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# Effect of maternal diet on mother's own milk pH and preterm infant intestinal inflammation

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BOSTON UNIVERSITY  
SCHOOL OF MEDICINE

Thesis

**EFFECT OF MATERNAL DIET ON MOTHER'S OWN MILK PH AND  
PRETERM INFANT INTESTINAL INFLAMMATION**

by

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Master of Science

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**ABSTRACT**

**BACKGROUND**

Quantitative evidence of direct links between dietary intake during pregnancy and maternal and infant outcomes in the preterm population is lacking in the literature. However, studies are starting to investigate relationships between them, and data shows that many of the adverse effects of poor maternal diet are linked to inflammatory response and dysbiosis of the microbiome in both the mother and her offspring.

**OBJECTIVE**

Our objective with this study was to analyze the relationships between sociodemographic factors, maternal diet, pH of breast milk, and infant inflammation. We aimed to assess the dietary pattern of our population of mothers who delivered preterm in comparison to other populations, as well as explore the possibility of using pH of mother's own milk in future research.

**METHODS**

We reviewed the Electronic Medical Records (EMRs) of participants to gather clinical and demographic characteristics (infants n = 53; mothers n = 45). Maternal

participants also completed the Dietary Screener Questionnaire (DSQ). Spearman's rank correlation and raw unadjusted linear regression analyses were used to investigate relationships between maternal diet and characteristics, mother's own milk pH, and infant urinary intestinal fatty acid binding protein (I-FABP). Kruskal Wallis analysis was used to analyze between group differences of maternal comorbidities.

## **RESULTS**

None of our maternal participants met the guidelines for dietary recommendations by the United States Department of Agriculture (USDA) for pregnant women. This follows the trend in national data for women who are pregnant. The greatest number of women met the recommendation for fiber intake ( $n = 12$ ). Maternal intake of fiber and whole grains was negatively correlated with pH of mother's own milk ( $p < 0.5$ ). We did not find any significant correlations between maternal characteristics and maternal diet or pH of mother's own milk. However, meeting the guidelines for added sugars differed by race ( $p = 0.03$ ). We found no statistically significant correlations between urinary I-FABP and pH of mother's own milk or maternal dietary intake. Urinary I-FABP values differed by infant sex ( $p = 0.03$ ) and infant feeding status ( $> 50\%$  formula or donor milk vs.  $< 50\%$  formula or donor milk,  $p = 0.03$ ). Analysis by groups showed statistically significant differences ( $p = 0.04$ ). Preeclamptic participants had a higher intake of whole grains (0.97 oz) than women without preeclampsia (0.69 oz). Mothers with gestational diabetes had a lower intake of sugar (14.4 tsp) compared to women without diabetes (17.9 tsp) ( $p =$

0.01). We found no other statistically significant results between groups for maternal diet, milk pH, or urinary I-FABP.

## **CONCLUSION**

Our findings suggest that additional research on mother's own milk pH may be warranted, and that continued education on the importance of a healthy diet and its benefits during pregnancy is needed. Areas of planned future research include fat intake calculations and inflammatory measures of the maternal dietary data.

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## LIST OF ABBREVIATIONS

BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
DASH	Dietary Approaches to Stop Hypertension
DGA	Dietary Guidelines for Americans
DOL	Day of Life
DSQ	Dietary Screener Questionnaire
EMR	Electronic Medical Record
FDA	Food and Drug Administration
HHS	Health and Human Services
I-FABP	Intestinal Fatty Acid-Binding Protein
NEC	Necrotizing Enterocolitis
NHANES	National Health and Nutrition Examination Survey
NICU	Neonatal Intensive Care Unit
NIH	National Institutes of Health
TP	Total Protein
USDA	United States Department of Agriculture

## INTRODUCTION

Complications from preterm birth, defined as birth of an infant before 37 weeks of gestation, are the leading cause of neonatal morbidity in the world (Liu et al., 2015). In the United States more than 1 in 10 infants is born premature and rates have been rising since 2014 (Preterm Birth | Maternal and Infant Health | Reproductive Health | CDC, 2020). Worldwide more than 15 million infants are born preterm each year (Purisch & Gyamfi-Bannerman, 2017). Preterm infants are at a significant disadvantage compared to term infants due to immature organ systems. Of interest to our research is immature gut development and difficulty breastfeeding, common to the preterm infant. These infants are born with lower diversity in their gut microbiome and may be unable to exclusively breastfeed, reducing the impact of the mother's milk, which is rich with bioactive factors including human milk oligosaccharides, microbes, and immune cells that help establish a healthy intestinal microbiome. It is well documented that exclusive breastfeeding is the safest and best route of nutrition for the majority of infants under six months of age. Breastfeeding provides life-long protections and benefits for both mother (decreased rates of breast and ovarian cancers, Type 2 diabetes mellitus, and osteoporosis) and child (including decreased rates of respiratory illness, food allergies, and increased intelligence) (Victora et al., 2016). A longitudinal study of 107 healthy mother-infant pairs found statistically significant evidence that mother's milk helps establish the infant gut microbiome (Pannaraj et al., 2017).

During pregnancy and after, the women's microbiota evolves to aid in the development of the fetus and keep the mother and child healthy. In mothers, vaginal, oral, and placental microbiota undergo changes in diversity from trimester to trimester, distinct from non-pregnant women (Mesa et al., 2020). Additionally, research continues to reveal that dysbiosis of microbiota in mothers may lead to an increased chance of preterm delivery, due to factors like placental inflammation (Chen et al., 2020; DiGiulio et al., 2015). A small study on extremely premature infants' stool samples found that those infants exposed to the external environment, e.g. through prolonged preterm premature rupture of the amniotic membrane (PPROM), had increased levels of pathogenic bacteria in their gut microbiome from birth through the first month of life (Chernikova et al., 2016). Preterm infants may, therefore, be at a second disadvantage if their mothers have unhealthy microbiota, since their intrauterine development is cut short, and their mothers may not have a healthy diversity of flora to compensate for preterm delivery. For example, a study analyzing breast milk samples from mothers with and without celiac disease found that mothers with celiac disease had lower concentrations of TGF- $\beta$ 1 and *Bifidobacterium* spp, possibly conferring fewer immunoprotective benefits to their infants (Olivares et al., 2015).

With disadvantages present before and after, it is important to identify possible mediators to reduce adverse outcomes in preterm births. Since breastfeeding is promoted by several regulatory and policy making entities (e.g. W.H.O., UNICEF, and AAP), studying the composition of breast milk in mothers of preterm infants, in addition to mothers' diets, and any effect on the gut microbiome of their infants will provide insight

on a possibly modifiable pathway. One of the major areas of interest is the high number of anti-inflammatory bioactive components found in breast milk. Preterm infants are especially vulnerable to the effects of inflammation, seen in the elevated cases of necrotizing enterocolitis (NEC) compared to their term counterparts, due to their underdeveloped intestinal and immune systems (Sharma & Hudak, 2013; Shulhan et al., 2017).

## **MATERNAL DIET**

There are multiple diets that are suggested for healthier eating patterns in women who are pregnant or lactating, but most of them suggest the same thing: high intake of fruits, vegetables, and whole grains and reduced intake of processed foods, as well as foods and drinks with a high sugar and/or high fat content. However, in a typical Western dietary pattern, the most common pattern in the United States population, we find a reversal of these recommendations. The traditional Western diet has been shown to lead to an imbalance in the gut microbiome, although calorie restriction and exercise can reverse the change (Duda-Chodak et al., 2015).

Maternal diet during pregnancy has been researched most often for its effect on birthweight and childhood obesity, but other studies found higher fruit, vegetable, whole grains intake and lower processed foods intake was especially significant in late pregnancy to increase bone mass in children (Cole et al., 2009); lower adherence to the Mediterranean Diet significantly increased the risk of spina bifida in infants (Vujkovic et al., 2009); a prudent diet may decrease the risk of neural tube defects and congenital heart

defects, even after adjusting for folic acid intake, versus a Western or Mexican diet (Sotres-Alvarez et al., 2013).

Research on survivors of the Dutch famine of 1944-45 suggests that mothers exposed to famine conditions during the third trimester, but not first trimester, gave birth to infants with lower birth weights, head circumferences, and length (Clausen et al., 2001). Other studies of people born during the Dutch famine analyzed the long-term effects, and found that exposure to famine at any point in gestation led to glucose intolerance and hypertension (Stein et al., 2006). Exposure in early gestation led to increased coronary heart disease, stress responsiveness, and obesity as adults, as well as increased risk of breast cancer in women. Mid-gestation famine exposure led to more obstructive airway diseases (T. Roseboom et al., 2006). Other research supports the importance of a healthy diet during early pregnancy for a reduced risk of small for gestational age infants (J. M. D. Thompson et al., 2010). Newer studies focusing on fetal programming suggest that diet during pregnancy affects the fetus in different ways at different points in the development and contributes to the development of diseases later in life, possibly due to dysbiosis established during the critical window from preconception to 1,000 days after birth (Robertson et al., 2019; T. J. Roseboom, 2019).

### **Maternal Diet And Preterm Birth**

As noted previously, preterm birth increases the risk for inflammation and associated morbidities in an infant, so studying mediators of preterm birth are important. Diets high in vegetables and low in carbohydrates or processed foods have been shown to

reduce the risk of preterm birth, which is of interest for our population (Mitku et al., 2020). Not all the literature supports this data. One study of 2,768 women in the Netherlands, 138 of whom spontaneously delivered preterm, found no statistically significant relationship between fruit or vegetable intake and preterm birth (Baron et al., 2017).

Assessment of dietary intake using a food frequency survey during weeks 26 through 29 of gestation of more than 3,000 mothers in the Pregnancy, Infection, and Nutrition (PIN) study in North Carolina identified four main dietary patterns in the cohort. They found that diets high in collard greens, cabbage or coleslaw, red meats and processed meats, cornbread, fried chicken and fish, eggs or egg biscuits, gravy, whole milk, and vitamin C-rich drinks led to greater risk of preterm birth. They also found that the odds for preterm birth decreased as adherence to the Dietary Approaches to Stop Hypertension (DASH) eating pattern increased when compared to the lowest quartile in adherence (Martin et al., 2015).

The DASH eating plan does not specify foods to eat or avoid, but instead outlines daily and weekly goals for nutritional intake. The plan advises eating vegetables, fruits, and whole grains, including low-fat or fat-free dairy, fish, poultry, beans, nuts, and vegetable oils, while limiting saturated and trans fats, sodium, coconut and palm oil products, and added sugars in food and drinks. It also promotes eating foods rich in potassium, calcium, magnesium, fiber, and protein (*DASH Eating Plan* | NHLBI, NIH, n.d.).

A study of 66,000 pregnant women in the Norwegian Mother and Child Cohort Study found similar results using slightly different dietary definitions (Englund-Ögge et al., 2014). They described three distinct dietary patterns: prudent, e.g. fruits, vegetables, whole grain cereals, bread high in fiber; Western, e.g. salty and sweet snacks, processed meats, white bread; traditional Norwegian, e.g. potatoes and fish. Their results show that the hazard ratio for preterm birth was significantly reduced for the highest third versus the lowest third in association with high scores in the prudent dietary pattern, which is similar to the DASH eating pattern, as well as the traditional Norwegian pattern. They did not find an independent association with the Western pattern and preterm birth. They state that their results indicate it is more important to focus on increasing intake of fruits, vegetables, whole grains, and fiber, rather than eliminating highly processed foods, including fast food and junk food. This study sample excluded mothers from the cohort with multiple gestation, diabetes, and previous participation.

Adherence to six targets of the Mediterranean Diet, including more than 12 servings each of fruits and vegetables per week, in the end of the first trimester of pregnancy in a randomized control trial was found to be associated with a reduction in risk for preterm birth, as well as a composite of maternal-fetal outcomes (CMFCs). CMFCs were defined as emergency C-section, perineal trauma, pregnancy related hypertension and preeclampsia, prematurity, large-for-gestational age and/or small for gestational age (Assaf-Balut et al., 2018).

A study of nearly 60,000 mothers in Denmark showed that a Western dietary pattern, defined as high intake of meats and fats with low intake of fruits and vegetables,

was associated with greater odds of preterm birth, due to increased induced preterm deliveries (OR = 1.66, 95% CI: 1.30, 2.11, comparing the highest to the lowest quintile) more than spontaneous preterm deliveries (OR = 1.18, 95% CI: 0.99, 1.39) (Rasmussen et al., 2014).

