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THE IMPACT OF MATERNAL NUTRITION DURING PREGNANCY ON INFLAMMATION AND BIRTH OUTCOMES

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THE IMPACT OF MATERNAL NUTRITION DURING PREGNANCY ON INFLAMMATION AND BIRTH OUTCOMES

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Nursing at the University of Kentucky

By
Lori Ellen Ogden

Lexington, Kentucky

Director: Dr. Kristen Ashford, Professor of Nursing

Lexington, Kentucky

2019

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THE IMPACT OF MATERNAL NUTRITION DURING PREGNANCY ON INFLAMMATION AND BIRTH OUTCOMES

More than 85% of American adults do not consume recommended amounts of fruits or vegetables. Preterm birth and hypertensive disorders of pregnancy are common adverse conditions affecting pregnancy and are leading causes of maternal and fetal morbidity and mortality. Preterm birth affects nearly 10% of all births in the United States and is on the rise, as are hypertensive disorders, which have increased by 25% over the last two decades. Pregnancy is a state of controlled inflammation, and dysregulation has been linked to preterm birth and other adverse gestational outcomes. A healthy diet is recommended in pregnancy, but little is known about the effect fruit and vegetable intake on perinatal outcomes. Omega-3 (n-3) fatty acids are essential dietary components and are known to affect inflammatory state, but little is known about how they affect inflammation in pregnancy. As current evidence is lacking, further research is needed to investigate the relationships between maternal nutrition in pregnancy, inflammation and birth outcomes.

The purposes of this dissertation were to: 1) to review and evaluate the current evidence on the relationship between n-3 fatty acids and inflammation in pregnancy; 2) to evaluate the current state of the science on the impact of maternal dietary consumption of fruits and vegetables on preterm birth, gestational diabetes, preeclampsia, small for gestational age, gestational weight gain and measures of inflammation or oxidative stress in pregnancy; and 3) to examine relationships between maternal dietary intake of fruits and vegetables, cytokine expression in early and mid-pregnancy, preterm birth and gestational hypertension.

A critical review of literature examining the relationship between inflammation and n-3 intake during pregnancy found that multiple inflammatory cytokines in maternal and fetal tissues were lower in women who received n-3 supplements. A second review of literature review supported an inverse relationship between fruit and vegetables and risk of preeclampsia and suboptimal fetal growth. The available evidence was insufficient to establish relationships between fruit and vegetable intake and gestational diabetes, preterm birth or inflammation. A study evaluating the relationships between maternal fruit and vegetable intake, inflammation and birth outcomes was conducted. This study
provided evidence supporting a relationship between first and second trimester cytokine expression and maternal dietary intake of fruits and vegetables. Those who met recommended vegetable intake in the first trimester had higher first trimester serum CRP, IL1-α, IL-6 and TNF-α and lower first trimester cervicovaginal IL-6 levels. Those who met recommendations for first trimester fruit intake had 56% lower risk for preterm birth. Those who met second trimester vegetable intake recommendations had more than twice the risk of developing gestational hypertension.

The results of this dissertation provide support for the beneficial effects of omega-3 fatty acids and fruit and vegetable intake in pregnancy. Maternal intake of these dietary components may promote optimal immune status during pregnancy. Supplementation of maternal omega-3 fatty acids may help regulate inflammation via the anti-inflammatory effects their bioactive eicosanoids exert. Fruit and vegetables have antioxidant and anti-inflammatory effects that may also help balance the inflammatory state during pregnancy. These dietary components may help promote favorable immune status during pregnancy and reduce risk of adverse perinatal outcomes such as poor fetal growth, hypertensive disorders of pregnancy and preterm birth.

KEYWORDS: Maternal Nutrition, Diet, Inflammation, Birth Outcomes, Cytokine

Lori Ellen Ogden
November 11, 2019
Date
THE IMPACT OF MATERNAL NUTRITION DURING PREGNANCY ON INFLAMMATION AND BIRTH OUTCOMES

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Lori Ellen Ogden

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November 11, 2019
This dissertation is dedicated to the memories of John and Marie Preston and Kate Blakeman, my grandparents for their unwavering love and support and to the memory of my son Brandon. Your presence continues to impact my life. I am ME because of YOU! I love you more!
ACKNOWLEDGMENTS

First and foremost, I thank Jesus Christ, my Lord and Savior for His work in my life. Without His grace and strength none of this would be possible. All that I have and all that I am is from Him. May my use of these gifts be pleasing to Him and bring glory to His name.

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Being your Mom is my greatest blessing. You have also sacrificed much for my successes-groceries, home cooked meals, snuggles, and time-because I was writing or studying. You are my HEART! My extended family and those who call me “Mimi”, I love you and am so grateful for your patience during this season of my life when you have been a little neglected and for all the hugs! As I have walked the path of grieving mother these last five years, I have not been alone. My “Love Mom” sisters and fellow travelers have been there always and in all ways. This group of amazing women made me believe I could keep going. Your strength and faith mean more than I can express. To my friends- thanks for hanging in there and your love and support these last several years. Thanks also to my “fellow nerds” in the PhD program- Karen, Stephanie, and Hartley-you all are the BEST!

*I can do all things through Christ who strengthens me.*

*Philippians 4:13*
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CHAPTER ONE: Introduction

The purpose of this dissertation was to examine relationships between maternal nutrition during pregnancy, maternal inflammatory state during pregnancy and adverse perinatal outcomes. Women are advised to eat a healthy diet during pregnancy. This healthy diet should include a variety of foods to meet the nutritional needs of both the mother and growing fetus. Pregnancy is a state of controlled inflammation, and alterations to this inflammatory state may contribute to poor outcomes including preterm birth, hypertension and poor fetal growth. The purpose was addressed in three manuscripts that comprise Chapters Two through Four. Chapter 2 is a review and evaluation of the current evidence on the relationship between maternal omega-3 fatty acid intake and inflammation in pregnancy. Chapter 3 is a report on the state of the science on the impact of maternal dietary consumption of fruits and vegetables on the incidence of preterm birth, gestational diabetes, preeclampsia, small for gestational age, gestational weight gain and measures of inflammation or oxidative stress in pregnancy. Chapter Four is a study of the relationships among maternal intake of fruits and vegetables, trimester specific cytokine expression in early and mid-pregnancy and birth outcomes. Chapter Five is a synthesis of the chapters with implications for future research and clinical practice integration.

Most Americans do not meet recommend intake for fruits, vegetables or dairy and exceed those for added sugar, saturated fats and sodium ("Facts & Statistics," 2017). Poor diet quality has been linked to increased risk of some cancers, cardiovascular disease, type 2 diabetes and stroke, while healthier dietary patterns may reduce risk of diseases (Casas, Sacanella, & Estruch, 2014; Griffiths et al., 2016; Micha et al., 2017).

Inflammation arises from the body’s response to injury and is the primary mechanism of signaling the body to defend and repair damaged tissues. Dysregulation in
inflammatory cascade may be responsible for development of chronic diseases such as cancer, cardiovascular disease and type 2 diabetes as well as inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease (Hunter, 2012; Kollias, Douni, Kassiotis, & Kontoyiannis, 1999; H. Li et al., 2017; Subirana et al., 2018). Diet related inflammation is one possible mechanism underlying the diet-disease connection.

Dysregulation of the tightly controlled inflammatory state during pregnancy has been implicated in adverse gestational outcomes. Preterm birth, or delivery prior to 37 weeks of gestation, is one adverse outcome that may be related to a dysregulated inflammatory state. Nearly 10% of births in the United States occur preterm (H. B. Martin JA, Osterman MJK, Driscoll AK, Drake P., 2018) and are a leading cause of infant morbidity and mortality (CDC, 2017b). Hypertensive disorders of pregnancy represent a group of disorders unique to pregnancy. Gestational hypertension is high blood pressure alone after 20 weeks gestation, while diagnosis of preeclampsia requires signs of other organ involvement. Hypertensive disorders are a leading cause of both maternal and infant morbidity and mortality worldwide (Foundation, 2013). Both preterm birth and hypertensive disorders are associated with dysregulation of immune function in pregnancy (Aggarwal et al., 2019; Brou et al., 2012; Cappelletti, Della Bella, Ferrazzi, Mavilio, & Divanovic, 2016; U. N. Das, 2006). Consequently, improved immune function regulation may promote healthier gestational outcomes.

Result from several studies support a link between nutrition and adverse gestational outcomes. Healthy dietary patterns rich in fruits and vegetables, whole grains, unprocessed meats and fish may reduce the risk of hypertensive disorders of pregnancy and preterm birth (Haugen et al., 2008; Mikkelsen et al., 2008; Raghavan et al., 2019; Schoenaker,
Soedamah-Muthu, Callaway, & Mishra, 2015), but little is known about how individual foods or food groups affect birth outcomes. Thus, the purpose of this dissertation was to examine the impact of dietary intake of fruits, vegetables and omega-3 fatty acids on maternal inflammation and birth outcomes.

**Theoretical framework**

The framework described by Ma (2013) guided this dissertation (Figure 1.1). The framework includes all the key concepts of this dissertation and provided a theoretical basis on which to explore the relationships among maternal nutrition, inflammation, and birth outcomes. This dissertation was focused on the influence of nutrition on biomarkers of stress and birth outcomes in the context of sociodemographic variables and health behaviors. Thus, the scope of this dissertation did not include the relationships of food environment or neighborhood characteristics.

According to the model, demographic variables including income or socioeconomic status, marital status, maternal age, and race or ethnicity affect health behaviors and may influence biological factors. Health behaviors include maternal dietary intake, exercise and physical activity level, and smoking status. They are potential mediators of the relationships between sociodemographic variables and maternal risk factors with birth outcomes. Maternal risk factors include stress, prior birth history, maternal weight and body mass index, gestational weight gain and infection, which influence both biological factors and birth outcomes. Biological factors may be affected by maternal factors and nutrition. Nutritional factors may mediate the effects of sociodemographic characteristics, health behaviors and maternal risk factors and affect
biological factors. Combined these relationships demonstrate how nutrition interventions could promote more positive birth outcomes.

Figure 1.1 Framework of food environment and birth outcomes (Ma, 2013)

Chapter summaries

Chapter Two is a systematic review that aims to fill an existing gap in the understanding of the effect of omega-3 fatty acids on inflammatory markers in pregnancy. Dysregulation of maternal inflammatory status during pregnancy has been linked with increased risk for preterm birth (Moghaddam Banaem, Mohamadi, Asghari Jaafarabadi, & Aliyan Moghadam, 2012; Ruiz et al., 2012), gestational diabetes and hypertensive disorders of pregnancy (U. N. Das, 2006; Harmon et al., 2016; Yu et al., 2017). Supplementation of omega-3 fatty acids has been shown to regulate inflammation (Wu & Schauss, 2012). Omega-3 fatty acids were supplemented in the maternal diet via salmon or
capsule preparation in the seven studies were included in the review. Biomarkers of inflammation were measured in maternal serum and adipose tissue as well as fetal cord blood and placental tissues. This review summarizes and evaluates the current evidence on the relationship between omega-3 fatty acid consumption and markers of inflammation in pregnancy.

Chapter Three is a review of the literature that evaluated and summarized evidence to address gaps that exist in relationship between fruit and vegetable intake, adverse gestational outcomes and inflammation/oxidative stress in pregnancy. Adverse pregnancy outcomes, including preeclampsia, preterm birth, gestational diabetes and infant growth restriction, are associated with dysregulation of inflammation and oxidative stress. Fruit and vegetable intake are also known to affect inflammation and oxidative stress and improve outcomes in other chronic diseases with proinflammatory pathologies. The review aimed to improve the understanding of the influence of dietary fruit and vegetable intake on perinatal outcomes and markers of inflammation and oxidative stress. Nineteen articles met inclusion criteria with fruit and/or vegetable intake as an independent variable and at least one of the outcomes of interest: preeclampsia, preterm birth, gestational diabetes, fetal growth, gestational weight gain or inflammation/oxidative stress.

