutr, 62*, 330-337.

Sugizaki, C. S. A., & Naves, M. M. V. (2018). Potential prebiotic properties of nuts and edible seeds and their relationship to obesity. *Nutrients, 10*(11). doi:10.3390/nu10111645

Sullivan, S. A., & Birch, L. L. (1994). Infant dietary experience and acceptance of solid foods. *Pediatrics, 93*(2).

Swinburn, B., & Vandevijvere, S. (2016). WHO report on ending childhood obesity echoes earlier recommendations. *Public Health Nutrition*, 19, 1 - 2. https://doi.org/10.1017/S1368980015003663

Szabo, L. (2014). *Diabetes rates skyrocket in kids and teens*. *USA TODAY*. Retrieved 3 August 2017, from https://www.usatoday.com/story/news/nation/2014/05/03/diabetes-rises-in-kids/8604213/

Tai, N., Wong, F., & Wen, L. (2015). The role of gut microbiota in the development of type 1, type 2 diabetes mellitus and obesity. *Reviews in Endocrine and Metabolic Disorders, 16(*1), 55-65. http://dx.doi.org/10.1007/s11154-015-9309-0

Tan, C. C., & Holub, S. C. (2010). Children's self-regulation in eating: Associations with inhibitory control and parents' feeding behavior. *Journal of Pediatric Psychology, 36*(3), 340-345. doi:10.1093/jpepsy/jsq089

Tanaka, S., Kobayashi, T., Songjinda, P., Tateyama, A., Tsubouchi, M., Kiyohara, C., . . . Nakayama, J. (2009). Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. *FEMS Immunol Med Microbiol, 56*(1), 80-87. doi:10.1111/j.1574-695X.2009.00553.x

Tang, W. H., Wang, Z., Levison, B. S., Koeth, R. A., Britt, E. B., Fu, X., . . . Hazen, S. L. (2013). Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med, 368*(17), 1575-1584. doi:10.1056/NEJMoa1109400

Taylor, R. W., Gold, E., Manning, P., & Goulding, A. (1997). Gender differences in body fat content are present well before puberty. *Int J Obes Relat Metab Disord, 21*(11), 1082-1084. doi:10.1038/sj.ijo.0800522.

Taylor, E. D., Theim, K. R., Mirch, M. C., Ghorbani, S., Tanofsky-Kraff, M., Adler-Wailes, D. C., . . . Yanovski, J. A. (2006). Orthopedic complications of overweight in children and adolescents. *Pediatrics, 117*(6), 2167-2174. doi:10.1542/peds.2005-183

Thabane, L., Ma, J., Chu, R., Cheng, J., Ismaila, A., Rios, L. P., Robson, R....Goldsmith, C. H. (2010). A tutorial on pilot studies: The what, why and how. *BMC Medical Research Methodology, 10*(1), 1-10.

Tilg, H., & Adolph, T. E. (2015). Influence of the human intestinal microbiome on obesity and metabolic dysfunction. *Current Opinion in Pediatrics, 27*(4), 496-501. doi:10.109

Tilg, H., & Moschen, A. R. (2015). Food, immunity, and the microbiome. *Gastroenterology, 148*(6), 1107-1119. doi:10.1053/j.gastro.2014.12.036

Tierney, B. T., Yang, Z., Luber, J. M., Beaudin, M., Wibowo, M. C., Baek, C., . . . Kostic, A. D. (2019). The landscape of genetic content in the gut and oral human microbiome. *Cell Host Microbe, 26*(2), 283-295 e288. doi:10.1016/j.chom.2019.07.00

Thingholm, L., Rühlemann, M., Koch, M., Fuqua, B., Laucke, G., Boehm, R., Bang, C., Franzosa, E., Hübenthal, M., Rahnavard, A., Frost, F., Lloyd-Price, J., Schirmer, M., Lusis, A., Vulpe, C., Lerch, M., Homuth, G., Kacprowski, T., Schmidt, C., Nöthlings, U., Karlsen, T., Lieb, W., Laudes, M., Franke, A., & Huttenhower, C. (2019). Obese Individuals with and without Type 2 Diabetes Show Different Gut Microbial Functional Capacity and Composition.. *Cell host & microbe*. https://doi.org/10.1016/j.chom.2019.07.004

