Maternal Overweight Prior to Pregnancy and its Impact on the Infant Gut Microbiome and Subsequent Child Overweight Risk.

by

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ABSTRACT

Background: Maternal overweight and obesity is a widespread problem in Canada that has been linked to many complications during pregnancy. The gut microbiome has been revealed to have origins during infancy, which might be influenced by maternal weight gain during pregnancy. It would be beneficial to study the microbiome as a possible link between maternal and child overweight risk.

Objective: To assess the impact of maternal overweight, prior to pregnancy, on the infant gut microbiome and child overweight.

Methods: Height and weight measurements of 1021 women, and their children were obtained from the birth chart in the Canadian Healthy Infant Longitudinal Development [CHILD] study. Information on the infant gut microbiome was acquired from fecal samples in diapers, where DNA was isolated with a commercial kit 'Qiagen QIAamp DNA stool Mini Kit', and extracted and amplified from the hypervariable V4 region of the 16S rRNA locus using a Miseq2 machine.

Results: Maternal prepregnancy overweight doubled the risk of child overweight at one year, independent of mode of delivery, exclusive breast feeding or formula feeding, and infant antibiotic exposure by three months. Exclusive breastfeeding on the other hand, lowered the risk of child overweight by 33%. Maternal pregnancy overweight is associated with a low relative abundance of Bacteroidaceae in the infant gut microbiome,.

Conclusions: Maternal pregnancy overweight doubles the risk of child overweight, and appears to lower the risk of the presence of Bacteroidaceae in the infant gut microbiome.

These findings offer further evidence of the necessity of establishing preventive measures in clinical practice to halt the harmful sequelae of overweight and obesity in pregnancy.

Preface

All the work presented henceforth is as a result of the multidisciplinary team work from investigators at the University of Alberta, Manitoba and Toronto. I was responsible for the statistical analysis of the maternal and child overweight data as well as the infant gut microbiome data of the CHILD study. All projects and associated methods were approved by the University of Manitoba's research ethics board.

DEDICATION

I lovingly dedicate this thesis to my husband who supported me each step of the way and to our daughter Zoe, the joy of my life.

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List of Symbols and Abbreviations

APA	Appropriate for gestational age
BMI	Body Mass Index
Chao1	Species richness
CHILD	Canadian Healthy Infant Longitudinal Development
Cl	Class
Fa	Family
FISH	Fluorescence in situ hybridization
GDM	Gestational Diabetes Mellitus
Ge	Genus
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
LGA	Large for gestational age
Or	Order
OTU	Operational Taxonomic Unit
OW	Overweight
SGA	Small for gestational age

Shannon Index	Species diversity
Sp	Species
SyMBIOTA	Synergy in Microbiota Research

CHAPTER 1 – INTRODUCTION

Background

Over the last three decades, the prevalence of maternal overweight and obesity has substantially increased in Canada. Currently, the Canadian Council of Food and Nutrition and the Canadian Community Health Survey (CCHS) indicates that 15.2% of toddlers, 30% of children and 60% of adults are obese. It also revealed that obesity related chronic conditions are responsible for $4.3 billion in direct and indirect costs to Canada and even this figure might be an underestimation. According to the 2006-2007 Canadian Maternity Experience Survey (MES), approximately one-third of Canadian women began their pregnancy either overweight or obese (13,14,40). Maternal obesity, separately and through association with type 2 diabetes, is a risk factor for childhood obesity (1,2). Studies have shown that there is an increase in body fat percentage of infants born to overweight mothers (7).

It has also been observed that overweight and normal weight individuals harbour different communities of intestinal microflora ("commensal microbes"). The gut microbiome is comprised of symbiotic microorganisms living in the human gastrointestinal tract. Recent studies show that the gut microbiome performs important biochemical functions for the human body. Disorders of the microbiome have been implicated in the pathogenesis of several diseases states, such as obesity, cardiovascular disease and inflammatory bowel disease (4). Gut microbiota compositional differences

are evident between overweight and lean children and originate in infants as early as 3 – 6 months of age (12,73,92,93).

Intestinal commensal microbes predictive of overweight may have origins during early infancy and be influenced by maternal weight gain during pregnancy. Vael et al (95) and White et al (96) reported stronger correlations between *Bacteroides fragilis* counts and infant growth at < 26 weeks but not later during infancy. Further, the gut microbiota remodels itself during pregnancy, with the first trimester bacteria being similar to those of healthy individuals and the third trimester bacteria resembling changes associatd with inflammation and typically seen in individuals with metabolic syndrome (49).

With new insights into the importance of the gut microbiome in human health obtained from new metagenomic approaches (4), studies of microbiome activity as a possible physiologic mediator between maternal and child obesity are critical. These studies will provide new and valuable evidence to health practitioners and mothers about the risks of maternal obesity to the infant. Once women are made aware of the health related implications of being obese during pregnancy, they might take action to achieve a normal body mass index before getting pregnant. Finally, evidence regarding the impact of maternal overweight on the infant gut microbiome, a critical period of development, has the greatest potential for interventions to reduce morbidity. Our study is the first to assess the impact of maternal overweight on the infant gut microbiome and subsequent child overweight riskamong Canadian mothers and their offspring., as early as age 1.

The Microbiome

The microbiome consists of bacteria, bacteriophage, fungi, protozoa and viruses (56), which exist inside and on the human body. Humans have 10 times more microbial cells than human cells. Carl von Linne established the hierarchy of biological classification of taxonomic ranks for the animal kingdom (57). The eight major ones are life, domain, kingdom, phylum, class, order, family, genus and species. Bacteria follows the same ranking from domain down to species.

The human body possesses over a trillion microbes and there is a growing awareness that is crucial to understand the significance of these microbes (58). The human microbiota is composed of different microbiomes at different sites and it constantly evolves throughout neonatal and childhood development and through healthy and disease states. The skin is dominated by Actinobacteria, Firmicutes or Proteobacteria. However, the major bacterial phyla in the vagina is Firmicutes. The adult gastrointestinal tract predominantly consists of Bacteroidetes and Firmicutes. The gastrointestinal tract of the newborn is dominated by aerobic facultative bacteria like *Eschericia coli*, *Enterococcus spp*, α-hemolytic Streptococci and *Staphylococcus spp*. After a few weeks, the infant gut microbiome is dominated by anaerobic bacteria like Bacteroides, Bifidobacterium and *Clostridium spp* (58).

Infants delivered by cesarean section have a reduced proportion of Bifidobacterium or Bacteroides spp. While the microbiota of vaginally delivered infants predominantly

consists of Lactobacillus, Prevotella, Escherichia, Bacteroides, Bifidobacterium and *Streptococcus spp*. Several studies (58) show a higher relative abundance of Bifidobacterium and Lactobacillus among breast fed infants and a higher relative abundance of *Clostridium spp*, *C difficile* in particular, among formula fed infants. The microbiome is further altered when children are weaned to solid food. Bacteroides is associated with ingestion of animal proteins and saturated fats while *Prevotella* is associated with ingestion of carbohydrates (58). *Akkermansia* is naturally present in the human digestive tract at 3-5% but reduces with obesity.

Outline of thesis

This thesis is presented in a traditional, monograph format and consists of four chapters. The first chapter outlines the background and states the objectives and hypotheses of this thesis. It also gives an overview of the microbiome. The second chapter reviews the scope of literature available on this thesis as well as the methodologies used in the thesis. The third chapter explores the results obtained from the analysis carried out and includes a comprehensive discussion. The fourth chapter highlights the main findings, future directions and clinical significance of these findings.

Objectives and Hypotheses

The purpose of this research is to identify the impact of maternal overweight prior to pregnancy, on the infant gut microbiota and subsequent childhood obesity. We hypothesized that: (1) Maternal overweight prior to pregnancy increases the risk of childhood obesity and (2) maternal overweight prior to pregnancy has an effect on the infant gut microbiota.

CHAPTER 2

Literature review

Maternal overweight during pregnancy is a growing epidemic that can adversely affect the unborn child. Prenatal maternal obesity increases the likelihood of stillbirth and macrosomia, meconium aspiration and shoulder dystocia in the newborn infant. Risks can persist into adolescence and adulthood, and include hypertension, type 2 diabetes, cardiovascular diseases and depression (3). Another potential complication of maternal overweight is gestational diabetes which is associated with its own risk of complications from spontaneous abortion to intrauterine fetal death and a higher risk of perinatal complications (8). The resulting endocrine disturbance may lead to 'the metabolic syndrome' which is characterized by insulin resistance, fetal hyperleptinemia and childhood obesity. The child also has a high propensity to develop 'the metabolic syndrome' and type 2 diabetes, cardiovascular complications and hypertension in later life (8). These adverse outcomes have been linked to alterations in the maternal and infant gut microbiome.

Maternal pre-pregnancy overweight and offspring overweight

The impact of maternal prenatal overweight on offspring overweight has been extensively studied. A systematic review and meta-analyses done by Weng et al (20) identified three studies showing that compared to children of normal weight mothers, children of overweight mothers were 1.37 times (95%CI: 1.18-1.58) more likely to be overweight at 3 years and 4.25 times (95%CI:2.86-6.32) more likely to be overweight at

7 years and 2.36 times (95%CI:2.36-8.85) more likely to be overweight between the ages of 9-14 years. Flores et al (21) came to the same conclusion when they found that children of overweight mothers were 1.9 times more likely to be obese (95% CI; 1.62-2.18) and children of obese mothers were 3.4 times more likely to be obese (95%CI; 2.96-4.00) between the ages of 5-11, compared to children of normal weight mothers.

Evidence is accumulating for the impact of pregnancy overweight on offspring overweight in the preschool years. Barroso et al (66), reported this associaton in children as young as 1-2 years old among US Mexicans (P=0.003). In their study of preschoolers, aged 2-4 years, in the US, Kitsantas et al (78) observed that children of overweight Hispanic mothers were twice as likely to be obese than children born to Hispanic mothers with a normal pre-pregnancy body mass index (BMI) (OR:2.74, 95%CI:1.60-4.69). Children of caucasian mothers were 1.4 times more likely be overweight compared to children born to mothers with normal pre-pregnancy BMI (OR:1.40, 95%CI:1.05-1.93). 46.1% of Hispanic mothers were overweight or obese prior to pregnancy in comparison to 34.8% of caucasian mothers. Li et al (79) conducted a study in the United States, on the interactions of maternal BMI and breastfeeding on childhood overweight between the ages of 2 -14. They found that children of mothers with prepregnancy obesity were 4 times more likely to become overweight than children of mothers with a normal prepregnancy BMI (OR:4.1; 95%CI:2.6-6.4). Breastfeeding for 4 or more months was associated with a lower risk of childhood overweight (OR:0.6, 95%CI:0.4-1.0, p=0.06). They also found that children of obese mothers who were never breast fed had the greates risk of becoming overweight (OR:6.1, 95%CI:2.9-13.1). As we become more aware of

the importance of the role the infant gut microbiome plays in future body programming, it is essential that it be studied as a possible link to the host's physiologic and pathogenic states, including future overweight.

Overweight and the gut microbiome

The gastrointestinal microbiota are microbes that exist in the intestines and regulate tolerance to commensal and dietary antigens, while remaining responsive to pathogenic stimuli (83). Any imbalance could result in an inflammatory response, which could lead to susceptibility to chronic diseases such as ulcerative colitis, crohns disease, celiac disease, irritable bowel disease, asthma and allergies as well as systemic diseases such as type 1 diabetes, type 2 diabetes and obesity (83).

As reviewed by Angelakis et al (94), several 16S rDNA pyrosequencing studies have reported a higher abundance of Bacteroidetes, specifically Prevotellaceae, in the gut microbiota of obese and overweight than in lean individuals. Backhed et al (38) summarized the evidence on the role of gut microbiota in regulating fat storage. Using conventionalized adult germ free (GF) mice with a normal microbial composition, they observed a 60% increase in body fat and although they reduced the food intake of the mice, they found increased insulin resistance within 2 weeks. They reported that gut microbes can contribute to new onset hepatic lipogenesis by promoting the absorption of monosaccharides from the gut lumen. They also found that the microbiota-induced (Bacteroides) increase in the epididymal fat pad mirrored adipocyte hypertrophy.

Another mechanism linking gut microbiota to obesity involves the translocation of lipopolysaccharides from the intestinal microbiota into the bloodstream. Lipopolysaccharides (LPS) are endotoxins that exist in the cell wall of gram-negative bacteria present in the gut microbiota and during lipid digestion are partially transported out of the gut and into the blood stream (73,74). This increases LPS plasma levels in the blood and contributes to the inflammatory process in the body. Therefore, some gram negative bacteria such as *Bacteroides* and Enterobacteriaceae have been implicated in obesity (73). Karlsson et al (73) conducted a study in the South of Sweden, assessing the gut micrbiota in normal weight and overweight preschool children aged 4-5 years, using quantitative polymerase chain reaction (qPCR), terminal restriction fragment length polymorphism and calprotectin measurements in feces. They found among other things, that the overweight children had a higher level of Enterobacteriaceae and lower levels of *Akkermansia muciphilia*-like bacteria compared to the normal weight children ($p=0.036$) and ($p=0.030$) respectively.

Before further summarizing the literature on microbiota mechanisms for future overweight in infants, a short overview is required of maternal gut microbiome changes during pregnancy, and of how gut microbiota are established and developed during infancy towards an adult profile.

i) development of the infant gut microbiome

The uterus was considered sterile according to evidence from culture-dependent methods that were used to check for the presence of bacteria in the amniotic fluid. However, Han et al (89) found that culture-independent methods such as PCR were estimated to increase bacterial detection in amniotic fluid by 30 – 50% and increased by up to five-fold, the number of detected species. He also identified 9 bacterial species present in almost half of all amniotic fluid samples that tested positive with PCR but were not cultivated. Among them, the cultivation resistant anaerobic bacteria Fusobacteriaceae, which is now recognized as a common inhabitant of amniotic fluid.

The intestine of an infant is gradually colonized by an enormous microbial community called the gut microbiota, which is initially comprised of Enterobacteria, Bifidobacteria and *Bacteroides* (15,16). This colonization varies from infant to infant depending on their unique postnatal exposure, which includes mode of delivery, infant diet and antibiotic exposure (17). Although the infant microbiome in the first 6 months of life is variable, it progressively becomes more stable and by the age of 2, is similar to the microbiome of an adult, with a high concentration of of *Bacteroides* and Firmicutes and a low concentration of Proteobacteria and gram-negative bacteria, as revealed in a study done by Palmer et al (5).

ii) maternal gut microbiome in pregnancy

Koren et al (49) observed a dramatic change in the gut microbiome of pregnant women from the first trimester to the third trimester. Using a culture independent approach, they compared the gut microbial communities of pregnant women in their first and third trimesters as well as postpartum. They noticed a significant microbiota diversity shift that occured in all women and was unrelated to pre-pregnancy BMI, gestational diabetes or the use of antibiotics during pregnancy. From the first trimester to the third, the number of bacterial taxa was dramatically reduced ($p=0.0001$) and the relative abundance of lactic acid bacteria, Proteobacteria, specifically Enterobacteriaceae ($p=0.0004$) and Actinobacteria ($p=0.003$) increased.

They also observed that while the gut microbial composition of women in their first trimester was similar to that of a healthy individual, the gut microbial composition of women in their third trimester was similar to adults with inflammatory changes resembling the metabolic syndrome. When the first and third trimester gut microbiota were transferred into healthy germ free wild type mice, they found that the mice colonized with first trimester microbota remained healthy, while the mice with third trimester microbiota had reduced insulin sensitivity, inflammation and excessive weight gain.

Maternal pre-pregnancy overweight and the gut microbiome

In the Koren et al (49) gut microbiome study of pregnant women, pre-pregnancy obesity had the lowest within subject (α) diversity at both in the first and third trimesters. Using FCM-FISH and qPCR methods, Collado et al (18) observed normal weight gain during pregnancy and pre-pregnancy body mass index (BMI) to be positively correlated with fecal concentrations of *Bacteroides spp.* in the third trimester (r=0.32 for BMI, p=0.023). Higher levels of *Bacteroides-Prevotella* and Staphylococcus were found in the first and in the third trimesters of women who were overweight pre-pregnancy.

They also observed that maternal overweight has the capacity to alter infant gut microbiota (18,19). They also found that children of overweight mothers had higher concentrations of *Staphylococcus* and *C.difficile* and lower concentrations of Bifidobacteria (r=0.467, P=0.017; 81.2%: OR: 14.30; 95%CI:3.25,63.00; r= -0.301, P=0.047 respectively) while the children of normal weight mothers had a high concentration of Bifidobacteria and low concentrations of *Staphylococcus* (r= -0.30, P= 0.050; r= 0.55, P= 0.004 respectively) and *C. difficile*.

Link between maternal gut microbiota during pregnancy and infant gut microbiota

There is a great deal of speculation about the mode of transmission of maternal gut microbiota into the fetal or neonatal gut microbiota. In an overview by Ido Solt (90), it was reported that some microorganisms can colonize both the gastrointestinal and the

reproductive tracts. For example, group B streptococci (GBS) which are predominantly found in the vagina can also be present in the rectum. Solt also noted that when culture independent techniques such as PCR were used, 44.4 % of bacterial species that were identified from either the vaginal or rectal microbiome of pregnant women were found in both the vaginal and rectal sites. Newborns can be colonized with these microbes while *in utero*, as well as during vaginal delivery. An example of migration of bacteria from the gastrointestinal tract to the reproductive tract is the maternal – fetal infection by Listeria monocytogenes.

Gohir et al (91) also pointed out that the presence of specific strains of maternal gut bacteria in the meconium of infants implies that the fetus is exposed to microbes from the maternal gut *in uetero*. Through mechanisms not fully understood, maternal immune cells (dendritic cells) may make contact with the paracellular space between the gut epithelial cells which opens up tight junctions and allows dendritic cells to directly sample microbiota from the maternal gut lumen and become internalized. Thereafter, internalized intestinal bacteria can be transported to different organ systems such as the placenta through the circulation. In addition, changes in maternal intestinal permeability during pregnancy could play a key role in bacterial translocation. The Gohir et al review also noted that maternal obesity may affect placental function and transfer of internalized microbes by increasing the infiltration of macrophages and pro-inflammatory cytokines.

Other possible mechanisms by which maternal overweight could affect the fetal/infant gut microbiome are through alterations in maternal gut microbiome via the metabolites that microbiota produce, such as folate and short chain fatty acids like butyrate. These metabolites have the capacity to influence epigenetic coding of intestinal cells and fat cells (adipocytes)(6). Microbiota metabolites affect the epigenome via DNA methylation and histone modification. Adequate DNA methylation is centered on one-carbon metabolism that requires folate and Bifidobacteria, producers of folate found in the gut microbiome (18,6). The gut microbiome of overweight women has a high concentration of the Firmicutes which increases butyrate production, affecting histone acetylation. Both epigenetic changes could lead to fat accumulation by affecting the genes responsible for cholesterol and lipid metabolism and storage in offspring (6).