Chapter Four is a secondary analysis of data. This study was conducted expand on the limited available evidence examining relationships between maternal dietary intake of fruits and vegetables during early and mid-pregnancy on preterm birth, gestational hypertension. In addition is the first to measure the impact of fruit and vegetable intake on trimester specific markers of inflammation in pregnancy. The sample consisted of 181 pregnant women 18 years or older with healthy singleton gestation. Those with complete
dietary intake, cytokine values and birth outcome data. The mean age of the women was 27.0 years (SD=5.4). More than half had less than $50,000 per year household income (52.8%), most had at least a high school education (90.6%), and were white (81.7%). The majority were non-smokers (79.4%), were married or cohabitating (81.1%) and were first time mothers (64.1%). More than half were overweight or obese prior to pregnancy (52.9%). Linear regression was used to address Aim 1, which was to determine whether meeting recommendations for fruit or vegetable intake in the first or second trimester predicted serum and cervicovaginal cytokine expression while controlling for factors affecting inflammation such as smoking. Aim 2 was addressed by a logistic regression to determine if fruit or vegetable intake predicted either preterm birth or gestational hypertension while controlling for common risk factors.

Chapter Five is a brief summary and synthesis of Chapters, 2, 3, and 4. The integration of the findings was used to develop recommendations for evidence-based clinical practice changes and to make recommendations for future research.
CHAPTER TWO: Systematic review of ability of omega 3 fatty acids role in suppressing inflammation in pregnant women

Introduction/Background

Adverse perinatal outcomes account for a small proportion of all births statistically, and a large proportion of healthcare expenditures. Conditions such as preterm birth (PTB), preeclampsia, gestational hypertension (GHTN), and gestational diabetes (GDM) result in increased maternal healthcare costs during pregnancy, at birth, and often for the newborn infant.

Premature birth occurs at <37 completed weeks gestation and is the focus of the March of Dimes, one of the leading sources of research and funding on prematurity (2016). The rate of preterm birth has increased for last three years following a seven year decline and totaled nearly 10% of all births (J. A. Martin, Hamilton, Osterman, Driscoll, & Drake, 2018). Premature birth and its sequelae are the largest contributors to infant death, and account for 36% of the more than 23,000 infant deaths occurring in 2013 (Dimes, 2016). The most recent estimate in 2005 of the economic burden of prematurity totaled more than $26.2 billion ("Preterm Birth: Causes, Consequences, and Prevention," 2006).

Hypertensive disorders of pregnancy include preeclampsia/eclampsia, and GHTN. Preeclampsia is a multisystem disorder of human pregnancy of unknown causes. Maternal systemic manifestations include hypertension, proteinuria or other organ involvement, while fetal abnormalities include growth restriction, low amniotic fluid and abnormal oxygenation and placental blood flow (B. Sibai, G. Dekker, & M. Kupferminc, 2005). Preeclampsia becomes eclampsia with the onset of maternal seizures. Gestational hypertension is elevated blood pressure that starts after 20 weeks gestation without signs of other organ involvement. Preeclampsia is closely associated with PTB, with one recent
analysis reporting a gestational age at delivery of approximately two weeks less for those with preeclampsia than those without (Stevens et al., 2017). They also report total maternal and fetal costs for those affected by preeclampsia in the United States of $2.18 billion over the usual costs of pregnancy and birth.

Altered glucose metabolism in pregnancy can result from pre-gestational diabetes (type 1 or 2) or arise during pregnancy (GDM). Known risks of GDM are older maternal age, obese/overweight, and non-white race. The prevalence of GDM is estimated to be as high as 9.2% in the United States in 2010, with prevalence at least one in 20 pregnancies affected (DeSisto, Kim, & Sharma, 2014). The estimated cost of GDM in 2007 was an additional $3,305 per pregnancy, or an increase in medical costs for maternal care during pregnancy, birth and postpartum of $506 million, and newborn costs of $40 million in the United States (Chen et al., 2009)

**Inflammation and Pregnancy**

The immune system is focused on recognition of antigens found on the surface of bacteria, viruses or fungi and mounting a cellular response to eliminate them and repair the damage caused. Inflammatory substances are released from damaged cells to promote swelling and isolate the damaged tissue, as well as recruit cells to remove the damaged tissue. Due to the constant death/damage and repair/replacement of body cells, the inflammatory response is dynamic and requires balance between pro- and anti-inflammatory regulators. Dysregulation of the immune or inflammatory response can lead to over or under reaction as well as destruction of the body’s own tissues.

During gestation the body must work to protect the “foreign” fetus from attack by these systems in order to maintain a healthy pregnancy by tightly regulating the balance
between both pro- and anti-inflammatory states during this time of controlled inflammation (Bowen, Chamley, Mitchell, & Keelan, 2002; Calder, 2010). Key cellular components of immune function are types of white blood cells called T helper cells (Th) and their precursors T lymphocytes. T helper cells produce signaling molecules called cytokines direct the inflammatory response. Type 1 Th are important in intracellular response to microbial invasion and promote the immune response. These cells produce interferon gamma (IFN-γ), tumor necrosis factor alpha (TNFα), and Interleukin (IL) 2. Type 2 Th cells function in extracellular immunity are more anti-inflammatory. They produce IL 4, IL-5, IL-6, IL-10, and IL-13. Type 2 immunity predominates during successful human pregnancy (Bowen, Chamley, Keelan, & Mitchell, 2002).

As in times of illness and disease, alterations in immune response may lead to adverse outcomes in pregnancy. Researchers recently examined the relationship between dysregulation of inflammation and pregnancy complications. Maternal serum C reactive protein (CRP) levels above four in the first half of pregnancy resulted in nearly nine times greater risk for preterm birth (Moghaddam Banaem et al., 2012) while elevated IL-1Ra in the second trimester resulted in 2.5 greater odds of preterm birth (Ruiz et al., 2012). Lower levels of anti-inflammatory IL-10 and or transforming growth factor-beta (TGF-β), have been reported in those who delivered preterm (Pereira et al., 2016). Recent data also supports a relationship between alterations in inflammatory cytokines with preeclampsia and gestational diabetes (U. N. Das, 2006; Harmon et al., 2016; Yu et al., 2017).

**Omega3 Fatty Acids and Inflammation**

Polyunsaturated fatty acids (PUFA) are one of four types of dietary fats. Alpha linolenic acid (ALA) and linoleic acid (LA) are n-3 and n-6 fats respectively, termed
“essential fatty acids” in the diet as they cannot be synthesized in the body from their precursor oleic acid (OA). Both n-6 and n-3 fats provide energy in the diet and are an essential component of the phospholipid cell membrane. Humans can synthesize saturated fatty acids and monounsaturated fatty acids through desaturation and elongation reactions. However, humans lack the necessary desaturase enzyme necessary for synthesizing n-3 and n-6, thus intake of n-3 ALA and n-6 LA is essential. Plant sources provide only ALA, while marine sources provide EPA and DHA. The long chain n-3 PUFA eicosapentaenoic acid (EPA) is synthesized from ALA. Further synthesis of EPA results in the n-3 long chain PUFA docosahexaenoic acid (DHA) (Jane Higdon, 2019).
These long chain PUFA promote fluidity in the cell membrane, regulate gene expression and inflammation. As components of cell membranes, they can interfere with receptors in the membrane and block or promote gene transcription directly. Omega-3 are given preference over n-6 for incorporation into cell membranes. In other cases, n-3 regulate the amount of transcription factors in the cell nucleus. These transcription factors are responsible for production of eicosanoids and cytokines (Jane Higdon, 2019).

In addition to cell membrane structure, long chain PUFA are involved in other biological functions. Chemical messengers called oxylipins are derived from PUFA. The most common oxylipins are eicosanoids. Eicosanoid production is stimulated when hormones and cytokines signal release of substrate PUFA from cell membranes. The series 2 prostanoids, thromboxanes, and series four leukotrienes arising from n-6’s are primarily pro-inflammatory. In opposition, the series 3 prostanoids, leukotrienes and neuroprotectins that arise from n-3 are less inflammatory than the n-6. Some eicosanoids derived from EPA antagonize those produced by AA promoting more anti-inflammatory state (Calder,
Other cell signaling substances that arise from n-3 PUFA are specialized pro-resolving mediators and include their precursors the resolvins, protectins and maresins. They are potent anti-inflammatory and immune resolving molecules (Figure 2.1).

The typical “western” diet includes large amounts of foods containing vegetable oils (corn oil, soybean oil, safflower oil), which are high in LA. Plants are also the source of ALA but LA tends to predominate. Plants high in ALA are less commonly consumed (chia seeds, hemp seed, walnut and flax). The “Western” diet contains higher n-6 and lower n-3 amounts, with a ratio of 10-25:1, with optimal ratios of 1-4:1 (Simopoulos, 2000). Improved ratios of n-2:n-6 PUFA can be altered by either decreasing the intake of n-6 fats and/or increasing the intake of n-3. Currently, the American Congress of Obstetricians and Gynecologists’ dietary guidelines for pregnant women do not include n-3 (2015). Fish intake is limited to 8-12 ounces per week; however, certain fish are excluded or limited due to concerns about contamination with mercury. This may contribute to worsening of the relative deficiency of n-3 in the Western diet for pregnant women.

Supplementing n-3 during pregnancy may affect inflammatory state by several mechanisms. First, the increased intake improves n-6:n-3 ratios. Improved n-3 intake allows more availability for incorporation into cell membranes as well as production of eicosanoids. This may result a more anti-inflammatory balance in eicosanoids via increased production of inflammation reducing resolvins and protectins and lower excretion of labor initiating PGE2. Omega-3 fatty acids have been also been shown to affect production of inflammatory proteins such as cytokines through production of transcription factors involved in cell signaling (Calder, 2010) (Figure 2.2). Therefore, the purpose of this paper
was to evaluate the current evidence for the relationship between maternal intake of omega-3 fatty acids and inflammation in pregnancy.

Figure 2.2 Pathways for omega-3 to impact placental oxidative stress and inflammation


**Methods**

Electronic searches were conducted using PubMed and CINAHL. Search terms were used in combination: *pregnan* OR *prenat*, *omega-3 OR PUFA, and inflammat* OR *infect* OR *cytokin* *. Original research articles in English were included if published within the last 10 years for most relevant and up to date sources. Title review of 155 articles excluded those not performed in humans or those where the pregnant woman and/or fetus were not the primary outcome of interest, as were duplications. Only those including maternal intake of n-3 fatty acids as the dependent variable were included, as cell cultures using exposure to fatty acids in the laboratory setting are not be representative of human
physiology and should be cautiously interpreted. Abstract and full text review screened for those meeting all inclusion criteria and seven articles were included in the full review (Figure 2.3).