Tommaso, N., Gasbarrini, A., & Ponziani, F. (2021). Intestinal Barrier in Human Health and Disease. *International Journal of Environmental Research and Public Health*, 18. https://doi.org/10.3390/ijerph182312836

Tomova, A., Bukovsky, I., Rembert, E., Yonas, W., Alwarith, J., Barnard, N. D., & Kahleova, H. (2019). The effects of vegetarian and vegan diets on gut microbiota. *Front Nutr, 6*, 47. doi:10.3389/fnut.2019.00047

Trasande, L., Blustein, J., Liu, M., Corwin, E., Cox, L. M., & Blaser, M. J. (2013). Infant antibiotic exposures and early-life body mass. *Int J Obes (Lond), 37*(1), 16-23. doi:10.1038/ijo.2012.132

Trude, A. C., Surkan, P. J., Anderson Steeves, E., Pollack Porter, K., & Gittelsohn, J. (2019). The impact of a multilevel childhood obesity prevention intervention on healthful food acquisition, preparation, and fruit and vegetable consumption on African American adult caregivers. *Public Health Nutr, 22*(7), 1300-1315. doi:10.1017

Trust for America's Health (TFAH). 2023. The state of obesity: Better policies for a healthier America, 2023. Special report: 20-year report anniversary retrospective. Retrieved from https://www.tfah.org/report-details/state-of-obesity-2023/#:~:text=West%20Virginia%20(41%25)%2C%20Louisiana,the%20lowest%20adult%20obesity%20rates

Tucker, L. A., Seljaas, G. T., & Hager, R. L. (1997). Body fat percentage of children varies according to their diet composition. *Journal of the American Dietetic Association, 97*(9), 981-986. doi:10.1016/s0002-8223(97)00237-x

Tudge, J. R. H., Mokrova, I., Hatfield, B. E., & Karnik, R. B. (2009). Uses and misuses of Bronfenbrenner's bioecological theory of human development. *Journal of Family Theory & Review, 1*(4), 198-210. doi:10.1111/j.1756-2589.2009.00026.x

Tun, H. M., Bridgman, S. L., Chari, R., Field, C. J., Guttman, D. S., Becker, A. B., . . . Kozyrskyj, A. L. (2018). Roles of birth mode and infant gut microbiota in intergenerational transmission of overweight and obesity from mother to offspring. *JAMA Pediatr, 172*(4), 368-377. doi:10.1001/jamapediatrics.2017.5535

Turnbaugh, P., Ley, R., Mahowald, M., Magrini, V., Mardis, E., & Gordon, J. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature, 444*(7122), 1027-131. http://dx.doi.org/10.1038/nature05414

Turnbaugh, P. J., Bäckhed, F., Fulton, L., & Gordon, J. I. (2008a). Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe, 3*(4), 213-223. doi:10.1016/j.chom.2008.02.015

Turnbaugh, P. J., & Gordon, J. I. (2009). The core gut microbiome, energy balance and obesity. *The Journal of physiology*, *587*(Pt 17), 4153–4158. https://doi.org/10.1113/jphysiol.2009.174136

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., . . . Gordon, J. I. (2008b). A core gut microbiome in obese and lean twins. *Nature, 457*, 480. doi:10.1038/nature07540. Retrieved from https://www.nature.com/articles/nature07540#supplementary-information

U. S. Department of Agriculture (USDA). (2014). *School meals: Healthy hungry-free kids act.* Retrieved from https://www.fns.usda.gov/cn/healthy-hunger-free-kids-act

U. S. Department of Agriculture (USDA), U. S. Department of Health and Human Services(USDHHS). (2015). *2015-2010 Dietary guidelines for Americans, 8th edition.* Retrieved from http:// https://www.dietaryguidelines.gov/sites/default/files/2019-05/2015-2020_Dietary_Guidelines.pdf