### **Maternal Diet And Milk Composition**

Comprehensive, direct assessment of maternal diet's effect on nutritional composition of breast milk has not been well studied and documented in the literature. A systematic review from 2016 found only 36 publications with quantitative data on both maternal diet and breast milk composition (Bravi et al., 2016). This review only assessed studies of healthy mothers of healthy term infants in original observational or experimental design studies. Seventeen of the studies focused on fatty acid in breast milk, while only five found a link between maternal diet and breast milk nutrients; three showed evidence for fish consumption and high docosahexaenoic acid (DHA) in breast milk, and two found a positive correlation between vitamin C intake and concentration in breast milk. A newer human cross-over study in lactating women has found that human milk oligosaccharides (HMO) levels, a bioactive component of milk, were altered by different diets and influenced the microbiome composition of milk as well (Seferovic et al., 2020).

## **pH Of Mother's Own Milk**

There are very few studies that include data on the pH of mother's milk. Human breast milk has been measured around neutral at a pH of 7 with changes throughout lactation (Ansell et al., 1977). A study of 309 samples collected from 52 women with term deliveries found that colostrum, the highly nutritious and immunoprotective breast milk produced in the first few days of life, had the highest mean pH (7.45) and then dropped to a pH of 7.04 over the next week, then increasing again to 7.4 at 10 months (Morriss et al., 1986). A study from 1972 looked at the pH of milk fed to infants in the first week of life; however, they were assessing the difference between mother's own milk and cow's milk. They did find that increasing the pH of cow's milk, which is naturally more acidic than human milk, produced stools with more *Lactobacilli* than *E. coli*, possibly indicating a change in the infants microbiome, and non-alkylated cow's milk fed to infants led to a decrease in infant weight (Harrison & Peat, 1972).

In a study on the effects of breast milk pH on gastric pH of preterm infants, the measured pH of breast milk from women who delivered preterm was on average lower, but not at a significant level, than term mothers' breast milk samples, although this was a very small sample of 16 preterm mother-infant dyads and six term dyads (Gan et al., 2019). They also documented a shift in pH of the infant gut after feeding on mother's milk. This pH shift is necessary for selective proteolysis of  $\alpha$ -lactalbumin by the breast milk enzyme cathepsin D. Variations in pH of the infant gut or mother's milk may affect metabolism of certain human milk nutrients that are important for the healthy infant microbiome.

## **MATERNAL COMORBIDITIES' RELATIONSHIP TO INFLAMMATION**

Preeclampsia, defined as late gestational hypertension and proteinuria after 20 weeks of gestation, often results in preterm birth, as treatment is commonly delivery of the placenta and fetus (Leeman & Fontaine, 2008). The cause of preeclampsia, the most common medical disorder of pregnancy, is still undetermined; however, systemic inflammation is one possibility in conjunction with a dysregulated placental microbiome (Dasinger et al., 2020; Lv et al., 2019). The Lv study also found that infants' APGAR scores and birth weight were negatively correlated with the additional genera of bacteria found in their preeclamptic mothers. Studies have shown that the placental microbiome is more closely associated at the taxonomic family level with the oral microbiome than the vaginal or gut microbiome, and that it serves in fatty acid metabolism, creating metabolites that supply energy to the developing fetus (Gomez-Arango et al., 2017). Mothers with gestational diabetes and their infants both showed similar variances in microbiota, presenting evidence for maternal microbiome changes being passed down from one generation to the next (Wang et al., 2018). These changes and lack of diversity in the microbiome, called the "missing microbe hypothesis," proposes that lack of certain microbiota causes suboptimal immune development with each successive generation (Forgie et al., 2020).

## **IFABP AS A FRACTION OF TOTAL INTESTINAL PROTEIN**

Intestinal fatty acid-binding protein (I-FABP), a non-invasive marker of gastrointestinal (GI) tract inflammation, is of diagnostic and prognostic value in GI

pathologies during infancy (Coufal et al., 2020; Gregory et al., 2014). The kidney readily excretes the small protein, so tests can detect I-FABP in both urine and plasma within hours of GI damage (Lieberman et al., 1997). During an intestinal mucosal injury, I-FABP is secreted into the bloodstream from mature enterocytes if the membrane of the cell is damaged (J. P. M. Derikx et al., 2007). Results from recent studies indicate I-FABP may be useful as a biomarker for diagnosis of diseases of the GI tract, specifically necrotizing enterocolitis (Bottasso Arias et al., 2015; Joep P. M. Derikx et al., 2009; Evennett et al., 2010; Gregory et al., 2014). The role of other FABPs, specifically adipocyte FABP and epidermal FABP, has been established in metabolic and inflammatory diseases such as diabetes, asthma, and atherosclerosis (Furuhashi & Hotamisligil, 2008).

## **SPECIFIC AIMS**

The goals of this paper are to assess the demographic factors affecting maternal diet and look for correlations between maternal diet, pH of mother's own milk, and intestinal inflammation in the preterm infant. We aim to use mother-infant dyads' data from the Dietary Screener Questionnaire completed by the maternal participants, mother's own milk samples collected from the feeds of the preterm infants, and urine samples from the preterm infants.

We will test the data on three levels: first, an analysis of the role of demographic factors on maternal diet; second, an analysis of the influence of maternal diet on the intestinal inflammation of the preterm infant as measured by urinary I-FABP as a fraction of total intestinal protein; third, an exploratory analysis of the pH of the mother's own milk samples, evaluating the influence of both maternal diet on pH and pH on the intestinal inflammation of the preterm infant by the same urinary I-FABP measure.

AIM 1: Compare the diet of women who give birth preterm to women who do not give birth preterm.

AIM 2: Explore milk pH as a crude measure of maternal diet's influence on mother's own milk, as compared to other established aspects of human milk.

AIM 3: Analyze various nutrition factors within the maternal diet and assess what, if any, influence they may have on preterm infant health outcomes.

AIM 4: Analyze the influence of the pH of human milk on preterm infant outcomes.

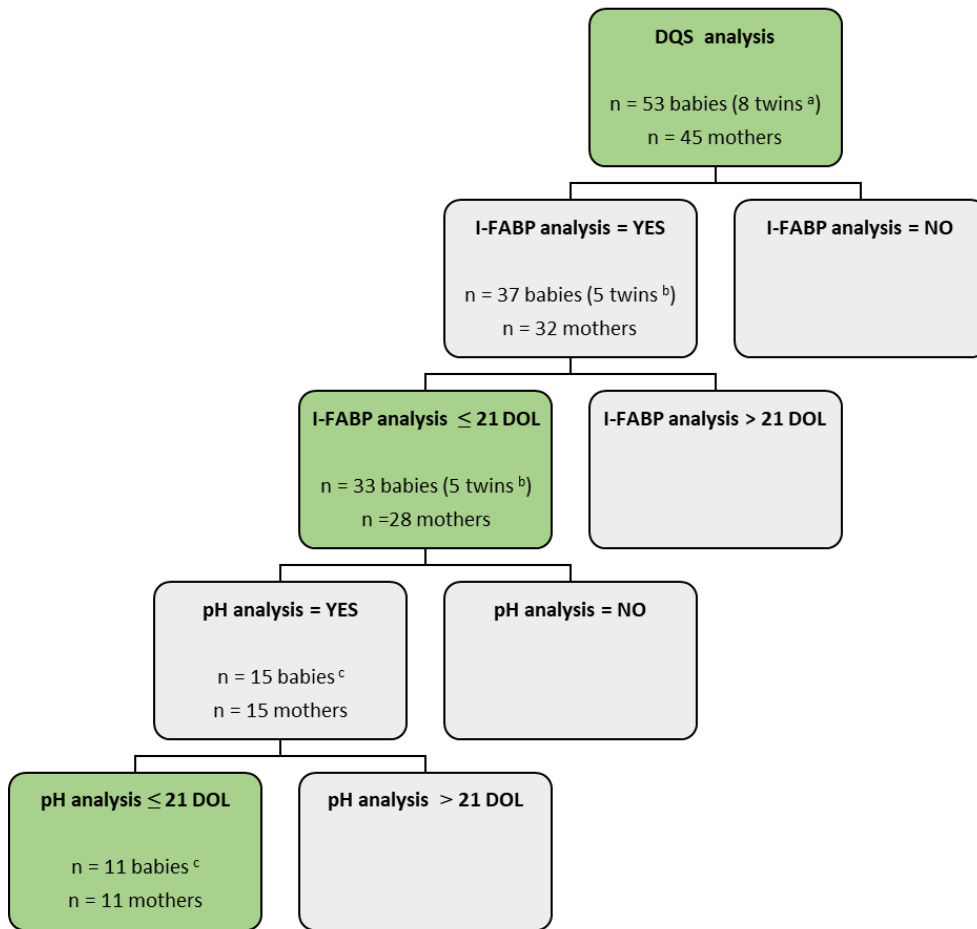
## **METHODS**

### **STUDY DESIGN AND POPULATION**

The study population consists of infants enrolled in the Baby Biome Tissue Bank Repository (Protocol # 2016P001020), who were hospitalized in the Neonatal Intensive Care Unit (NICU) at a large New England hospital between September 2019 and January 2020, and their mothers. All mothers were eligible to complete the 26-question Dietary Screener Questionnaire (DSQ), and any mother who finished the questionnaire and returned it to the study team is included in the descriptive analysis (n = 45). Mothers were given the DSQ after enrollment in the Baby Biome study, anytime in the two weeks after birth and infant admission to the NICU. Instructions on the DSQ stated, “These questions are about foods you ate or drank during the past month, that is, the past 30 days. When answering, please include meals and snacks at home, at work or school, in restaurants, and anyplace else;” however, we specified when distributing the questionnaire that mothers should try to recall dietary intake during the 30 days leading up to delivery. Mothers completed the DSQ on their own, so it is possible that some mothers included diet after delivery, as well. We checked each questionnaire for completion and noted inconsistencies or missing information when the data were entered into spreadsheets for analysis.

Recruitment of participants was limited to infants in the NICU and their mothers given the tissue bank repository is dedicated to the investigation of problems associated with preterm birth. Inclusion criteria for the Baby Biome repository is a live born infant

admitted to the NICU and at a gestational age less than or equal to 34 weeks. An infant was excluded if he or she was not expected to survive beyond 48 hours following birth, or if the infant was expected to be discharged prior to 48 hours following birth for care at an outside institution. Of the 53 infants whose mothers completed a DSQ, 37 infants (five sets of twins) were also included in a longitudinal microbiome case-control study funded by the National Institute of Health (NIH). The inclusion criteria for the microbiome cohort was enrollment in the Baby Biome repository and at least two of each feeding, urine, and blood sample per hospitalization, with each sample collected once every two weeks. A case was defined as predominantly or exclusively (> 50%) fed mother's own milk, and a control as predominantly or exclusively (> 50%) fed human donor milk and/or formula. Blood sample analysis was not performed in time for inclusion in this paper. Of the 37 infants in both the NIH microbiome cohort and DSQ cohort, there were 27 case infants and 10 control infants. Our final subgroups for analysis consisted of 33 infants (five sets of twins) with a qualifying urine sample and 11 infants with a qualifying mother's own milk sample. Eight of the infants were included in both the urine and pH analysis subgroups (Figure 1).



**Figure 1. Schema for Population Subgroups.** The infant of any mother who completed a Dietary Screener Questionnaire was included in the descriptive analysis. Two further subgroups were identified within this cohort based on availability of a urine sample for I-FABP analysis and/or a mother's own milk sample for pH analysis.

## CLINICAL ABSTRACTION

We abstracted from the electronic medical records (EMR; Epic Systems, Inc. Madison, WI) a limited dataset for the infant participants, which included demographic and clinical characteristics: race and ethnicity, gestational age, sex, birth weight, mode of delivery, primiparity, multiple gestation, SNAP-II and SNAPPE-II scores, weight for age

percentage and z-scores, and length of stay in the NICU. The dataset for the maternal participants included the following demographic and clinical characteristics: race and ethnicity, age, BMI at the time of delivery, diabetes status and type, chronic hypertension, and preeclampsia diagnosis. In the cases where infant race and/or ethnicity were not documented in the EMR, the mother's race and/or ethnicity, if documented, were recorded as the infant's race and/or ethnicity.

## **DIETARY SCREENER QUESTIONNAIRE DATA PROCESSING AND SCORING**

The Dietary Screener Questionnaire (DSQ) is a validated and reliable tool to estimate daily dietary intake. We used the data processing procedures outlined on the DSQ website (F. E. Thompson et al., 2017), which are based on the National Health and Nutrition Examination Survey 2009-2010 (NHANES) 24-hour dietary recall and DSQ data. More than 7500 Americans, including children as young as two years old through adults 69 years old, were surveyed for the NHANES 2009-2010. Members of the National Cancer Institute (NCI) research team developed scoring algorithms to convert an individual's DSQ responses to estimates of dietary intake of the exposures for fruits and vegetables (cup equivalents), dairy (cup equivalents), added sugars (teaspoon equivalents), whole grains (ounce equivalents), fiber (g), and calcium (mg).