All articles were assessed for quality and assigned scores. The method used to evaluate the quality of randomized controlled trials for brain cancer and includes items that, when omitted, result in increased bias (Lai R, 2006). The evaluation includes 15 key items from the Consolidated Standards of Reporting Trials recommended for use in all randomized controlled trials. The range of possible scores is zero-15, with the included studies scores ranging from nine to 15, and mean of 11.7. A matrix table was developed to organize the most relevant data on the relationship between n-3 intake and inflammation in pregnancy. The headings used were author and year, sample participants and setting, sources of inflammatory measures, type of intervention (supplement) used, and key findings, with a separate column for scores (Table 2.1).
Number of articles retrieved = 267

Number of articles after remove duplicates and apply inclusion criteria = 196

Number of articles for title review = 155

Number of articles for full text review = 36

Number of articles included in final review = 7

Number excluded based on title review = 119

Exclusion criteria applied for full text review:
- Only humans
- Omega- as dependent variable
- Pregnant women =/or fetus as population of interest
Results

Sample Characteristics/Interventions

The studies were randomized controlled trials published between 2008 and 2016 and enrolled between 40 and 343 pregnant women (Garcia-Rodriguez et al., 2017; Maricela Haghiaj et al., 2015; Harper et al., 2013; Jamilian et al., 2016; Keelan et al., 2015; Krauss-Etschmann et al., 2008; Warstedt, Furuhjelm, Duchen, Falth-Magnusson, & Fageras, 2009). Two studies were conducted in the United States (M. Haghjiaj et al., 2015; Harper et al., 2013). One each in the United Kingdom (Garcia-Rodriguez et al., 2012), Australia (Keelan et al., 2015), Sweden (Warstedt et al., 2009), and Iran (Jamilian et al., 2016). One study included subjects from Spain, Hungary and Germany (Krauss-Etschmann et al., 2008). All studies sought to examine the association between intake of n-3 and markers of inflammation/immunity in pregnant women both with and without specific conditions (such as GDM, obesity, allergies, or PTB). The studies used salmon (Garcia-Rodriguez et al., 2012), oral capsules (M. Haghjaiaj et al., 2015; Harper et al., 2013; Jamilian et al., 2016; Keelan et al., 2015; Warstedt et al., 2009) or a milk-based supplement (Krauss-Etschmann et al., 2008) to provide the omega-3 or placebo to participants. Fish diet history was also assessed at enrollment in one instance to provide a measure of dietary n-3 intake prior to treatment (Harper et al., 2013). The dosages of EPA ranged from 163mg to 1,500mg per day, while DHA ranged from 331mg to 3,700mg per day. Gestational age at treatment initiation ranged from 10 to 25 weeks gestation at onset for the included studies, with all except M. Haghjiaj et al. (2015) starting in the second trimester. Only overweight or obese participants were enrolled in one study (M. Haghjiaj et al., 2015); two included women with allergies (Keelan et al., 2015; Warstedt et al., 2009), and one only included women
with gestational diabetes (Jamilian et al., 2016). All these maternal states may affect or alter the inflammatory response in addition to pregnancy. Other inclusion criteria for the pregnant women were to have otherwise healthy singleton pregnancies (i.e. non-smokers, no pre-existing diabetes, hypertension, or other significant health problems) and did not previously take fish oil or n-3 supplements.

**Measures/Outcomes Used in Included Studies**

**Inflammatory biomarkers.** The most common pro-inflammatory biomarkers measured were IL-6, TNF-α, IL-1, IL1-β, IL-8, and C reactive protein (CRP). Common anti-inflammatory biomarkers measured were: IL-10, and IL-4. Other measures used to assess inflammatory status were prostaglandin E₂ (PGE₂), which is an eicosanoid generated from AA, as well as those initiating, mediating, or suppressing the immune response (specialized pro resolving mediators [SPM]). Serum cytokine concentrations and cytokine gene expression were used to measure inflammation. Maternal blood was used for cytokine analysis in all included studies (Garcia-Rodriguez et al., 2012; M. Haghiac et al., 2015; Harper et al., 2013; Jamilian et al., 2016; Keelan et al., 2015; Krauss-Etschmann et al., 2008; Warstedt et al., 2009). Cord blood was utilized for inflammatory biomarker analysis once (Keelan et al., 2015), while placental tissue was used in three studies (M. Haghiac et al., 2015; Keelan et al., 2015; Melody et al., 2015). One study also included maternal adipose tissue for evaluation (M. Haghiac et al., 2015).

**Impact of fatty acid intervention on inflammation.** The researchers used change over time in the same participants or differences between groups (supplement vs placebo) to evaluate trends in cytokine expression and the effect of the intervention on the individual biomarkers. Investigators in three studies reported utilizing regression analysis to assess
the relationship between treatment group and inflammatory markers (Garcia-Rodriguez et al., 2012; Harper et al., 2013; Keelan et al., 2015).

**Findings and Data Analysis of Included Studies**

Maricela Haghiac et al. (2015) reported maternal serum CRP levels decreased over time for those who received n-3 (-3.2 ±5.2µg/ml) and increased for those who did not (+.82 ±6.9 µg/ml, p<.05); as did Jamilian et al. (2016) (-3375.7 ±4863.8 ng/ml vs 82.8±3149.7ng/ml, p.001). Lower geometric means of maternal IL-1 and TNF-α levels were reported at delivery in those who received treatment (.69 vs .80, p<.00; .054±.03 vs .65±.05, p<.00) by Krauss-Etschmann et al. (2008). Jamilian et al. (2016) reported downregulated gene expression of IL-1 and TNF-α in those who were supplemented as well (p=.007 and p=.001). Maternal adipose cells had significantly lower gene expression of IL-6, IL-8, and TNF-α mRNA in those women given n-3 supplementation during pregnancy (<.001) (Maricela Haghiac et al., 2015).

Cord blood and placental cytokine levels also differed for those who received treatment with n-3. Krauss-Etschmann et al. (2008) reported lower cord levels of IL-4 (.54 vs .64, p<.00) and IL-13 (.61 vs .85, p<.00) and IL-1 (.81 vs .85, p<.00) for those who received fish oil supplement compared to those who did not. Placental trophoblast cells expressed lower levels of IL-6, IL-8 and TNF-α in those in the treatment group than those who were not (Maricela Haghiac et al., 2015).
Table 2.1 Characteristics of included studies on omega-3 intake and inflammation

<table>
<thead>
<tr>
<th>Author (Date); Participants</th>
<th>Sample/Setting</th>
<th>Measures</th>
<th>Intervention</th>
<th>Key Findings</th>
<th>Consort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garcia-Rodriguez, et al. (2012).</td>
<td>N=123 United Kingdom</td>
<td>Maternal blood samples were collected at baseline (20 weeks) and at 34 and 38 weeks for fatty acid analysis and biomarker analysis.</td>
<td>Tx: 2 portions/week of farmed salmon containing a total of 1.14g EPA and 2.32g DHA, 28mcg vitamin D, and 86 mcg selenium, from 20 weeks gestation through delivery. Control: continue usual diet</td>
<td>No differences in plasma biomarkers based on treatment received.</td>
<td>9</td>
</tr>
<tr>
<td>Haghiac, et al. (2015).</td>
<td>N=72 overweight/obese pregnant women United States</td>
<td>-Visit 1(8-16 weeks) and visit 2 (34-36 weeks): height/weight, maternal blood sample (glucose, insulin, fatty acid, and cytokines). -Placental tissue, cord blood at delivery. -Maternal adipose tissue was collected from subset (n=16) women with singleton pregnancy at term undergoing an elective cesarean section for isolation of adipose cells.</td>
<td>Tx: 2 capsules 2 x/day containing 800mg DHA and 1200mg EPA (total 2,000mg PUFA). Control: matching placebo capsules. Given from week 10-16 until delivery.</td>
<td>Lower maternal CRP expression in treatment group than control group and decreased significantly over time; control group levels increased. Treatment group had lower IL-6, IL-8, TNFα, TLR4 in adipose and placental tissue.</td>
<td>15</td>
</tr>
<tr>
<td>Harper, et al. (2013).</td>
<td>N=343 United States</td>
<td>Maternal blood samples were collected between 16-22 weeks gestation before starting treatment, and again between 25-28 weeks for cytokine analysis in women with a history of prior preterm birth.</td>
<td>All participants received weekly injections 17 OHPC (250mg). Tx: daily supplement of 2,000mg omega-3 PUFAs. Control: matching placebo capsules. Treatment initiated at enrollment (16-22 weeks).</td>
<td>No differences in cytokine expression based on supplementation.</td>
<td>11</td>
</tr>
</tbody>
</table>
### Table 2.1 (continued) Characteristics of included studies on omega-3 intake and inflammation

<table>
<thead>
<tr>
<th>Author (Date); Participants</th>
<th>Sample/Setting</th>
<th>Measures</th>
<th>Intervention</th>
<th>Key Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Krauss-Etschmann, S., et al. 2008).</strong></td>
<td>N=197 pregnant women. Spain, Hungary, Germany</td>
<td>Maternal blood collected at study entry, 30 weeks and at delivery. Cord blood was also collected delivery for phospholipid and cytokine analysis.</td>
<td>Treatment: Milk based supplement containing vitamins and minerals plus either fish oil (0.5g DHA and 0.15 g EPA), MTHF (400 mcg 5-MTHF), or both. Control: Milk based supplement. Treatment given from 22 weeks gestation until delivery.</td>
<td>1. Lower maternal expression of IFN-γ, IL-1, CRTH2 and CCR4 and higher TGFB levels at delivery for those who received fish oil. 2. Lower cord blood expression of CCR4, IL-13, IL-4, CXCR3 and IL-1 and higher TGF-β with omega 3 supplement.</td>
</tr>
<tr>
<td><strong>Keelan, et al. (2015).</strong></td>
<td>N=51 allergic pregnant women Australia</td>
<td>Maternal food frequency questionnaire completed at 20- and 30-weeks gestation. Maternal blood samples were collected at 36 weeks, cord blood was collected at birth, and placental tissue was obtained within 5-10 minutes of delivery.</td>
<td>Tx: 4 capsules/day containing 3.7g n-3 PUFA (2.07g DHA, 1.02g EPA). Control group: 4 capsules olive oil/day. Treatment from 20 weeks until delivery.</td>
<td>Specialized pro-resolving mediator precursors in placenta increased in those supplemented.</td>
</tr>
<tr>
<td><strong>Jamilian, M., Samimi, M., Kolahdooz, F., Khalaji, F., Razavi, M., &amp; Asemi, Z. (2016).</strong></td>
<td>N=40 with gestational diabetes. Iran</td>
<td>Maternal blood samples collected at enrollment and 6-weeks after treatment.</td>
<td>Tx: 1000mg fish oil capsules (180mg EPA, 120mg DHA) twice daily for 6 weeks. (mean gestational age at onset 25.3 weeks for placebo, 25.4 weeks for treatment, p=.89).</td>
<td>Maternal expression of CRP, TNFα, and IL-1 were lower after treatment compared to placebo.</td>
</tr>
<tr>
<td>Author (Date); Participants</td>
<td>Sample/Setting</td>
<td>Measures</td>
<td>Intervention</td>
<td>Key Findings</td>
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<tr>
<td>Warstedt, et al. (2009).</td>
<td>N=99 Sweden</td>
<td>At enrollment a 3-day food diary and maternal blood sample. Medical data obtained from medical record. Maternal adipose tissue was collected if cesarean section. Maternal blood samples obtained again within one week of delivery.</td>
<td>Tx: 9 capsules/ day (1.6g EPA, 1.1g DHA) and 23mg α-tocopherol. Control: 9 soybean oil capsules containing. From 25 weeks gestation continued through pregnancy.</td>
<td>Maternal PMBC expression of PGE_2 secretion decreased over time for those supplemented; increased for placebo group.</td>
</tr>
</tbody>
</table>

Legend: Tx-treatment; EPA- eicosapentaenoic acid; DHA- docosahexaenoic acid; g-grams; mg-milligrams; mcg-micrograms; PUFA-polyunsaturated fatty acid; PMBC-; MTHF-methyl –tetra-hydrofolic acid; IL-Interleukin; TNF-α-tumor necrosis factor alpha; TLR4-toll like receptor 4; 17 OHPC-17- hydroxyprogesterone caproate; CCR4-chemokine receptor 4; CXCR3-chemokine receptor 3; TGF-β-transforming growth factor beta; CRP-c reactive protein; IFN-γ-interferon gamma; CRTH2- chemoattractant receptor-homologous molecule expressed on Th2 cells.
**Strengths of the review**

This systematic review has several strengths. Randomized controlled trials were the only type of study included, which is the most rigorous research design. The combined evidence provides a higher level of evidence than the individual research studies. Weaknesses also exist as this review is only as strong as the rigor of the individual studies. As discussed previously, there are issues with the ability to interpret the quality and methodology used by the researchers. Wide variation among the studies existed for timing of treatment initiation, dosages of n-3, and the vehicle used to administer it which impact the ability to both interpret and generalize findings. In addition, publication bias cannot be excluded or evaluated, as it is not possible to know if preference was given to specific authors or institutions. One final limitation for this review is the limited amount of published results available for inclusion with only seven trials available.