Priyadarshini et al (85) conducted a study to investigate if short chain fatty acids were directly associated with metabolic parameters in mothers and newborns. Serum levels of short chain fatty acids (acetate, propionate and butyrate), maternal adipokines, maternal glucose and C- peptide were measured at 36 -38 weeks of gestation. They found among other things that serum acetate levels were associated with maternal weight gain and maternal adiponectin levels and that serum propionate correlated negatively with newborn length and body weight (85).

Finally, in the Cox et al (86) review of pregnancy-induced obesity, two pathways were proposed: i) microbe independent effects during a normal physiological condition like

pregnancy, which are capable of promoting weight gain and increasing adipose tissue, and ii) microbe-dependent effects through increased energy harvest, and altered metabolic signaling and inflammation of gut microbiota. In addition to maternal overweight, they also noted that microbe-induced obesity can involve events that disrupt the microbial community during pregnancy and birth, such as cesarean section delivery and maternal/infant antibiotic exposure. These confounding factors will be discussed in the subsequent sections.

Confounding factors

Several covariates were included in this study because of their capacity to affect either the exposure variable or outcome variable or both. They include maternal prenatal diet, gestational diabetes, fetal smoke exposure and birth weight, mode of delivery, antibiotic exposure and infant diet.

i) maternal prenatal diet

There is evidence that cellular degenerative changes such as dyslipidemia and atherosclerosis which are known triggers for cardiovascular disease states, can occur in childhood (5). Added sugar in particular is a well known culprit that has been associated with an increase in HDL (high density lipoprotein) cholesterol levels. Using the RISCK (Reading, Imperial, Surrey, Cambridge and King's) food exchange model, Fava et al (75) assessed the impact of dietary fat and carbohydrate on gut microbiome and short-chain

fatty acid excretion in a metabolic syndrome 'at-risk' population. Among several results, they found that participants in the high carbohydrate/high glycemic index diet had high levels of *Bacteroides spp* compared to baseline levels (p=0.038); this change was associated with a decrease in body weight, BMI and waist circumference (r=-0.64, r=-0.64, r=-0.45) respectively.

Increasingly, we are becoming aware that the onset of diet-induced changes has its origins during infancy and the establishment of the infant gut microbiota. Changes in prenatal diet can lead to pathophysiological changes in the mother such as inflammation, insulin resistance, endothelial dysfunction and oxidative stress, and alter the maternal gut microbiome, for example, increasing the presence of *Bacteroides spp* and *Clostridium spp*. (6)

Rodriguez et al (77) examined the effect of fructose (added sugar) intake during pregnancy in a rodent model. They tested whether fructose intake altered lipidemia in pregnant rats and caused corresponding changes in offspring by administering 10% wt/vol of fructose in the drinking water of rats for the entire duration of their pregnancy. These rats developed hypertriglyceridemia during pregnancy, unlike the glucose fed or control mothers. The offspring of fructose-fed rats developed hypotriglyceridemia and had high hepatic triglyceride levels compared to the offspring of the glucose or control mothers. Their results also revealed that fructose intake during pregnancy reduces maternal leptin response to fasting and re-feeding, and alters the leptin signal in the

offspring (77). In the Japanese macaque model, Ma et al (37) conducted a study of a high-fat pre or postnatal maternal diet and the abundance of microbial species in intestinal samples of offspring. Bacteroidetes, specifically *Prevotella spp.* dominated the gut profiles of offspring born to mothers fed a high-fat diet during pregnancy. These animal model findings implicate a role for the maternal prenatal diet in the establishment of the infant gut microbiome and future overweight.

ii) gestational diabetes

Gestational diabetes, independent of maternal overweight has been associated with the increased risk of child overweight and obesity. Nehring et al (33) found that in a recent study done among 5 year old children, 21% of children whose mothers had gestational diabetes were overweight, while 10.4% of the children of healthy mothers became overweight. Another impact of maternal overweight is its effect on neonatal outcomes. An article by Scott-Pillai et al (36) examined women in different body mass index groups and found that women in the overweight and obese class 1 groups had a greater risk of gestational diabetes (OR:3.7, 99%CI:2.8-5.0; OR:1.7, 99%CI:1.3-2.3), induction of labour, cesarean section and macrosomia compared to normal weight women. This supports findings by Nehring et al that maternal gestational diabetes was associated with child overweight.

iii) fetal tobacco exposure and birth weight

Karlsson et al (34) found that Lactobacillus was present in all neonates, however higher concentrations of Gram-negative Proteobacteria were found in large for gestational age (LGA) infants and higher concentrations of Gram-positive Firmicutes were found in appropriate for gestational age (APA) infants. This study shows that infant gut microbial composition is related to weight at birth. In Weng et al's (20) systematic review, seven studies showed a positive association between large for gestational age babies (LGA) and childhood overweight.

In Weng et al's (20) systematic review, seven studies found a positive association between childhood overweight and maternal prenatal smoking. While many infants exposed to tobacco smoke *in utero* are born low birth weight and small for their gestational age, they experience a change in appetite and body composition that leads to an increase in central, peripheral and total subcutaneous fat mass (9). Janjua et al (35) supported this with their finding that the pre-pregnancy BMI of the mother and maternal smoking, along with infant birth weight were positively associated with childhood overweight at the age of 5. Several studies have shown that prenatal smoking increases the risk of a higher body mass index in children aged 5-11 (28,29,30) and other studies, including the one by Braun et al (31) have identified that prenatal secondhand smoke exposure carries the same risk as prenatal smoking in predisposing children to a higher body mass index at age 2 - 3 years.

iv) mode of delivery

As reported in several recent studies (71,72), evidence is accumulating that cesarean section delivery predisposes children to a higher risk of developing overweight. For example, Huh et al (25) found that children born through cesarean section had a higher odds of being obese at the age of 3 (OR 2.10, 95% CI 1.36-3.23) compared to vaginally born infants. Several new studies have also revealed that alterations in infant gut microbiota, as a result of mode of delivery, could be in the pathway to the development of child overweight.

The microbiome of infants differs by mode of delivery and Azad et al (23) found that infants born by cesarean section had low levels of *Escherichia-Shigella* and *Bacteroides* levels were undetectable. Makino et al (87) conducted a study to investigate if mother-to-infant transmission of intestinal bifidobacterial strains has an impact on the early development of vaginally delivered infant's microbiota. Mother's feces and infant's feces, including meconium was collected and strains of Bifidobacteria was isolated and genotyped using a multilocus sequencing typing. Bifidobacteria counts in infant's feces were analyzed with quantitative PCR. They found that 11 out of 12 vaginal delivered infants carried at least one monophyletic strain of Bifidobacteria while the C-section delivered infants did not carry any monophyletic strain. They concluded that mothers colonize the intestines of vaginally delivered infants with several Bifidobacterial strains, shortly after birth (87).

Jakobsson et al (80) conducted a study in Sweden on the decreased gut microbiota diversity, delayed Bacteroidetes colonization and reduced Th-1 responses in infants delivered by cesarean section. They used pyrosequencing of 16S rRNA genes at 1 week and 1,3,6,12 and 24 months after birth to identify the components of the intestinal microbiota. They found that the microbiome of infants in the first year of life, depending on if they were delivered vaginally or by cesarean section, was dominated by Firmicutes (74~71%), Bacteroidetes (16~13%), Bifidobacteria (7~12%), Proteobacteria (3~2%) and Verrucomicrobia (1~2%). However, their main findings were that infants born through cesarean section had a lower microbial diversity ($p=0.047$), delayed Bacteroidetes colonization ($p=0.002$) and reduced Th-1 response in the first 2 years of life (80).

v) antibiotic exposure

The role early (< 6 months) antibiotic exposure of the infant plays in the alteration of the developing gut microbiome is demonstrated in a study by Ajslev et al (26) who looked at pre-pregnancy weight, antibiotic use and childhood overweight at 7 years. They found that antibiotic use was protective for children of overweight mothers and led to an increased risk of overweight in children of normal weight mothers. Trasande et al (27) conducted a similar study on infant antibiotic use and early life body mass and found that any exposure to antibiotics in the first 6 months of life was associated with a consistent increase in body mass from 10-38 months ($P=0.001$, $P=0.029$, respectively).

vi) infant diet

Breast milk, formula and solid foods provide nutrients for the infant, shaping gut microbial composition and programming growth. In Weng et al's (20) systematic review and meta-analysis, breastfeeding was shown to protect against childhood overweight, although five studies found no associations with breastfeeding. Based on older culture methods, the gut microbiota of breastfed infants is characterized as less diverse, with a predominance of bifidobacteria and lactobacilli, and less frequent colonization with *Clostridium species* than the microbiota of infants fed formula (22).

Using high-throughput DNA sequencing methods, Fan et al (81) conducted a study in the Hebei province of China on different patterns of feeding infants aged 3-6 months. Breastfed infants had a predominance of Firmicutes ($p=0.035$) and Actinobacteria ($p=0.047$) in their gut microbiota, while infants fed formulas (containing prebiotics) had a predominance of Proteobacteria ($p=0.0019$). The dominant bacteria for breast fed infants at the family taxonomic level were Veillonellaceae (Firmicutes) ~32.4% and Enterobacteriaceae (Proteobacteria) ~21.1%.

The article by Azad et al (23) is the only existing information on the gut microbiome in Canadian infants and they found that bottle-fed infants had a high microbial diversity with higher concentrations of *C. difficile* while breast fed infants had a low bacterial diversity. Follow up analysis were done and trend testing revealed that exclusivity of breastfeeding was negatively correlated with the prevalence of *C. difficile*.

Luoto et al (12) examined how the initial dietary and microbiological environments deviate in normal-weight children compared to overweight children at 10 years of age, who had been followed up from birth. They measured the maternal colostrum (first breast milk) adiponectin concentration and through fluorescent in situ hybridization, analyzed the gut microbiome at 3 months. They found that bifidobacteria in the gut microbiome and maternal adiponectin concentrations were higher in normal weight children compared to the overweight children; excessive weight gain was already evident in the first months of life in the overweight children (P=0.001). This study demonstrates that early gut microbial and dietary environments can have a long lasting effect on the metabolic programming of a child.

Summary

The animal and human studies described above, support the hypothesis of how pregnancy overweight and alterations in the gut microbiome could influence childhood obesity. Among the few studies that evaluate pre-pregnancy overweight and overweight risk in preschool children, such as the one carried out by Kitsantas et al (78), results are compelling enough to require further study in this age group. Some aspects of my topic "maternal overweight and obesity prior to pregnancy and its impact on the infant gut microbiota and subsequent childhood overweight risk" have been studied but not among Canadian infants. My proposed research would address this gap in the literature.

Methods

Overview of study design

This was a prospective cohort study. We were able to observe the infants from birth to the age of 1 and be aware of their exposure and determine the incidence of their outcomes. Our study population consisted of overweight and normal weight pregnant women and their infants in Canada who were enrolled in the Canadian Healthy Infant Longitudinal Development study (CHILD). Our exposed group were obese or overweight mothers and our unexposed group were normal weight mothers. We observed them to determine our outcome of interest, which was, variations in the intestinal microbiome of infants at 3 months, specifically looking at, Bacteroidaceae, Bififdobacteriaceae, Staphylococcacae, *Akkermansia*, *C. difficile*, Chao1 richness and Shannon index diversity and subsequent childhood overweight at age 1.

The CHILD study (59) is a longitudinal, population-based birth cohort study of 5,000 children recruited from four sites in Canada (Vancouver, Edmonton, Winnipeg and Toronto). Women are recruited at 20 weeks of gestation with the following inclusion criteria: a live birth at 36 weeks gestation or greater, with birth weight of 2,500 g or more. Infant exposures during birth and hospitalization are extracted from hospital records. Information from these records is obtained on mode of delivery; cesarean section or vaginal delivery and infant birth weight. Records are also retrieved on the use of maternal intrapartum antibiotics or if the infant was directly exposed to antibiotics. Maternal diet and exposures during pregnancy, and type of infant feeding is determined from parent

report. Biological material such as feces, is assayed both in real time and/or stored for later analyses (59). Microbiota is profiled through high through- put signature gene sequencing. The CHILD study has received Research Ethics Board approval and is underway.

Exposure variable:

Anthropometric assessment

Maternal prepregnancy overweight was defined as maternal overweight prior to pregnancy and it was determined by body mass index (BMI) which was measured as weight in kilograms divided by height in meters squared. Body mass index was categorized into normal weight: ≥18.5 ≤24.9; and overweight: ≥25.0. Maternal overweight status during pregnancy was determined from height and weight measurements obtained in the prenatal history section of the birth chart in the CHILD pregnancy cohort.

Outcome variables:

Anthropometric assessment

Child overweight risk at one year was defined as an infant with length and weight measurements in the 85th percentile. Length and weight at age 1 was obtained from data already collected for the CHILD cohort (59).

Gut microbiota measures

Infant gut microbiota was defined as the commensal microorganisms living in the gut of infants. We specifically measured the relative abundance of the following specific microbes at the family and genus levels; Fa Bacteroidaceae, Fa Bifidobacteriaceae, Fa Clostridiaceae, Fa Staphylococcaceae and *Ge Akkermansia*. Microbiome diversity (mean, median) and species richness were measured using the Shannon index and the Chao1 estimator, respectively (44). Infant meconium and fecal samples were collected from infants at 3 months of age and repeated at 1 year of age. Samples consisting of 5–10 g stool (1–2 g for meconium) were collected aseptically from a freshly soiled diaper using a sterile spatula, divided into aliquots and stored at –80°C in sterile containers (59).

To provide sufficient taxonomic resolution for the proposed hypotheses and accommodate the anticipated sample size, high through-put "signature gene" sequencing was selected for this study (62,63). The signature gene is 16S rRNA, the gene encoding the small subunit of ribosomal RNA. At the nucleotide level, the 16S rRNA gene consists of eight, highly conserved regions which are invariant across all bacteria. Between these invariant segments, there are nine variable regions whose sequences can be used to characterize many bacteria to the species level. Once determined, nucleotide sequences in the variable regions (V3-V7) of the 16S rRNA gene can be compared to sequences in public databases to identify individual bacteria (59).

Bacterial community DNA isolation and amplification (Scott Laboratory)

Frozen fecal samples were thawed and homogenized in phosphate buffered saline (PBS) at pH 7.4 in preparation for DNA isolation following Nechvatal et al (60) and Scholtens et al (61). Total DNA was isolated from this aliquot using a commercial kit (Qiagen QIAamp DNA Stool Mini kit) following manufacturer instructions and the stool bacterial DNA isolation optimization recommendations given by Nechvatal et al (60). Subregions of the bacterial 16S rRNA gene were amplified from isolated DNA using primer sequences that target conserved nucleotide segments flanking the variable V4 region.

High through-put signature gene sequencing (Guttman Laboratory)

The V4 hyper-variable region from the 16S rRNA locus was sequenced using 150nt paired-end sequencing on an Illumina MiSeq2 machine (62,63). Samples were barcoded and multiplexed during each sequencing run to generate between 250K – 1M paired-reads per multiplexed sample. Paired reads were de-multiplexed using QIIME software (Quantitative Insights into Microbial Ecology) and quality filtered with Quake to improve taxonomic resolution.

Sequence alignment, taxonomic assignment and designation of OTU's.

Taxonomic classification of microbes detected in fecal samples into OTUs (Operational Taxonomic Units), through alignment of gene sequences with existing databases (i.e. Greengenes), was achieved with QIIME software.

Nutritional assessment

Prenatal maternal and postnatal infant nutrition were assessed as maternal diet during pregnancy and breastfeeding status of the infant at age 3 months (59). A Food Frequency Questionnaire (FFQ), developed by the nutritional epidemiologists at the Fred Hutchinson Cancer Research Center in Seattle, WA and based on the questionnaires used in two large NIH funded studies, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) and the VITamins and Lifestyle study (VITAL) was modified to reflect Canadian ethnic food choices. In addition, the database developed by the University of Minnesota Nutrition Data Systems for Research (NDSR) for data entry and nutrient analysis was "Canadianized" for use in CHILD. This questionnaire is a self-administered FFQ (59) asking pregnant mothers to report the frequency of consumption and portion size of approximately 175 line items during their current pregnancy. These questionnaires were completed by the mother at the time of enrollment. According to the literature, added sugar in maternal diet was strongly associated with overweight so we specifically assessed it in our study population. Added sugar was the only component of maternal diet assessed in this study and the American Heart Association recommended an added sugar limit of no more than 100 calories per day (24 grams of sugar) for women and no more than 150 calories per day (36 grams of sugar) for men (84). Infant diet was assessed at 3 months and mothers reported if their children were exclusively breast fed, partially breast fed or formula fed. The weight of an infant at birth specifically high birth weight and low birth weight, can contribute to child overweight. However, in this study, low birth weight was excluded because only 2 study participants had low birth weight in a study of 1021. Maternal weight was categorized into normal weight and overweight only because when

it was further subdivided into an obese category, no statistically significant results were obtained.

Breast-feeding status was assessed by questionnaire at 3, 6, and 12 months of age. Items on the questionnaire included duration of exclusive breast-feeding, age of first supplementation, and the age of first introduction of solids.

Other covariates

Mode of delivery was defined as the method used to deliver an infant which can be either through vaginal delivery or cesarean section and this was determined from birth chart records. Gestational diabetes occurs when women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy. Information on gestational diabetes was determined prenatally between 24 to 28 weeks of gestation and was retrieved from the birth chart. Birth weight was defined as the weight of an infant at birth and this information was retrieved from birth chart records and categorized into high [>4kg] and normal [<= 4kg]. Antibiotics were used to treat bacterial infections and antibiotic use was determined from the CHILD medication questionnaire. It was categorized into no exposure, indirect or maternal exposure and direct exposure.

Smoking is the inhalation of the smoke of burning tobacco encased in cigarettes. Smoke exposure was obtained from the prenatal hospital records and CHILD questionnaires. It was categorized into no exposure, maternal prenatal and postnatal exposure and only

postnatal exposure. From the CHILD questionnaire on the mother's health coding legend, the following questions were asked to determine prenatal smoke exposure; "At the present time, how often do you smoke? During this pregnancy, did you stop smoking completely? If yes, at what week of pregnancy did you stop smoking?" Environmental tobacco smoke exposure was assessed on the comprehensive prenatal and postnatal CHILD questionnaires (59). From the CHILD questionnaire on the mother's health coding legend, the following question was asked to determine environmental smoke exposure; "Does anybody, at present, smoke at your home? {Yes] [No]".

Statistical analysis

Our data was analyzed with the use of purposeful stepwise logistic regression modelling with SPSS 21.0 software. Our infant gut microbiota data was divided into quartiles for each bacteria. We selected the lowest quartile for Bacteroidaceae, Chao 1 richness and Shannon diversity index and we selected the highest quartile for Bifidobacteriaceae, Clostridiaceae, Staphylococcaceae and Akkermansia. An association between maternal overweight prior to pregnancy, and infant gut microbiota diversity and composition was determined using logistic regression analysis. Logistic regression analyses was also run to determine associations between pregnancy overweight and overweight in offspring.