Participants reported frequency of intake on the DSQ using a rate and time unit ranging from options of "Never" and "1 time last month" to "6 or more times per day" with some variation between food and drink items. Using the provided conversions, we transformed all frequency responses to a common unit of time (times per day). Then we

compared all participants' converted frequency responses for each food item against the maximum acceptable daily frequency values as calculated by the NCI research team. We identified no extreme values in our questionnaire responses.

Next we categorized the two most frequently consumed cereal types reported by each participant into a tertile for four dimensions: density of added sugars, whole grains, fiber, and calcium. Each dimensional category tertile has distinct portion size and regression coefficients for the scoring algorithm. Respondents were provided with a list of 391 cereals, both generic and name brand, with 283 of the listed cereals considered distinct entities. We then weighted each cereal frequency by the order in which it was reported on the DSQ. The first reported cereal was weighted at 0.75 and the second at 0.25. For participants who reported more than two cereals, only the first two were considered in the scoring algorithm.

The scoring algorithm utilizes a regression model where the dependent variable is the dietary intake of each exposure from the NHANES 24-hour recall, mentioned above, and the independent variables are, for each food item, the intake reported by individuals on the DSQ and sex-age specific portion size estimates. We used the provided variable for the adult age group (18-69 years) in our scoring calculations, as well as the age-sex specific median portion size and sex-specific regression coefficients for each food item. After converting all responses to estimates of dietary intake for fruits and vegetables, dairy, added sugars, whole grains, fiber, and calcium, we compared the participants' estimated daily intake values with recommended values for sex-age specific groups, 19-30 years and 31-50 years of age.

We used the *Dietary Guidelines for Americans, 2020-2025* (DGA, 2020-2025), published by the U.S. Department of Agriculture (USDA) and U.S. Department of Health and Human Services (HHS), as our source for recommended values (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2020). Of note, we administered the DGA, 2020-2025 to participants from August 2019 to January 2020, but new dietary guidelines, which are updated every five years, were released in December 2020. Additionally, recommendations for women who are pregnant or lactating were included in the 2020-2025 edition, the first time this distinction has been made since the USDA began publishing the DGA in 1980. Results from the questionnaires were compared to the 2015-2020 recommendations for women by age group and the newly released 2020-2025 recommendations specifically for pregnant women in their third trimester by age group. Since the DGA classifies recommended added sugars intake as a percentage of daily calories consumed, we converted the caloric intake value to teaspoons for comparison with our data. Therefore, 260 calories (10% of a 2600 calorie diet recommended for pregnant women in the third trimester in the 18-30 age group) converted to 15.6 teaspoons. For pregnant women in the third trimester in the 31-50 age group a 2400 calorie diet is recommended, which we converted to 14.4 teaspoons.

## **pH MEASUREMENT**

Frozen mother's own milk samples (2 ml) were thawed overnight in a refrigerator at 4 °C, after which they were centrifuged at 15,000 x g at room temperature. A sterile cotton-tipped swab was used to remove the fat layer, and the supernatant was transferred

into a new microcentrifuge tube. The tubes were submerged in a water bath at 37 °C to simulate normal physiological temperature. The pH measurements of the milk supernatant were made using a digital pH-meter (HI5221, Hanna Instruments, Smithfield, RI) equipped with an extended length glass electrode with a micro bulb and calibrated with pH 4.0 and 7.0 buffers.

### **INTESTINAL INFLAMMATORY PROTEIN MEASUREMENT**

As described in previous research from our laboratory, urine samples were collected by bedside nurses in the NICU according to the IRB approved protocol (Shelly et al., 2021). Clean cotton balls in sample bags were distributed by the study team each week and placed by NICU nurses in clean diapers. Saturated cotton balls were placed in a specimen bag, refrigerated at 4 °C during storage, then processed by the research team (< 48 hours). During processing, urine samples (< 2.0 mL) were collected into a microcentrifuge tube using aseptic techniques, then frozen immediately (-80 °C) awaiting analysis. All frozen samples were thawed at room temperature in preparation for analysis.

The R&D Systems enzyme linked immunosorbent assay (ELISA) kit (Minneapolis, MN) was used to measure urinary intestinal fatty acid binding protein (I-FABP) in the infant urine samples. First, all samples were diluted five-fold in a buffer supplemented with 1% bovine serum albumin (R&D Systems). To obtain accurate measurements, any samples with levels that fell outside the assay detection range were repeatedly tested: undiluted, for levels below the detection range, or diluted up to 500-fold, for levels above the detection range. The Pierce bicinchoninic acid (BCA) assay

(Thermo Scientific, Rockford, IL; Gregory et al., 2014) was used to normalize results of each urine analyte by adjusting for total protein (TP) concentrations ( $\mu\text{g/mL}$ ), so they could be screened for TP at 20-fold dilution in phosphate buffered saline (PBS) (Gibco, Thermo Scientific, Rockford, IL). As before, samples with levels that fell below or above the assay detection range were repeatedly tested undiluted or diluted up to 200-fold, respectively. The adjusted I-FABP levels were expressed as pg/mg urinary TP.

## **DATA SELECTION AND STATISTICAL ANALYSIS**

Urinary I-FABP and pH of mother's own milk values were obtained from data generated using samples from an existing tissue bank repository. Urine samples collected within the first 21 days of life (DOL) were used for analysis (mean  $10.1 \pm 4.9$  days). For infants with more than one urine sample within the timeframe of 21 days, the sample in closest proximity to the mean DOL (mean  $10.2 \pm 6.0$  days), calculated from infants with only one sample  $\leq 21$  DOL, was chosen. Each infant had only one mother's own milk sample that was both expressed (mean  $10.3 \pm 5.9$  days) and fed (mean  $11.8 \pm 6.4$  days) to an infant within the first 21 days.

Stata<sup>®</sup> version 14.2 (College Station, TX; Stata) was used to perform statistical analysis. Results are reported as mean (standard deviation) for normally distributed data and median (interquartile range [IQR]) for skewed data. Categorical variables are reported as number (percentage). Normality was assessed using Shapiro-Wilk test. Mann-Whitney U test was used to evaluate differences between groups with continuous

variables. Chi-square test was used to assess differences between groups for categorical variables with a  $p$ -value of 0.03.

Spearman's Correlations were carried out to assess relationships between DSQ equivalents and urinary I-FABP levels, as well as pH of mother's own milk samples. The level of statistical significance was determined by a  $p$ -value of  $<0.05$ . Preliminary unadjusted linear regressions were used to explore relationships between DSQ equivalents and both pH of mother's milk as well as log transformed urinary I-FABP concentrations. We ran Kruskal Wallis tests to determine if there were differences in milk pH, maternal dietary intake, and urinary I-FABP between maternal participants with a maternal comorbidity (preeclampsia or gestational diabetes) and participants without.

## RESULTS

### STUDY POPULATION

The mean age of the maternal population was 34 years old (Table 1a). The majority of mothers reported race as White (60%) in their EMRs, as well as a majority (80%) reporting ethnicity as not Hispanic or Latino. For about half (51%) of the mothers, this was their first birth, and a third of the population delivered by Caesarean section. Nine of the mothers had multiple gestations (20%), but one mother suffered an intrauterine fetal demise of one twin, so our infant study population consists of only eight sets of twins. Two mothers had Type 2 Diabetes (4%) and seven mothers had Gestational Diabetes (7%). preeclampsia was diagnosed in nine mothers (20%) and chronic hypertension in three (7%) with one mother falling into both categories. The mean BMI at delivery was 30.1, recorded from the anesthesiology note in the EMR, or the delivery summary if no anesthesia note was available.

The mean gestational age of preterm infants was 30 weeks and 3 days with a mean birth weight of 1464.7 grams  $\pm$  452 grams (Table 1b). The gestational age of infants ranged from 25 weeks and 1 day old to 33 weeks and 6 days old with a slightly negative skew of -0.2. The infant population consisted of 29 females (55%) and 24 males (45%) and eight sets of twins. The race and ethnicity of the infants were the same as their mothers' race and ethnicity, except for one set of twins whose race was reported as more than one race in the EMR and whose mother's race was white.

**Table 1a. Demographic and Clinical Characteristics of Maternal Participants.** The mean (standard deviation) is defined for the continuous study variables and marked with an asterisk. The categorical variables are defined by number (percent).

Characteristic	Participants, n = 45
	<i>n (%)</i>
Age*	34.1 (5.8)
Race	
Asian	4 (8.9)
Black or African American	8 (17.7)
White	27 (60.0)
Not Reported/Unknown	6 (13.3)
Ethnicity	
Hispanic/Latino	6 (13.3)
Not Hispanic/Latino	36 (80.0)
Not Reported/Unknown	3 (6.7)
Primigravida	23 (51.1)
Multiple Gestation	9 (20.0)
Caesarean section	30 (66.7)
Previous Preterm Birth	9 (20.0)
Maternal Comorbidities	
Diabetes	9 (20.0)
Type 2	2 (4.4)
Gestational	7 (15.6)
preeclampsia	9 (20.0)
Chronic Hypertension	3 (6.7)
GBS at Delivery	12 (26.7)
Previous or Current Tobacco Use	4 (8.9)
BMI at Delivery*	30.1 (5.8)

**Table 1b. Demographic and Clinical Characteristics of Neonatal Participants.** The mean (standard deviation) is defined for the continuous study variables that are normally distributed and marked with an asterisk. The median [interquartile range] is reported for skewed data and marked with two asterisks. The categorical variables are defined by number (percent).

Characteristic	Participants, n=53
	<i>n (%)</i>
Gestational Age of Infant (weeks)*	30.4 (2.5)
Sex	
Female	29 (54.7)
Male	24 (45.3)
Race	
Asian	5 (9.4)
Black or African American	8 (15.1)
White	30 (56.6)
More than One Race	2 (3.8)
Not Reported/Unknown	8 (15.1)
Ethnicity	
Hispanic/Latino	7 (13.2)
Not Hispanic/Latino	43 (81.1)
Not Reported/Unknown	3 (5.7)
Birth Weight (grams)*	1464.7 (451.8)
Olsen Weight for Age Percentage*	42.8 (27.7)
Weight for Age z-score*	-0.2 (0.9)
SNAP-II Score**	6.8 [9]
SNAPPE-II Score**	13 [19]
Sepsis or Late Onset Sepsis	5 (9.4)
Length of Stay (days)*	62.1 (32.2)
Disposition at Discharge	
Home	49 (92.5)
Other Facility	4 (7.5)

## **DIETARY SCREENER QUESTIONNAIRE**

None of the maternal participants met all the recommended guidelines surveyed for by the DSQ (Table 2). For the categories of vegetables, whole grains, and fiber, all of the maternal participants (n = 45) fell below the recommendation. Only one participant met the recommendation for fruits and a single separate participant met the recommendation for dairy. The nutritional goal for calcium was met by the greatest number (n = 12) of maternal participants, which was calculated from estimated daily intake of 25 foods and drinks with both positive correlations for intake, such as fruit, salad, milk, and cheese, and negative correlations, such as fruit juices, salsa, and soda. Nine of our mothers reported drinking only almond milk and no dairy milks, and five mothers reported never drinking dairy or other non-dairy milks. Additionally, only 5 mothers consumed less than 10 percent of their caloric intake in the form of sugar.

Red meat and processed meat intake are collected by the DSQ, but not included in the scoring procedure. Eight of our mothers answered they never ate red meat in the month before delivery, and 13 reported never eating processed meats. Six mothers reported never eating either red meat or processed meats. On the high end of consumption, two mothers reported eating processed meats 3-4 times per week, and six mothers reported eating red meat 3-4 times per week, with one mother reporting eating red meat once per day.

**Table 2. Dietary Intake of Maternal Participants Prior to Preterm Delivery.** The daily nutritional goals are based on the *Dietary Guidelines for Americans, 2020-2025* age-trimester specific values for women who are pregnant or lactating from the U.S. Department of Agriculture and U.S. Department of Health and Human Services. The range of values for added sugars, fiber, and whole grains goals reflects the different recommendations based on age group. All other variables did not differ by age. As defined by the USDA, a one ounce-equivalent (oz-equiv) of 100% whole grains has 16 grams of whole grains. The median [interquartile range] is reported for non-normal distributed data. N = 45.