**Weaknesses of evidence**

Sources of bias cannot be eliminated in any research study. The authors did not thoroughly describe the study protocol, including sampling technique, randomization, study site, or qualifications of those involved in the research which limits the ability to fully evaluate whether other sources of bias may exist. Sampling bias could have affected the results of the studies with most using voluntary recruitment and relatively small samples. The largest sample size was 343, the smallest only 4. Pregnant women are considered vulnerable and subject to more rigorous protection measures than adults in general which makes study design and recruitment more challenging. These are relatively small samples for randomized trials and only two conducted power analyses to justify size and ensure confidence in the findings (Jamilian et al., 2016; Warstedt et al., 2009).
Voluntary recruitment was the default for all included studies and may have resulted in sampling bias. Random assignment was not always blinded to both participants and researchers, with limited descriptions often not detailed enough to evaluate the reliability of this crucial process. Minority inclusion was not given priority in any included studies. Minorities, specifically African Americans, are disproportionately affected by prematurity, which could affect results obtained as well ("Preterm Birth: Causes, Consequences, and Prevention," 2006).

Other factors may have influenced findings such as compliance and study variables. Compliance with the intervention could have also affected results as pregnant women may have difficulty with taking the supplement due to gastrointestinal issues common to pregnancy and was one of the main sources of dropout reported among included studies. Not all investigators measured the impact of supplementation on maternal fatty acid status, and only one study measured maternal dietary intake prior to treatment. One study included obese/overweight women, one included only those having/at risk for atopy and one only included those with GDM, which are conditions that can also affect immune function and impact maternal inflammatory state and response to n-3. These studies did not compare affected women to normal controls in order to evaluate group differences based on supplementation. This would allow broader understanding of which circumstances n-3 supplementation affects disease related inflammation in pregnancy as well.

The lack of theoretical framework included to guide study design and interpretation is a weakness to for all included studies. Inclusion of a framework would have guided timing of interventions and cytokine measures. Only three of seven studies reported reliability and validity measures for the biochemical analyses such as coefficient of
variances (Maricela Haghiac et al., 2015; Jamilian et al., 2016; Warstedt et al., 2009). This limits the ability to determine the precision of the laboratory performance and consistency of the findings for the phospholipid and cytokine analyses that are key to these studies.

**Summary of Research**

The evidence provided by most studies supported a decrease in inflammation in response to n-3 fatty acids supplementation. The research in five of seven studies supported differences between immune biomarkers based on treatment status exist (M. Haghiac et al., 2015; Jamilian et al., 2016; Keelan et al., 2015; Krauss-Etschmann et al., 2008; Warstedt et al., 2009) and two did not (Garcia-Rodriguez et al., 2012; Harper et al., 2013).

Maternal levels of some proinflammatory cytokines were different between those who received supplements containing n-3 than those who did not. Levels of proinflammatory CRP were significantly lower in women who received n-3 than those who did not (M. Haghiac et al., 2015; Jamilian et al., 2016). Levels of CRP also decreased over time in the treatment group, and increased from mid to late pregnancy for those who received placebo (Maricela Haghiac et al., 2015). Maternal levels of inflammatory TNFα and IL-1 were also lower for those supplemented, as was interferon (IFN)-γ (Jamilian et al., 2016; Krauss-Etschmann et al., 2008). Maternal adipose tissue also had lower levels of inflammatory cytokines IL-6, IL-8, and TNF-α for those women in the treatment group than those in the control group (Maricela Haghiac et al., 2015).

Fetal levels of some pro-inflammatory cytokines were lower for those who received supplementation than those who did not. Cord blood IL-1 was lower in those supplemented (Krauss-Etschmann et al., 2008). Placental cytokine expression of IL-8 and IL-6 was also lower for the treatment group than the control group (Maricela Haghiac et al., 2015).
Findings were conflicting for placental TNF-α, with lower levels reported by M. Haghaic et al. (2015), and 14 fold higher expression reported by Keelan et al. (2015). However, the findings from two studies did not support a difference based on fish intake or supplementation (Garcia-Rodriguez et al., 2012; Harper et al., 2013). Garcia-Rodriguez et al. (2012) provided the supplementation in the form of salmon two times per week and was the lowest dosage of all included studies. This may account for the lack of differences as the dosing was too low to affect inflammation. Despite higher dosages in other studies, there were no significant adverse events reported attributable to the intake of n-3. Harper et al. (2013) provided a daily supplement of n-3, but also administered weekly 17-hydroxyprogesterone injections to the women with prior history of PTB. The progesterone supplement is an approved treatment to reduce the risk of recurrent spontaneous PTB. Progesterone production in pregnancy promotes Th2 mediated immunity and production of IL-4 and IL-5, which in turn suppresses Th1 mediated immunity (Piccinni, Scaletti, Maggi, & Romagnani, 2000). This may not only explain how 17-hydroxyprogesterone reduces PTB, but why there were no additional differences bases on supplementation with n-3.

Levels of anti-inflammatory cytokines were either not changed at all, or lower in those treated. Cord levels of IL-4 and IL-13 were lower in cord blood among mothers in the treatment group, as were higher placental levels of SPM precursors (Keelan et al., 2015). The lack of measurable effects of n-3 on pro-inflammatory cytokines was not unexpected as Graham et al. (2017) reported that anti-inflammatory cytokines did not change significantly in pregnancy in general.
Prostaglandin production also affects pregnancy. This eicosanoid arises from n-6 AA and induces both IL-6 and IL-8 production. These cytokines induce cellular changes in the amniotic membranes that may lead to ruptured membranes and PTB (Bagga, Wang, Farias-Eisner, Glaspy, & Reddy, 2003; Bowen, Chamley, Keelan, et al., 2002). Prostaglandin E2 (PGE2) production is enhanced by IL-1, IL-6 and TNF-α, and is a potent stimulatory of uterine contractions and is a crucial element to the initiation of labor (Bowen, Chamley, Keelan, et al., 2002). Secretion of PGE2 decreased in the majority of women supplemented with n-3 (64%), and increased in most of those who did not receive it (77%, p=.002), despite no significant differences in cytokine expression (Warstedt et al., 2009). This study only included women with allergies, which is a known altered state of immune function, which may have unknown effects on maternal immune and inflammatory status. Another study including only women with allergies only explored inflammatory factors expressed in placental tissues which prevents comparisons, but the potential impact of allergic status on PGE2 cannot be eliminated.

Implications

Recent studies confirm the effect of n-3 fatty acid supplementation on some proinflammatory cytokines in pregnancy. While increasing the amount of n-3 intake in pregnancy is likely beneficial for pregnant women, there is much that remains unclear. Further research is needed for clear guidelines and recommendations to emerge. A recent review by Calder (2015) cited available evidence supporting a dosage between 1.35 and 2.7 g EPA/day as optimal for anti-inflammatory effect in adults. Due to high fetal demands during gestation, the optimal dosage may be insufficient to meet both maternal and fetal demand and promote improved n-3:n-6 ratios needed for inflammatory modulation. Both
DHA and EPA were administered in the included studies, but the effects may differ for them alone or in differing ratios. Future studies should aim to more clearly define the optimal dosage of DHA and/or EPA and timing of treatment, and the optimal vehicle to administer them in.

Maternal blood was the most widely used medium and utilized several different types of analyses including serum, red blood cell, and gene expression measures. The operational definition of inflammation included a wide range of biomarkers. Maternal biomarker patterns during gestation are poorly understood, making comparisons among the included studies difficult. Greater understanding of inflammation in pregnancy is needed to establish optimal biomarkers to measure inflammation in pregnancy.

Samples that are more inclusive and representative of the population are needed. Grouping based on characteristics such as BMI, race, age, or perinatal risks may aid in determining those who may benefit most. Further research is essential to develop a broader understanding of inflammation in pregnancy and when or how n-3 supplements may result in the most beneficial outcomes.

Clinically, there is good reason to recommend inclusion of n-3 supplements for all pregnant women. The Western diet includes high amounts of n-6 fats and insufficient amounts of n-3. This is more problematic for pregnant women with the current restrictions on intake of fish. The included studies did not report any significant adverse effects attributable to supplementation except for stomach upset.

**Conclusions**

During the last decade the rate of PTB has changed little and has increased in recent years. Gestational diabetes and hypertensive disorders of pregnancy, along with preterm
birth represent significant numbers of affected pregnancies, with significant associated monetary costs. There are no current recommendations from the American College of Obstetricians and Gynecologists to include n-3 supplements for women who are pregnant. A recent Cochrane review supports the ability of n-3 supplement to reduce the risk of PTB, early PTB (<34 weeks) and low birth weight infants due to high quality evidence. It is time to align practice with the evidence and recommend all pregnant women receive omega-3 supplements.
CHAPTER THREE: Systematic Review Supports Improved Perinatal Outcomes with Higher Intake of Fruits and Vegetables

Introduction

Pregnancy and birth outcomes are generally positive in the U.S., but increasing proportions are affected by adverse maternal or neonatal outcomes. Preeclampsia and gestational hypertension (GHTN) affects 912.4 per 10,000 pregnancies, and has increased steadily in recent years (CDC, 2017a). Preterm birth affects nearly 10% of all births in the U.S. and has risen the last two years following a steady decline since 2007 (H. B. Martin JA, Osterman MJK, 2017). Gestational diabetes mellitus (GDM) is another adverse outcome for pregnant women that increases maternal and infant morbidity with prevalence as high as 9.2% in the U.S. (DeSisto et al., 2014). Fetal growth restriction (weight below 10th percentile for gestational age) affects approximately 10% of the nearly four million births nationwide (Hamilton, 2017), with both immediate and long-term risks associated. The percentage of infants with low birthweight (less than 2,500 grams) has increased the last two years following a seven year decrease (Hamilton, 2017).

Background

Preeclampsia is defined as hypertension after 20 weeks gestation with proteinuria or signs of organ involvement, where GHTN only involves hypertension after 20 weeks. Maternal risk factors for preeclampsia include pre-pregnant obesity, being nulliparous, family history of hypertensive disorder in pregnancy, diabetes, and maternal age and are similar to cardiovascular disease risks (Ramsay, Stewart, Greer, & Sattar, 2003). It is the leading cause of maternal and fetal morbidity and mortality in pregnancy and affects nearly 10 million pregnancies worldwide (Foundation, 2013; Baha Sibai, Gus Dekker, & Michael
Kupferminc, 2005). Preeclampsia accounts for one-third of preterm deliveries, one-fourth of growth restricted infants, and 15-20% of maternal mortality in developed countries with associated costs in the U.S in 2012 at $2.18 billion (Foundation, 2013; Baha Sibai et al., 2005; Stevens et al., 2017). Women in developing countries are seven times more likely to develop preeclampsia (Foundation, 2013). Preeclampsia also increases both maternal and offspring risks for future hypertension, heart disease, stroke and type 2 diabetes (Ramsay et al., 2003; Baha Sibai et al., 2005).

Another adverse pregnancy outcome is preterm birth (PTB) which occurs between 22 and 37 completed weeks of gestation. Early PTB occurs less than 34 weeks, while late PTB is 34-36 weeks. Extremely preterm is <28 weeks, and very preterm 28-32 weeks gestation. About half of all PTB are spontaneous, which occurs after spontaneous labor or premature rupture of membranes. Provider initiated PTB may occur for medical (maternal or fetal compromise) or non-medical (convenience or maternal request) reasons. The most recent estimate reports costs of PTB at $26 billion per year in the U.S. ("Preterm Birth: Causes, Consequences, and Prevention," 2006). It is a leading cause of infant mortality and lifelong disability (Bolton, Bush, Hurst, Kotecha, & McGarvey, 2015; CDC, 2017b; Heinonen et al., 2015; Luyckx, 2017; Nuyt, Lavoie, Mohamed, Paquette, & Luu, 2017). Maternal history of prior PTB is the most significant risk factor for subsequent PTB, but other known risks are maternal smoking, non-Hispanic black race, lower socioeconomic status, pre-pregnant obesity, maternal infection, and maternal stress.

Gestational diabetes is characterized by insulin resistance and hyperglycemia in pregnancy and increases maternal risk for preeclampsia, cesarean delivery and birth trauma, infant risk of being large for gestational age, PTB, birth defects or respiratory
distress (Catalano et al., 2012; C. Wang & Yang, 2016). It is typically diagnosed by oral glucose tolerance test at 24-28 weeks gestation. Maternal risk factors for GDM are older age, family history of diabetes, being overweight or obese, excessive weight gain during pregnancy, and Asian, Pacific Islander, American Indian or non-Hispanic black race/ethnicity (Pu et al., 2015). Long term risk for Type 2 diabetes is higher with pregnancy complicated by GDM for both mother and offspring (DeSisto et al., 2014).