We also pursued a systematic approach to modeling by selecting covariates that have had a significant effect on our outcome variables of interest; child overweight and the infant gut microbiome. Logistic regression was used to test the significance of these covariates. In the first model, the unadjusted crude odds ratio was obtained for each covariate. The

second model was adjusted for other maternal pregnancy variables such as maternal pregnancy overweight, maternal gestational diabetes, mode of delivery, maternal diet and smoke exposure. The rationale for adding these covariates was that they might have an impact on maternal pregnancy overweight. The third model was adjusted for birth and postnatal infant variables such as birth weight, infant diet and antibiotic exposure. The rationale for adding these covariates was that they might have an impact on the child overweight and the infant gut microbiome (17,20). Covariates that were highly correlated with each other were excluded from the final model (Table 1D), such as, between gestational diabetes and infant antibiotic exposure, and maternal prenatal smoking and infant diet. So, our final model consisted of maternal overweight, mode of delivery, infant diet and antibiotic exposure.

Sample size calculation

Published data (Collado et al (19): p1026) estimated that the prevalence of *C. difficile* was lower in 1 month old infants of normal weight women {3.8%} than in 1 month old infants of overweight women {31.2%}. I needed 60 patients to be enrolled in my study. Due to the length of the study, I estimated a 15% drop out rate, therefore I planned to enroll 69.

Sample size calculation for the comparison of two proportions .

$P=(p_1 + p_2)/2$ $N= 2PI^2 * \{P(1-P)/(p_1 - p_2)^2\}$

$P= (0.038+0.312)/2$

$P= 0.175.$

$N= 2\ (2.80)^2 * \{(0.175*0.825)/0.038-0.312)^2\}$

$N= 31.36*\{0.1444/0.075076\}$

$N= 31.36*\{1.9234\}$

N= 60.

Table 1: Definition and source of study variables

Study variables	**Operational definitions**	**Data source**
Exposure variable: 1. Maternal prepregnancy overweight	Maternal prepregnancy weight was defined as maternal weight prior to pregnancy and it was determined by body mass index (BMI) which was measured as weight in kilograms divided by height in meters squared. Body mass index was categorized into normal weight: ≥18.5 ≤24.9; and overweight: ≥25.0	Height and weight were obtained from the hospital charts of all Canadian Healthy Infant Longitudinal Development [CHILD] study mothers and BMI was calculated from these measures. (39)
Outcome variables: 1. Child overweight risk at 1 year	Infant with length and weight measurements in the 85th percentile was at risk for being overweight.	Length and weight were obtained for all CHILD infants at 1 year. (39,42)
2. Infant gut microbiota at 3 months	Infant gut microbiota are the commensal microorganisms living in the gut of infants. We specifically measured; 1. Relative abundance of specific microbes at the family and genus levels. 2.Microbiome diversity (mean, median) using the Shannon index and species	Fecal samples were aseptically acquired from diapers at 3 months by the CHILD study. This outcome measure was subsequently evaluated. (44)

	richness using the Chao1 estimator (44)	
Covariates: 1. Mode of delivery	Mode of delivery was the method used to deliver infants which can be either 1. Vaginally 2. Cesarean section	Mode of delivery was determined from birth chart records by the CHILD study
2. Infant diet	At 3 months: 1. Exclusively breast fed, 2.Partially breast fed or 3.formula fed	Verbal report from mothers
3. Maternal diet	Maternal diet during pregnancy specifically 'added sugar'. [<=24g/day: normal, >24g/day: high]	Food Frequency Questionnaires (FFQ) to mothers from the CHILD study
4. Birth weight	The weight of an infant at birth. [<=4kg:normal & >4kg: high]	Birth weight was determined from birth chart records by the CHILD study
5. Gestational diabetes	Women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy	Gestational diabetes was determined from birth chart records by the CHILD study.
6. Antibiotic use	The use of antibiotics to treat bacterial infections. Antibiotic use was categorized into 1. None, 2. Indirect exposure (maternal), 3. Direct exposure.	Antibiotic use was determined from the CHILD medication questionnaire.
7. Smoke exposure	Smoking is the inhalation of the smoke of burning tobacco encased in cigarettes. Smoking was categorized into 1.None, 2. Maternal prenatal + postnatal, 3.Postnatal only	Smoke exposure was determined from prenatal hospital records and CHILD questionnaires during pregnancy and postnatally.

CHAPTER 3

Results

In a study of 1021 Canadian women and their offspring from the Winnipeg site of the Canadian Healthy Infant Longitudinal Development (CHILD) cohort, 822 women had complete data on pregnancy overweight; this sample size fell to 734 due to missing data on birth method (Appendix-Figure 11). 49% of these women were overweight, 3.1% had gestational diabetes, 93% had an excessive amount of added sugar in their diet and 18.3% were delivered by cesarean section (Appendix-Figure 9). From the 895 children with complete data on overweight status, 106 children needed to be excluded due to missing data on birth method, which reduced the sample size to 789 (Appendix-Figure 11). 21.0% were overweight, 18.9% were delivered by cesarean section and 54.6% of our study population were exclusively breastfed. 12.0% of our study population were exposed to maternal prenatal and postnatal smoke, while 29.8% were exposed to postnatal smoke alone. 31.7% of our study population had indirect antibiotic exposure (maternal) and 6.0% had direct antibiotic exposure. 12.5% of our study population were born with high birth weights (Appendix-Figure 8).

The results from our study population are comparable to the whole Winnipeg population (64). To prove this, we statistically compared our study percentages to the Winnipeg population percentages by generating a confidence interval from our study percentages for estimating population rates. The prevalence of smoking during pregnancy from the

Winnipeg cohort was 18%, exclusive breastfeeding was 51.0% and cesarean delivery was 19.9% (64,65).

Table 2: Comparison of cohorts to Winnipeg population

	Overweight Analysis Cohort	Microbiota Analysis Cohort	Winnipeg Cohort	95% CI [Prevalence rate]
Prenatal smoking	12.0%	6.6%	18.0%	11 – 16% [12.0]
Exclusive breastfeeding	54.6%	50.3%	51.0%	50 – 56% [54.6]
Cesarean section	18.9%	22.2%	19.9%	16 - 21% [18.9]

In table 2, the prevalence of cesarean section among the children in our study population was 18.9% and using this prevalence rate, we obtained a confidence interval. The Winnipeg cohort reported a prevalence of 19.9%, which falls within our 95% confidence interval range of 16 – 21%. The prevalence of exclusive breastfeeding among the children in our study population was 54.6% and using this prevalence rate, we obtained a confidence interval. The Winnipeg cohort reported a prevalence of 51.0%, which falls within our 95% confidence interval range of 50 – 56%. The prevalence of women who reported smoking during pregnancy was 12.0% in our study population and using this prevalence rate, we obtained a confidence interval. The Winnipeg cohort reported a

prevalence of 18%, however this result was slightly out of our 95% confidence interval range of 11 – 16%.

At 13%, exclusively breastfed infants were half as likely to develop overweight at age 1 than exclusively formula-fed infants (Table 3, $p<0.0001$). A significantly higher percentage of exclusively formula fed infants (59%) were also born to overweight mothers ($p<0.0001$). 41% of infants who were born to mothers with gestational diabetes become overweight at age 1, compared to 17% of infants born to mothers without gestational diabetes ($p=0.001$). 88% of infants whose mothers had gestational diabetes were also overweight ($p<0.0001$). 32% of infants born with high birth weights were overweight at age 1 compared to 15% of children born to normal birth ($p<0.0001$). 58% of infants who had high birth weights were born to overweight mothers ($p=0.01$). 29% of infants who were directly exposed to antibiotics and 19% of infants who were indirectly exposed to antibiotics, were overweight at age 1 compared to 13% of infants not exposed to antibiotics ($p<=0.02$). 58% of infants who were exposed to maternal pre and postnatal smoking were born to overweight mothers ($p=0.004$).

In table 4, 43% of infants delivered by cesarean section had a low relative abundance Bacteroidaceae in their infant gut microbiome compared to 8% of infants who were born vaginally ($p<0.0001$). 63% of infants indirectly exposed to antibiotics had a low relative abundance of Bacteroidaceae in their infant gut microbiome ($p<0.0001$) compared to infants not exposed to antibiotics.

Table 3:

Frequency distribution of study covariates according to pregnancy and child overweight

	Total	Mom OW	n = 400		Total	Child OW	n=166	
	Count	n	%	P	Count	n	%	P
Cesarean section								
Yes	134	71	53.0	0.11	149	30	20.1	0.35
No	600	272	45.3		640	108	16.9	
Breast feeding								
Exclusive	442	178	40.3	0.0001	489	63	12.9	0.0001
Partial	213	111	52.1		228	46	20.2	
Zero	167	99	59.3		178	49	27.5	
Maternal GDM								
Yes	24	21	87.5	0.0001	27	11	40.7	0.001
No	741	334	45.1		799	134	16.8	
Smoke exposure								
None	420	177	42.1	0.004	459	69	15.0	0.12
Pre+Postnatal	89	52	58.4		95	22	23.2	
Postnatal	212	111	52.4		235	44	18.7	
Antibiotic exposure								
None	264	113	42.8	0.06	271	35	12.9	0.02
Indirect	145	75	51.7		150	28	18.7	
Direct	48	28	58.3		49	14	28.6	
Prenatal diet-added sugar								
High	733	340	46.4	0.24	805	145	18.0	0.61
Normal	53	29	54.7		58	12	20.7	
Birth weight								
High	101	59	58.4	0.01	112	36	32.1	0.0001
Normal	650	289	44.5		700	106	15.1	

Table 4:

Frequency distribution of study covariates according to pregnancy and the infant gut microbiome (Fa Bacteroidaceae)

	Total	Mom OW	n = 400		Total	Bacteroidaceae	n=74	
	Count	**n**	**%**	**P**	**Count**	**n**	**%**	**P**
Cesarean section								
Yes	134	71	53.0	0.11	39	31	42.5	0.0001
No	600	272	45.3		136	8	7.8	
Breast feeding								
Exclusive	442	178	40.3	0.0001	88	42	47.7	0.33
Partial	213	111	52.1		53	19	35.8	
Zero	167	99	59.3		34	13	38.2	
Maternal GDM								
Yes	24	21	87.5	0.0001	6	3	50.0	0.69
No	741	334	45.1		170	71	41.8	
Smoke exposure								
None	420	177	42.1	0.004	99	44	44.4	0.46
Pre+Postnatal	89	52	58.4		10	5	50.0	
Postnatal	212	111	52.4		41	14	34.1	
Antibiotic exposure								
None	264	113	42.8	0.06	86	20	23.3	0.0001
Indirect	145	75	51.7		62	39	62.9	
Direct	48	28	58.3		18	10	55.6	
Prenatal diet-added sugar								
High	733	340	46.4	0.24	147	58	39.5	0.47
Normal	53	29	54.7		12	6	50.0	
Birth weight								
High	101	59	58.4	0.01	28	10	35.7	0.46
Normal	650	289	44.5		148	64	43.2	

In table 5, 36% of infants who were exclusively breastfed had a high relative abundance of Bifidobacteria in their infant gut microbiome compared to 11% of formula-fed infants ($p=0.002$). In table 6, 43% of infants delivered by cesarean section had a high relative abundance Clostridiaceae in their infant gut microbiome compared to 22% of infants who had vaginal deliveries ($p=0.01$). 40% of infants indirectly exposed to antibiotics had a low relative abundance of Clostridiaceae in their infant gut microbiome ($p=0.02$) compared to infants not exposed to antibiotics.

In table 7, 38% of infants who were exclusively breastfed had a high relative abundance of Staphylococcaceae in their infant gut microbiome compared to 6% of formula-fed infants ($p<0.0001$). In table 8, 34% of infants who were not exposed to antibiotics had a high relative abundance of *Akkermansia* compared to infants who were either directly (11%) or indirectly (19%) exposed to antibiotics ($p=0.05$).

Though not statistically significant, 29.5% of exclusively breastfed infants (table 9) showed a trend of a low Chao 1 richness in their infant gut microbiome compared to 14% of formula-fed infants ($p=0.21$). In table 10, 32% of infants who were exclusively breastfed had a low Shannon index diversity in their infant gut microbiome compared to 3% of formula-fed infants ($p=0.004$).

Table 5:

Frequency distribution of study covariates according to pregnancy and the infant gut microbiome (Fa Bifidobacteriaceae)

	Total	**Mom OW**	**n = 400**		**Total**	**Bifido bacteriaceae**	**n=44**	
	Count	**n**	**%**	**P**	**Count**	**n**	**%**	**P**
Cesarean section								
Yes	134	71	53.0	0.11	38	8	21.1	0.51
No	600	272	45.3		137	36	26.3	
Breast feeding								
Exclusive	442	178	40.3	0.0001	88	32	36.4	0.002
Partial	213	111	52.1		52	8	15.4	
Zero	167	99	59.3		35	4	11.4	
Maternal GDM								
Yes	24	21	87.5	0.0001	5	0	0.0	0.19
No	741	334	45.1		171	44	25.7	
Smoke exposure								
None	420	177	42.1	0.004	99	26	26.3	0.17
Pre+Postnatal	89	52	58.4		10	0	0.0	
Postnatal	212	111	52.4		41	9	22.0	
Antibiotic exposure								
None	264	113	42.8	0.06	86	23	26.7	0.85
Indirect	145	75	51.7		61	14	23	
Direct	48	28	58.3		18	5	27.8	
Prenatal diet-added sugar								
High	733	340	46.4	0.24	147	37	25.2	0.51
Normal	53	29	54.7		12	2	16.7	
Birth weight								
High	101	59	58.4	0.01	28	7	25.0	1.00
Normal	650	289	44.5		148	37	25.0	

Table 6:

Frequency distribution of study covariates according to pregnancy and the infant gut microbiome (Fa Clostridiaceae)

	Total	Mom OW	n = 400		Total	Clostridiaceae	n=44	
	Count	n	%	P	Count	n	%	P
Cesarean section								
Yes	134	71	53.0	0.11	37	16	43.2	0.01
No	600	272	45.3		127	28	22.0	
Breast feeding								
Exclusive	442	178	40.3	0.0001	84	27	32.1	0.17
Partial	213	111	52.1		47	8	17.0	
Zero	167	99	59.3		33	9	27.3	
Maternal GDM								
Yes	24	21	87.5	0.0001	6	1	16.7	0.57
No	741	334	45.1		159	43	27.0	
Smoke exposure								
None	420	177	42.1	0.004	92	24	26.1	0.89
Pre+Postnatal	89	52	58.4		9	3	33.3	
Postnatal	212	111	52.4		39	10	25.6	
Antibiotic exposure								
None	264	113	42.8	0.06	81	15	18.5	0.02
Indirect	145	75	51.7		57	23	40.4	
Direct	48	28	58.3		16	4	25.0	
Prenatal diet-added sugar								
High	733	340	46.4	0.24	137	33	24.1	0.94
Normal	53	29	54.7		12	3	25.0	
Birth weight								
High	101	59	58.4	0.01	27	7	25.9	0.92
Normal	650	289	44.5		138	37	26.8	

Table 7:

Frequency distribution of study covariates according to pregnancy and the infant gut microbiome (Fa Staphylococcaceae)

	Total	Mom OW	n = 400		Total	Staphylococcaceae	n=44	
	Count	**n**	**%**	**P**	**Count**	**n**	**%**	**P**
Cesarean section								
Yes	134	71	53.0	0.11	39	12	30.8	0.30
No	600	272	45.3		137	31	22.6	
Breast feeding								
Exclusive	442	178	40.3	0.0001	88	33	37.5	0.0001
Partial	213	111	52.1		53	8	15.1	
Zero	167	99	59.3		35	2	5.7	
Maternal GDM								
Yes	24	21	87.5	0.0001	6	1	16.7	0.66
No	741	334	45.1		171	42	24.6	
Smoke exposure								
None	420	177	42.1	0.004	100	26	26.0	0.50
Pre+Postnatal	89	52	58.4		10	1	10.0	
Postnatal	212	111	52.4		41	9	22.0	
Antibiotic exposure								
None	264	113	42.8	0.06	86	17	19.8	0.21
Indirect	145	75	51.7		62	20	32.3	
Direct	48	28	58.3		18	4	22.2	
Prenatal diet-added sugar								
High	733	340	46.4	0.24	148	35	23.6	0.45
Normal	53	29	54.7		12	4	33.3	
Birth weight								
High	101	59	58.4	0.01	28	7	25.0	0.92
Normal	650	289	44.5		149	36	24.2	

Table 8:

Frequency distribution of study covariates according to pregnancy and the infant gut microbiome (*Ge Akkermansia*)

	Total	**Mom OW**	**n = 400**		**Total**	***Akker mansia***	**n=44**	
	Count	**n**	**%**	**P**	**Count**	**n**	**%**	**P**
Cesarean section								
Yes	134	71	53.0	0.11	39	7	17.9	0.25
No	600	272	45.3		137	37	27.0	
Breast feeding								
Exclusive	442	178	40.3	0.0001	88	17	19.3	0.28
Partial	213	111	52.1		53	16	30.2	
Zero	167	99	59.3		35	10	28.6	
Maternal GDM								
Yes	24	21	87.5	0.0001	6	0	0.0	0.15
No	741	334	45.1		171	44	25.7	
Smoke exposure								
None	420	177	42.1	0.004	100	23	23.0	0.96
Pre+Postnatal	89	52	58.4		10	2	20.0	
Postnatal	212	111	52.4		41	10	24.4	
Antibiotic exposure								
None	264	113	42.8	0.06	86	29	33.7	0.05
Indirect	145	75	51.7		62	12	19.4	
Direct	48	28	58.3		18	2	11.1	
Prenatal diet-added sugar								
High	733	340	46.4	0.24	148	34	23.0	0.42
Normal	53	29	54.7		12	4	33.3	
Birth weight								
High	101	59	58.4	0.01	28	6	21.4	0.65
Normal	650	289	44.5		149	38	25.5	

Table 9:

Frequency distribution of study covariates according to pregnancy and the infant gut microbiome (Chao1: Richness)

	Total	Mom OW	n = 400		Total	Chao1	n=44	
	Count	**n**	**%**	**P**	**Count**	**n**	**%**	**P**
Cesarean section								
Yes	134	71	53.0	0.11	39	12	30.8	0.35
No	600	272	45.3		137	32	23.4	
Breast feeding								
Exclusive	442	178	40.3	0.0001	88	26	29.5	0.21
Partial	213	111	52.1		53	13	24.5	
Zero	167	99	59.3		35	5	14.3	
Maternal GDM								
Yes	24	21	87.5	0.0001	6	1	16.7	0.64
No	741	334	45.1		171	43	25.1	
Smoke exposure								
None	420	177	42.1	0.004	100	26	26.0	0.18
Pre+Postnatal	89	52	58.4		10	0	0.0	
Postnatal	212	111	52.4		41	10	24.4	
Antibiotic exposure								
None	264	113	42.8	0.06	86	16	18.6	0.14
Indirect	145	75	51.7		62	17	27.4	
Direct	48	28	58.3		18	7	38.9	
Prenatal diet-added sugar								
High	733	340	46.4	0.24	148	36	24.3	0.19
Normal	53	29	54.7		12	5	41.7	
Birth weight								
High	101	59	58.4	0.01	28	7	25.0	0.99
Normal	650	289	44.5		149	37	24.8	