U. S. Department of Agriculture (USDA) and U. S. Department of Health and Human Services (USDHHS). (2020). *Dietary Guidelines for Americans, 2020-2025* (9th edition). Retrieved from https://www.dietaryguidelines.gov/sites/default/files/2020-12/Dietary_Guidelines_for_Americans_2020-2025.pdf

U. S. Department of Agriculture and National Institute of Food and Agriculture. (n.d.). *Regional nutrition education and obesity prevention centers of excellence.* Retrieved from https://www.nifa.usda.gov/grants/programs/regional-nutrition-education-obesity-prevention-centers-excellence-rnece

Valdes. A. M., Walter, J., Segal, E., & Spector, D. E. (2018). Role of gut microbiota in nutrition and health. *BMJ, 361*, k2179. doi: 10.1136/bmj.k2179

Valerio, G., Galle, F., Mancusi, C., Di Onofrio, V., Guida, P., Tramontano, A., . . . Liguori, G. (2012). Prevalence of overweight in children with bone fractures: A case control study. *BMC Pediatr, 12,* 166. doi:10.1186/1471-2431-12-166.

van Baarlen, P., Troost, F., van der Meer, C., Hooiveld, G., Boekschoten, M., Brummer, R. J. M., Kleerebezem, M. (2011). Human mucosal in vivo transcriptome responses to three lactobacilli indicate how probiotics may modulate human cellular pathways. *PNAS, 108*(1), 4562-4569. https://doi.org/10.1073/pnas.1000079107

Vandevijvere, S. (2014). Why a Global Convention to Protect and Promote Healthy Diets is timely. *Public Health Nutrition*, 17, 2387 - 2388. https://doi.org/10.1017/S1368980014002031.

Vaughan, E. E., de Vries, M. C., Zoetendel, E. G., Ben-Amor, K., Akkermans, A. D. L. & de Vos, W. M. (2002). The intestinal labs. *Antonie van Leeuwenhoek, 82*, 341-352.

Vauthier, J. M., Lluch, A., Lecomte, E., Artur, Y., & Herbeth, B. (1996). Family resemblance in energy intake and macronutrient intakes: The Stanislas family study. *International Journal of epidemiology*, *25*(5), 1030-1037.

Vrieze, A., Van Nood, E., Holleman, F., Salojarvi, J., Kootte, R. S., Bartelsman, J. F., . . . Nieuwdorp, M. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology*, *143*(4), 913-916.e917. doi:10.1053/j.gastro.2012.06.031

Venn, A. J., Thomson, R. J., Schmidt, M. D., Cleland, V.J., Curry, B. A., Gennat, H. C. & Dwyer, T. (2007). Overweight and obesity from childhood to adulthood: A follow-up of participants in the 1985 Australian schools health and fitness survey. *MJA, 186*(9), 458-460.

Vinciguerra, F., Tumminia, A., Roppolo, F., Romeo, L., Spina, N., Baratta, R., Parrino, C., Sciacca, L., Vigneri, R., & Frittitta, L. (2019). Impact of unhealthy childhood and unfavorable parents' characteristics on adiposity in schoolchildren. *Diabetes/Metabolism Research and Reviews*, 35. https://doi.org/10.1002/dmrr.3199

von Kries, R., Koletzko, B., Sauerwald, T., von Mutius, E. Barnert, D., Grunert, V., von Voss, H. (1999). Breastfeeding and obesity: Cross sectional study. *British Medical Journal, 319*, 147-150.

Wall, R., Ross, R. P., Ryan, C. A., Hussey, S., Murphy, B., Fitzgerald, G. F. (2009). Role of gut microbiota in early infant development. *Clinical Medicine:Pediatrics, 3,* 45-54.