Daily Nutritional Goals for Third Trimester	Maternal Intake	Meeting Goal
	<i>Median [IQR]</i>	<i>n (%)</i>
Dairy (3 cups)	1.6 [0.6]	1 (2.2)
Fruits (2 cups)	1.0 [0.6]	1 (2.2)
Vegetables (3 cups)	1.4 [0.4]	0 (0.0)
Added Sugar (14.4-15.6 tsp)	17.1 [4.7]	5 (11.1)
Fiber (31-34 g)	18.3 [5.5]	0 (0.0)
Calcium (1000 mg)	867.4 [190.9]	12 (26.7)
Whole Grains (3.5-4 oz-equiv)	.707 [0.3]	0 (0.0)

## pH OF MOTHER’S OWN MILK

The mean pH of breast milk samples from our population was  $6.77 \pm 0.2$  with a range from pH 6.16 to 7.29 (Table 3). We collected additional data on the DOL mothers expressed the breast milk sample (mean  $9.4 \pm 5.1$  days) and the DOL it was fed to their infant (mean  $10.7 \pm 5.4$  days), which is the same day the sample was collected for this study. Both expression and collection dates were recorded for analysis at the maternal and infant level.

**Table 3. Mother’s Own Milk Samples.** For this study the day of birth is considered 1 day of life. The mean (standard deviation) is defined for the continuous study variables that are normally distributed. N = 11.

Mother’s Own Milk Measures	Mean (SD)
Day of Life Expressed	9.4 (5.1)
Day of Life Collected and Fed	10.7 (5.4)
pH at 37 °C	6.77 (0.2)

### URINARY I-FABP

The mean DOL urine samples were collected was  $10.1 \pm 4.9$  days (Table 4). Raw urinary I-FABP (mean  $4087.4 \pm 11,761.3$  pg/mL) and urinary total protein (mean  $13.8 \pm 7.1$  mg/mL) are reported, as well as the log transformed data. The concentration of I-FABP per TP was  $5.5$  pg/mg  $\pm 1.6$ . The amount of urinary I-FABP measured covered a very wide distribution from 7.63 pg/mL to 62,822 pg/mL.

**Table 4. Urine Samples.** The mean (standard deviation) is defined for the continuous study variables that are normally distributed. The median [interquartile range] is reported for skewed data and marked with an asterisk. Urinary I-FABP and Urinary I-FABP per Total Protein data was log-transformed for analysis due to a lack of normal distribution. N = 33.

Urinary I-FABP and Total Protein Measures	Mean (SD)	Median [IQR]
Day of Life Collected	10.1 (4.9)	
Urinary Total Protein (mg/mL)*		13.8 [7.1]
Urinary I-FABP (pg/mL)*		4087.4 [11761.3]
ln-Transformed Urinary I-FABP	8.1 (1.9)	
Urinary I-FABP per Total Protein (pg/mg)*		248.7 [675.56]
ln-Transformed Urinary I-FABP per Total Protein	5.5 (1.6)	

## **RELATIONSHIPS BETWEEN MATERNAL DIET, pH, AND I-FABP**

Whether or not a mother met the guidelines for added sugars intake differed by maternal race ( $p = 0.03$ ) for those mothers whose race was specified in the EMR. We found no statistically significant correlations between maternal diet and any other maternal characteristics.

There were no differences in pH of mother's own milk based on maternal characteristics. However, statistically significant negative correlations were found between the pH of mother's breast milk and maternal intake of fiber and whole grains, as well as positive correlations between added sugars and fruit intake and fiber and vegetable intake ( $r = 0.20$ ,  $p < 0.05$ ) (Table 5a).

We found no statistically significant correlations between pH of mother's own milk and urinary I-FABP. Urinary I-FABP values differed by infant sex ( $p = 0.03$ ) and infant feeding status ( $> 50\%$  formula or donor milk vs.  $< 50\%$  formula or donor milk,  $p = 0.03$ ). We did not find any significant correlations between maternal dietary intake and I-FABP (Table 5b).

Other statistically significant correlations were found in the Spearman's correlations, as might be expected, between intake of the different DSQ food groups. In Table 5b, fiber and vegetable intake and whole grains and fiber intake were significantly positively correlated ( $r = 0.26$ ,  $p < 0.05$ ). In Table 5c our results show that fruit intake was positively correlated with vegetable, fiber, and whole grain intake, vegetable intake was positively correlated with fiber and whole grain intake, and added sugars and whole grains intake was positively correlated with fiber intake ( $r = 0.17$ ,  $p < 0.05$ ).

**Table 5a. Spearman's Rank Correlation Coefficients for pH of Mother's Own Milk at 37 °C.** Statistically significant correlations between pH and daily intake as measured by the Dietary Screener Questionnaire are marked with an asterisk. Spearman rho is 0.203 and the *p*-value is <0.05.

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) pH at 37 °C	1.000							
(2) Dairy	0.154	1.000						
(3) Fruit	-0.382	-0.245	1.000					
(4) Vegetables	-0.497	0.077	0.350	1.000				
(5) Added Sugars	-0.266	-0.322	0.634*	0.007	1.000			
(6) Fiber	-0.622*	-0.273	0.273	0.783*	0.287	1.000		
(7) Calcium	-0.371	-0.217	0.067	-0.147	0.238	0.140	1.000	
(8) Whole Grains	-0.790*	-0.175	0.389	0.385	0.538	0.559	0.203	1.000

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**Table 5b. Spearman's Rank Correlation Coefficients for Urinary I-FABP per Total Protein.** Statistically significant correlations between urinary I-FABP and daily intake as measured by the Dietary Screener Questionnaire are marked with an asterisk. Spearman rho is 0.2641 and the *p*-value is <0.05.

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) I-FABP/TP	1.000							
(2) Dairy	-0.243	1.000						
(3) Fruit	0.073	0.214	1.000					
(4) Vegetables	0.170	0.332	0.284	1.000				
(5) Added Sugars	0.288	-0.009	-0.006	-0.107	1.000			
(6) Fiber	0.193	0.131	0.235	0.501*	0.295	1.000		
(7) Calcium	0.302	0.111	0.063	0.270	0.300	0.263	1.000	
(8) Whole Grains	-0.180	0.042	0.112	0.228	0.219	0.548*	0.264	1.000

**Table 5c. Spearman's Rank Correlation Coefficients for Maternal BMI at Time of Delivery.** Statistically significant correlations between maternal BMI and daily intake as measured by the Dietary Screener Questionnaire are marked with an asterisk. Spearman rho is 0.171 and the  $p$ -value is  $<0.05$ .

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) Maternal BMI	1.000							
(2) Dairy	0.198	1.000						
(3) Fruit	-0.260	0.185	1.000					
(4) Vegetables	-0.198	0.213	0.400*	1.000				
(5) Added Sugars	-0.191	0.014	0.217	-0.013	1.000			
(6) Fiber	-0.172	0.054	0.424*	0.585*	0.328*	1.000		
(7) Calcium	-0.186	0.163	0.034	0.158	0.291	0.213	1.000	
(8) Whole Grains	-0.077	0.105	0.328*	0.309*	0.283	0.480*	0.171	1.000

In the raw unadjusted regression analysis in Table 6a, mother's own milk pH was inversely associated with intake of fiber ( $p < 0.05$ , with an  $R^2$  of 0.47) and whole grain ( $p < 0.01$ , with an  $R^2$  of 0.65), as in the Spearman's correlation analysis, in addition to an inverse relationship with vegetable intake ( $p < 0.1$ , with an  $R^2$  of 0.25). We have no significant results to report for the analysis of the log transformed urinary I-FABP data and DSQ food groups (Table 6b). Dairy and whole grains intake were the only two negative relationships, although not statistically significant.

Results from Kruskal Wallis tests, analyzed by groups (preeclampsia vs healthy, diabetes vs healthy) showed a statistically significant difference ( $p = 0.04$ ) between maternal participants diagnosed with preeclampsia, who had a higher intake of whole grains (0.97 oz), and women without preeclampsia (0.69 oz). Mothers with gestational diabetes had a lower intake of sugar (14.4 tsp) compared to women without diabetes (17.9 tsp), which was statistically significant ( $p = 0.01$ ). We found no other statistically significant results between groups for maternal diet, milk pH, or urinary I-FABP.

**Table 6a. Raw Unadjusted Regression of pH of Mother’s Own Milk at 37 °C and Daily Intake Groups.** Statistically significant relationships between pH and daily intake as measured by the Dietary Screener Questionnaire are marked with an asterisk. Standard errors are listed in parentheses. *p*-values are as follows: \*\*\*  $p < 0.01$ , \*\*  $p < 0.05$ , \*  $p < 0.1$ .

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
	pH at 37 °C	pH at 37 °C	pH at 37 °C	pH at 37 °C	pH at 37 °C	pH at 37 °C	pH at 37 °C
Dairy	.126 (.094)						
Fruit		.123 (.173)					
Vegetables			-.408* (.224)				
Added Sugars				.005 (.032)			
Fiber					-.045** (.015)		
Calcium						0 (.001)	
Whole Grains							-1.233*** (.287)
Constant	6.521*** (.169)	6.58*** (.214)	7.339*** (.349)	6.633*** (.57)	7.503*** (.271)	7.048*** (.605)	7.481*** (.185)
Coefficient							
Observations	12	12	12	12	12	12	12
R-squared	.152	.048	.248	.002	.47	.029	.648

**Table 6b. Raw Unadjusted Regression of ln-Transformed Urinary I-FABP per Total Protein Measurements and Daily Intake Groups.** The urinary I-FABP data were log transformed due to lack of normality. There were no statistically significant relationships between ln-transformed I-FABP and daily intake as measured by the Dietary Screener Questionnaire. Standard errors are listed in parentheses. *p*-values are as follows: \*\*\*  $p < 0.01$ , \*\*  $p < 0.05$ , \*  $p < 0.1$ .

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
	I-FABP/TP	I-FABP/TP	I-FABP/TP	I-FABP/TP	I-FABP/TP	I-FABP/TP	I-FABP/TP
Dairy	-.44 (.733)						
Fruit		.211 (.698)					
Vegetables			1.6 (1.021)				
Added Sugars				.093 (.085)			
Fiber					.062 (.093)		
Calcium						.003 (.002)	
Whole Grains							-.223 (1.057)
Constant	6.149***	5.224***	3.067*	3.843**	4.292**	2.989*	5.636***
Coefficient	(1.17)	(.881)	(1.564)	(1.51)	(1.792)	(1.577)	(.837)
Observations	27	27	27	27	27	27	27
R-squared	.014	.004	.089	.046	.018	.093	.002

## DISCUSSION

Our objective with this study was to analyze at multiple levels the relationships between sociodemographic factors, maternal diet, pH of breast milk, and infant inflammation. We also wanted to assess the dietary pattern of our population of mothers who delivered preterm in comparison to other populations, as well as explore the possibility of using pH of mother's own milk in future research.

## DEMOGRAPHICS

The estimated race and ethnicity for the population of Boston in 2019 as reported by The U.S. Census Bureau is 52.8% White, 25.2% Black or African American, and 9.7% Asian with 5.3% of the population estimated as more than one race. The estimated population that is Hispanic and/or Latino is 19.8% (*U.S. Census Bureau QuickFacts*, n.d.). Estimates are extrapolated from self-reported data. Our demographic make-up is 60% White, 18% Black or African American, and 9% Asian with 13% of the respondents reporting Hispanic and/or Latino ethnicity in their EMR.