Table 10:

Frequency distribution of study covariates according to pregnancy and (Shannon Diversity Index)

	Total	Mom OW	n = 400		Total	Shannon Index	n=44	
	Count	n	%	P	Count	n	%	P
Cesarean section								
Yes	134	71	53.0	0.11	39	9	23.1	0.81
No	600	272	45.3		136	34	25.0	
Breast feeding								
Exclusive	442	178	40.3	0.0001	88	28	31.8	0.004
Partial	213	111	52.1		53	15	28.3	
Zero	167	99	59.3		34	1	2.9	
Maternal GDM								
Yes	24	21	87.5	0.0001	6	2	33.3	0.63
No	741	334	45.1		170	42	24.7	
Smoke exposure								
None	420	177	42.1	0.004	100	28	28.0	0.15
Pre+Postnatal	89	52	58.4		10	0	0.0	
Postnatal	212	111	52.4		40	11	27.5	
Antibiotic exposure								
None	264	113	42.8	0.06	86	18	20.9	0.21
Indirect	145	75	51.7		62	15	24.2	
Direct	48	28	58.3		17	7	41.2	
Prenatal diet-added sugar								
High	733	340	46.4	0.24	147	37	25.2	0.99
Normal	53	29	54.7		12	3	25.0	
Birth weight								
High	101	59	58.4	0.01	28	6	21.4	0.63
Normal	650	289	44.5		148	38	25.7	

Table 11:

Descriptive table of associations between covariates

		Abx-I	Abx-D			BF-P	BF-E			C/S	
	Count	**n (%)**	**n(%)**	**p**	**Count**	**n(%)**	**n(%)**	**p**	**Count**	**n(%)**	**p**
Maternal GDM											
Yes	18	8(5.2)	6(11.5)	0.001	29	12(5.5)	8(1.8)	0.02	30	9(5.7)	0.11
No	474	146(94.8)	46(88.5)		816	205(94.5)	447(98.2)		822	150(94.3)	
Prenatal diet-added sugar											
High	426	135(92.5)	41(89.1)	0.25	821	214(92.6)	440(94.6)	0.26	720	129(87.2)	0.003
Normal	29	11(7.5)	5(10.9)		58	17(7.4)	25(5.4)		56	19(12.8)	
Smoke exposure											
None	244	71(51.8)	21(46.7)	0.07	463	113(57.4)	296(67.6)	0.0001	401	75(55.1)	0.69
Pre+Postnatal	42	18(13.1)	7(15.6)		102	24(12.2)	27(6.2)		88	20(14.7)	
Post	140	48(35.0)	17(37.8)		239	60(30.5)	115(26.3)		213	41(30.1)	
Birth weight											
High	70	22(14.4)	8(16.3)	0.92	111	21(9.8)	65(14.5)	0.21	113	21(13.5)	0.97
Normal	416	131(85.6)	41(83.7)		719	193(90.2)	383(85.5)		727	134(86.5)	

Highly correlating co-variates:

GDM *Abx – Indirect & Direct (p=0.001)

GDM *BF – Partial & Exclusive (p=0.02)

Mat Diet *C/S (p=0.003)

Smoking*BF – Partial & Exclusive (p=0.0001)

In table 11, gestational diabetes was highly associated with indirect and direct antibiotic exposure (P=0.001). It was also more likely to precede partial and exclusive breastfeeding (p=0.02). Maternal prenatal diet with high added suagr was more likely to result in cesarean delivery (p=0.003). Maternal prenatal and postnatal smoking was highly associated with partial and exclusive breastfeeding (p<0.0001).

Table 12:

Risk of child overweight subsequent to maternal pregnancy overweight, unadjusted and adjusted for covariates

Outcome variable = Child Overweight

		Model 1	**Model 2**	**Model 3**	**Model 4**
Odds Ratio (OR), 95%CI	**Reference category**	Unadjusted crude OR	Adjusted for other maternal pregnancy variables	Adjusted for birth & postnatal infant variables	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	2.04 (1.41–2.95)*	2.00 (1.27-3.16) *	1.64 (0.94-2.86)	1.88 (1.08-3.26) *
Maternal gestational diabetes	**No GDM**	3.41 (1.55-7.52)*	3.13 (1.24-7.89) *		
Cesarean section	**Vaginal delivery**	1.24 (0.79-1.95)	1.09 (0.63-1.90)		1.88 (0.87-4.05)
Maternal diet (added sugar >24g)	**≤24g/day**	0.84 (0.44-1.63)	0.99 (0.43-2.32)		
Smoking – pre+postnatal	**No smoking**	1.70 (0.99-2.93)*	1.49 (0.77-2.89)		
Smoking – postnatal only	**No smoking**	1.30 (0.86-1.97)	0.96 (0.58-1.59)		
High birth weight (>4kg)	**Normal BW**	2.65 (1.70-4.15)*		3.23 (1.70-6.14) *	
Exclusive breastfeeding	**Formula fed**	0.62 (0.50-0.77)*		0.64 (0.46-0.89) *	0.67 (0.48-0.93) *
Antibiotic exposure: Indirect	**No antibiotics**	1.55 (0.90-2.66)		1.30 (0.72-2.37)	0.88 (0.42-1.85)
Antibiotic exposure: Direct	**No antibiotics**	2.70 (1.32-5.51)*		2.62 (1.19-5.75) *	2.15 (0.94-4.91)

Maternal pregnancy overweight doubled the risk of child overweight at age 1 (table 12). Adjustment for gestational diabetes, a high-sugar prenatal diet, pre/postnatal smoking exposures and birth mode, did not change this risk. However, adjustment for high birth weight status, exclusivity of breastfeeding and antibiotic exposure by three months, made it non significant. In a final model which included mode of delivery, exclusive breast feeding, and infant antibiotic exposure, we found that maternal prepregnancy overweight independently doubled the risk of child overweight at age 1 (OR:1.88, 95%CI:1.08-3.26, $p<0.05$). Exclusive breastfeeding on the other hand, lowered the risk of child overweight by 33% at age 1. Adjustment for maternal overweight, high birth weight status and antibiotic exposure by three months did not change this risk. In a final model, after adjusting for maternal pregnancy overweight, mode of delivery and antibiotic exposure, exclusive breastfeeding lowered the risk of child overweight by 33% at age 1 (OR: 0.67, 95%CI: 0.48-0.93, $p=0.02$).

We also found that maternal gestational diabetes tripled the risk of child overweight at age 1 (model 2), after adjusting for maternal overweight, a high-sugar prenatal diet, pre/postnatal smoking exposures and birth mode (OR:3.13, 95% CI:1.24-7.89, $p<0.05$). High birth weight also tripled the risk of child overweight at age 1 after adjusting for maternal overweight, exclusivity of breastfeeding and antibiotic exposure by three months (OR:3.23, 95%CI:1.70-6.14, $p<0.05$). Another result from table 12, showed that direct antibiotic exposure doubled the risk of child overweight at age 1, after adjusting for maternal overweight, high birth weight and exclusivity of breastfeeding (OR:2.62, 95%CI:1.19-5.75, $p<0.05$).

Table 13:

Risk of lowest relative abundance of infant gut Bacteroidaceae subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates

Outcome variable = Bacteroidaceae

		Model 1	**Model 2**	**Model 3**	**Model 4**
Odds Ratio (OR), 95%CI	**Reference category**	Unadjusted crude OR	Adjusted for other maternal pregnancy variables	Adjusted for birth & postnatal infant variables	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.62(0.34-1.14)	0.46 (0.22-0.96)*	0.48(0.23-0.99)*	0.41(0.19-0.88)*
Maternal gestational diabetes	**No GDM**	1.39 (0.27-7.11)	0.84 (0.11-6.32)		
Cesarean section	**Vaginal delivery**	8.67 (3.68-20.46)*	6.59(2.48-17.51)*		5.79 (2.10-15.99)*
Maternal diet (added sugar >24g)	**≤24g/day**	0.65 (0.20-2.12)	0.69 (0.19-2.59)		
Smoking – pre+postnatal	**No smoking**	1.25 (0.34-4.59)	0.55 (0.11-2.81)		
Smoking – postnatal only	**No smoking**	0.65 (0.30-1.38)	0.71 (0.31-1.66)		
High birth weight (>4kg)	**Normal BW**	0.73 (0.32-1.69)		0.85 (0.32-2.24)	
Exclusive breastfeeding	**Formula fed**	1.28 (0.86-1.89)		1.17 (0.74-1.87)	1.18 (0.72-1.94)
Antibiotic exposure: Indirect	**No antibiotics**	5.60 (2.73-11.48)*		6.25(2.90-13.46)*	3.03 (1.27-7.25)*
Antibiotic exposure: Direct	**No antibiotics**	4.13 (1.44-11.86)*		5.26(1.74-15.94)*	2.48 (0.72-8.59)

Maternal pregnancy overweight lowered the risk of a low relative abundance of Bacteroidaceae in the infant gut microbiome at 3months (Table 13). Adjustment for gestational diabetes, a high-sugar prenatal diet, pre/postnatal smoking exposures and birth mode, did not change this risk. Neither did adjustment for high birth weight status, exclusivity of breastfeeding and antibiotic exposure by three months. In a final model which included mode of delivery, exclusive breast feeding, and infant antibiotic exposure, we found that maternal prepregnancy overweight independently lowered the risk of a low relative abundance of Bacteroidaceae in the infant gut microbiome at 3months (OR:0.41, 95%CI: 0.19-0.88, p= 0.02). However, we couldn't find evidence that maternal overweight had an impact on the infant gut microbiota measures of species richness, diversity and the relative abundance of Bifidobacteriaceae, Clostridiaceae, Staphylococcaceae and Akkermansia.

Other results from table 13 reveal that infants born by cesarean section were 6 times more likely to have a low relative abundance of Bacteroidaceae. Adjustment for gestational diabetes, a high-sugar prenatal diet, pre/postnatal smoking exposures and birth mode, did not change this risk. In a final model which included maternal overweight, exclusive breast feeding and antibiotic exposure, we found that infants born by cesarean section were 6 times more likely to have a low relative abundance of Bacteroidaceae ($p<0.05$). Indirect infant antibiotic exposure was 3 times more likely to be associated with a low relative abundance of Bacteroidaceae (Table 13). Adjustment for maternal overweight, high birth weight status and exclusivity of breastfeeding by three months did not change this risk. In a final model, which included maternal overweight, exclusive breast feeding

at three months, and mode of delivery, we found that Indirect infant antibiotic exposure was 3 times more likely to be associated with a low relative abundance of Bacteroidaceae ($p<0.05$).

Exclusively breastfed infants were 2.5 times more likely to have a high relative abundance of Bifidobacteria in their infant gut microbiome (Table 14). Adjustment for prepregnancy overweight, high birth weight status, and antibiotic exposure by three months did not change this risk. In a final model, which included prepregnancy overweight, mode of delivery, and antibiotic exposure at three months, we found that exclusively breastfed infants were 2.5 times more likely to have a high relative abundance of Bifidobacteria (OR:2.54, 95%CI: 1.39-4.63, $p<0.05$).

Indirect infant antibiotic exposure was 3 times more likely to have a high relative abundance of Clostridiaceae (Table 15). Adjustment for maternal overweight, high birth weight status and exclusivity of breastfeeding by three months did not change this risk. In a final model, which included maternal overweight, exclusive breast feeding at three months, and mode of delivery, we found that Indirect infant antibiotic exposure was 3 times more likely to be associated with a low relative abundance of Clostridiaceae (OR:2.66, 95%CI:1.05-6.72, $p<0.05$).

Table 14:

Risk of highest relative abundance of infant gut Bifidobacteriaceae subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates

Outcome variable = Bifidobacteriaceae

		Model 1	**Model 2**	**Model 3**	**Model 4**
Odds Ratio (OR), 95%CI	**Reference category**	Unadjusted crude OR	Adjusted for other maternal pregnancy variables	Adjusted for birth & postnatal infant variables	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.70 (0.35-1.40)	0.76 (0.34-1.68)	0.78 (0.37-1.67)	0.81 (0.38-1.73)
Maternal gestational diabetes	**No GDM**	Non estimable	Non estimable		
Cesarean section	**Vaginal delivery**	0.75 (0.31-1.78)	0.96 (0.34-2.76)		0.57 (0.19-1.69)
Maternal diet (added sugar >24g)	**≤24g/day**	1.68 (0.35-8.03)	1.43 (0.28-7.26)		
Smoking – pre+postnatal	**No smoking**	Non estimable	Non estimable		
Smoking – postnatal only	**No smoking**	0.79 (0.33-1.87)	0.83 (0.34-2.04)		
High birth weight (>4kg)	**Normal BW**	1.00 (0.392.54)		0.98 (0.34-2.81)	
Exclusive breastfeeding	**Formula fed**	2.37 (1.38-4.05)*		2.47 (1.36-4.48)*	2.54 (1.39-4.63)*
Antibiotic exposure: Indirect	**No antibiotics**	0.82 (0.38-1.75)		0.71 (0.32-1.61)	0.90 (0.36-2.27)
Antibiotic exposure: Direct	**No antibiotics**	1.05 (0.34-3.28)		1.29 (0.39-4.33)	1.79 (0.49-6.46)

Table 15:

Risk of highest relative abundance of infant gut Clostridiaceae subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates

Outcome variable = Clostridiaceae

		Model 1	**Model 2**	**Model 3**	**Model 4**
Odds Ratio (OR), 95%CI	**Reference category**	Unadjusted crude OR	Adjusted for other maternal pregnancy variables	Adjusted for birth & postnatal infant variables	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.92 (0.46-1.84)	0.87 (0.39-1.96)	1.01 (0.47-2.16)	0.99 (0.47-2.11)
Maternal gestational diabetes	**No GDM**	0.54 (0.06-4.75)	0.58 (0.06-5.66)		
Cesarean section	**Vaginal delivery**	2.69 (1.24-5.84)*	1.90 (0.73-4.95)		1.40 (0.54-3.63)
Maternal diet (added sugar >24g)	**≤24g/day**	0.95 (0.24-3.72)	1.08 (0.27-4.38)		
Smoking – pre+postnatal	**No smoking**	1.42 (0.33-6.11)	1.59 (0.32-7.89)		
Smoking – postnatal only	**No smoking**	0.98 (0.42-2.30)	1.27 (0.52-3.13)		
High birth weight (>4kg)	**Normal BW**	0.96 (0.37-2.45)		0.72 (0.25-2.07)	
Exclusive breastfeeding	**Formula fed**	1.25 (0.79-1.97)		1.05 (0.64-1.74)	1.07 (0.65-1.77)
Antibiotic exposure: Indirect	**No antibiotics**	2.98 (1.38-6.43)*		3.25 (1.46-7.24)*	2.66 (1.05-6.72)*
Antibiotic exposure: Direct	**No antibiotics**	1.47 (0.42-5.19)		1.53 (0.43-5.50)	1.44 (0.37-5.68)

Exclusively breastfed infants were 3 times more likely to have high relative abundance of Staphylococcous in their infant gut microbiome (Table 16). Adjustment for prepregnancy overweight, high birth weight status, and antibiotic exposure by three months did not change this risk. In a final model, which included prepregnancy overweight, mode of delivery, and antibiotic exposure at three months, we found that exclusively breastfed infants were 3 times more likely to have a high relative abundance of Staphylococcaceae (OR:3.35, 95%CI: 0.60-6.46, $p<0.05$).

In this study, *Akkermansia* (table 17) did not have any statistically significant results. In model 3, (Table 18), infants directly exposed to antibiotics are 3.15 times more likely to be have low species richness independent of maternal overweight, high birth weight and infant diet ($p<0.05$).

Exclusively breastfed infants were 2 times more likely to have a low Shannon index diversity in their infant gut microbiome (Table 19). Adjustment for prepregnancy overweight, high birth weight status, and antibiotic exposure by three months did not change this risk. In a final model, which included prepregnancy overweight, mode of delivery, and antibiotic exposure at three months, we found that exclusively breastfed infants were 2 times more likely to have a low Shannon index diversity (OR:2.33, 95%CI: 1.28-4.26, $p<0.05$).