Walters, W. A., Xu, Z., & Knight, R. (2014). Meta-analyses of human gut microbes associated with obesity and ibd. *FEBS Letters, 588*(22), 4223-4233. doi:https://doi.org/10.1016/j.febslet.2014.09.039

Wan, Y., Wang, F., Yuan, J., Li, J., Jiang, D., Zhang, J., . . . Li, D. (2019). Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: A 6-month randomized controlled-feeding trial. Gut, 68(8), 1417-1429. doi:10.1136/gutjnl-2018-317609

Wang, L., Morelen, D., & Alamian, A. (2022). Abstract 077: Association Between Key Family And Individual Factors And The Risk Of Obesity In Offspring: Findings From A Prospective Cohort Study. *Circulation*. https://doi.org/10.1161/circ.145.suppl_1.077.

Wang, W. L., Xu, S.-Y., Ren, Z.-G., Tao, L., Jiang, J.-W., & Zheng, S.-S. (2015). Application of metagenomics in the human gut microbiome. *World J Gastroenterol, 21*(3), 803. doi:10.3748/wjg.v21.i3.803

Ward, Z. J., Bleich, S. N., Long, M. W., & Gortmaker, S. L. (2021). Association of body mass index with health care expenditures in the United States by age and sex. *PLOS ONE, 16*(3), e0247307. https://doi.org/10.1371/journal.pone.0247307

Wardle, J., & Beales, S. (1986). Restraint, body image and food attitudes in children from 12 to 18 years. *Appetite, 7*(3), 209-217. doi:10.1016/s0195-6663(86)80026-5

Wardle, J., Cooke, L. J., Gibson, E. L., Sapochnik, M., Sheiham, A., & Lawson, M. (2003). Increasing children's acceptance of vegetables; a randomized trial of parent-led exposure. *Appetite, 40*(2), 155-162. doi:10.1016/s0195-6663(02)00135-6

Warren, M., Beck, S., & Delgado, D. (2020). *The state of obesity 2020: Better policies for a healthier America* (September 2020 Issue Report). Retrieved from Trust for America's Health website: https://www.tfah.org/report-details/state-of-obesity-2020/

Wilding, J. P. H., Batterham, R. L., Calanna, S., Davies, M., Van Gaal, L. F., Lingvay, I., . . . Kushner, R. F. (2021). Once weekly semaglutide in adults with overweight or obesity. *New England Journal of Medicine*, *384*(11), 989-1002. doi:10.1056/NEJMoa2032183

Whitaker, R. C., Wright, J. A., Pepe, M. S., Seidel, K. D., & Dietz, W. H. (1997). Predicting obesity in young adulthood from childhood and parental obesity. *N Engl J Med*, *337*(13), 869-873. doi:10.1056/nejm199709253371301

Whitaker, R. C., Pepe, M. S., Wright, J. A., Seidel, K. D. & Dietz, W. H. (1998). Early adiposity rebound and the risk of adult obesity. *Pediatrics*, *101*(5), 1-6.

Wilding, J. P. H., Batterham, R. L., Calanna, S., Davies, M., Van Gaal, L. F., Lingvay, I., McGowan, B. M., Rosenstock, J., Tran, M. T. D., Wadden, T. A., Wharton, S., Yokote, K., Zeuthen, N., Kushner, R. F., & STEP 1 Study Group (2021). Once-Weekly Semaglutide in Adults with Overweight or Obesity. *The New England journal of medicine*, *384*(11), 989–1002. https://doi.org/10.1056/NEJMoa2032183

Whitman, E. (2016). *Obesity In America: As Healthcare Costs Rise, Hospitals Weigh New Ways Of Caring For Larger Patients*. *International Business Times*. Retrieved 3 August 2017, from http://www.ibtimes.com/obesity-america-healthcare-costs-rise-hospitals-weigh-new-ways-caring-larger-patients-2325147

Wolstein, J., Babey, S. H., Diamant, A. L. (2015). Obesity in California. Los Angeles, CA: UCLA Center for Health Policy Research