Less than optimal fetal or newborn growth is another common adverse pregnancy outcome. Small for gestational age (SGA) are defined as below 10th percentile for gestation. Growth restriction is present in about 10% of births in developed countries, and about six times higher in developing countries (Saleem et al., 2011). In 2010, 27% of live births, or 32.4 million infants, were born SGA in low and middle-income countries, with 10.6 million born term low birthweight (Lee et al., 2013). Poor uteroplacental perfusion and fetal undernutrition are the pathophysiological causes for poor fetal growth. Growth restricted fetuses are at increased risk for fetal or neonatal death, cognitive delay in childhood and chronic diseases in adulthood (Salam, Das, & Bhutta, 2014). Growth restricted newborns are at increased risk of complications such as hypoglycemia, hypothermia, hyperbilirubinemia, seizure, and respiratory distress ("ACOG Practice bulletin no. 134: fetal growth restriction," 2013). Risk factors for growth restriction include pregnancy related hypertension (GHTN or preeclampsia), pre-gestational diabetes, tobacco, drug or alcohol use during pregnancy, multiple gestation, African American race, lower socioeconomic status and maternal infection (L. McCowan & Horgan, 2009). Diabetes during pregnancy, whether onset is before pregnancy or after, is a risk factor for both growth restriction and growth excess. Large for gestational age (LGA) is defined as above
the 10th percentile for gestational age, and macrosomia is more than 4,000 grams (about 9 pounds) at birth regardless of gestational age. Large infants are at increased risk of birth trauma, and hypoglycemia while maternal risk is increased for birth trauma and cesarean delivery.

One prominent risk factor for adverse pregnancy outcomes is adequacy of gestational weight gain. Gestational weight gain is simply how much weight the mother gains over the course of pregnancy. The Institute of Medicine published guidelines in 2009 for appropriate GWG. Recommendations for appropriate GWG are based on pre-pregnant body mass index (BMI) for underweight, normal weight, overweight or obese women. More than optimal gains are associated with increased pregnancy risks such as preeclampsia, GDM, and macrosomia/LGA, especially for overweight or obese women (Flick, Brookfield, Tudela, Duthely, & González-Quintero, 2010; Frederick, Williams, Sales, Martin, & Killien, 2008; Spiegler et al., 2013). Women who were underweight and/or had suboptimal GWG had higher risk of SGA and PTB (Goldstein et al., 2017; Liu, Dai, Dai, & Li, 2012).

Dysregulation of inflammatory state and oxidative stress are associated with adverse gestational outcomes. Women with preeclampsia and preterm birth (PTB) have higher expression of inflammatory cytokines and oxidative stress during pregnancy (Brou et al., 2012; Bullen et al., 2013; Burton & Jauniaux, 2004; Cobo et al., 2013; Coussons-Read et al., 2012; Undurti N. Das, 2015; Han, Ha, Park, Kim, & Lee, 2011; Harmon et al., 2016; Hecht et al., 2008; Moghaddam Banaem et al., 2012; Ruiz et al., 2012). Obesity prior to pregnancy along with excess maternal weight gain also alters maternal inflammatory status in pregnancy and increases risk for both PTB, gestational diabetes (GDM) and
preeclampsia (Bullen et al., 2013; Cnattingius et al., 2013; Dong et al., 2017; Han et al., 2011). Excess GWG is associated with altered inflammation and increased risk for PTB (Chavan et al., 2015). Hyperglycemia and insulin resistance in pregnancy trigger endothelial dysfunction and inflammation and increase the risk of preeclampsia (Catalano et al., 2012; Ramsay et al., 2003).

Fruit and vegetable intake have been implicated in chronic disease incidence and management. Intake of fruits and vegetables has been demonstrated to protect against type 2 diabetes, cancer and cardiovascular disease (Carter, Gray, Troughton, Khunti, & Davies, 2010; Cooper et al., 2012; He et al., 2004) possibly related to the antioxidant and anti-inflammatory properties they possess (Wu & Schauss, 2012; Zhang et al., 2015). Though fruits and vegetables alone or as part of a dietary pattern are known to impact other health problems related to inflammation and oxidation, less is known about their influence on pregnancy outcomes. Therefore, the purpose of this systematic review is to evaluate the current state of the science on the impact of maternal dietary consumption of fruits and/or vegetables on the incidence of PTB, GDM, preeclampsia, SGA, as well as GWG and measures of inflammation or oxidative stress in pregnancy.
Methods

The purpose of this systematic review is to investigate the influence of dietary fruit and/or vegetable intake on perinatal outcomes and associated inflammation or oxidative stress. The aims are to: 1. evaluate the relationship between fruit and vegetable intake and adverse pregnancy and birth outcomes. 2. evaluate the relationship between fruit and vegetable intake and inflammatory/oxidative stress biomarkers.

Databases searched were PubMed, CINAHL, and Medline. Search terms for dietary intake were “fruit” or “vegetable”. The population of interest was during pregnancy terms with modifiers were: “pregnan*”, “prenat*”, “gestat*”, or “maternal” were included. Based on outcomes of interest the terms “preterm”, “premature”, “preeclampsia”, “diabetes”, “inflammat*”, “oxidat*”, “IUGR”, “SGA”, “weight”, and “gain” were used. Searches were connected by Boolean operators “AND” and “OR” to retrieve all possible relevant articles. Articles were filtered to include the last 15 years, English only, and in humans only. Primary experimental, observational, cross-sectional and case-control study designs were included. There were 271 articles retrieved after duplicates were removed. Title and abstract review were used to select 28 articles based on the following criteria for inclusion: whole fruit or vegetable intake (not nutrients or extracts) alone as an independent variable; one or more of the outcomes of interest as dependent variables (PTB, GWG, fetal growth (IUGR/SGA), preeclampsia/GHTN, GDM, or inflammation/oxidative stress). Those meeting inclusion criteria were saved in EndNote with full text publication obtained and attached to the reference. Exclusion criteria applied to the remaining 28 articles upon full article review were: an educational intervention was the independent variable (n=3), “fruit drink”, fruit extract or macronutrient intake was used instead of fruit (n=4), or outcomes
other than those of interest (n=2). In total, 19 articles were included in this systematic review (Figure 3.1).

A data table was used to organize and synthesize the included studies based on study location, sample type and size, study purpose, dietary measures, pregnancy outcome, and key findings (Table 3.1). The outcomes of interest for this review were fruit and or vegetable intake and the pregnancy outcomes of interest (PTB, GWG, fetal growth (IUGR/SGA), preeclampsia/GHTN, GDM, or inflammation/oxidative stress). Data were extracted and analyzed based on gestational outcome.

Quality assessment of all included publications was assessed based on study type (observational cohort, cross-sectional and case-control studies). Newcastle-Ottawa scale for case-control (Appendix A) and cohort studies (Appendix B) were used for their respective study types, as well as a modified version for cross-sectional studies (Appendix C). These scales evaluate quality based on participant selection, comparability of groups, and outcome. Scores range from zero to nine with lower scores representing lower quality observational studies. For the purposes of this review, high quality studies were scored seven to nine, moderate quality four to six, and poor quality zero to three.

**Dietary intake and birth outcome variables**

*Preeclampsia.* All included studies evaluating preeclampsia as an outcome of interest used the same criteria for diagnosis of preeclampsia: Hypertension with systolic blood pressure of 140mmHg or higher and or diastolic blood pressure of 90 or greater on two separate occasions at least four hours apart and occurring after 20 weeks gestation and prior to labor, or after delivery, along with either proteinuria or other signs of multiorgan involvement. Signs of multiorgan involvement were: acute renal insufficiency (defined as
a new increase in serum creatinine concentration ≥100 µmol/L antepartum or >130 µmol/L postpartum); liver dysfunction (defined as high transaminases, severe right upper quadrant or epigastric pain or liver rupture); neurological symptoms (eclampsia, severe headache, hyperreflexia, visual disturbance, or cerebral hemorrhage); blood disorders such as thrombocytopenia (platelets <100,000), disseminated intravascular coagulation, or hemolysis.

_Fetal growth_. Small for gestational age (SGA) was most often used to describe infants with poor growth in utero. The infant weight at birth below the 10th percentile was the primary characteristic and was usually adjusted for gestational age and sex of the infant. In some instances, it was also adjusted based on maternal height, pre-pregnant weight, or ethnicity. Low birthweight is sometimes used to define infant growth and is defined as infants born weighing less than 2,500g regardless of gestational age. Individual anthropometric measures such as birth weight, length or head circumference were used by Loy et al to describe infant growth in relation to gestation (2011).

_Gestational weight gain/adequacy_. Gestational weight gain was calculated as the maternal weight at delivery minus maternal weight prior to pregnancy. Maternal pre-pregnant weight was either self-reported or the measured weight at first prenatal visit was used. Weight prior to delivery was either performed on a scale, or by patient self-report. Pre-pregnant BMI was calculated in order to determine adequacy of weight gain and used pre-pregnant weight and either measured height, self-report or medical record to determine height. The formula for BMI is weight in kilograms divided by height(meters) squared. The Institute of Medicine (IOM) guidelines for GWG were used in all included studies to determine adequacy of maternal gain (Figure 3.2).
Preterm birth. Gestational age was calculated from either the last menstrual period of the mother or based on ultrasound measures. First trimester ultrasound is considered the most accurate at estimating gestational age and was used to adjust due dates if this measure varied from last menstrual period dates by more than a week. Preterm birth occurred if gestational age was less than 37 weeks. One study focused on a specific category of PTB with gestational age 32 weeks to 36 weeks 6 days and called late to moderate preterm birth (LMPT) (Smith et al., 2015)

Inflammation/Oxidative stress. The two studies that measured inflammation in pregnancy used maternal serum biochemical assays to evaluate the inflammatory state in pregnancy. Maternal samples were collected at 14-16 weeks in one study (Vieira et al., 2017), the other collected samples at approximately 30 weeks gestation (Hrolfsdottir et al., 2016). Six biomarkers were measured by Hrolfsdottir et al. (2016), and 54 were included by Vieira et al (2017). There were no studies evaluating oxidative stress and diet in pregnancy.

Gestational Diabetes Mellitus. Impaired glucose tolerance meeting criteria for GDM was defined as maternal fasting glucose ≥95mg/dl (high glucose) or adequate if level was below 95mg/dl, or as glucose tolerance test level of ≥140mg/dl compared to normal or adequate if glucose was less than 140mg/dl. The American Congress of Obstetricians and Gynecology (ACOG) supports two step testing, first with screening with 50 g oral glucose followed by serum glucose measure an hour later ("ACOG Practice Bulletin No. 190: Gestational Diabetes Mellitus," 2018). Abnormal results are considered above 140mg/dl and indicate further testing using a 100g three-hour oral glucose challenge test. Abnormal results on two or more values for either fasting (above 95-105mg/dl), hour one
(above 180-190mg/dl), hour two (above 155-165mg/dl), or hour 3(above 140-145mg/dl) indicate GDM is present. Values used vary based on different laboratory standards and which of two criteria are used for comparison.

**Dietary intake of fruits and vegetables.** Significant heterogeneity of dietary measures existed among studies. Intake was measured most often using food frequency questionnaire (FFQ), but also included four-day food record or other investigator determined methods. They were either self-administered or by interview. Validated dietary tools were used in less than half the included studies. Timing of administration of questionnaires occurred through all trimesters and postpartum; the time period of interest could be from a few days up to a full year prior to the assessment. Dietary intake was evaluated as continuous intake or categorized based on volume or frequency of intake and adequacy.

**Results**

**Study Characteristics**

Studies included in the review were published 2004-2017 (Table 3.1). There were 12 cohort studies (quality score range 6-8; mean=6.8), four cross-sectional studies (quality score range 6-7; mean=6.8), three case-control studies (quality score range 5-9; mean=6.3) (Appendix E.).