Table 16:

Risk of highest relative abundance of infant gut Staphylococcaceae subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates

Outcome variable = Staphylococcaceae

		Model 1	**Model 2**	**Model 3**	**Model 4**
Odds Ratio (OR), 95%CI	**Reference category**	Unadjusted crude OR	Adjusted for other maternal pregnancy variables	Adjusted for birth & postnatal infant variables	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	1.13 (0.57-2.25)	0.99 (0.45-2.18)	1.60 (0.74-3.46)	1.65 (0.77-3.54)
Maternal gestational diabetes	**No GDM**	0.61 (0.07-5.41)	0.77 (0.08-7.92)		
Cesarean section	**Vaginal delivery**	1.52 (0.69-3.35)	1.60 (0.62-4.12)		1.11 (0.41-3.00)
Maternal diet (added sugar >24g)	**≤24g/day**	0.62 (0.18-2.18)	0.62 (0.17-2.29)		
Smoking – pre+postnatal	**No smoking**	0.32 (0.04-2.62)	0.30 (0.03-2.63)		
Smoking – postnatal only	**No smoking**	0.80 (0.34-1.90)	0.86 (0.35-2.13)		
High birth weight (>4kg)	**Normal BW**	1.05 (0.41-2.66)		0.98 (0.35-2.78)	
Exclusive breastfeeding	**Formula fed**	3.24 (1.76-5.97)*		3.24 (1.69-6.22)*	3.35 (1.74-6.46)*
Antibiotic exposure: Indirect	**No antibiotics**	1.93 (0.91-4.10)		1.60 (0.72-3.55)	1.51 (0.60-3.83)
Antibiotic exposure: Direct	**No antibiotics**	1.16 (0.34-3.97)		1.25 (0.34-4.62)	1.43 (0.36-5.61)

Table 17:

Risk of highest relative abundance of infant gut *Akkermansia* subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates

Outcome variable = *Akkermansia*

		Model 1	**Model 2**	**Model 3**	**Model 4**
Odds Ratio (OR), 95%CI	**Reference category**	Unadjusted crude OR	Adjusted for other maternal pregnancy variables	Adjusted for birth & postnatal infant variables	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.83 (0.42-1.65)	0.86 (0.39-1.92)	0.83 (0.39-1.76)	0.84 (0.40-1.75)
Maternal gestational diabetes	**No GDM**	Non estimable	Non estimable		
Cesarean section	**Vaginal delivery**	0.59 (0.24-1.46)	0.56 (0.18-1.72)		0.98 (0.32-3.05)
Maternal diet (added sugar >24g)	**≤24g/day**	0.60 (0.17-2.10)	0.47 (0.13-1.79)		
Smoking – pre+postnatal	**No smoking**	0.84 (0.17-4.22)	1.36 (0.23-7.96)		
Smoking – postnatal only	**No smoking**	1.08 (0.46-2.53)	1.16 (0.48-2.82)		
High birth weight (>4kg)	**Normal BW**	0.80 (0.30-2.11)		1.01 (0.36-2.85)	
Exclusive breastfeeding	**Formula fed**	0.75 (0.48-1.15)		0.66 (0.41-1.05)	0.66 (0.41-1.06)
Antibiotic exposure: Indirect	**No antibiotics**	0.47 (0.22-1.02)		0.52 (0.24-1.16)	0.53 (0.20-1.38)
Antibiotic exposure: Direct	**No antibiotics**	0.25 (0.05-1.14)		0.23 (0.05-1.09)	0.24 (0.05-1.24)

Table 18:

Risk of low Chao1 richness subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates

Outcome variable = Chao1 {Richness}

		Model 1	Model 2	Model 3	Model 4
Odds Ratio (OR), 95%CI	**Reference category**	Unadjusted crude OR	Adjusted for other maternal pregnancy variables	Adjusted for birth & postnatal infant variables	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.57 (0.29-1.15)	0.67 (0.30-1.48)	0.59 (0.28-1.28)	0.61 (0.29-1.30)
Maternal gestational diabetes	**No GDM**	0.60 (0.07-5.24)	0.71 (0.06-7.94)		
Cesarean section	**Vaginal delivery**	1.46 (0.66-3.22)	2.04 (0.79-5.30)		1.20 (0.45-3.15)
Maternal diet (added sugar >24g)	**≤24g/day**	0.45 (0.14-1.51)	0.42 (0.12-1.54)		
Smoking – pre+postnatal	**No smoking**	Non estimable	Non estimable		
Smoking – postnatal only	**No smoking**	0.92 (0.40-2.13)	0.95 (0.39-2.31)		
High birth weight (>4kg)	**Normal BW**	1.01 (0.40-2.56)		1.02 (0.36-2.86)	
Exclusive breastfeeding	**Formula fed**	1.51 (0.94-2.42)		1.35 (0.80-2.27)	1.39 (0.83-2.36)
Antibiotic exposure: Indirect	**No antibiotics**	1.65 (0.76-3.60)		1.61 (0.72-3.60)	1.47 (0.58-3.74)
Antibiotic exposure: Direct	**No antibiotics**	2.78 (0.93-8.30)		3.15 (1.02-9.75)*	3.26 (0.97-10.94)

Table 19:

Risk of low Shannon diversity index subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates

Outcome variable = Shannon Index {Diversity}

		Model 1	**Model 2**	**Model 3**	**Model 4**
Odds Ratio (OR), 95%CI	**Reference category**	Unadjusted crude OR	Adjusted for other maternal pregnancy variables	Adjusted for birth & postnatal infant variables	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.85 (0.43-1.68)	0.95 (0.44-2.05)	0.93 (0.43-2.00)	0.87 (0.41-1.87)
Maternal gestational diabetes	**No GDM**	1.52 (0.27-8.62)	0.87 (0.08-9.38)		
Cesarean section	**Vaginal delivery**	0.90 (0.39-2.09)	1.24 (0.47-3.28)		0.79 (0.28-2.20)
Maternal diet (added sugar >24g)	**≤24g/day**	1.01 (0.26-3.93)	1.08 (0.26-4.39)		
Smoking – pre+postnatal	**No smoking**	Non estimable	Non estimable		
Smoking – postnatal only	**No smoking**	0.98 (0.43-2.21)	0.85 (0.36-2.04)		
High birth weight (>4kg)	**Normal BW**	0.79 (0.30-2.09)		0.70 (0.23-2.12)	
Exclusive breastfeeding	**Formula fed**	2.19 (1.29-3.71)*		2.44 (1.33-4.47)*	2.33 (1.28-4.26)*
Antibiotic exposure: Indirect	**No antibiotics**	1.21 (0.55-2.63)		1.04 (0.46-2.36)	1.15 (0.45-2.93)
Antibiotic exposure: Direct	**No antibiotics**	2.64 (0.88-7.92)		3.09 (0.95-10.03)	2.94 (0.83-10.46)

Gender analysis were additional analyses conducted. In the final model (Appendix-Table 20), boys born to overweight mothers, were 3 times more likely to become overweight at age 1, independent of mode of delivery, infant diet and antibiotic exposure. (OR:3.12, 95%CI:1.43-6.81) p=0.004. In the final model (Appendix-Table 21), there were no statistically significant results among the girls.

In the final model (Appendix-Table 22), the risk of a low relative abundance of Bacteroidaceae at 3 months, was lowered in boys born to overweight mothers by 76%, independent of mode of delivery, infant diet and antibiotic exposure. (OR:0.24, 95%CI:0.08-0.75) p=0.02. On the other hand, the risk of a low relative abundance of Bacteroidaceae at 3 months, in boys who were delivered by cesarean section was increased by 7 times, independent of maternal overweight, infant diet and antibiotic exposure. (OR:6.55, 95%CI:1.51-28.41) p=0.01. In the final model (Appendix-Table 23), the risk of a low relative abundance of Bacteroidaceae at 3 months, in girls who were delivered by cesarean section was increased by 7.5 times, independent of maternal overweight, infant diet and antibiotic exposure. (OR:6.55, 95%CI:1.51-28.41) p=0.01. The risk of a low relative abundance of Bacteroidaceae at 3 months, in girls who were indirectly exposed to antibiotics was tripled, independent of maternal overweight, mode of delivery and infant diet. (OR:3.33, 95%CI: 0.99-11.13) p=0.05.

In the final model (Appendix-Table 24), the risk of a high relative abundance of

Bifidobacteria in boys who were exclusively breast fed was 2.61, independent of maternal overweight, mode of delivery and antibiotic exposure. (OR:2.61, 95%CI:1.04-6.59) p=0.04. In the final model (Appendix-Table 25), the risk of a high relative abundance of Bifidobacteria in girls who were exclusively breast fed was trippled, independent of maternal overweight, mode of delivery and antibiotic exposure. (OR:2.96, 95%CI:1.20-7.33) p=0.02. In the final model (Appendix-Table 26), there were no statistically significant results among the boys. In the final model (Appendix-Table 27), the risk of a high relative abundance of Clostridiaceae in girls who had been indirectly exposed to antibiotics was 3.67, independent of maternal overweight, mode of delivery and infant diet. (OR:3.67, 95%CI:1.01-13.30) p=0.05.

In the final model (Appendix-Table 28), the risk of a high relative abundance of Staphylococcaceae in boys who were exclusively breast fed was trippled, independent of maternal overweight, mode of delivery and antibiotic exposure. (OR:3.27, 95%CI:1.24-8.62) p=0.02. In the final model (Appendix-Table 29), the risk of a high relative abundance of Staphylococcaceae in girls who were exclusively breast fed was trippled, independent of maternal overweight, mode of delivery and antibiotic exposure. (OR:3.26, 95%CI:1.23-8.61) p=0.02. In the final model (Appendix-Table 30), the risk of a high relative abundance of Akkermansia in boys born to overweight mothers was lowered by 74%, independent of mode of delivery, infant diet and antibiotic exposure. (OR:0.26, 95%CI:0.07-0.93) p=0.04. In the final model (Appendix-Table 31), there were no statistically significant

results among the girls.

In the final model (Appendix-Table 32), the risk of a low Chao 1 richness in boys who had been directly exposed to antibiotics was increased by 6 times, independent of maternal overweight, mode of delivery and infant diet. (OR:6.05, 95%CI:1.16-31.52) p=0.03. In the final model (Appendix-Table 33), there were no statistically significant results among the girls. In the final model (Appendix-Table 34), there were no statistically significant results among the boys. In the final model (Appendix-Table 35), the risk of a low Shannon diversity index in girls who were exclusively breast fed was trippled, independent of maternal overweight, mode of delivery and antibiotic exposure. (OR:2.94, 95%CI:1.18-7.35) p=0.02.

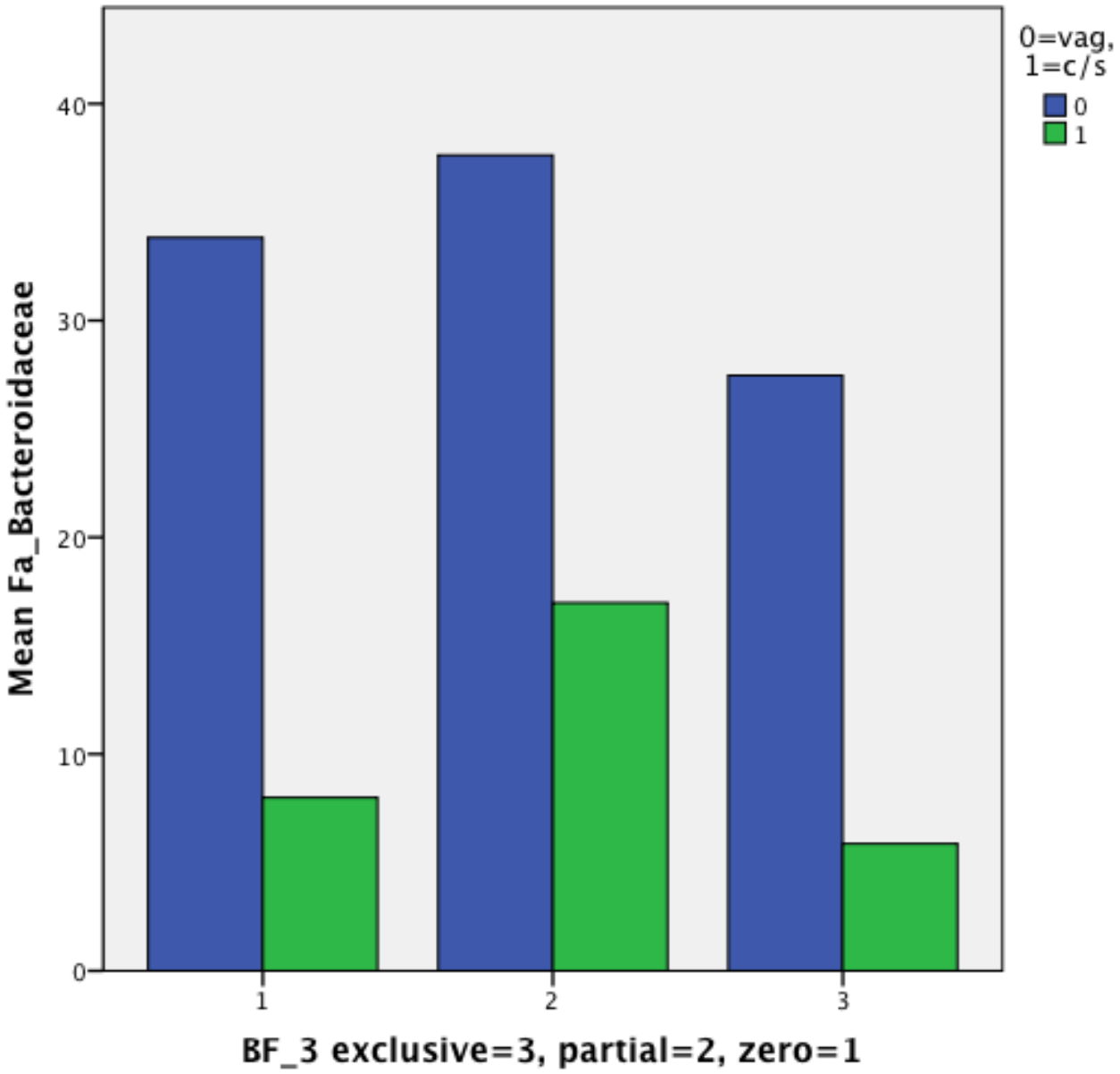

Figure 1: Mean relative abundance of Bacteroidaceae in infants at 3 months

Bacteroidaceae is significantly higher among vaginally delivered infants compared to infants delivered by cesarean section. This is similar to results obtained by Jakobsson et al (80).

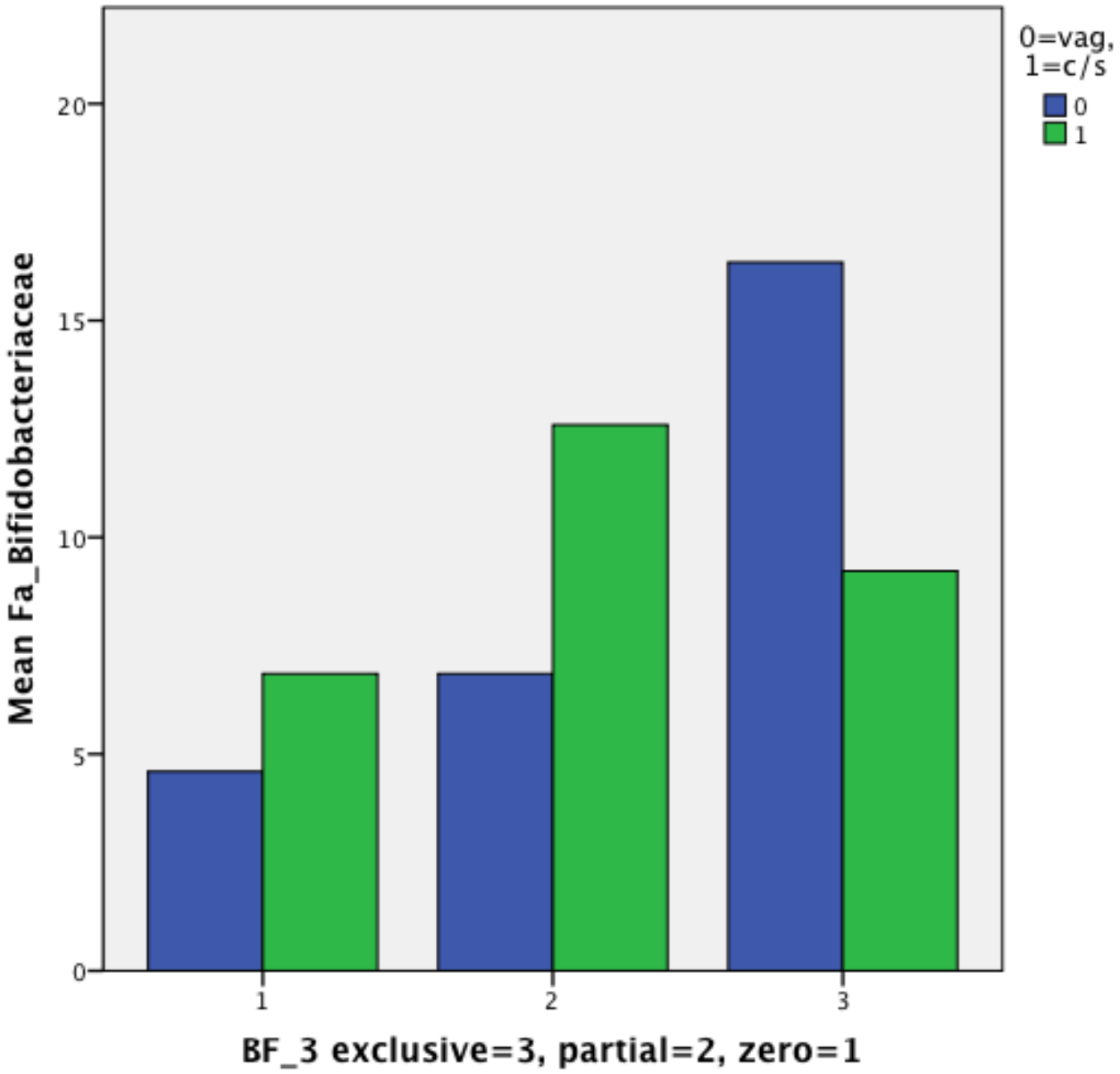

Figure 2: Mean relative abundance of Bifidobacteriaceae in infants at 3 months

Bifidobacteria is significantly higher in vaginally delivered infants who were exclusively breast fed compared to mixed or formula fed infants. This is similar to results obtained by Fan et al (82).

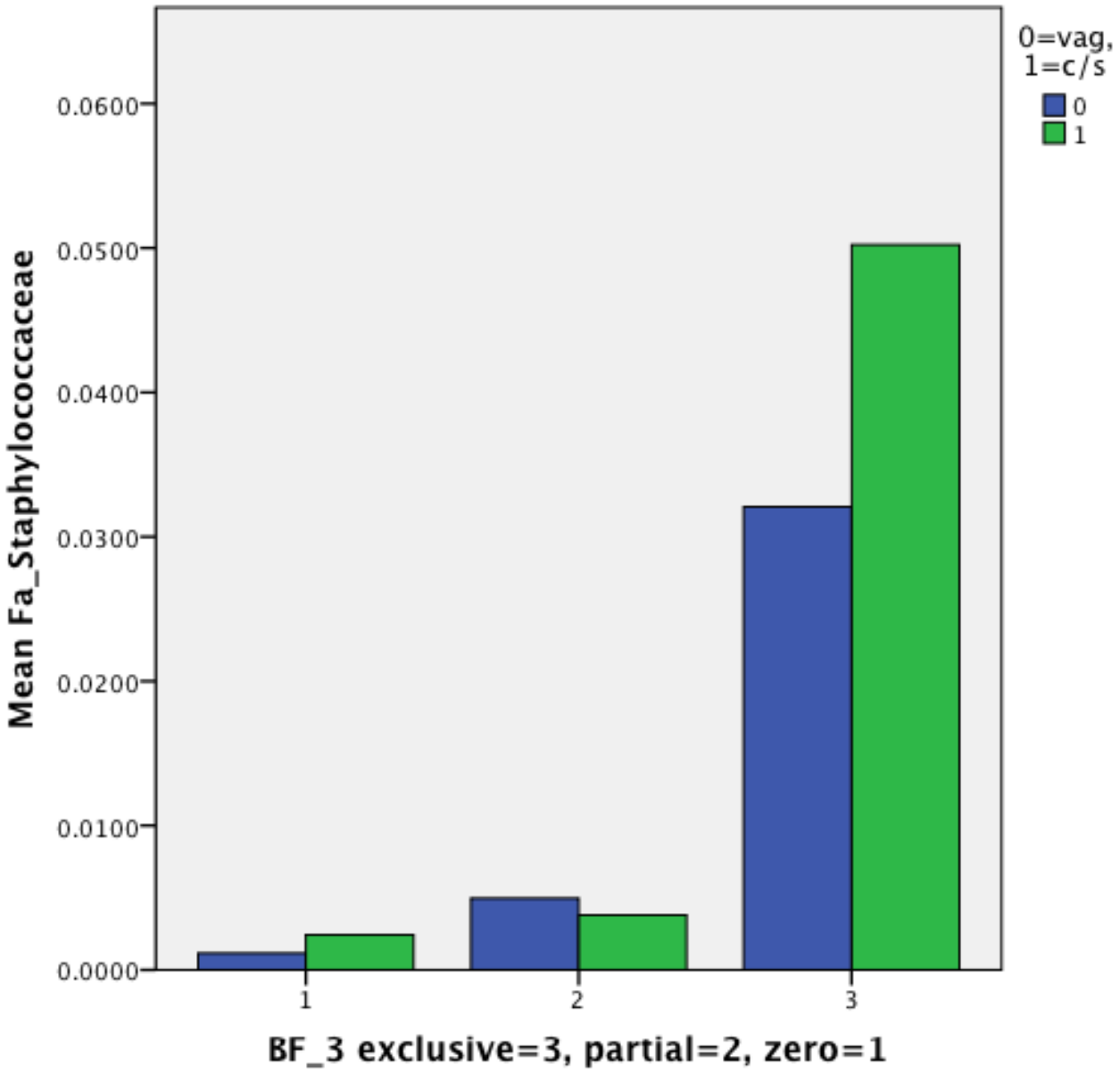

Figure 3: Mean relative abundance of Staphylococcaceae in infants at 3 months

At 3 months, Staphylococcaceae is hardly detectable in infants, however, they are present to a higher degree in vaginally delivered, exclusively breast fed infants compared to mixed or formula fed infants.