The rate of preeclampsia in our population was 20%, which is much higher than the reported 3% to 7% of all pregnancies (*Preeclampsia: MedlinePlus Medical Encyclopedia*, n.d.). One study of 238 women, reported 87 women diagnosed with preeclampsia delivered preterm (36.6%) at a mean gestational age of  $33.3 \pm 3.4$  weeks, which is even higher than in our population (Johnson et al., 2015). The percentage of

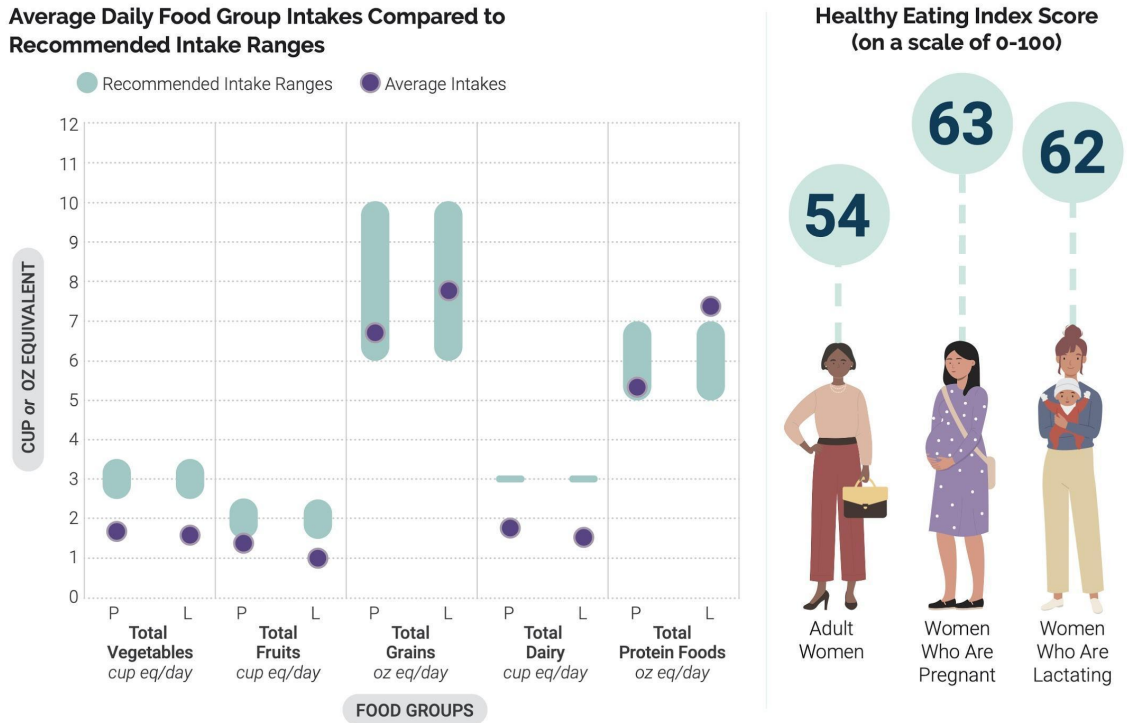
mothers in our population with gestational diabetes was also higher than the 6% to 9% reported by the CDC (*Diabetes During Pregnancy | Maternal Infant Health | Reproductive Health | CDC*, 2019). One study found, however, that rates of preterm birth before 32 weeks was higher in women without gestational diabetes (Leon et al., 2016). Our modest sample size and restriction of population to the NICU may account for these differences.

## **DIETARY GUIDELINES**

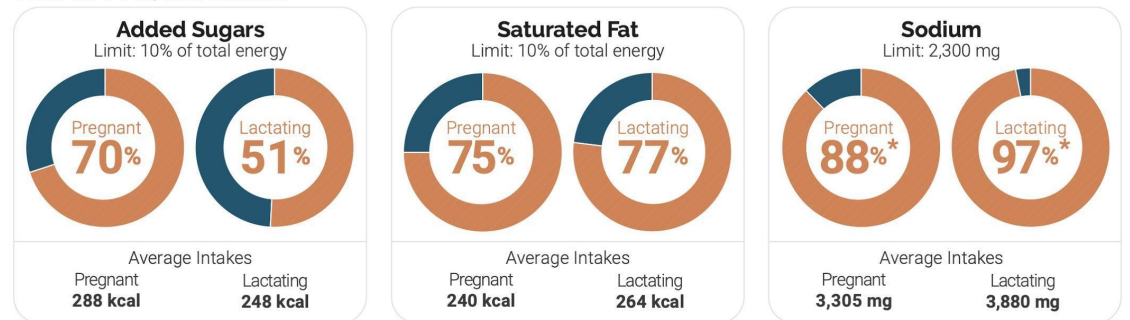
The *Dietary Guidelines for Americans, 2020-2025* (DGA, 2020-2025) recommends the Healthy U.S.-Style Eating Pattern, which “is based on the types and proportions of foods Americans typically consume but in nutrient-dense forms and appropriate amounts” (USDA and HHS, 2020). Recommendations for the Healthy Vegetarian and Mediterranean-Style Eating Patterns are also included in Appendix 3. As with our population, the current average intakes reported in the DGA, 2020-2025, show that pregnant women surveyed for the NHANES 2013-2016 fall below the recommended intake ranges for vegetables, fruits, dairy and whole grains (Figures 2a-b). As the DSQ is a self-reported measure that asks for retrospective data, some amount of recall bias is expected. On average, our participants consumed half of what was recommended, except for whole grains (mean  $0.707 \pm 0.3$  oz-equiv; recommended 3.5-4 oz-equiv) and added sugars, which they exceeded. The percentage of mothers in our population not exceeding the added sugars recommendation (11.1%) was much lower than the 30% reported in the DGA, 2020-2025.

Figure 5-1

## Current Intakes: Women Who Are Pregnant or Lactating



### Percent Exceeding Limits of Added Sugars, Saturated Fat, and Sodium

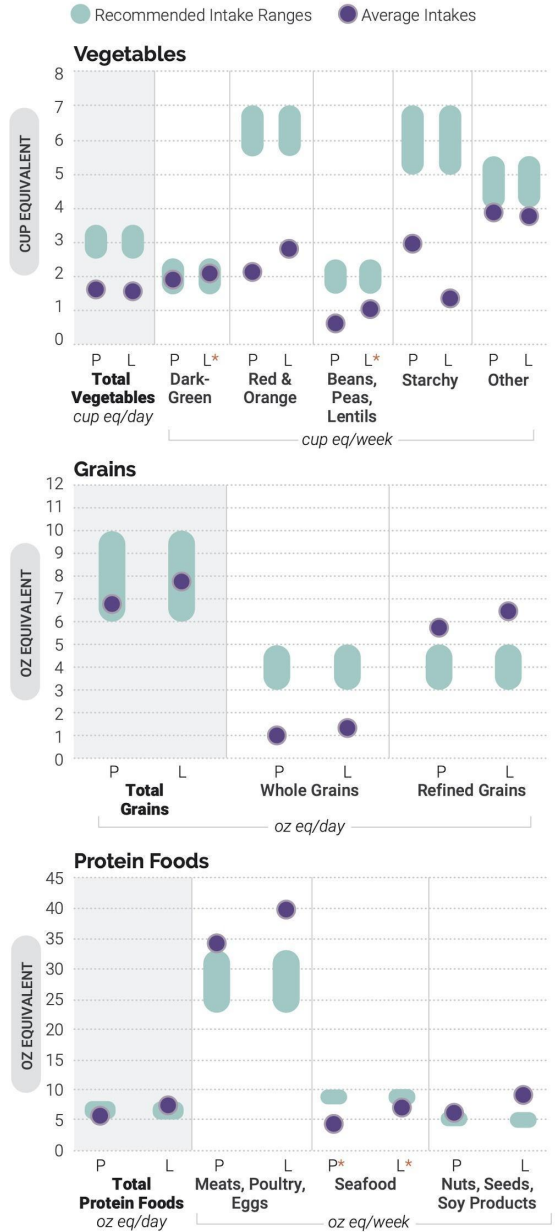


\*NOTE: Estimates may be less precise than others due to small sample size and/or large relative standard error.

Data Sources: Average Intakes and HEI-2015 Scores: Analysis of What We Eat in America, NHANES 2013-2016, women ages 20-44, day 1 dietary intake data, weighted. Recommended Intake Ranges: Healthy U.S.-Style Dietary Patterns (see Appendix 3). Percent Exceeding Limits: What We Eat in America, NHANES 2013-2016, 2 days dietary intake data, weighted.

**Figure 2a. Current Intakes: Women Who Are Pregnant or Lactating.** Recommended intake ranges and current average intakes are shown for total vegetables, fruits, grains, dairy, and protein foods, as well as added sugars, saturated fats, and sodium. Source: *Dietary Guidelines for Americans, 2020-2025*.

Figure 5-2  
**Average Intakes of Subgroups Compared to Recommended Intake Ranges: Women Who Are Pregnant or Lactating**



**Figure 2b. Average Intake of Subgroups Compared to Recommended Intake Ranges: Women Who Are Pregnant or Lactating.** Recommended intake ranges and current average intakes are shown for total vegetables and subgroups, total grains and subgroups, and total protein foods and subgroups. Source: *Dietary Guidelines for Americans, 2020-2025*.

Our results also line up with evidence presented in the literature that most women are not meeting recommended guidelines for nutrition during pregnancy. The Prenatal Health Project asked 2313 pregnant women in London, Ontario about their diet using a FFQ and found that only 3.5% met the guidelines set out by the 2007 *Eat Well with Canada's Food Guide*. Of the four food groups specified, fruit and vegetables, grain products, milk and alternatives, and meat and alternatives, 15.3% did not meet the recommendations for any (Fowler et al., 2012).

The NHANES is a more comprehensive survey than the DSQ, so information about protein, saturated fats, and sodium intake are included in the average intake data for women who are pregnant and lactating. Data for specific types of vegetables, grains, and protein foods is included, which is also not collected in the DSQ. Future research could utilize a more detailed survey to collect information about intake in more food groups and micronutrients and their effects on maternal and infant outcomes, as used in other studies (Martin et al., 2015; Waksmańska et al., 2020; Wang et al., 2018). There is also the possibility for evaluating maternal diet preconception. Research has shown that higher intake of protein-rich foods, fruit, and whole grains during the year leading up to conception, as reported retrospectively by mothers, is associated with a reduced likelihood of preterm delivery (Grieger et al., 2014).

### **Maternal Diet and Characteristics**

The literature shows that pregnant women who are older, more educated, non-white, exercise more, or have higher incomes have healthier dietary patterns.

Pregnant women who are younger, smokers, overweight, or have higher parity have a less healthy diet (Northstone et al., 2008; Wall et al., 2016). A major limitation in this study is that we did not collect education attainment data from our maternal participants at the time of enrollment, although we plan to in the future. Maternal education level is a primary predictor of birth outcomes in the United States, such as preterm delivery and infant mortality (Ely & Driscoll, 2019).

The recommendation for calcium intake (1000 mg) was met by the largest number (n = 12) of maternal participants in this study. The mean intake, however, was only 867.4 ± 190.9 mg. Thirteen studies included in a Cochrane meta-analysis showed that a high-dose calcium supplement ( $\geq 1 \text{ g/day}$ ) reduced the risk of preeclampsia in women with low calcium diets (Hofmeyr et al., 2014). The greatest reduction was found in women with higher risk of preeclampsia. Our analysis of preeclamptic mothers versus healthy mothers in our population showed no statistically significant difference in between the groups in calcium intake.

The only significant difference we found between groups was whole grains intake; maternal participants diagnosed with preeclampsia had a higher mean intake (0.97 oz-equiv) than women without preeclampsia (0.69 oz-equiv). Both of these groups fall far below the recommendation of 3.5-4 oz-equiv daily. Whole grains are recommended in almost all dietary guidelines, and meta-analyses and systematic reviews have found that diets high in whole grains lowered the risk for preterm birth, and a Norwegian study found lower risk of preeclampsia and preterm birth (Chia et al., 2019; Gete et al., 2020;

Hillesund et al., 2014). The results may reflect an attempt by participants to improve dietary intake after a preeclampsia diagnosis, rather than any causal link.

Whether or not a mother met the guidelines for added sugars intake differed by maternal race ( $p = 0.03$ ). We also found a statistically significant difference between intake of added sugars in mothers with gestational diabetes, who had a lower intake (14.4 tsp), compared to women without diabetes (17.9 tsp). It is likely that our results reflect the fact that gestational diabetes is often managed with dietary intervention. Future research could include more detailed interviews with maternal participants about any changes to their diet due to a medical condition diagnosed during pregnancy.

### **pH of Mother's Own Milk**

To our knowledge, this is the first study to investigate pH of mother's own milk in relation to maternal diet and intestinal inflammation in preterm infants. The results from our exploratory analysis of a small sample size ( $n = 11$ ) show that the pH of the samples of mother's own milk (mean  $6.77 \pm 0.2$ ) fall within the reported range for preterm breast milk from one previously published study (Gan et al., 2019). In our analysis, we found that whole grains and fiber intake was significantly inversely related to mother's own milk pH. All other food groups were inversely correlated at non-statistically significant levels with pH, except for dairy. There were no differences in pH of mother's own milk based on maternal characteristics or between groups (preeclampsia vs healthy; diabetic vs healthy).

Differences in fiber intake have been shown to modify the maternal gut microbiome in overweight and obese women who were pregnant (Gomez-Arango et al., 2018). Women with a low fiber intake had increased levels of *Collinsella* in their guts, which was associated with more lactate fermenting bacteria versus the abundance of short-chain fatty acid-producing bacteria seen in high fiber diets. Inclusion of maternal milk microbiome analysis in future studies may be useful to explore the correlation between fiber intake and milk pH. Measurements of vaginal pH have been correlated with cervical neoplasm severity and microbiome changes, although in an animal model, milk pH was not indicative of infection, even though elevated pH levels were observed in disease states (Kandeel et al., 2019; Łaniewski et al., 2018).