All but four of the included studies were conducted in developed countries (Asefa & Nemomsa, 2016; Endeshaw, Abebe, Bedimo, & Asart, 2015; Longo-Mbenza, Kadima-Tshimanga, Buassa-bu-Tsumbu, & M'Buyamba, 2008; Loy et al., 2011). Sample sizes ranged from 95 to 43,585 pregnant women. Dietary data were collected from early pregnancy to after delivery and primarily self-reported. About half the researchers used
validated dietary tools, with most of those not validated for use in pregnant women. Though 19 publications were included in this review, four used a single database from New Zealand (SCOPE) (Kenny et al., 2014; L. M. McCowan et al., 2010; North et al., 2011; Vieira et al., 2017). Five studies originated in Scandinavian countries (Bärebring et al., 2016; Hrolfsdottir et al., 2016; Merkx, Ausems, Bude, de Vries, & Nieuwenhuijze, 2015; Mikkelsen, Osler, Orozova-Bekkevold, Knudsen, & Olsen, 2006; Myhre et al., 2013). Two studies were conducted in Ethiopia (Asefa & Nemomsa, 2016; Endeshaw et al., 2015). These two plus Longo-Mbenza et al. (2008) originated in African countries. Two each from Asia (Loy et al., 2011; J. Wang et al., 2017) and Europe (Ramon et al., 2009; Smith et al., 2015) were included as well. Six studies evaluated gestational outcomes that included preeclampsia or hypertension in pregnancy; five included GWG and six fetal growth. Four studies included PTB, two measured inflammation, and a single study evaluated maternal glucose tolerance/GDM.
<table>
<thead>
<tr>
<th>Reference</th>
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<th>Dietary assessment</th>
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<th>Quality/ type</th>
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</thead>
<tbody>
<tr>
<td>Asefa, F., &amp; Nemomsa, D. (2016).</td>
<td>Ethiopia.</td>
<td>Women delivering at healthcare facilities in Ethiopia (N=411).</td>
<td>Assess weight gain during pregnancy and its associated factors.</td>
<td>Interview after delivery to evaluate dietary pattern average throughout pregnancy.</td>
<td>GWG</td>
<td>Those who consumed fruit and vegetables and meat at least weekly were more likely to have adequate gains.</td>
<td>7; cs</td>
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<tr>
<td>Bärebring, L., Brembeck, P., Löf, M., Brekke, H. K., Winkvist, A., &amp; Augustin, H. (2016).</td>
<td>Sweden.</td>
<td>Swedish pregnant women who perceive themselves as healthy, having an uncomplicated pregnancy, in gestational week 35–37 at the study visit, between 25 and 40 years of age (N=95).</td>
<td>Investigate if food intake is associated with gestational weight gain (GWG) in Swedish women</td>
<td>At enrollment (35-37 weeks) 4-day food record (four consecutive days, including at least 1 weekend day).</td>
<td>GWG</td>
<td>No significant relationships between GWG and fruit and vegetable intake.</td>
<td>7; coh</td>
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<tr>
<td>Endeshaw, M., Abebe, F., Bedimo, M., &amp; Asart, A. (2015).</td>
<td>Ethiopia</td>
<td>Pregnant women who stayed in the region at least 6 months and received prenatal care or delivered in public health facilities (N=453).</td>
<td>Determine the effects of dietary habits on preeclampsia.</td>
<td>Dietary data collected at enrollment (20 weeks gestation to 48 hours postpartum) by a trained midwife using pretested tools.</td>
<td>PE</td>
<td>Intake of fruit or vegetables at least 3 times/week was protective against PE (AOR .46 and .51 respectively).</td>
<td>9; cc</td>
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<td>Guilloty, N. I., Soto, R., Anzalota, L., Rosario, Z., Cordero, J. F., &amp; Palacios, C. (2015).</td>
<td>Puerto Rico</td>
<td>Pregnant women age 18-40 with healthy pregnancies and had completed dietary data (n=160).</td>
<td>Describe the dietary patterns in healthy pregnant woman and determine the association between diet factors, pre-pregnancy BMI and socio-demographic characteristics with GWG.</td>
<td>Dietary assessment 20-28 weeks gestation using self-administered FFQ.</td>
<td>GWG</td>
<td>No differences in GWG based on fruit or vegetable intake.</td>
<td>6; coh</td>
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<td>*Hrolfsdottir, L„ Schalkwijk, C. G„ Birgisdottir, B. E„ Gunnarsdottir, I„ Maslova, E„ Granström, C„ . . . Halldorsson, T. I. (2016).</td>
<td>Denmark</td>
<td>Pregnant women with singleton gestation and received care at a clinic in Aarhus (N=965).</td>
<td>Examine the relationship between GWG, diet and inflammation in pregnancy.</td>
<td>Dietary intake was collected by self- administered FFQ and face to face interview in gestational week 30.</td>
<td>GWG and maternal serum markers of inflammation.</td>
<td>Plasma CRP and SAA were 3% higher and IL-8 was 2% lower for each 1kg increase in GWG the first 30 weeks of pregnancy. Intake of plant protein was inversely associated with CRP and SAA compared to animal protein intake.</td>
<td>6; cs</td>
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<td>Kenny, L. C„ Black, M. A„ Poston, L„ Taylor, R„ Myers, J. E„ Baker, P. N„ . . . North, R. A. (2014).</td>
<td>New Zealand, Australia, United Kingdom, and Ireland. Part of SCOPE cohort.</td>
<td>Healthy nulliparous women with singleton pregnancies (N=5,623).</td>
<td>Develop a multivariable predictive model for the prediction of preeclampsia; and to develop multivariable models to predict specific types of preeclampsia.</td>
<td>Dietary information using FFQ was collected 14-16 weeks.</td>
<td>PE and timing of onset.</td>
<td>High intake of fruit (≥ 3 pieces of fruit/day) in mid pregnancy was protective against PE and term PE. Endoglin was higher in those with early-onset PE. IL-1Ra was lower in those with preterm PE.</td>
<td>7; coh</td>
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<tr>
<td>Longo-Mbenza, B„ Kadima-Tshimanga, B„ Buassa-bu-Tsumbu, B„ &amp; M'Buyamba, K„ Jr. (2008).</td>
<td>Democratic republic of Congo</td>
<td>Pregnant women who did not have chronic hypertension (N=238).</td>
<td>Assess the incidence of GHTN, and if vegetable intake and physical activity are protective against GHTN among rural women from the Democratic republic of Congo.</td>
<td>First trimester dietary questionnaire.</td>
<td>GHTN/PE</td>
<td>Consuming at least 3 servings of vegetables per day was protective against preeclampsia.</td>
<td>6; coh</td>
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<td>Loy, S. L., Marhazlina, M., Azwany, Y. N., &amp; Hamid Jan, J. M. (2011).</td>
<td>Malaysia</td>
<td>Healthy pregnant women with singleton gestation who were Malaysian citizen/ethnicity age 19-40 and planned to give birth at Universiti Sains Malaysia Hospital (N=121).</td>
<td>Investigate the effect of maternal diet on birth size among Malays.</td>
<td>Maternal interview was used to complete FFQ at enrollment (28-38 weeks).</td>
<td>SGA</td>
<td>Leafy vegetables were positively associated with head circumference. Tuberous vegetables were positively associated with birth length and head circumference. Fruit intake was positively associated with larger babies.</td>
<td>6; cs</td>
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<td>McCowan, L. M., Roberts, C. T., Dekker, G. A., Taylor, R. S., Chan, E. H., Kenny, L. C., . . . North, R. A. (2010).</td>
<td>New Zealand, Australia, the United Kingdom, and Ireland Database is part of SCOPE trial.</td>
<td>Healthy nulliparous women with singleton pregnancy at 15 ± 1 weeks of gestation (N = 3560).</td>
<td>Identify risk factors among healthy nulliparous women for SGA.</td>
<td>Dietary information collected at 15±1 week and 20±1 week.</td>
<td>SGA</td>
<td>Those with normotensive SGA were less likely to eat 3 servings/day of green leafy vegetables prior to pregnancy vs normotensive non-SGA. Those eating fruit less than once weekly pre-pregnancy had twice the risk of normotensive SGA.</td>
<td>8; coh</td>
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<td>Merkx, A., Ausems, M., Bude, L., de Vries, R., &amp; Nieuwenhuijze, M. J. (2015).</td>
<td>Netherlands September 2012-November 2012.</td>
<td>Healthy pregnant women of all gestational ages who agreed to participate and completed questionnaires (N=550). Excess GWG n=173.</td>
<td>Gain knowledge of healthy pregnant women's GWG and identify factors associated with healthy GWG.</td>
<td>Dietary intake of fruit, vegetables and fish by either mail or phone interview using FFQ.</td>
<td>GWG</td>
<td>Pre-pregnant BMI nor diet were associated with adequacy of GWG.</td>
<td>7; cs</td>
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<td>Mikkelsen, T. B., Osler, M., Orozova-Bekkevold, I., Knudsen, V. K., &amp; Olsen, S. F. (2006).</td>
<td>Denmark, Part of Danish National Birth Cohort (MoBa).</td>
<td>Pregnant women who had singleton live births were included in the analyses (N=43,585).</td>
<td>Examine the relationship between fetal growth and fruit and vegetable intake.</td>
<td>Dietary data collected at 25 weeks using validated self-administered FFQ.</td>
<td>Birthweight</td>
<td>Higher green leafy vegetable intake was associated with increase in birth weight.</td>
<td>6; coh</td>
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<tr>
<td>Mitchell, E. A., Robinson, E., Clark, P. M., Becroft, D. M., Glavish, N., Pattison, N. S., Wild, C. J. (2004).</td>
<td>New Zealand</td>
<td>Pregnant women who delivered singleton gestation at term (n=844 SGA; n=870 AGA).</td>
<td>Evaluate the effect of maternal diet during pregnancy and the risk of delivering an SGA baby.</td>
<td>FFQ completed at enrollment in early pregnancy (mean 8.2± 1.9 weeks gestation), and after delivery (mean 1.4±1.3) weeks.</td>
<td>SGA</td>
<td>No significant effect of diet on fetal growth.</td>
<td>5; cc</td>
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<tr>
<td>Myhre, R., Brantsaeter, A. L., Myking, S., Eggesbo, M., Meltzer, H. M., Haugen, M., &amp; Jacobsson, B. (2013).</td>
<td>Norway, Part of Norwegian Mother and Child Cohort (MoBa).</td>
<td>Healthy pregnant women age 20-35 with singleton gestation with complete dietary and general health questionnaires (N=18,888). Spontaneous PTB n=950.</td>
<td>Evaluate the influence of foods with antimicrobial and prebiotic components on risk of spontaneous PTB.</td>
<td>Valid dietary FFQ self-administered at approximately 17-22 weeks gestation.</td>
<td>PTB</td>
<td>Higher intake of allium and dried fruit lowered risk of PTB.</td>
<td>7; coh</td>
</tr>
<tr>
<td>North, R. A., McCowan, L. M., Dekker, G. A., Poston, L., Chan, E. H., Stewart, A. W., ... Kenny, L. C. (2011).</td>
<td>New Zealand, Australia, United Kingdom, Ireland. Part of SCOPE dataset.</td>
<td>Healthy nulliparous women with singleton gestation pregnancy (N=4,961).</td>
<td>Develop a predictive model for preeclampsia based on clinical risk factors for nulliparous women and to identify a subgroup at increased risk.</td>
<td>Dietary FFQ was collected 14-16 weeks gestation.</td>
<td>PE</td>
<td>Consuming more than 3 servings/day of fruit was protective against PE.</td>
<td>6; coh</td>
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<td>Ramon, R., Ballester, F., Iniguez, C., Rebagliato, M., Murcia, M., Esplugues, A., . . . . Vioque, J. (2009).</td>
<td>Spain.</td>
<td>Healthy pregnant women at 10-13 weeks gestation at least 16 years old with singleton gestation (n=787). SGA weight 11.5%; SGA length 5.7%.</td>
<td>Determine if fruit and vegetable intake during pregnancy was associated with fetal size at birth.</td>
<td>FFQ completed first trimester at 10-13 weeks and in the third trimester 28-32 weeks</td>
<td>Infant birth size (length, weight, SGA).</td>
<td>Low first and third trimester vegetable intake was associated with lower mean birthweight vs those with higher intake. Higher vegetable intake during the first trimester lowered SGA risk for weight. Higher vegetable intake during the third trimester for was associated with lower risk for SGA for length.</td>
<td>8; coh</td>
</tr>
<tr>
<td>Smith, L. K., Draper, E. S., Evans, T. A., Field, D. J., Johnson, S. J., Manktelow, B. N., . . . . Boyle, E. M. (2015).</td>
<td>United Kingdom.</td>
<td>All births 32-36+6 weeks (N=1,887).</td>
<td>Evaluate the association between lifestyle factors and LMPT.</td>
<td>Maternal interview soon after delivery established dietary intake.</td>
<td>LMPT birth (32+0 to 36+6 weeks gestation).</td>
<td>Those who ate 5 portions of fruits and vegetables per day less than weekly had higher risk for LMPT birth than those who ate them more frequently.</td>
<td>6; coh</td>
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<td>*Soto, R., Guilloty, N., Anzalota, L., Rosario, Z., Cordero, J. F., &amp; Palacios, C. (2015).</td>
<td>Puerto Rico.</td>
<td>Pregnant women 18-45 years old, &lt;20 weeks gestation with spontaneous healthy, uncomplicated pregnancy who lived in northern karst area (N=180).</td>
<td>Describe dietary patterns of Puerto Rican pregnant women and analyze the association between protective and non-protective diet factors and pregnancy related measurements.</td>
<td>Dietary data collected 20-24 weeks using validated self-administered FFQ.</td>
<td>Maternal glucose tolerance, blood pressure, and length of gestation.</td>
<td>Those who ate vegetables at least weekly were more likely to have high 1-hour glucose test than those who ate them less often.</td>
<td>7; coh</td>
</tr>
<tr>
<td>Vieira, M. C., Poston, L., Fyfe, E., Gillett, A., Kenny, L. C., Roberts, C. T., . . . Pasupathy, D. (2017).</td>
<td>New Zealand, Australia, United Kingdom, and Ireland. Part of SCOPE cohort.</td>
<td>Healthy nulliparous pregnant women with singleton gestation who were normal weight (n=3,106) or obese (n=834) prior to pregnancy (N=3,940).</td>
<td>Compare early pregnancy clinical risks and biomarkers between women with obesity and those with normal BMI to determine risk factors for later preeclampsia.</td>
<td>Dietary assessment at 14-16 weeks by maternal interview.</td>
<td>PE and markers of inflammation.</td>
<td>Eating 3 or more pieces of fruit per day predicted significantly lower odds of developing preeclampsia.</td>
<td>8; coh</td>
</tr>
<tr>
<td>*Wang, J., Zeng, Y., Ni, Z. M., Wang, G., Liu, S. Y., Li, C., . . . Nie, S. F. (2017).</td>
<td>China</td>
<td>Singleton newborn babies without birth defects born at Women and Children Medical. matched with normal birth weight controls (n=708), as were PTB cases (n=472) with term (n=708) controls.</td>
<td>Explore factors associated with PTB and low birth weight.</td>
<td>Interview by a trained investigator after delivery to estimate intake of fruits and vegetables</td>
<td>Birth weight and gestational age.</td>
<td>Consuming 250 g or more of fruit per day in the first trimester increased odds of LBW.</td>
<td>5; cc</td>
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Index: GWG-gestational weight gain; FFQ-food frequency questionnaire, CRP-c reactive protein; SAA-serum amyloid A; PE-preeclampsia; IL-1Ra-interleukin 1 receptor agonist; GHTN-gestational hypertension; SGA-small for gestational age; BMI-body mass index; cc-case control study; coh-cohort study; cs-case study.
Preeclampsia