Discussion

In a study of 1021 Canadian women, we found that maternal pregnancy overweight doubled the risk of child overweight at one year (OR:1.88, 95%CI: 1.08-3.26), independent of mode of delivery, exclusive breast feeding or formula feeding, and infant antibiotic exposure by three months. Our findings are consistent with studies completed by Flores et al (21) and Barroso et al (66) which, both indicate that maternal pregnancy overweight significantly increases the risk of child overweight at 5-11 and 1-2 years of age, respectively, among Mexican and Mexican- American children. In Weng et al's (20) systematic review and meta-analyses of risk factors for childhood overweight identifiable during infancy, the association between prenatal maternal overweight and child overweight was seen at three different time points, ages 3, 7 and 9-14. Similar studies were done by Sridhar et al (67), and they observed that maternal gestational weight gain increased the risk of child overweight at 2-5 years of age (OR:1.46, 95%CI:1.17-1.83) (67). To the best of our knowledge, this is the first Canadian study to report on the impact of maternal overweight on child overweight and the infant gut microbiome in Canadian women and children. While other studies have been done on pregnancy overweight and child overweight in Canada (68), this is the first study to incorporate the infant gut microbiome as a possible intermediate pathway between maternal overweight and child overweight.

It was important to adjust for the effect different covariates might have on the infant gut microbiome and child overweight. Several studies concur that mode of delivery (vaginal or cesarean), antibiotic exposure and infant diet (breast fed or formula fed), can significantly alter the infant gut microbiome (23,69). The same covariates have been reported to play a role in the development of child overweight (26,20). In view of this, we decided to adjust for the effect of these covariates on the infant gut microbiome and child overweight. Using logistic regression, we adjusted for maternal pregnancy variables such as gestational diabetes, mode of delivery, maternal diet and smoking. Prepregnancy overweight was statistically significant in its association with child overweight risk. However, in the following model, we adjusted for birth and postnatal infant variables such as birth weight, exclusive breastfeeding and antibiotic exposure at 3 months and found that prepregnancy overweight became non significant. This could have occurred as a result of the inclusion of highly correlated covariates. Thus, in our final model, we only adjusted for mode of delivery, exclusive breast feeding and antibiotic exposure and pre-pregnancy overweight was independently associated with child overweight risk.

Further in our study, after adjusting for mode of delivery, exclusive breastfeeding or formula feeding and infant antibiotic exposure, maternal pregnancy overweight lowered the risk of a low relative abundance of Bacteroidaceae in the infant gut microbiome at 3 months (OR:0.41, 95%CI: 0.19-0.88, p= 0.02). While this finding is consistent with the Collado maternal microbiota study (25), where they

found that high *Bacteroides* concentrations were associated with excessive weight gain during pregnancy (P=0.014), it was inconsistent with the Collado infant gut microbiome study (19), which found that maternal pre-pregnancy overweight was associated with lower concentrations of *Bacteroides* in infants at one month. The first reason for the discrepancy between the two results lies in the differences between the microbiota profiling method. Using qPCR and the FISH method, the Collado team measured counts of *Bacteroides* in infant fecal samples, while, our gene sequencing, measured the relative abundance of Bacteroidaceae, which refers to the proportion of Bacteroidaceae relative to the other microbial families in fecal samples. Secondly, there was a notable difference in the taxa classification between the two results. Collado et al reported on the combined group of genus *Bacteroides* and *Prevotella*, while our research used the family of Bacteroidaceae, which does not include *Prevotella*. Another difference between the two results is that a greater percent of infants in the pregnancy overweight group were delivered by cesarean section, which means that the children in this category would have very low levels of *Bacteroides* in their gut microbiome. Some studies have found the *Bacteroides* level of cesarean born infants to be undetectable (23). Collado et al did not adjust for mode of delivery in their paper, but we did. Finally, their results are for one month old infants, who would have been predominantly exclusively breastfed, while our data are from 3-month old infants, some of which were formula fed.

In a primate model study conducted by Ma et al (37), the relative abundance of different bacterial species in the intestinal samples of offspring relegated to either the control group or high fat diet group was measured. The dominance of Bacteroidetes in the profile of the high fat fed dams was as a result of the increased relative abundance of *Prevotella*. This supports our earlier statement that the most likely reason why maternal overweight was not associated with the family of Bacteroidaceae was because it does not contain *Prevotella*, which is a component of the order of Bacteriodales.

Though our analysis of Bacteroidaceae was statistically significant, we could not find evidence that maternal overweight had an impact on the infant gut microbiota measures of species richness, diversity and the relative abundance of Bifidobacteriaceae, Clostridiaceae, Staphylococcaceae and genus *Akkermansia*. However, the Collado infant gut microbiome study found that maternal overweight was associated with higher concentrations of the genus of *Bacteroides*, *Clostridium* and *Staphylococcus* and lower concentrations of the genus *Bifidobacterium* (25). In our study, the lack of association with other microbiota could be as a result of the profiling methods we used in analyzing our microbiota. High through put sequencing is optimal in describing whole microbial communities in fecal samples but only to the genus level. In other studies, by for example, Collado et al, fecal sample sequencing was followed with qPCR to identify specific species. The reason an association was found with Bacteroidaceae and not the others could perhaps be attributed to the differences in

relative abundances of different species. While the same sequencing method was used for all the families, the proportion of *Bacteroides* relative to the other microbial families, might have been low enough to make it statistically significant.

Our study also revealed that exclusive breastfeeding lowered the risk of child overweight at one year by 33%, (OR:0.67, 95% CI:0.48-0.93). The benefits of breast milk have been well documented and McCroy et al (70) found that being breastfed for 26 weeks or more was associated with a 51% reduction in the risk of obesity at nine years. This might be explained by the actions of the infant gut microbiota, because a study by Munoz-Quezada et al (24) revealed that three supernatants isolated from the faeces of breast milk fed infants (*Lactobacillus paracasei, Bifidobacterium breve* and *Lactobacillus rhamnosus*), were capable of inhibiting enterobacteria (*Salmonella, Shigella* and *E.coli*) by 40,55 and 81% respectively.

Although not statistically significant, children who received a course of antibiotics for suspected sepsis at birth were twice as likely to become overweight (OR:2.15, 95%CI:0.94-4.91) . Initially, the unadjusted crude odds ratio in model 1 (Table 2), for direct infant antibiotic exposure was statistically significant at 2.70, and remained significant at 2.62, even after adjusting for birth and postnatal infant variables (model 3). Nevertheless, the significance was lost in the final model. A

study by Ajslev et al (26) who examined pre-pregnancy weight, antibiotic use in infancy and childhood overweight at age 7, found that antibiotic use was protective for children of overweight mothers and led to an increased risk of overweight in children of normal weight mothers. Trasande et al (27) conducted a similar study on infant antibiotic use and early life body mass and found that any exposure to antibiotics in the first 6 months of life was associated with a consistent increase in body mass from 10-38 months ($p=0.001$, $p=0.029$, respectively).

Strengths

This research has many strengths. The total number of women that participated in our study and their offspring was quiet large, in comparison to other existing studies by Collado (19,25). Expert CHILD investigators retrieved birth anthropometric data from birth chart reviews. Physical assessments and questionnaires on maternal and child body mass index and pre and postnatal nutrition were utilized. Comprehensive questionnaires on breastfeeding and smoking were also used. These measurements are standardized, following precise and detailed measurement protocol (39,43). The World Health Organization (WHO) child growth standards based on length/height, weight and age was used to classify the body mass index of children (43) and the WHO body mass index (BMI) classification was used to classify the BMI of women (39). Infant gut microbiome is obtained from the infant at 3 months by analyzing the fecal samples. The DNA analysis process consists of several stages. However, all the

equipment is standardized and the investigators are well trained. Surveys were mailed out and written in lay language, so the public would easily understand and respond to, without fear of judgement. However, this might have introduced respondent bias, so we compared the sociodemographics of the mothers in our study population to all the mothers at the Winnipeg site and found the results to be quite similar. The prevalence of cesarean section among overweight children was 18.9%, which was slightly lower than the Winnipeg cohort that reported a prevalence of 20.2%. The prevalence of exclusive breastfeeding among overweight children was 54.6%, which was lower than the Winnipeg cohort, which reported a prevalence of 65.0%. The prevalence of prenatal smoking was 12.0% among overweight children, which was lower than the Winnipeg cohort that reported a prevalence of 18.0%. Information on weight, height, diet, smoke exposure, blood pressure and blood glucose is routinely collected for the duration of the pregnancy and up to 3 years after, so there is no time lag and this reduces recall bias.

We also pursued a systematic approach to modeling by selecting covariates that have had a significant effect on our outcome variables of interest; child overweight and the infant gut microbiome. Logistic regression was used to test the significance of these covariates. In the first model, the unadjusted crude odds ratio was obtained for each covariate. The second model was adjusted for other maternal pregnancy variables such as maternal pregnancy overweight, maternal gestational diabetes, mode of delivery, maternal diet and smoke exposure. The

rationale for adding these covariates was that they might have an impact on maternal pregnancy overweight. The third model was adjusted for birth and postnatal infant variables such as birth weight, infant diet and antibiotic exposure. The rationale for adding these covariates was that they might have an impact on the child overweight and the infant gut microbiome (17,20). Covariates that were highly correlated with each other were excluded from the final model (Table 1D), such as, between gestational diabetes and infant antibiotic exposure, and maternal prenatal smoking and infant diet. So, our final model consisted of maternal overweight, mode of delivery, infant diet and antibiotic exposure. The infant gut microbiome is influenced by 3 major confounding factors; mode of delivery (vaginal or cesarean), antibiotic therapy and infant feeding (breast or bottle-fed). Therefore, to control for it, a multivariate analysis was done which was simultaneously adjusted for several variables.

Limitations

On the other hand, this study has several limitations. The literature has shown that maternal overweight has an impact on child overweight, so data on maternal overweight was derived from the prenatal record in hospital birth charts. Height and weight measurements in centimeters and kilos, respectively were retrieved from the charts and body mass index was calculated using the formula 'mass in kilograms divided by the square of the height in meters' (kg/m^2). WHO body mass index (BMI) classification was used to classify the BMI of women (39). There could be inaccuracies in the transcription of those results due to human

error, by investigators. A problem we encounter is that fecal samples obtained from infants at 3 months of age are small and this will be further aliquoted (divided), before analysis begin. We used high throughput sequencing to profile our gut microbiota which is desirable for identifying whole communities but only to the genus level. However, we did not follow it up with qPCR to identify specific species, as some other studies have done. Infant gut microbiome is obtained from the infant at 3 months and 1 year by analyzing the fecal samples and working under the assumption that it is an accurate representation of the gut microbiome. Ideally, we should be examining samples of the gut but that would be extremely invasive.

CHAPTER 4: Conclusion

Main findings

In this research, we found that maternal pregnancy overweight doubled the risk of child overweight at one year, independent of mode of delivery, exclusive breast feeding or formula feeding, and infant antibiotic exposure by three months (OR:1.88, 95%CI: 1.08-3.26, p= 0.03). Exclusive breastfeeding on the other hand, lowered the risk of child overweight by 33% after adjusting for maternal pregnancy overweight, mode of delivery and antibiotic exposure (OR: 0.67, 95%CI: 0.48-0.93, p=0.02). Although not statistically significant, children who received a course of antibiotics at birth were twice as likely to become overweight after adjusting for maternal overweight, mode of delivery and infant diet (OR: 2.15, 95%CI:0.94-4.91, p=0.07).

We found that maternal pregnancy overweight lowers the risk of a low relative abundance of Bacteroidaceae in the infant gut microbiome at 3months, independent of mode of delivery, exclusive breastfeeding or formula feeding and infant antibiotic exposure (OR:0.41, 95%CI: 0.19-0.88, p= 0.02).

Clinical Relevance

Our study is unique because it is done among Canadians and as early as age 1, which is younger than most articles report in the literature. Maternal overweight and obesity is a rapidly growing problem that carries not just immediate consequences for the newborn, such as shoulder dystocia and macrosomia, but our study contributed to evidence of its long-term impact on the development of

cardiovascular disease and hypertension by increasing the risk of overweight already at age 1. Bacteroidaceae is the only gut microbiota persistently associated with overweight and even Collado et al linked it to maternal overweight. Without promoting breastfeeding in infancy, or a timely appropriate intervention in older children, such as a healthy diet and an active lifestyle, they could end up becoming overweight or obese adults. Family physicians can council women who would like to start a family about how achieving a normal body mass index prior to pregnancy would be beneficial to their child and will reduce the risk of child overweight.

Future directions

As recent genomic sequencing approaches have provided new insights on being able to map microbiome variability between different species, individuals and populations (22), future research should investigate further the role the gut microbiome plays as a physiologic mediator and the link between maternal overweight and child obesity. This information would be very beneficial in any clinical practice. Future research should also explore the presence of gut microbiota in amniotic fluid as as a determinant of infant gut microbiota alterations having origins in-utero.

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Appendix

Figure 4: Conceptual Framework

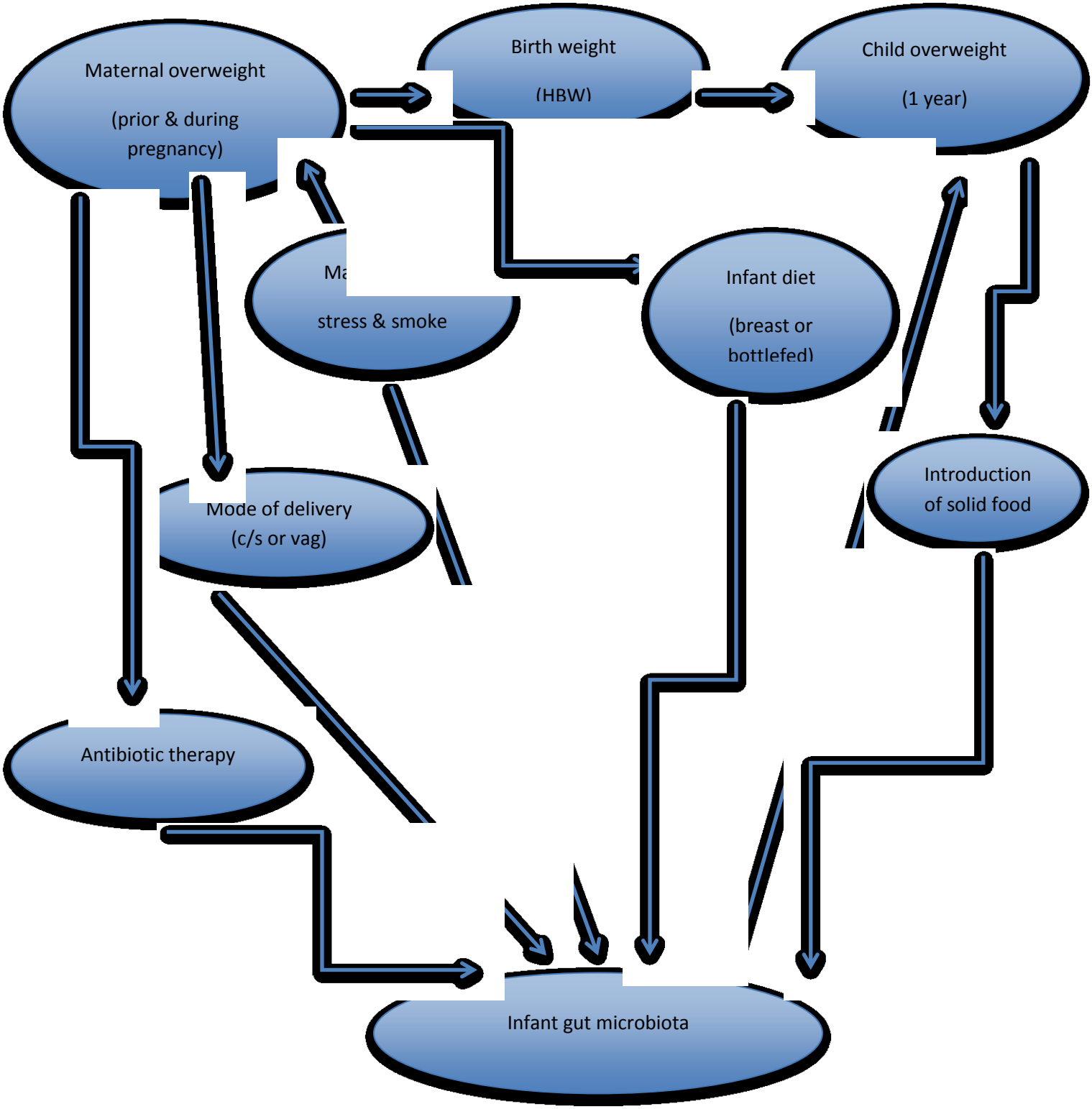


Figure 5: 8 Major taxonomic ranks

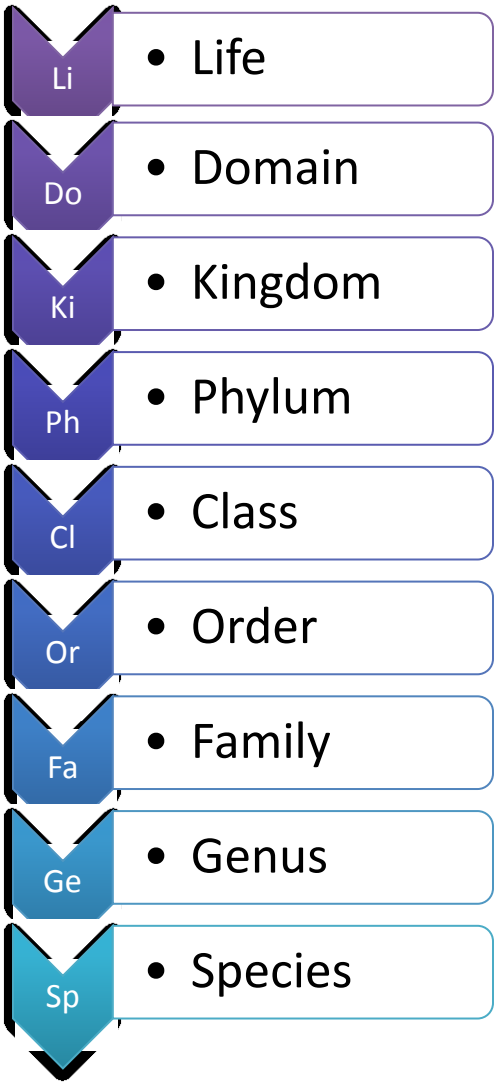


Figure 6: The taxonomy of Bacteroidaceae, Bifidobacteriaceae and Staphylococcaceae.