We found no significant relationships between pH of breast milk and urinary I-FABP. We did find that urinary I-FABP values differed by infant sex ( $p = 0.03$ ) and infant feeding status ( $> 50\%$  formula or donor milk vs.  $< 50\%$  formula or donor milk,  $p = 0.03$ ). This is in line with previous research published by our laboratory. We showed negative correlations, which were not statistically significant, between infants who were fed a higher proportion of mother's own milk and levels of I-FABP (Shelly et al., 2021). Further exploration of the connection between diet and milk pH and milk pH and infant inflammation is needed.

## **LIMITATIONS AND FUTURE RESEARCH**

A major limitation in our study is the lack of complete sociodemographic information on our participants, including educational attainment and pre-pregnancy

BMI. When studying diet in populations it is important to consider the social, cultural, and economic factors that may affect food availability and dietary patterns. Research done in multiple countries and various communities shows that there are consistent trends in the factors that influence dietary intake in women and mothers. Evidence for positive health effects from diets high in fruits, vegetables, and whole grains was described earlier; however, studies of socioeconomic influences on dietary intake indicate that certain factors increase the likelihood for adherence to more nutritious diets.

A diet high in fruits, salads, fish, eggs, white meat, and whole grain breads, considered the health conscious pattern in a 2008 study of more than 12,000 British women surveyed in 1991-1992 in their third trimester of pregnancy, was positively associated with increasing education levels and age (Northstone et al., 2008).

Demographic factors with a negative association with the health conscious pattern were increased parity, women who smoked, and women who were overweight pre-pregnancy.

A Welsh cross-sectional study, which identified Western and health conscious dietary patterns in a cohort of 303 mothers undergoing elective Cesarean sections, found maternal BMI, age, education, income, and exercise were all significantly associated with the health conscious pattern (Garay et al., 2019). A three year study of women delivering a singleton at a single hospital in northern England (n = 5083) identified three dietary patterns, snack and processed foods, meat and fish, and grains and starches, in three age groups, aged  $\leq 19$ , 20–34, and  $\geq 35$  (Marvin-Dowle et al., 2018). While the authors found no differences between age groups within the meat and fish pattern, women in the oldest age group had significantly higher principal component analysis scores for the

grains and starches pattern. Women in the youngest age group had higher scores in the snack and processed food pattern, and their intake of sugar-sweetened sodas was higher, while their intake of fruit and vegetables was lower. The authors also reported higher mean BMI and overweight and obese mothers in the oldest age group.

A Brazil study published in 2016 found that adherence to a healthy diet pattern during pregnancy was more likely in older women and women with a higher monthly per capita income. Women with higher parity showed decreased adherence to the healthy diet pattern (de Castro et al., 2016). A second study from Brazil of 454 women found that higher maternal education attainment is associated with a diet of lean meats and seasonal vegetables, as well as a diet of snacks, sandwiches, sweets, and sodas, although only 10.3% of their participants had more than eight years of formal education (Teixeira et al., 2018).

A New Zealand study, specifically focused on a multi-ethnic society context, found four dietary patterns in women surveyed prior to delivery (Wall et al., 2016). Positive associations were found between higher scores in the Junk and Traditional/White Bread patterns and younger age, less education, and smoking, in addition to higher scores in the Health Conscious and Fusion/Protein patterns and older age, lower pre-pregnancy BMI, and non-smokers. This study showed that the greatest odds of adherence to the New Zealand National Food and Nutrition Guidelines for Healthy Pregnant Women were associated with the Health Conscious pattern.

A Polish study of second trimester women (n = 1306) also found that older age, more education, were positive determinants of a healthier diet characterized by higher

consumption of fruit, vegetables, legumes, whole grains, poultry, and low-fat dairy (Wesołowska et al., 2019). However, they found that being overweight or obese before pregnancy was also a positive determinant of a healthier diet. Parity was significantly associated with a more Western pattern, defined as increased consumption of refined grain, processed meat, potatoes, and a rather low intake of whole grains.

An analysis of women and children in the southern United States enrolled from 2006 to 2011 in the Conditions Affecting Neurocognitive Development and Learning in Early Childhood study (n = 1222) found that mothers who received the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) food package with revisions had infants with greater length-for-age *z* scores at one year of age and better Bayley Scales of Infant Development cognitive composite scores at two years (Guan et al., 2021). The food package was updated in October 2009 to be more in line with the DGA, 2020-2025. Previous studies in low-income households had shown that increased healthy food access, quality of diet, and birth outcomes were associated with the revised package (Hamad et al., 2019; Schultz et al., 2015).

Another limitation is our modest sample size and the exploratory nature of this research, which limited our ability to perform a more robust analysis. A larger sample size and longitudinal collection of dietary habits and infant outcomes will provide data necessary to further explore the correlations we found.

Future analyses of this dataset in collaboration with other groups are planned to assess the dietary inflammation index (DII) and fat intake for the maternal participants. A DII is a tool that can indicate inflammatory or anti-inflammatory potential from answers

to a food intake survey like the DSQ we used (Steck et al., 2014). More information about dietary inflammatory potential in the maternal participants will allow for better investigation into the possibility of intergenerational inflammatory responses. Calculating fat intake is of interest to us for the ability to compare maternal fat intake to the fat content of mother's own milk and the levels of urinary I-FABP, as well as microbiome changes, in the preterm infant (Chu, Meyer, et al., 2016).

We are interested in long-chain polyunsaturated fatty acids (LCPUFA), including the ratio of arachidonic acid (AA) to docosahexaenoic acid (DHA), given the essential functions these fatty acids play in placental and fetal development, breast milk composition, and infant nutrition (Duttaroy & Basak, 2020; Innis et al., 2002). Levels of DHA in breast milk from preeclamptic mothers have been shown to be higher than in normotensive women (Dangat et al., 2014). Additionally, evidence exists that a high fat diet during pregnancy decreased the genera *Bacteroides*, known to aid in mucosal immunity maturation, in the infant gut microbiome (Chu, Antony, et al., 2016).

In mice models, microbial species have been shown to spread through the blood from oral cavity to placenta, many of which are associated with adverse pregnancy outcomes in humans (Fardini et al., 2010). This similarity between oral and placental microbiome may indicate future research opportunities for maternal diets effects on the various human microbiomes and inflammation, as the maternal gut microbiome has been shown to increase inflammation in pregnant mothers leading to preeclampsia (Amarasekara et al., 2015). If mothers have elevated levels of inflammation causing dysbiosis of their placental microbiome, the placenta may not protect the infant from a

type of inheritance of inflammation. There are already studies that show preterm birth is associated with intrauterine infections and decreased diversity of the vaginal microbiome (Witkin, 2015). New research shows there may be a correlation between the maternal and neonatal oral microbiome, transmitted through the placenta and amniotic fluid (Wu et al., 2021). Oral transmission through blood to the fetus has been demonstrated in sheep studies (Yu et al., 2021).

## **CONCLUSIONS**

We show that there are statistically significant correlations between maternal dietary intake of fiber and whole grains during the month before delivery of a preterm infant and pH of mother's milk. We also show between group differences in whole grains intake in maternal participants diagnosed with preeclampsia and those not, as well as differences in added sugars intake between participants with gestational diabetes and without. Our data also supports the national data that pregnant women are not meeting the guidelines for a healthy diet. These findings suggest that additional research on mother's own milk pH may be warranted, and that continued education on the importance of a healthy diet and its benefits during pregnancy is needed.

## REFERENCES

- Amarasekara, R., Jayasekara, R. W., Senanayake, H., & Dissanayake, V. H. W. (2015). Microbiome of the placenta in pre-eclampsia supports the role of bacteria in the multifactorial cause of pre-eclampsia. *The Journal of Obstetrics and Gynaecology Research*, *41*(5), 662–669. <https://doi.org/10.1111/jog.12619>
- Ansell, C., Moore, A., & Barrie, H. (1977). Electrolyte pH changes in Human Milk. *Pediatric Research*, *11*(12), 1177–1179. <https://doi.org/10.1203/00006450-197712000-00002>
- Assaf-Balut, C., García de la Torre, N., Fuentes, M., Durán, A., Bordiú, E., Del Valle, L., Valerio, J., Jiménez, I., Herraiz, M. A., Izquierdo, N., Torrejón, M. J., de Miguel, M. P., Barabash, A., Cuesta, M., Rubio, M. A., & Calle-Pascual, A. L. (2018). A High Adherence to Six Food Targets of the Mediterranean Diet in the Late First Trimester is Associated with a Reduction in the Risk of Materno-Foetal Outcomes: The St. Carlos Gestational Diabetes Mellitus Prevention Study. *Nutrients*, *11*(1). <https://doi.org/10.3390/nu11010066>
- Baron, R., te Velde, S. J., Heymans, M. W., Klomp, T., Hutton, E. K., & Brug, J. (2017). The Relationships of Health Behaviour and Psychological Characteristics with Spontaneous Preterm Birth in Nulliparous Women. *Maternal and Child Health Journal*, *21*(4), 873–882. <https://doi.org/10.1007/s10995-016-2160-4>
- Bottasso Arias, N. M., García, M., Bondar, C., Guzman, L., Redondo, A., Chopita, N., Córscico, B., & Chirido, F. G. (2015). Expression Pattern of Fatty Acid Binding Proteins in Celiac Disease Enteropathy. *Mediators of Inflammation*, *2015*, e738563. <https://doi.org/10.1155/2015/738563>
- Bravi, F., Wiens, F., Decarli, A., Dal Pont, A., Agostoni, C., & Ferraroni, M. (2016). Impact of maternal nutrition on breast-milk composition: A systematic review. *The American Journal of Clinical Nutrition*, *104*(3), 646–662. <https://doi.org/10.3945/ajcn.115.120881>
- Chen, X., Li, P., Liu, M., Zheng, H., He, Y., Chen, M.-X., Tang, W., Yue, X., Huang, Y., Zhuang, L., Wang, Z., Zhong, M., Ke, G., Hu, H., Feng, Y., Chen, Y., Yu, Y., Zhou, H., & Huang, L. (2020). Gut dysbiosis induces the development of pre-eclampsia through bacterial translocation. *Gut*, *69*(3), 513–522. <https://doi.org/10.1136/gutjnl-2019-319101>
- Chernikova, D. A., Koestler, D. C., Hoen, A. G., Housman, M. L., Hibberd, P. L., Moore, J. H., Morrison, H. G., Sogin, M. L., Zain-ul-abideen, M., & Madan, J. C. (2016). Fetal exposures and perinatal influences on the stool microbiota of premature infants. *The Journal of Maternal-Fetal & Neonatal Medicine*, *29*(1), 99–105.

<https://doi.org/10.3109/14767058.2014.987748>

- Chia, A.-R., Chen, L.-W., Lai, J. S., Wong, C. H., Neelakantan, N., van Dam, R. M., & Chong, M. F.-F. (2019). Maternal Dietary Patterns and Birth Outcomes: A Systematic Review and Meta-Analysis. *Advances in Nutrition, 10*(4), 685–695. <https://doi.org/10.1093/advances/nmy123>
- Chu, D. M., Antony, K. M., Ma, J., Prince, A. L., Showalter, L., Moller, M., & Aagaard, K. M. (2016). The early infant gut microbiome varies in association with a maternal high-fat diet. *Genome Medicine, 8*. <https://doi.org/10.1186/s13073-016-0330-z>
- Chu, D. M., Meyer, K. M., Prince, A. L., & Aagaard, K. M. (2016). Impact of maternal nutrition in pregnancy and lactation on offspring gut microbial composition and function. *Gut Microbes, 7*(6), 459–470. <https://doi.org/10.1080/19490976.2016.1241357>
- Clausen, T., Slott, M., Solvoll, K., Drevon, C. A., Vollset, S. E., & Henriksen, T. (2001). High intake of energy, sucrose, and polyunsaturated fatty acids is associated with increased risk of preeclampsia. *American Journal of Obstetrics and Gynecology, 185*(2), 451–458. <https://doi.org/10.1067/mob.2001.116687>
- Cole, Z. A., Gale, C. R., Javaid, M. K., Robinson, S. M., Law, C., Boucher, B. J., Crozier, S. R., Godfrey, K. M., Dennison, E. M., & Cooper, C. (2009). Maternal dietary patterns during pregnancy and childhood bone mass: A longitudinal study. *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research, 24*(4), 663–668. <https://doi.org/10.1359/jbmr.081212>
- Coufal, S., Kokesova, A., Tlaskalova-Hogenova, H., Frybova, B., Snajdauf, J., Rygl, M., & Kverka, M. (2020). Urinary I-FABP, L-FABP, TFF-3, and SAA Can Diagnose and Predict the Disease Course in Necrotizing Enterocolitis at the Early Stage of Disease. *Journal of Immunology Research, 2020*. <https://doi.org/10.1155/2020/3074313>
- Dangat, K., Kilari, A., Mehendale, S., Lalwani, S., & Joshi, S. (2014). Preeclampsia alters milk neurotrophins and long chain polyunsaturated fatty acids. *International Journal of Developmental Neuroscience, 33*(1), 115–121. <https://doi.org/10.1016/j.ijdevneu.2013.12.007>
- DASH Eating Plan* | NHLBI, NIH. (n.d.). Retrieved March 16, 2021, from <https://www.nhlbi.nih.gov/health-topics/dash-eating-plan>