There were six articles included in the review examining the influence of fruit and vegetable intake with risk for preeclampsia or maternal blood pressure (Endeshaw et al., 2015; Kenny et al., 2014; Longo-Mbenza et al., 2008; Soto et al., 2015; Vieira et al., 2017). Five were cohort and one was case control. Fruit or vegetable intake alone was generally associated with lower risk for preeclampsia in multivariate analyses. Specifically, higher vegetable intake decreased odds of preeclampsia by 20-54% (Brantsaeter et al., 2009; Endeshaw et al., 2015; Longo-Mbenza et al., 2008; Torjusen et al., 2014). Consuming three or more pieces of fruit per day was also protective against preeclampsia (Kenny et al., 2014), with researchers in one study reporting that eating fruit at least three times weekly lowered the odds of preeclampsia by about half (Endeshaw et al., 2015). High maternal blood pressure was not related to dietary intake based on bivariate analyses by Soto et al (2015). This was only included study not using multivariate analyses including covariates.

Results from all studies investigating the effect of maternal diet on preeclampsia using multivariate analyses, support the beneficial effects of vegetables in maternal diet at reducing risk for development of this dangerous condition. Consumption of at least three servings per day of either fruit or vegetables was the most common amount reported to reduce risk. No increased risk of preeclampsia was reported based on intake of fruit or vegetable in any of the included studies. These findings are consistent with a recent meta-analysis of observational studies examining the effect of diet on hypertensive disorders of pregnancy (Schoenaker, Soedamah-Muthu, & Mishra, 2014). Their findings suggested that higher intakes of fruits and vegetables were beneficial on preeclampsia.
Fetal growth

Six articles reported on the impact of fruit and vegetable intake on fetal growth (Loy et al., 2011; L. M. McCowan et al., 2010; Mikkelsen et al., 2006; Mitchell et al., 2004; Ramon et al., 2009; J. Wang et al., 2017). Three cohort studies, two case control and one case study comprise the group evaluating this outcome. Pre-pregnant fruit intake was associated with two-fold lower risk of SGA in women with normal blood pressure (L. M. McCowan et al., 2010). Birthweight also significantly increased with higher fruit intakes (Loy et al., 2011; Mikkelsen et al., 2006), as did birth length and head circumference (Mikkelsen et al., 2006). Maternal intake of at least three servings of green leafy vegetables per day resulted in a greater than 50% reduction in risk of SGA (L. M. McCowan et al., 2010). Loy et al (2011) reported a 1.78 centimeter increase in head circumference for every 10 gram increase in green leafy vegetable intake (p=.04). Those who had highest vegetable intake in their first and third trimester had lower risk for SGA overall (OR=5.5), as well as adjusted for length and weight (first trimester: length OR=1.6, weight OR=3.7; third trimester: length OR=5.5, weight OR=2.1) compared to those with the lowest intakes (Ramon et al., 2009). However, Wang, et al. reported a 70% increased risk of LBW with 300 or more grams of fruit intake per day in the first trimester of pregnancy (2017). Low birthweight may not be representative of optimal growth as it is not gestational age adjusted. No differences were reported in incidence of SGA based on intake by Mitchell et al. (2004).

Overall, improved fetal growth and reduction in risk for SGA was supported by the inclusion of fruit or vegetables in maternal diet by most of the studies, which also had moderate to high quality scores (range 6-8). The two studies that reported either increased
risk or no difference represented the lowest quality scores of those included in this review (five for both). Intake of fruit less than weekly increased risk of SGA, while higher intakes of fruit was beneficial in increasing birth size and reducing risk for SGA. Intake of green leafy vegetables and onion type vegetables also predicted of lower risk of SGA. These findings are consistent with a prior review on the impact of maternal diet on birthweight which supported the ability of dietary patterns including fruits and vegetables to promote optimal birthweight (Grieger & Clifton, 2014).

**Gestational Weight Gain**

Five articles reported findings of the effect of fruit and/or vegetable intake on GWG (Asefa & Nemomsa, 2016; Bärebring et al., 2016; Guilloty et al., 2015; Hrolfsdottir et al., 2016; Merkx et al., 2015). Three case studies and two cohort studies evaluated GWG. In four of the five included studies, one-fourth to half the women exceeded the IOM guidelines for GWG (Bärebring et al., 2016; Guilloty et al., 2015; Hrolfsdottir et al., 2016; Merkx et al., 2015). Substituting energy from plant protein for animal protein reduces risk of excess GWG by 32%, but no differences were reported based solely on vegetable intake (Hrolfsdottir et al., 2016). Combined intake of fruit and vegetables at least once a week decreased the odds of inadequate GWG for women in Ethiopia (AOR 2.7, CI 1.2-6.6, p=.05), as did weekly meat intake (Asefa & Nemomsa, 2016). This study was conducted in Ethiopia and inadequate GWG was more concerning than excessive gains (69.3% inadequate vs 2.7% excessive). Adequacy, excess or inadequacy of GWG did not differ significantly based solely on fruit or vegetable intake in pregnant women in four of the five studies (Bärebring et al., 2016; Guilloty et al., 2015; Hrolfsdottir et al., 2016; Merkx et al., 2015).
Recent studies confirm that appropriate GWG was not predicted by maternal fruit or vegetable intake. A single study suggests a beneficial effect for fruit intake at least three times weekly in those at risk of inadequate GWG.

**Preterm Birth**

Researchers in four studies evaluated the relationship between fruit and vegetable intake and PTB (Myhre et al., 2013; Smith et al., 2015; Soto et al., 2015; J. Wang et al., 2017). Intake of dried fruit and onion-type vegetables was associated with 18% lower odds of PTB (Myhre et al., 2013). The intake of onion-type vegetables also significantly lowered the odds of early PTB (28-32 weeks)(OR .47), as well as late PTB (32-37 weeks)(OR=.83) (Myhre et al., 2013). Those who ate five servings per day of fruits and vegetables less than once a week had 31% higher risk of PTB (Smith et al., 2015). However, intake of fruits and vegetables did not affect risk for PTB based on findings from two included studies (Soto et al., 2015; J. Wang et al., 2017). Soto et al. based findings on bivariate analyses only and was one of the two lowest studies in quality rankings included in the review (score=5). Two studies collected dietary data after delivery which could introduce recall bias (Smith et al., 2015; J. Wang et al., 2017).

The evidence supporting lower risk of PTB with increased maternal intake of fruits or vegetables is inconclusive. The findings from two studies are promising, but more high-quality evidence is needed to either support or refute benefits.

**Gestational Diabetes**

A single publication included glucose metabolism as an outcome of interest and fruit or vegetable intake as independent variables (Soto et al., 2015). This cohort study used bivariate analyses to evaluate associations between dietary factors and specific
outcomes. No bivariate associations between fruit and or vegetable intake and fasting glucose levels were found. High one-hour glucose challenge tests (>140mg/dl) were more common among those eating vegetables at least weekly (60.5%) than those who consumed them no more than monthly (39.5%, p=.008). The results of this single study are insufficient to base any conclusions on.

**Inflammation**

There were no publications that evaluated the effect of fruit or vegetable intake on biomarkers of inflammation or oxidative stress.

**Discussion**

This review shows a limited amount of evidence existing on the impact of fruit or vegetable intake on pregnancy and birth outcomes. The few available observational studies support potential benefits to improved maternal intakes of these foods on healthier pregnancy and better birth outcomes. Recent studies confirm that increased fruit or vegetable intake is beneficial at reducing risk of preeclampsia and promoting optimal fetal growth. There was no dietary impact on GWG from fruit or vegetable intake. The benefits of fruit or vegetable intake on GDM, PTB and inflammation is inconclusive either due to conflicting findings or lack of available evidence.