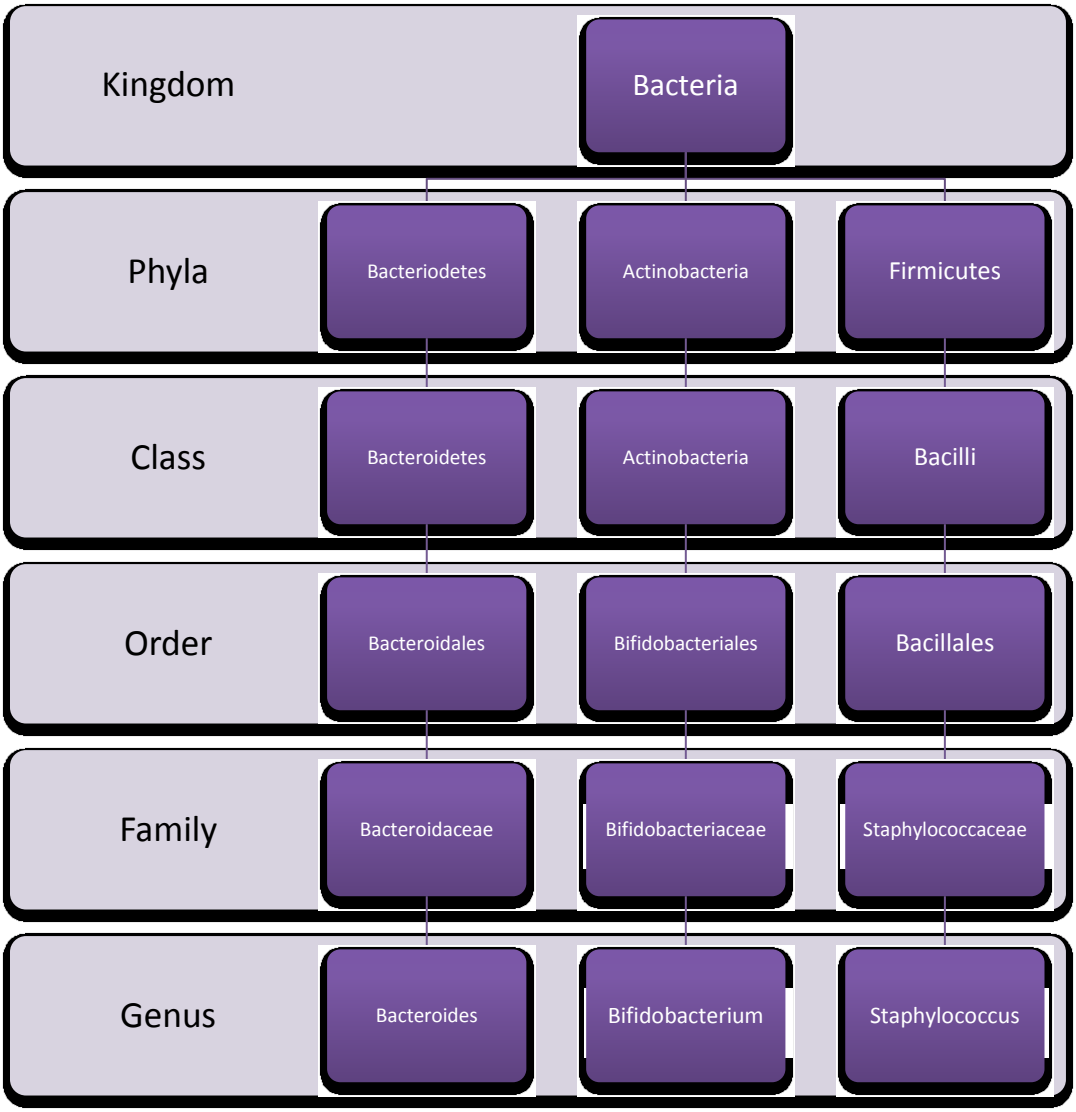


Figure 7: The taxonomy of Clostridiaceae and *Akkermansia*

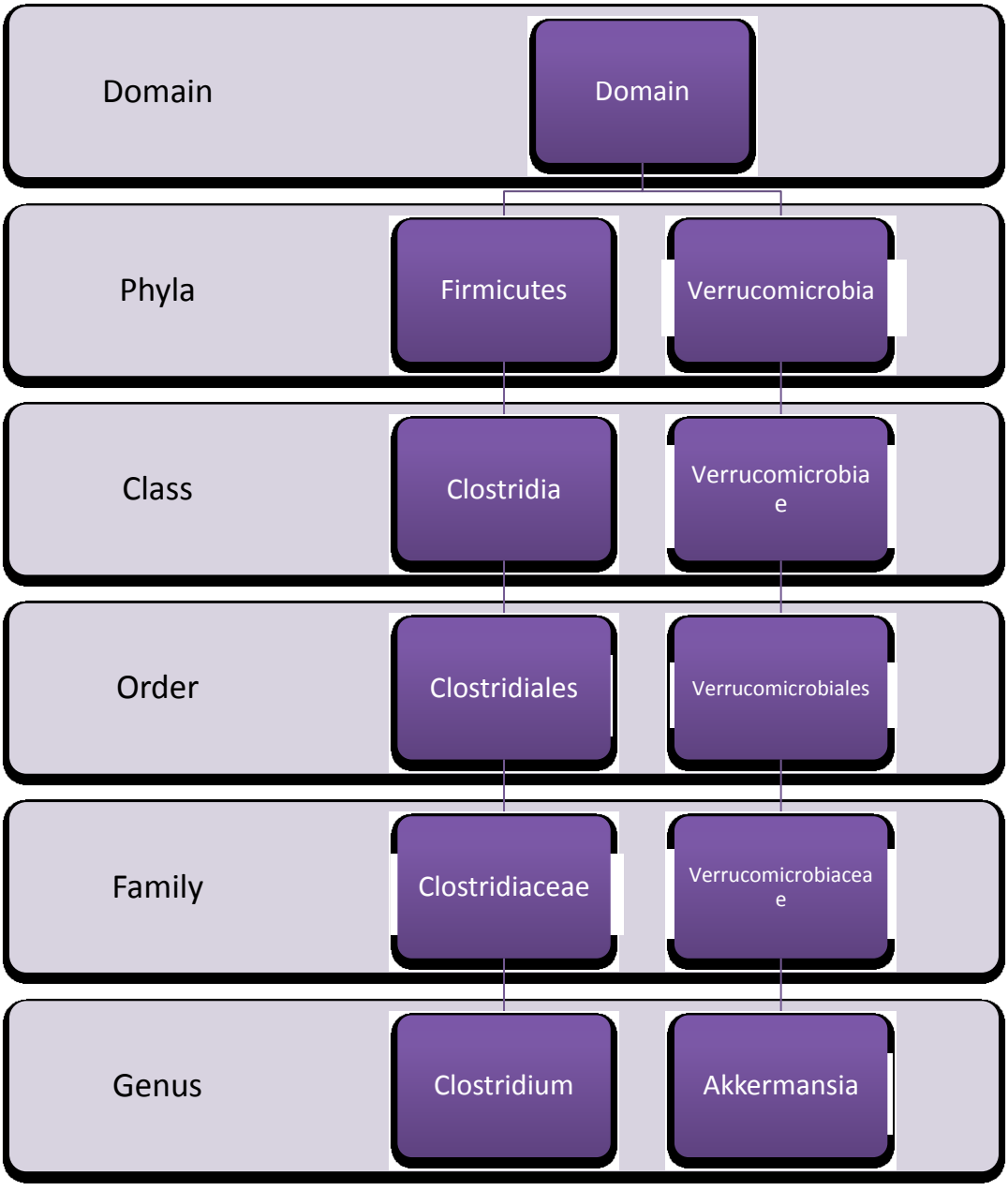


Figure 8: Demographic of child overweight (%)

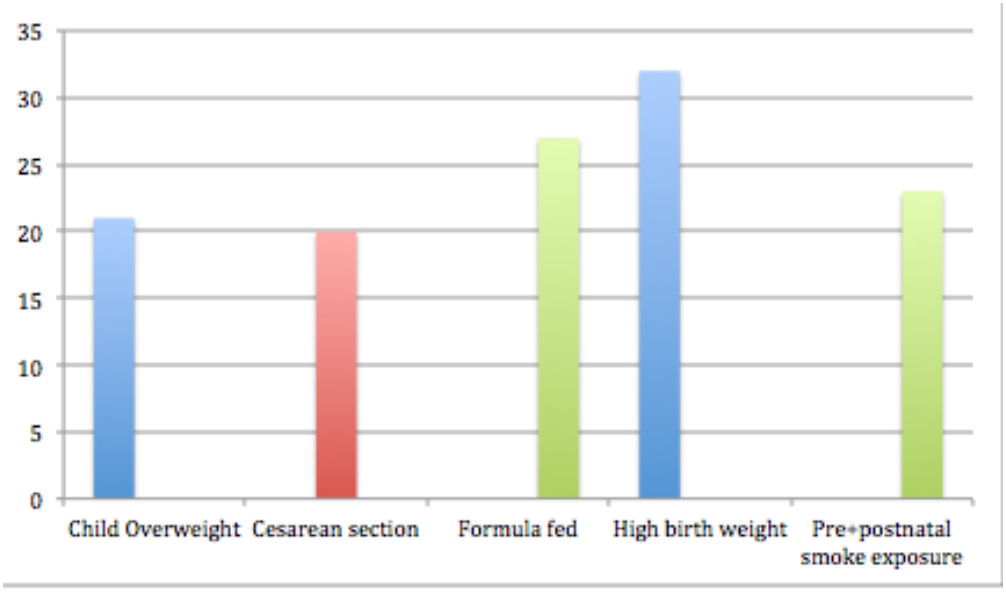


Figure 9: Demographic of maternal overweight (%)

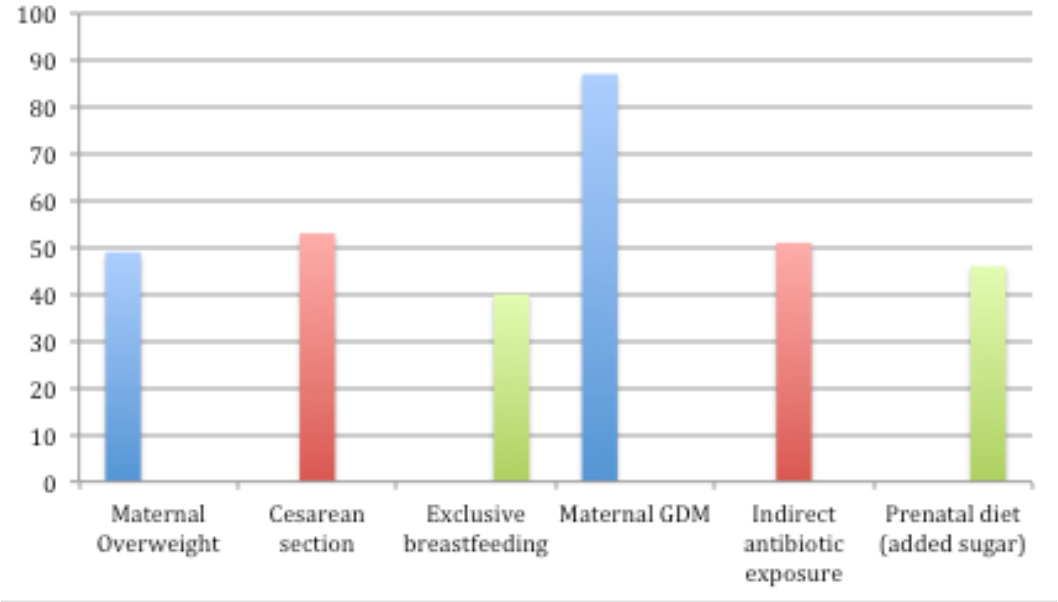


Figure 10: Demographic of infant Bacteroidaceae (%)

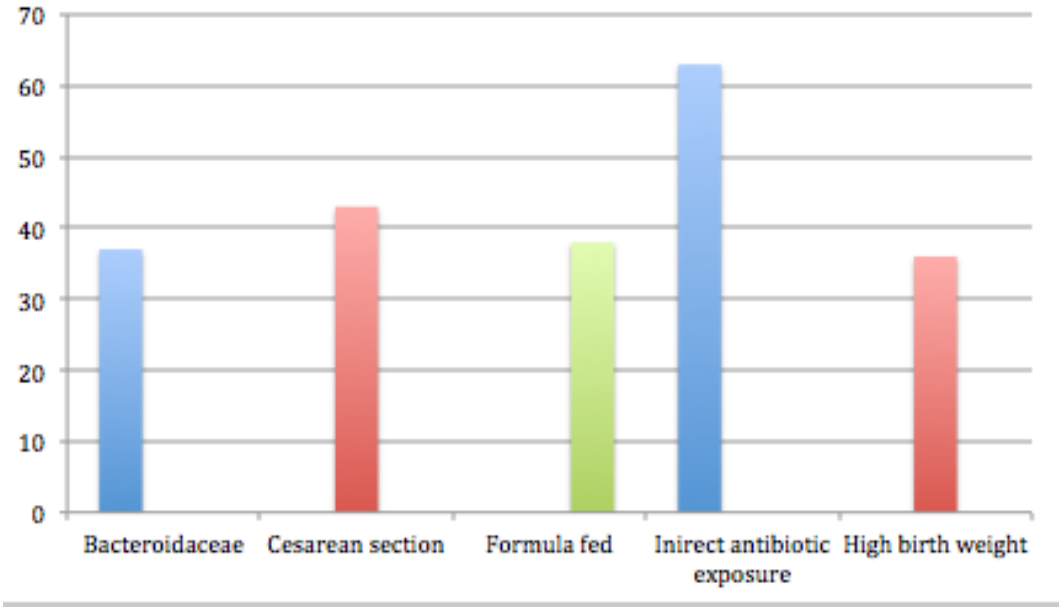
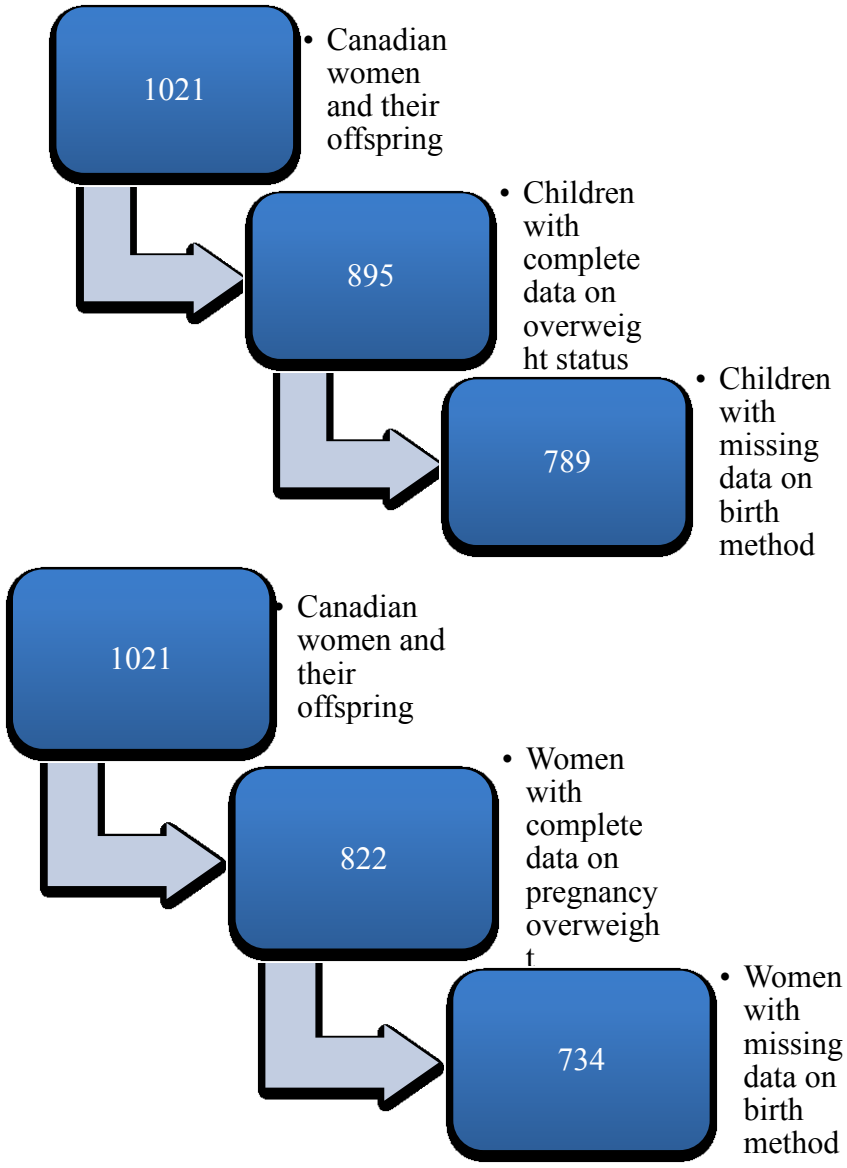


Figure 11

Flow diagram of study numbers



Gender specific analysis

Table 20

Risk of child overweight subsequent to maternal pregnancy overweight, unadjusted and adjusted for covariates for **boys**

Outcome variable = Child overweight

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	2.62 (1.56 – 4.41) *	3.12 (1.43 – 6.81) *
Cesarean section	**Vaginal delivery**	1.22 (0.66 – 2.24)	2.18 (0.74 – 6.41)
Exclusive breastfeeding	**Formula fed**	0.62 (0.46 – 0.83) *	0.65 (0.41 – 1.04)
Antibiotic exposure: Indirect	**No antibiotics**	1.22 (0.56 – 2.64)	0.54 (0.17 – 1.64)
Antibiotic exposure: Direct	**No antibiotics**	2.58 (1.09 – 6.13) *	1.69 (0.62 – 4.65)

In the final model (table 20), boys born to overweight mothers, were 3 times more likely to become overweight at age 1, independent of mode of delivery, infant diet and antibiotic exposure. (OR:3.12, 95%CI:1.43-6.81) p=0.004.