World Health Organization (WHO). (2015, January). WHO-led commission on ending childhood obesity meets to develop global responses to obesity epidemic. In M. C. Director- General, Commission on ending childhood obesity. Second meeting of commission on ending childhood obesity. World Health Organization, Geneva, Switzerland. Retrieved from https://www.who.int/end-childhood-obesity/news/echo-second-meeting/en/

World Health Organization (WHO). (2021). Obesity and overweight fact sheet. Retrieved from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Whitaker, R. C., Wright, J. A., Pepe, M. S., Seidel, K. D., & Dietz, W. H. (1997). Predicting obesity in young adulthood from childhood and parental obesity. *N Engl J Med, 337*(13), 869-873. doi:10.1056/nejm199709253371301

Woo, K. S., Chook, P., Yu, C. W., Sung, R. Y., Qiao, M., Leung, S. S., . . . Celermajer, D. S. (2004). Effects of diet and exercise on obesity-related vascular dysfunction in children. *Circulation, 109*(16), 1981-1986. doi:10.1161/01.CIR.0000126599.47470.BE

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., . . . Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science, 334*(6052), 105-108. doi:10.1126/science.1208344

Wu, Y.-X., Fan, H.-L., Dai, J., Wu, H.-L., Yang, J.-Y., Wang, Y., . . . Zhang, M.-X. (2023). Analysis of association between eating behaviours and childhood obesity among pre-school children: A cross-sectional study. *Frontiers in Pediatrics, 10*. doi:10.3389/fped.2022.1073711

Xu, S., & Xue, Y. (2015). Pediatric obesity: Causes, symptoms, prevention, and treatment (Review). *Experimental a Therapeutic Medicine*. http://dx.doi.org/10.3892/etm.2015.2853

Xu, Z., & Knight, R. (2014). Dietary effects on human gut microbiome diversity. *British Journal of Nutrition, 113*(S1), S1-S5. http://dx.doi.org/10.1017/s0007114514004127

Yackobovitch-Gavan, M., Nagelberg, N., Demol, S., Phillip, M., & Shalitin, S. (2008). Influence of weight-loss diets with different macronutrient compositions on health-related quality of life in obese youth. *Appetite, 51*(3), 697-703. doi:10.1016/j.appet.2008.06.010

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., . . . Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature, 486*(7402), 222-227. doi:10.1038/nature11053

Zhang, L., Chen, F., Zeng, Z., Xu, M., Sun, F., Yang, L., . . . Xie, Y. (2021). Advances in metagenomics and its application in environmental microorganisms. *Front Microbiol, 12*. doi:10.3389/fmicb.2021.766364

Zhao, L., Zhang, F., Ding, X., Wu, G., Lam, Y. Y., Wang, X., . . . Zhang, C. (2018). Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science, 359*(6380), 1151-1156. doi:10.1126/science.aao5774

Zhang, Z., Kahn, H. S., Jackson, S. L., Steele, E. M., Gillespie, C., & Yang, Q. (2022). Associations between ultra- or minimally processed food intake and three adiposity indicators among US adults: NHANES 2011 to 2016. *Obesity (Silver Spring, Md.), 30*(9), 1887–1897. https://doi.org/10.1002/oby.23507

Zilanawala, A., Davis-Kean, P., Nazroo, J., Sacker, A., Simonton, S., & Kelly, Y. (2014). Race/ethnic disparities in early childhood BMI, obesity and overweight in the United

Kingdom and United States. *International Journal of Obesity*, *39*(3), 520-529. http://dx.doi.org/10.1038/ijo.2014.171

Zocca, J. M., Shomaker, L. B., Tanofsky-Kraff, M., Columbo, K. M., Raciti, G. R., Brady, S. M., . . . Yanovski, J. A. (2011). Links between mothers' and children's disinhibited eating and children's adiposity. *Appetite, 56*(2), 324-331. doi:10.1016/j.appet.2010.12.014

Zoetendal, E. G., et al. (2015). Obesity Alters the Microbial Community Profile in Korean Adolescents. Plos One 10(7): e0134333.