- Dasinger, J. H., Abais-Battad, J. M., & Mattson, D. L. (2020). Influences of environmental factors during preeclampsia. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 319(1), R26–R32. <https://doi.org/10.1152/ajpregu.00020.2020>
- de Castro, M. B. T., Freitas Vilela, A. A., de Oliveira, A. S. D., Cabral, M., de Souza, R. A. G., Kac, G., & Sichieri, R. (2016). Sociodemographic characteristics determine dietary pattern adherence during pregnancy. *Public Health Nutrition*, 19(7), 1245–1251. <https://doi.org/10.1017/S1368980015002700>
- Derikx, J. P. M., Evennett, N. J., Degraeuwe, P. L. J., Mulder, T. L., Bijnen, A. A. van, Heurn, L. W. E. van, Buurman, W. A., & Heineman, E. (2007). Urine based detection of intestinal mucosal cell damage in neonates with suspected necrotising enterocolitis. *Gut*, 56(10), 1473–1475. <https://doi.org/10.1136/gut.2007.128934>
- Derikx, Joep P. M., Vreugdenhil, A. C. E., Van den Neucker, A. M., Grootjans, J., van Bijnen, A. A., Damoiseaux, J. G. M. C., van Heurn, L. W. E., Heineman, E., & Buurman, W. A. (2009). A Pilot Study on the Noninvasive Evaluation of Intestinal Damage in Celiac Disease Using I-FABP and L-FABP. *Journal of Clinical Gastroenterology*, 43(8), 727–733. <https://doi.org/10.1097/MCG.0b013e31819194b0>
- Diabetes During Pregnancy | Maternal Infant Health | Reproductive Health | CDC.* (2019, January 16). <https://www.cdc.gov/reproductivehealth/maternalinfanthealth/diabetes-during-pregnancy.htm>
- DiGiulio, D. B., Callahan, B. J., McMurdie, P. J., Costello, E. K., Lyell, D. J., Robaczewska, A., Sun, C. L., Goltsman, D. S. A., Wong, R. J., Shaw, G., Stevenson, D. K., Holmes, S. P., & Relman, D. A. (2015). Temporal and spatial variation of the human microbiota during pregnancy. *Proceedings of the National Academy of Sciences*, 112(35), 11060–11065. <https://doi.org/10.1073/pnas.1502875112>
- Duda-Chodak, A., Tarko, T., Satora, P., & Sroka, P. (2015). Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: A review. *European Journal of Nutrition*, 54(3), 325–341. <https://doi.org/10.1007/s00394-015-0852-y>
- Duttaroy, A. K., & Basak, S. (2020). Maternal dietary fatty acids and their roles in human placental development. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 155, 102080. <https://doi.org/10.1016/j.plefa.2020.102080>

- Ely, D. M. P., & Driscoll, A. K. P. (2019). Infant Mortality in the United States, 2017: Data From the Period Linked Birth/Infant Death File. *National Vital Statistics Reports*, 68(10), 20.
- Englund-Ögge, L., Brantsæter, A. L., Sengpiel, V., Haugen, M., Birgisdottir, B. E., Myhre, R., Meltzer, H. M., & Jacobsson, B. (2014). Maternal dietary patterns and preterm delivery: Results from large prospective cohort study. *BMJ (Clinical Research Ed.)*, 348, g1446. <https://doi.org/10.1136/bmj.g1446>
- Evennett, N. J., Hall, N. J., Pierro, A., & Eaton, S. (2010). Urinary intestinal fatty acid-binding protein concentration predicts extent of disease in necrotizing enterocolitis. *Journal of Pediatric Surgery*, 45(4), 735–740. <https://doi.org/10.1016/j.jpedsurg.2009.09.024>
- Fardini, Y., Chung, P., Dumm, R., Joshi, N., & Han, Y. W. (2010). Transmission of diverse oral bacteria to murine placenta: Evidence for the oral microbiome as a potential source of intrauterine infection. *Infection and Immunity*, 78(4), 1789–1796. <https://doi.org/10.1128/IAI.01395-09>
- Forgie, A. J., Drall, K. M., Bourque, S. L., Field, C. J., Kozyrskyj, A. L., & Willing, B. P. (2020). The impact of maternal and early life malnutrition on health: A diet-microbe perspective. *BMC Medicine*, 18(1), 135. <https://doi.org/10.1186/s12916-020-01584-z>
- Fowler, J. K. MSc., R. D., Evers, S. E. P., & Campbell, M. K. P. (2012). Inadequate Dietary Intakes: Among Pregnant Women. *Canadian Journal of Dietetic Practice and Research*. <https://doi.org/10.3148/73.2.2012.72>
- Furuhashi, M., & Hotamisligil, G. S. (2008). Fatty acid-binding proteins: Role in metabolic diseases and potential as drug targets. *Nature Reviews Drug Discovery*, 7(6), 489–503. <https://doi.org/10.1038/nrd2589>
- Gan, J., Zheng, J., Krishnakumar, N., Goonatilleke, E., Lebrilla, C. B., Barile, D., & German, J. B. (2019). Selective proteolysis of  $\alpha$ -lactalbumin by endogenous enzymes of human milk at acidic pH. *Molecular Nutrition & Food Research*, 63(18), e1900259. <https://doi.org/10.1002/mnfr.201900259>
- Garay, S. M., Savory, K. A., Sumption, L., Penketh, R., Janssen, A. B., & John, R. M. (2019). The Grown in Wales Study: Examining dietary patterns, custom birthweight centiles and the risk of delivering a small-for-gestational age (SGA) infant. *PloS One*, 14(3), e0213412. <https://doi.org/10.1371/journal.pone.0213412>
- Gete, D. G., Waller, M., & Mishra, G. D. (2020). Effects of maternal diets on preterm birth and low birth weight: A systematic review. *The British Journal of Nutrition*, 123(4), 446–461. <https://doi.org/10.1017/S0007114519002897>

- Gomez-Arango, L. F., Barrett, H. L., McIntyre, H. D., Callaway, L. K., Morrison, M., & Nitert, M. D. (2017). Contributions of the maternal oral and gut microbiome to placental microbial colonization in overweight and obese pregnant women. *Scientific Reports*, 7(1), 2860. <https://doi.org/10.1038/s41598-017-03066-4>
- Gomez-Arango, L. F., Barrett, H. L., Wilkinson, S. A., Callaway, L. K., McIntyre, H. D., Morrison, M., & Dekker Nitert, M. (2018). Low dietary fiber intake increases *Collinsella* abundance in the gut microbiota of overweight and obese pregnant women. *Gut Microbes*, 9(3), 189–201. <https://doi.org/10.1080/19490976.2017.1406584>
- Gregory, K. E., Winston, A. B., Yamamoto, H. S., Dawood, H. Y., Fashemi, T., Fichorova, R. N., & Van Marter, L. J. (2014). Urinary intestinal fatty acid binding protein predicts necrotizing enterocolitis. *The Journal of Pediatrics*, 164(6), 1486–1488. <https://doi.org/10.1016/j.jpeds.2014.01.057>
- Grieger, J. A., Grzeskowiak, L. E., & Clifton, V. L. (2014). Preconception dietary patterns in human pregnancies are associated with preterm delivery. *The Journal of Nutrition*, 144(7), 1075–1080. <https://doi.org/10.3945/jn.114.190686>
- Guan, A., Hamad, R., Batra, A., Bush, N. R., Tylavsky, F. A., & LeWinn, K. Z. (2021). The Revised WIC Food Package and Child Development: A Quasi-Experimental Study. *Pediatrics*, 147(2). <https://doi.org/10.1542/peds.2020-1853>
- Hamad, R., Collin, D. F., Baer, R. J., & Jelliffe-Pawlowski, L. L. (2019). Association of Revised WIC Food Package With Perinatal and Birth Outcomes: A Quasi-Experimental Study. *JAMA Pediatrics*, 173(9), 845–852. <https://doi.org/10.1001/jamapediatrics.2019.1706>
- Harrison, V. C., & Peat, G. (1972). Significance of Milk pH in Newborn Infants. *British Medical Journal*, 4(5839), 515–518.
- Hillesund, E. R., Øverby, N. C., Engel, S. M., Klungsøyr, K., Harmon, Q. E., Haugen, M., & Bere, E. (2014). Associations of adherence to the New Nordic Diet with risk of preeclampsia and preterm delivery in the Norwegian Mother and Child Cohort Study (MoBa). *European Journal of Epidemiology*, 29(10), 753–765. <https://doi.org/10.1007/s10654-014-9948-6>
- Hofmeyr, G. J., Lawrie, T. A., Atallah, A. N., Duley, L., & Torloni, M. R. (2014). Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. *The Cochrane Database of Systematic Reviews*, 6, CD001059. <https://doi.org/10.1002/14651858.CD001059.pub4>

- Innis, S. M., Adamkin, D. H., Hall, R. T., Kalhan, S. C., Lair, C., Lim, M., Stevens, D. C., Twist, P. F., Diersen-Schade, D. A., Harris, C. L., Merkel, K. L., & Hansen, J. W. (2002). Docosahexaenoic acid and arachidonic acid enhance growth with no adverse effects in preterm infants fed formula. *The Journal of Pediatrics*, *140*(5), 547–554. <https://doi.org/10.1067/mpd.2002.123282>
- Johnson, A., Federico, C., Martinez, M., Tran, K.-A., Kao, E., Hooshvar, N., Tice, D., Wu, G., Gambala, C., Pridjian, G., & Dola, C. (2015). [192-POS]: Term and preterm preeclampsia: Are there two distinct phenotypes? *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health*, *5*(1), 97. <https://doi.org/10.1016/j.preghy.2014.10.198>
- Kandeel, S. a., Megahed, A. a., Ebeid, M. h., & Constable, P. d. (2019). Ability of milk pH to predict subclinical mastitis and intramammary infection in quarters from lactating dairy cattle. *Journal of Dairy Science*, *102*(2), 1417–1427. <https://doi.org/10.3168/jds.2018-14993>
- Łaniewski, P., Barnes, D., Goulder, A., Cui, H., Roe, D. J., Chase, D. M., & Herbst-Kralovetz, M. M. (2018). Linking cervicovaginal immune signatures, HPV and microbiota composition in cervical carcinogenesis in non-Hispanic and Hispanic women. *Scientific Reports*, *8*(1), 1–13. <https://doi.org/10.1038/s41598-018-25879-7>
- Leeman, L., & Fontaine, P. (2008). Hypertensive disorders of pregnancy. *American Family Physician*, *78*(1), 93–100.
- Leon, M. G., Moussa, H. N., Longo, M., Pedroza, C., Haidar, Z. A., Mendez-Figueroa, H., Blackwell, S. C., & Sibai, B. M. (2016). Rate of Gestational Diabetes Mellitus and Pregnancy Outcomes in Patients with Chronic Hypertension. *American Journal of Perinatology*, *33*(8), 745–750. <https://doi.org/10.1055/s-0036-1571318>
- Lieberman, J. M., Sacchetti, J., Marks, C., & Marks, W. H. (1997). Human intestinal fatty acid binding protein: Report of an assay with studies in normal volunteers and intestinal ischemia. *Surgery*, *121*(3), 335–342. [https://doi.org/10.1016/S0039-6060\(97\)90363-9](https://doi.org/10.1016/S0039-6060(97)90363-9)
- Liu, L., Oza, S., Hogan, D., Perin, J., Rudan, I., Lawn, J. E., Cousens, S., Mathers, C., & Black, R. E. (2015). Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: An updated systematic analysis. *Lancet (London, England)*, *385*(9966), 430–440. [https://doi.org/10.1016/S0140-6736\(14\)61698-6](https://doi.org/10.1016/S0140-6736(14)61698-6)