Table 21

Risk of child overweight subsequent to maternal pregnancy overweight, unadjusted and adjusted for covariates for **girls**

Outcome variable = Child overweight

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	1.54 (0.90 – 2.63)	0.98 (0.43 – 2.23)
Cesarean section	**Vaginal delivery**	1.28 (0.65 – 2.52)	1.65 (0.51 – 5.32)
Exclusive breastfeeding	**Formula fed**	0.63 (0.47 – 0.84) *	0.69 (0.42 – 1.12)
Antibiotic exposure: Indirect	**No antibiotics**	1.95 (0.91 – 4.20)	1.44 (0.52 – 3.99)
Antibiotic exposure: Direct	**No antibiotics**	2.20 (0.55 – 8.73)	1.68 (0.30 – 9.43)

In the final model (table 21), there were no statistically significant results.

Table 22

Risk of lowest relative abundance of infant gut Bacteroidaceae subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **boys**

Outcome variable = Bacteroidaceae

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.48 (0.20 – 1.12)	0.24 (0.08 – 0.75) *
Cesarean section	**Vaginal delivery**	6.21 (2.18 – 17.74) *	6.55 (1.51 – 28.41) *
Exclusive breastfeeding	**Formula fed**	1.34 (0.74 – 2.45)	1.70 (0.75 – 3.84)
Antibiotic exposure: Indirect	**No antibiotics**	6.06 (2.20 – 16.75) *	3.03 (0.80 – 11.50)
Antibiotic exposure: Direct	**No antibiotics**	2.20 (0.56 – 8.69)	2.52 (0.47 – 13.48)

In the final model (table 22), the risk of a low relative abundance of Bacteroidaceae at 3 months, was lowered in boys born to overweight mothers by 76%, independent of mode of delivery, infant diet and antibiotic exposure. (OR:0.24, 95%CI:0.08-0.75) p=0.02. On the other hand, the risk of a low relative abundance of Bacteroidaceae at 3 months, in boys who were delivered by cesarean section was increased by 7 times, independent of maternal overweight, infant diet and antibiotic exposure. (OR:6.55, 95%CI:1.51-28.41) p=0.01.

Table 23

Risk of lowest relative abundance of infant gut Bacteroidaceae subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **girls**

Outcome variable = Bacteroidaceae

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.79 (0.32 – 1.94)	0.66 (0.22 – 2.02)
Cesarean section	**Vaginal delivery**	14.58 (2.97 – 71.62) *	7.50 (1.26 – 44.70) *
Exclusive breastfeeding	**Formula fed**	1.20 (0.71 – 2.04)	0.84 (0.42 – 1.68)
Antibiotic exposure: Indirect	**No antibiotics**	5.06 (1.80 – 14.21) *	3.33 (0.99 – 11.13) *
Antibiotic exposure: Direct	**No antibiotics**	10.28 (1.71 – 61.83) *	4.25 (0.51 – 35.51)

In the final model (table 23), the risk of a low relative abundance of Bacteroidaceae at 3 months, in girls who were delivered by cesarean section was increased by 7.5 times, independent of maternal overweight, infant diet and antibiotic exposure. (OR:6.55, 95%CI:1.51-28.41) p=0.01. The risk of a low relative abundance of Bacteroidaceae at 3 months, in girls who were indirectly exposed to antibiotics was tripled, independent of maternal overweight, mode of delivery and infant diet. (OR:3.33, 95%CI: 0.99-11.13) p=0.05.

Table 24

Risk of highest relative abundance of infant gut Bifidobacteriaceae subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **boys**

Outcome variable = Bifidobacteriaceae

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.82 (0.32 – 2.10)	0.92 (0.33 – 2.59)
Cesarean section	**Vaginal delivery**	0.58 (0.19 – 1.77)	0.61 (0.15 – 2.43)
Exclusive breastfeeding	**Formula fed**	2.33 (1.06 – 5.11) *	2.61 (1.04 – 6.59) *
Antibiotic exposure: Indirect	**No antibiotics**	0.88 (0.31 – 2.54)	0.98 (0.26 – 3.67)
Antibiotic exposure: Direct	**No antibiotics**	1.51 (0.37 – 6.18)	3.06 (0.58 – 16.13)

In the final model (table 24), the risk of a high relative abundance of Bifidobacteria in boys who were exclusively breast fed was 2.61, independent of maternal overweight, mode of delivery and antibiotic exposure. (OR:2.61, 95%CI:1.04-6.59) p=0.04.

Table 25

Risk of highest relative abundance of infant gut Bifidobacteriaceae subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **girls**

Outcome variable = Bifidobacteriaceae

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.57 (0.20 – 1.62)	0.60 (0.19 – 1.90)
Cesarean section	**Vaginal delivery**	1.01 (0.25 – 4.08)	0.50 (0.08 – 3.23)
Exclusive breastfeeding	**Formula fed**	2.40 (1.14 – 5.08) *	2.96 (1.20 – 7.33) *
Antibiotic exposure: Indirect	**No antibiotics**	0.74 (0.24 – 2.25)	0.81 (0.21 – 3.04)
Antibiotic exposure: Direct	**No antibiotics**	0.47 (0.05 – 4.34)	0.55 (0.05 – 6.42)

In the final model (table 25), the risk of a high relative abundance of Bifidobacteria in girls who were exclusively breast fed was trippled, independent of maternal overweight, mode of delivery and antibiotic exposure. (OR:2.96, 95%CI:1.20-7.33) p=0.02.

Table 26

Risk of highest relative abundance of infant gut Clostridiaceae subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **boys**

Outcome variable = Clostridiaceae

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	1.11 (0.42 – 2.95)	1.49 (0.50 – 4.44)
Cesarean section	**Vaginal delivery**	2.08 (0.74 – 5.83)	1.58 (0.39 – 6.42)
Exclusive breastfeeding	**Formula fed**	1.95 (0.89 – 4.28)	1.57 (0.66 – 3.73)
Antibiotic exposure: Indirect	**No antibiotics**	2.95 (0.98 – 8.85) *	2.05 (0.50 – 8.33)
Antibiotic exposure: Direct	**No antibiotics**	0.55 (0.06 – 5.17)	0.52 (0.05 – 5.60)

In the final model (table 26), there were no statistically significant results.

Table 27

Risk of highest relative abundance of infant gut Clostridiaceae subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **girls**

Outcome variable = Clostridiaceae

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.75 (0.28 – 2.04)	0.72 (0.24 – 2.17)
Cesarean section	**Vaginal delivery**	4.20 (1.23 – 14.39) *	1.62 (0.36 – 7.23)
Exclusive breastfeeding	**Formula fed**	0.95 (0.54 – 1.69)	0.73 (0.37 – 1.45)
Antibiotic exposure: Indirect	**No antibiotics**	3.01 (1.02 – 8.91) *	3.67 (1.01 – 13.30) *
Antibiotic exposure: Direct	**No antibiotics**	3.28 (0.61 – 17.65)	4.40 (0.61 – 31.62)

In the final model (table 27), the risk of a high relative abundance of Clostridiaceae in girls who had been indirectly exposed to antibiotics was 3.67, independent of maternal overweight, mode of delivery and infant diet. (OR:3.67, 95%CI:1.01-13.30) p=0.05.

Table 28

Risk of highest relative abundance of infant gut Staphylococcaceae subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **boys**

Outcome variable = Staphylococcaceae

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	1.43 (0.55 – 3.71)	1.95 (0.66 – 5.82)
Cesarean section	**Vaginal delivery**	0.81 (0.28 – 2.36)	0.50 (0.12 – 2.00)
Exclusive breastfeeding	**Formula fed**	3.15 (1.31 – 7.54) *	3.27 (1.24 – 8.62) *
Antibiotic exposure: Indirect	**No antibiotics**	2.00 (0.69 – 5.77)	2.56 (0.66 – 9.95)
Antibiotic exposure: Direct	**No antibiotics**	1.50 (0.32 – 6.97)	2.88 (0.49 – 16.77)

In the final model (table 28), the risk of a high relative abundance of Staphylococcaceae in boys who were exclusively breast fed was trippled, independent of maternal overweight, mode of delivery and antibiotic exposure. (OR:3.27, 95%CI:1.24-8.62) p=0.02.

Table 29

Risk of highest relative abundance of infant gut Staphylococcaceae subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **girls**

Outcome variable = Staphylococcaceae

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.87 (0.32 – 2.37)	1.31 (0.42 – 4.11)
Cesarean section	**Vaginal delivery**	3.67 (1.07 – 12.64) *	3.79 (0.70 – 20.65)
Exclusive breastfeeding	**Formula fed**	3.36 (1.41 – 8.01) *	3.26 (1.23 – 8.61) *
Antibiotic exposure: Indirect	**No antibiotics**	1.85 (0.63 – 5.41)	0.84 (0.21 – 3.36)
Antibiotic exposure: Direct	**No antibiotics**	0.69 (0.07 – 6.43)	0.27 (0.02 – 3.95)

In the final model (table 29), the risk of a high relative abundance of Staphylococcaceae in girls who were exclusively breast fed was trippled, independent of maternal overweight, mode of delivery and antibiotic exposure. (OR:3.26, 95%CI:1.23-8.61) p=0.02.

Table 30

Risk of highest relative abundance of infant gut *Akkermansia* subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **boys**

Outcome variable = *Akkermansia*

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.31 (0.10 – 0.95) *	0.26 (0.07 – 0.93) *
Cesarean section	**Vaginal delivery**	0.64 (0.19 – 2.16)	1.32 (0.22 – 8.07)
Exclusive breastfeeding	**Formula fed**	1.00 (0.47 – 2.12)	0.84 (0.34 – 2.10)
Antibiotic exposure: Indirect	**No antibiotics**	0.47 (0.15 – 1.53)	0.48 (0.09 – 2.66)
Antibiotic exposure: Direct	**No antibiotics**	0.26 (0.03 – 2.31)	0.33 (0.03 – 3.55)

In the final model (table 30), the risk of a high relative abundance of Akkermansia in boys born to overweight mothers was lowered by 74%, independent of mode of delivery, infant diet and antibiotic exposure. (OR:0.26, 95%CI:0.07-0.93) p=0.04.

Table 31

Risk of highest relative abundance of infant gut *Akkermansia* subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **girls**

Outcome variable = *Akkermansia*

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	1.78 (0.70 – 4.52)	1.97 (0.72 – 5.35)
Cesarean section	**Vaginal delivery**	0.67 (0.17 – 2.65)	1.65 (0.29 – 9.42)
Exclusive breastfeeding	**Formula fed**	0.66 (0.39 – 1.13)	0.62 (0.34 – 1.13)
Antibiotic exposure: Indirect	**No antibiotics**	0.50 (0.18 – 1.40)	0.47 (0.14 – 1.60)
Antibiotic exposure: Direct	**No antibiotics**	0.26 (0.03 – 2.34)	0.27 (0.02 – 3.33)

In the final model (table 31), there were no statistically significant results.

Table 32

Risk of low Chao1 richness subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **boys**

Outcome variable = Chao1 {Richness}

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.64 (0.26 – 1.62)	0.53 (0.19 – 1.53)
Cesarean section	**Vaginal delivery**	1.43 (0.54 – 3.82)	1.32 (0.36 – 4.92)
Exclusive breastfeeding	**Formula fed**	1.56 (0.78 – 3.11)	1.74 (0.76 – 4.02)
Antibiotic exposure: Indirect	**No antibiotics**	2.36 (0.79 – 7.01)	2.01 (0.51 – 7.94)
Antibiotic exposure: Direct	**No antibiotics**	3.93 (0.93 – 16.58)	6.05 (1.16 – 31.52) *

In the final model (table 32), the risk of a low Chao 1 richness in boys who had been directly exposed to antibiotics was increased by 6 times, independent of maternal overweight, mode of delivery and infant diet. (OR:6.05, 95%CI:1.16-31.52) p=0.03.

Table 33

Risk of low Chao1 richness subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **girls**

Outcome variable = Chao1 {Richness}

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.47 (0.16 – 1.40)	0.63 (0.20 – 1.95)
Cesarean section	**Vaginal delivery**	1.18 (0.29 – 4.83)	0.95 (0.18 – 5.13)
Exclusive breastfeeding	**Formula fed**	1.44 (0.75 – 2.79)	1.32 (0.64 – 2.72)
Antibiotic exposure: Indirect	**No antibiotics**	1.07 (0.34 – 3.41)	1.04 (0.28 – 3.88)
Antibiotic exposure: Direct	**No antibiotics**	0.59 (01.64 – 9.89)	1.81 (0.24 – 13.98)

In the final model (table 33), there were no statistically significant results.

Table 34

Risk of low Shannon diversity index subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **boys**

Outcome variable = Shannon Index {Diversity}

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.92 (0.35 – 2.38)	1.02 (0.36 – 2.91)
Cesarean section	**Vaginal delivery**	0.79 (0.27 – 2.31)	1.35 (0.32 – 5.72)
Exclusive breastfeeding	**Formula fed**	1.84 (0.86 – 3.93)	1.98 (0.80 – 4.87)
Antibiotic exposure: Indirect	**No antibiotics**	1.81 (0.27 – 2.43)	0.60 (0.15 – 2.52)
Antibiotic exposure: Direct	**No antibiotics**	1.29 (0.28 – 5.94)	1.55 (0.29 – 8.41)

In the final model (table 34), there were no statistically significant results.

Table 35

Risk of low Shannon diversity index subsequent to exposure to maternal overweight, unadjusted and adjusted for covariates for **girls**

Outcome variable = Shannon Index {Diversity}

		Model 1	**Model 4**
Odds Ratio (OR), 95%CI	**Reference Category**	Unadjusted crude OR	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.77 (0.29 – 2.09)	0.73 (0.23 – 2.29)
Cesarean section	**Vaginal delivery**	1.01 (0.25 – 4.08)	0.35 (0.06 – 1.92)
Exclusive breastfeeding	**Formula fed**	2.55 (1.21 – 5.41) *	2.94 (1.18 – 7.35) *
Antibiotic exposure: Indirect	**No antibiotics**	1.81 (0.59 – 5.52)	2.11 (0.59 – 7.58)
Antibiotic exposure: Direct	**No antibiotics**	6.33 (1.18– 33.98) *	6.53 (0.78 – 54.46)

In the final model (table 35), the risk of a low Shannon diversity index in girls who were exclusively breast fed was trippled, independent of maternal overweight, mode of delivery and antibiotic exposure. (OR:2.94, 95%CI:1.18-7.35) p=0.02.

Table 36

Frequency distribution of First Nation ethnicity according to maternal overweight and the infant gut microbiome (Bacteroidaceae)

	Total	**Mom OW =384**			**Total**	**Bacteroidaceae =73**		
	Count	n	%	p	Count	n	%	p
First Nation								
Yes	28	12	3.1	0.67	5	2	2.7	0.91
No	792				167			

Table 37

Risk of maternal overweight subsequent to being of First Nation descent.

Odds Ratio (OR), 95%CI	**Reference category**	**Unadjusted crude OR**	**P**
Maternal pregnancy OW	**Maternal normal weight**	1.18 (0.55 – 2.53)	0.67

Table 38

Risk of lowest relative abundance of infant gut Bacteroidaceae subsequent to being of First Nation descent.

Odds Ratio (OR), 95%CI	**Reference category**	**Unadjusted crude OR**	**P**
Maternal pregnancy OW	**Maternal normal weight**	1.11 (0.18 – 6.82)	0.91

Table 39

Risk of child overweight subsequent to maternal pregnancy overweight and obesity, adjusted for covariates

Outcome variable = Child overweight

		Model 4
Odds Ratio (OR), 95%CI	**Reference category**	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	2.18 (1.17-4.05)*
Maternal pregnancy obesity	**Maternal normal weight**	1.54 (0.76-3.08)
Antibiotic exposure: Indirect	**No antibiotics**	0.88 (0.42-1.86)
Antibiotic exposure: Direct	**No antibiotics**	2.13 (0.93-4.88)
Cesarean section	**Vaginal delivery**	1.95 (0.90-4.23)
Exclusive breastfeeding	**Formula fed**	0.67 (0.48-0.93)*

Table 40

Risk of lowest relative abundance of infant gut Bacteroidaceae subsequent to exposure to maternal overweight and obesity, adjusted for covariates

Outcome variable = Bacteroidaceae

		Model 4
Odds Ratio (OR), 95%CI	**Reference category**	Adjusted for pre and postnatal variables
Maternal pregnancy OW	**Maternal normal weight**	0.42 (0.17-1.07)
Maternal pregnancy obesity	**Maternal normal weight**	0.39 (0.15-1.02)
Antibiotic exposure: Indirect	**No antibiotics**	3.03 (1.27-7.24)*
Antibiotic exposure: Direct	**No antibiotics**	2.48 (0.72-8.58)
Cesarean section	**Vaginal delivery**	5.86 (2.09-16.47)*
Exclusive breastfeeding	**Formula fed**	1.18 (0.72-1.95)

Mother's Health Coding Legend [PRNMH18W_91]

4.2 **Diabetes (high blood sugar):**

[2] Currently

[1] In the past

[0] Never

4.2a **If so, are you taking or did you take medication(s)?**

[2] Currently

[1] In the past

[0] Never

5.1 Have you had any of the following during your pregnancy?

[1]/[0] High Blood Pressure

[1]/[0] Urinary Infection

[1]/[0] Severe morning sickness after your 1st trimester

[1]/[0] Diarrhea

[1]/[0] High Cholesterol

[1]/[0] Convulsions

[1]/[0] Diabetes (high blood sugar)

[1]/[0] Fever

[1]/[0] Cold(s) or Flu

[1]/[0] Cold sores

[1]/[0] Chest Infection/Pneumonia

[1]/[0] Sexually Transmitted Disease

[1]/[0] Yeast Infection

[1]/[0] None of these conditions

[1]/[0] Other (specify below)

35. **At the present time, how often do you smoke?**

[1] Daily or occasionally

[0] Not at all

35.1 **How many cigarettes per day do you now smoke on average?**

36. **Have you ever smoked for as long as one year?**

[1] Yes

[0] No

36.1 **How old were you when you began tosmoke regularly?**

36.2 **PRIOR TO this pregnancy, had you stoppedand not restarted smoking?**

[1] Yes

[0] No

[8] N/A

36.2a **If Yes, at what age did you stop smoking?**

36.3 **DURING this pregnancy, did you completely stop smoking?**

[1] Yes

[0] No

[8] N/A

36.3a **If Yes, at what week of pregnancy did you stop smoking?**

37. **Does anybody, at present, smoke AT your home?**

[1] Yes [0] No

Child Nutrition 3 Months Coding Legend [NUTR3M_230]

1. **Did you breastfeed your child for any duration (more than a few days) since birth?**

[1] Yes

[0] No

1.1 **If Yes, are you currently breastfeeding your child (whether or not feedings are supplemented)?**

[1] Yes

[0] No

4. **If you are not currently breastfeeding, how old was your child when you stopped breastfeeding?**

5. **Are you currently giving your child any infant formula?**

[1] Yes

[0] No

5.1 **How old was your child when you started giving him/her any type of infant formula?**

6. **If you are not currently giving your child formula, how old was s/he when you stopped feeding formula?**

13. **Do you give your child any solid food?**

[1] Yes

[0] No