- Lv, L.-J., Li, S.-H., Li, S.-C., Zhong, Z.-C., Duan, H.-L., Tian, C., Li, H., He, W., Chen, M.-C., He, T.-W., Wang, Y.-N., Zhou, X., Yao, L., & Yin, A.-H. (2019). Early-Onset Preeclampsia Is Associated With Gut Microbial Alterations in Antepartum and Postpartum Women. *Frontiers in Cellular and Infection Microbiology*, *9*, 224. <https://doi.org/10.3389/fcimb.2019.00224>
- Martin, C. L., Sotres-Alvarez, D., & Siega-Riz, A. M. (2015). Maternal Dietary Patterns during the Second Trimester Are Associated with Preterm Birth. *The Journal of Nutrition*, *145*(8), 1857–1864. <https://doi.org/10.3945/jn.115.212019>
- Marvin-Dowle, K., Kilner, K., Burley, V., & Soltani, H. (2018). Differences in dietary pattern by maternal age in the Born in Bradford cohort: A comparative analysis. *PloS One*, *13*(12), e0208879. <https://doi.org/10.1371/journal.pone.0208879>
- Mesa, M. D., Loureiro, B., Iglesia, I., Fernandez Gonzalez, S., Llubra Olivé, E., García Algar, O., Solana, M. J., Cabero Perez, M. J., Sainz, T., Martínez, L., Escuder-Vieco, D., Parra-Llorca, A., Sánchez-Campillo, M., Rodríguez Martínez, G., Gómez Roig, D., Perez Gruz, M., Andreu-Fernández, V., Clotet, J., Sailer, S., ... Cabañas, F. (2020). The Evolving Microbiome from Pregnancy to Early Infancy: A Comprehensive Review. *Nutrients*, *12*(1). <https://doi.org/10.3390/nu12010133>
- Mitku, A. A., Zewotir, T., North, D., Jeena, P., & Naidoo, R. N. (2020). Modeling Differential Effects of Maternal Dietary Patterns across Severity Levels of Preterm Birth Using a Partial Proportional Odds Model. *Scientific Reports*, *10*. <https://doi.org/10.1038/s41598-020-62447-4>
- Morriss, F. H., Brewer, E. D., Spedale, S. B., Riddle, L., Temple, D. M., Caprioli, R. M., & West, M. S. (1986). Relationship of Human Milk pH During Course of Lactation to Concentrations of Citrate and Fatty Acids. *Pediatrics*, *78*(3), 458–464.
- Northstone, K., Emmett, P., & Rogers, I. (2008). Dietary patterns in pregnancy and associations with socio-demographic and lifestyle factors. *European Journal of Clinical Nutrition*, *62*(4), 471–479. <https://doi.org/10.1038/sj.ejcn.1602741>
- Olivares, M., Albrecht, S., De Palma, G., Ferrer, M. D., Castillejo, G., Schols, H. A., & Sanz, Y. (2015). Human milk composition differs in healthy mothers and mothers with celiac disease. *European Journal of Nutrition*, *54*(1), 119–128. <https://doi.org/10.1007/s00394-014-0692-1>

- Pannaraj, P. S., Li, F., Cerini, C., Bender, J. M., Yang, S., Rollie, A., Adisetiyo, H., Zabih, S., Lincez, P. J., Bittinger, K., Bailey, A., Bushman, F. D., Sleasman, J. W., & Aldrovandi, G. M. (2017). Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome. *JAMA Pediatrics*, *171*(7), 647–654. <https://doi.org/10.1001/jamapediatrics.2017.0378>
- Preeclampsia: MedlinePlus Medical Encyclopedia*. (n.d.). Retrieved March 19, 2021, from <https://medlineplus.gov/ency/article/000898.htm>
- Preterm Birth | Maternal and Infant Health | Reproductive Health | CDC*. (2020, October 30). <https://www.cdc.gov/reproductivehealth/maternalinfanthealth/pretermbirth.htm>
- Purisch, S. E., & Gyamfi-Bannerman, C. (2017). Epidemiology of preterm birth. *Seminars in Perinatology*, *41*(7), 387–391. <https://doi.org/10.1053/j.semperi.2017.07.009>
- Rasmussen, M. A., Maslova, E., Halldorsson, T. I., & Olsen, S. F. (2014). Characterization of dietary patterns in the Danish national birth cohort in relation to preterm birth. *PloS One*, *9*(4), e93644. <https://doi.org/10.1371/journal.pone.0093644>
- Robertson, R. C., Manges, A. R., Finlay, B. B., & Prendergast, A. J. (2019). The Human Microbiome and Child Growth – First 1000 Days and Beyond. *Trends in Microbiology*, *27*(2), 131–147. <https://doi.org/10.1016/j.tim.2018.09.008>
- Roseboom, T., de Rooij, S., & Painter, R. (2006). The Dutch famine and its long-term consequences for adult health. *Early Human Development*, *82*(8), 485–491. <https://doi.org/10.1016/j.earlhumdev.2006.07.001>
- Roseboom, T. J. (2019). Epidemiological evidence for the developmental origins of health and disease: Effects of prenatal undernutrition in humans. *The Journal of Endocrinology*, *242*(1), T135–T144. <https://doi.org/10.1530/JOE-18-0683>
- Schultz, D. J., Byker Shanks, C., & Houghtaling, B. (2015). The Impact of the 2009 Special Supplemental Nutrition Program for Women, Infants, and Children Food Package Revisions on Participants: A Systematic Review. *Journal of the Academy of Nutrition and Dietetics*, *115*(11), 1832–1846. <https://doi.org/10.1016/j.jand.2015.06.381>
- Seferovic, M. D., Mohammad, M., Pace, R. M., Engevik, M., Versalovic, J., Bode, L., Haymond, M., & Aagaard, K. M. (2020). Maternal diet alters human milk oligosaccharide composition with implications for the milk metagenome. *Scientific Reports*, *10*(1), 22092. <https://doi.org/10.1038/s41598-020-79022-6>

- Sharma, R., & Hudak, M. L. (2013). A Clinical Perspective of Necrotizing Enterocolitis. *Clinics in Perinatology*, 40(1), 27–51. <https://doi.org/10.1016/j.clp.2012.12.012>
- Shelly, C. E., Filatava, E. J., Thai, J., Pados, B. F., Rostas, S. E., Yamamoto, H., Fichorova, R., & Gregory, K. E. (2021). Elevated Intestinal Inflammation in Preterm Infants With Signs and Symptoms of Gastroesophageal Reflux Disease: *Biological Research For Nursing*. <https://doi.org/10.1177/1099800420987888>
- Shulhan, J., Dicken, B., Hartling, L., & Larsen, B. M. (2017). Current Knowledge of Necrotizing Enterocolitis in Preterm Infants and the Impact of Different Types of Enteral Nutrition Products. *Advances in Nutrition*, 8(1), 80–91. <https://doi.org/10.3945/an.116.013193>
- Sotres-Alvarez, D., Siega-Riz, A. M., Herring, A. H., Carmichael, S. L., Feldkamp, M. L., Hobbs, C. A., Olshan, A. F., & National Birth Defects Prevention Study. (2013). Maternal dietary patterns are associated with risk of neural tube and congenital heart defects. *American Journal of Epidemiology*, 177(11), 1279–1288. <https://doi.org/10.1093/aje/kws349>
- Steck, S., Shivappa, N., Tabung, F., Harmon, B., Wirth, M., Hurley, T., Hebert, J., & Sc.D. (2014). The Dietary Inflammatory Index: A New Tool for Assessing Diet Quality Based on Inflammatory Potential. *The Digest*, 49, 1–9.
- Stein, A. D., Zybert, P. A., van der Pal-de Bruin, K., & Lumey, L. H. (2006). Exposure to famine during gestation, size at birth, and blood pressure at age 59 y: Evidence from the dutch famine. *European Journal of Epidemiology*, 21(10), 759–765. <https://doi.org/10.1007/s10654-006-9065-2>
- Teixeira, J. A., Castro, T. G., Grant, C. C., Wall, C. R., Castro, A. L. da S., Francisco, R. P. V., Vieira, S. E., Saldiva, S. R. D. M., & Marchioni, D. M. (2018). Dietary patterns are influenced by socio-demographic conditions of women in childbearing age: A cohort study of pregnant women. *BMC Public Health*, 18(1), 301. <https://doi.org/10.1186/s12889-018-5184-4>
- Thompson, F. E., Midthune, D., Kahle, L., & Dodd, K. W. (2017). Development and Evaluation of the National Cancer Institute’s Dietary Screener Questionnaire Scoring Algorithms. *The Journal of Nutrition*, 147(6), 1226–1233. <https://doi.org/10.3945/jn.116.246058>
- Thompson, J. M. D., Wall, C., Becroft, D. M. O., Robinson, E., Wild, C. J., & Mitchell, E. A. (2010). Maternal dietary patterns in pregnancy and the association with small-for-gestational-age infants. *The British Journal of Nutrition*, 103(11), 1665–1673. <https://doi.org/10.1017/S0007114509993606>

- U.S. Census Bureau QuickFacts: Boston city, Massachusetts. (n.d.). Retrieved March 19, 2021, from <https://www.census.gov/quickfacts/fact/table/bostoncitymassachusetts/PST045219>
- U.S. Department of Agriculture and U.S. Department of Health and Human Services. (2020). *U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2020-2025. 9th Edition. December 2020. Available at DietaryGuidelines.gov.* DietaryGuidelines.gov
- Victora, C. G., Bahl, R., Barros, A. J. D., França, G. V. A., Horton, S., Krasevec, J., Murch, S., Sankar, M. J., Walker, N., & Rollins, N. C. (2016). Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. *The Lancet*, 387(10017), 475–490. [https://doi.org/10.1016/S0140-6736\(15\)01024-7](https://doi.org/10.1016/S0140-6736(15)01024-7)
- Vujkovic, M., Steegers, E. A., Looman, C. W., Ocké, M. C., van der Spek, P. J., & Steegers-Theunissen, R. P. (2009). The maternal Mediterranean dietary pattern is associated with a reduced risk of spina bifida in the offspring. *BJOG: An International Journal of Obstetrics and Gynaecology*, 116(3), 408–415. <https://doi.org/10.1111/j.1471-0528.2008.01963.x>
- Waksmańska, W., Bobiński, R., Ulman-Włodarz, I., & Pielesz, A. (2020). The differences in the consumption of proteins, fats and carbohydrates in the diet of pregnant women diagnosed with arterial hypertension or arterial hypertension and hypothyroidism. *BMC Pregnancy and Childbirth*, 20(1), 29. <https://doi.org/10.1186/s12884-019-2711-y>
- Wall, C. R., Gammon, C. S., Bandara, D. K., Grant, C. C., Atatoa Carr, P. E., & Morton, S. M. B. (2016). Dietary Patterns in Pregnancy in New Zealand-Influence of Maternal Socio-Demographic, Health and Lifestyle Factors. *Nutrients*, 8(5). <https://doi.org/10.3390/nu8050300>
- Wang, J., Zheng, J., Shi, W., Du, N., Xu, X., Zhang, Y., Ji, P., Zhang, F., Jia, Z., Wang, Y., Zheng, Z., Zhang, H., & Zhao, F. (2018). Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus. *Gut*, 67(9), 1614–1625. <https://doi.org/10.1136/gutjnl-2018-315988>
- Wesołowska, E., Jankowska, A., Trafalska, E., Kałużny, P., Grzesiak, M., Dominowska, J., Hanke, W., Calamandrei, G., & Polańska, K. (2019). Sociodemographic, Lifestyle, Environmental and Pregnancy-Related Determinants of Dietary Patterns during Pregnancy. *International Journal of Environmental Research and Public Health*, 16(5). <https://doi.org/10.3390/ijerph16050754>

- Witkin, S. S. (2015). The vaginal microbiome, vaginal anti-microbial defence mechanisms and the clinical challenge of reducing infection-related preterm birth. *BJOG: An International Journal of Obstetrics and Gynaecology*, *122*(2), 213–218. <https://doi.org/10.1111/1471-0528.13115>
- Wu, S., Yu, F., Ma, L., Zhao, Y., Zheng, X., Li, X., Li, Z., He, X., & Zhou, J. (2021). Do Maternal Microbes Shape Newborn Oral Microbes? *Indian Journal of Microbiology*, *61*(1), 16–23. <https://doi.org/10.1007/s12088-020-00901-7>
- Yu, K., Rodriguez, M., Paul, Z., Gordon, E., Gu, T., Rice, K., Triplett, E. W., Keller-Wood, M., & Wood, C. E. (2021). Transfer of oral bacteria to the fetus during late gestation. *Scientific Reports*, *11*(1), 708. <https://doi.org/10.1038/s41598-020-80653-y>

## CURRICULUM VITAE