These findings are consistent with other reviews that support a relationship between diet and health. Chronic diseases such as cardiovascular disease, hypertension, obesity and type 2 diabetes are often treated, at least in part, through dietary management. These diseases are associated with chronic inflammation in the body that may promoted by high intake of refined grains and sugars, processed foods, and red meat (Barbaresko, Koch, Schulze, & Nothlings, 2013), while those high in fruits, vegetables, whole grains, low fat
dairy and lean meats reduce inflammation and risk for chronic diseases and death (Barbaresko et al., 2013; Carter et al., 2010; Casas et al., 2014; Cooper et al., 2012; Ledoux, Hingle, & Baranowski, 2011; B. Li, Li, Wang, & Zhang, 2016; Liyanage et al., 2016; McMacken & Shah, 2017). Diets high in fruits and vegetables are rich in phytochemicals that may attenuate the inflammatory response in chronic diseases (Griffiths et al., 2016; Islam et al., 2016; Salaritabar et al., 2017). Obesity incites inflammation and oxidative stress in the body as well, which may also be diminished by intake of foods rich in flavonoids (Sharma, Chung, Kim, & Hong, 2016; Yeon, Kim, & Sung, 2012b). Increased intake of fruits and vegetables may also promote healthier weight, and aid weight loss (Bertoia et al., 2016; He et al., 2004; Ledoux et al., 2011). However, a paradoxical relationship may exist between fruit intake and obesity, with increased intake being detrimental to weight (Sharma et al., 2016).

**Strengths**

This review is the first to evaluate the impact of fruit and or vegetable intake only on multiple gestational outcomes and inflammation in pregnancy. This is a comprehensive review including 15 years of research. The exclusion of dietary patterns allows generalizations not possible with inclusion of multiple foods and food groups.

**Limitations**

Some limitations of the research included in this review are the availability of observational studies only. Observational studies provide evidence to support future controlled trials to confirm or refute causal relationships and randomized controlled trials are often not feasible in this population. Study enrollment is limited until after diagnosis of pregnancy, and often longer if study exclusions include multiple gestation or miscarriage.
The outcome interest often occurs well into pregnancy or even after delivery. Preeclampsia, PTB, GDM, GWG, and poor fetal growth are infrequent occurrences and necessitate either large sample sizes to have enough statistical power, or retrospective analyses to identify cases. In addition, most of the included studies failed to adequately control for known risk factors.

Bias in study design is another limitation. Sampling was most often based on a geographical location or ethnic group with voluntary recruitment which introduces sampling bias. Samples were primarily non-Hispanic white women from developed countries where nutritional deficiencies are uncommon. The researchers often failed to control for other factors affecting both intake and birth outcomes. Dietary data collection often used non-validated instruments, performed at differing gestational ages often remote from the period of interest which promotes both random and systematic measurement errors. A single baseline dietary assessment may not adequately reflect the adaptations women make to their diet to either improve outcomes or reduce symptoms such as nausea. Dietary assessment varied and used FFQ, interview, recall and food record. Variation also existed in how intake was quantified and included use of quantiles, means, medians or established dietary recommendations used to determine adequacy.

Implications of findings for practice/education

Improved maternal intake of fruits and vegetables has potential to reduce risk for some adverse perinatal outcomes. Dietary assessment early in pregnancy should be performed to identify those with low intakes. Pregnant women, especially those at risk for preeclampsia or poor fetal growth, should be encouraged to eat a diet rich in fruits and
vegetables. Fruit intake should be encouraged, or supplemental food provided, for those at risk of inadequate GWG.

The small number of available studies on maternal dietary fruit and vegetable intake, gestational outcomes and inflammation is evidence of need for future research in this area. Further research is needed using ethnically diverse populations in non-European countries, especially the U.S., which is disproportionately affected by adverse maternal and fetal outcomes and was minimally represented in this review. More representative samples including those with both high and low risk for adverse outcomes are needed. African American pregnancies are affected by preeclampsia and PTB at much higher rates than their peers, and Hispanic immigrants tend to have better birth outcomes than native born Americans (CDC, 2017a, 2017b; Hamilton, 2017; H. B. Martin JA, Osterman MJK, 2017). Those from differing marital and socioeconomic backgrounds require evaluation as well to determine the dietary effects for other risk profiles as well. Multiple assessments of maternal dietary intake and biomarkers over the course of pregnancy would allow researchers to examine the associations between diet and inflammation over time toward the outcome of pregnancy. Confirmatory findings in a more representative population could enable clinical interventions and policy recommendations to improve nutrition in pregnant women and decrease risk for adverse gestational and birth outcomes. The paucity of available evidence necessitates future studies on dietary factors affecting perinatal outcomes.

**Conclusion**

This review evaluated the relationship between maternal fruit and vegetable intake and adverse pregnancy and birth outcomes and determined that increased intake is
associated with decreased risk for preeclampsia and poor infant growth. However, the paucity of evidence necessitates interpreting the overall impact of diet on gestational outcomes with caution. The impact of maternal intake on PTB, GDM is inconclusive due to limited evidence. Fruit and vegetable intake did not affect GWG adequacy. In addition, the relationship between fruit and vegetable intake on regulation of inflammatory and oxidative stress biomarkers was not evaluated due to lack of available evidence. Further research is needed using more consistent procedures and measures in more diverse populations to confirm or refute these findings. The impact of fruit or vegetable intake on inflammation and oxidative stress is also a future pathway for research as inflammation may play a crucial role in healthy gestational outcomes.
Figure 3.1 Flow of articles

271 relevant articles retrieved - PubMed, CINAHL, Medline

Title review, remove duplicates

71-abstract review

Inclusion criteria: fruit or vegetable intake as independent variable; gestational outcomes as dependent variable (PTB, GDM, GWG, PE, fetal growth, inflammation)

27 full text assessed

Articles not meeting criteria:
- Fruit drinks: 4
- Effect of intervention: 3
- Other outcomes: 2

19 articles included in review of fruit or vegetable intake and pregnancy (* articles included in >1 category)

- Gestational diabetes: *N=1
- Gestational weight gain: N=4
- Preterm birth: *N=4
- Fetal growth/size: *N=6
- Preeclampsia: *N=6
- Inflammation/oxidative stress: *Fruit/veg=3
Figure 3.2 Institute of Medicine Weight Gain Recommendations for Pregnancy

<table>
<thead>
<tr>
<th>Prepregnancy Weight Category</th>
<th>Body Mass Index*</th>
<th>Recommended Range of Total Weight (lb)</th>
<th>Recommended Rates of Weight Gain† in the Second and Third Trimesters (lb) (Mean Range [lb/wk])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>Less than 18.5</td>
<td>28–40</td>
<td>1 (1–1.3)</td>
</tr>
<tr>
<td>Normal Weight</td>
<td>18.5–24.9</td>
<td>25–35</td>
<td>1 (0.8–1)</td>
</tr>
<tr>
<td>Overweight</td>
<td>25–29.9</td>
<td>15–25</td>
<td>0.6 (0.5–0.7)</td>
</tr>
<tr>
<td>Obese (includes all classes)</td>
<td>30 and greater</td>
<td>11–20</td>
<td>0.5 (0.4–0.6)</td>
</tr>
</tbody>
</table>

*Body mass index is calculated as weight in kilograms divided by height in meters squared or as weight in pounds multiplied by 703 divided by height in inches.

†Calculations assume a 1.1–4.4 lb weight gain in the first trimester.

CHAPTER FOUR: Fruit and Vegetable Intake in Pregnancy is Associated with Inflammation, and Risks for Preterm Birth and Gestational Hypertension

Introduction

Fruit and vegetable intake are considered important components of a healthy diet and are especially important during pregnancy. Consuming enough amounts of fruits and vegetables may reduce the risk of adverse birth outcomes. A diet rich in fruit and vegetable intake may regulate inflammation in the body as well.

In 2015, only 12.2% of adults met fruit intake recommendations while 9.3% met vegetable intake recommendations (Lee-Kwan, Moore, Blanck, Harris, & Galuska, 2017). Nutritional deficiencies due to poor maternal intake may increase risk of poor gestational outcomes such as preterm birth, gestational diabetes and preeclampsia (Kibret, Chojenta, Gresham, Tegegne, & Loxton, 2018; Schoenaker et al., 2014; Shin, Lee, & Song, 2015).

Diet also influences immune function (Barbaresko et al., 2013; Wu & Schauss, 2012). During pregnancy, dysregulation in immune functions increases susceptibility of perinatal infections as well as other adverse birth outcomes including preterm birth and hypertension in pregnancy. Due to the breadth of this topic, this paper will examine these two commonly occurring perinatal outcomes (preterm birth and hypertensive disorders) in relation to maternal fruit and vegetable intake during pregnancy. These adverse perinatal outcomes have also been linked to inflammation (Aggarwal et al., 2019; Harmon et al., 2016; Lyon et al., 2010), likely affected by dietary intake (Wu & Schauss, 2012).

Background

Research examining the nutritional influence of fruit and vegetable intake on hypertensive disorders of pregnancy is limited. Hypertensive disorders of pregnancy are a group of disorders exclusive to pregnancy (preeclampsia/eclampsia, preeclampsia
superimposed on chronic hypertension, and gestational hypertension). Gestational hypertension is defined as maternal systolic blood pressure of 140 or higher or diastolic of 90 or higher from two separate readings at least four hours apart after 20 weeks gestation. Preeclampsia occurs when gestational hypertension criteria are present as well as proteinuria or other signs of multiorgan involvement. Signs of multiorgan involvement are acute renal insufficiency (defined as a new increase in serum creatinine concentration ≥100 \( \mu \text{mol/L} \) antepartum or >130 \( \mu \text{mol/L} \) postpartum), blood disorders such as low platelets (<100,000 mg/dl), disseminated intravascular coagulation, or hemolysis, liver dysfunction (transaminases two times normal limits, severe right upper quadrant/epigastric pain or liver rupture), central nervous system involvement (eclampsia, severe headache, hyperactive reflexes, visual disturbance, or cerebral bleeding). Preeclampsia may occur either after 20 weeks gestation and prior to the onset of labor or up to six weeks postpartum.

Preeclampsia affects 2-5% of pregnancies in industrialized nations worldwide, while women in developing countries have nearly seven times the risk (Foundation, 2013). Preeclampsia costs an estimated $2.18 billion in healthcare costs over usual pregnancy care costs annually in the United States (Stevens et al., 2017), and affects 10 million pregnancies worldwide (Foundation, 2013). Hypertensive disorders of pregnancy were responsible for approximately 7% of pregnancy related deaths between 2011-2015 in the U. S. according to the CDC (Petersen et al., 2019), and account for 76,000 of maternal deaths and 500,000 fetal deaths per year worldwide (Foundation, 2013). Preeclampsia also accounts for approximately 15 % of preterm births in the United States according to the March of Dimes (2017). Women who have preeclampsia may also be at increased risk for heart attack or stroke in the future (Ramsay et al., 2003).
Of the few studies examining the relationship between maternal diet and hypertension, the majority focus on increased consumption of fruits and vegetables decreasing odds of preeclampsia by 20-54% (Brantsaeter et al., 2009; Endeshaw et al., 2015; Longo-Mbenza et al., 2008; Torjusen et al., 2014). Eating fruit at least three times weekly also lowered the odds of preeclampsia by nearly half (Endeshaw et al., 2015). Conversely, research by Soto et al (2015) did not find an association between fruit or vegetable intake and maternal blood pressure.

Preterm birth occurs prior to 37 completed weeks of gestation and may occur either spontaneously or for medical reasons. Preterm birth occurs in about 10% of all births in the U. S. with healthcare costs of $26 billion based on the most recent estimate from 2006 ("Preterm Birth: Causes, Consequences, and Prevention," 2006). Preterm birth rates have risen in recent years in the U. S. following a nearly decade of steady decline (J. A. Martin & Osterman, 2018). Immediate health issues that are more common among infants born premature include respiratory issues, thermoregulation, feeding difficulties, jaundice and sepsis that may require initial admission to intensive care as well as increase risk of rehospitalizations (Saigal & Doyle, 2008). An inverse relationship between length of gestation and infant morbidity and mortality exists. These risks may persist throughout life for those born premature. Current evidence supports links between being born prematurely and increased risk of asthma and respiratory disorders, obstructive sleep apnea, hypertension, learning difficulties, mental health and behavioral disorders, cardiovascular disease, as well as insulin resistance and diabetes that persist throughout childhood, adolescence and into adulthood (Raju et al., 2017). Women who deliver preterm also have
increased risk of later cardiovascular disease (Bonamy, Parikh, Cnattingius, Ludvigsson, & Ingelsson, 2011).

The influence of fruit and vegetable intake on preterm birth is inconclusive. Participants had 18% lower odds of preterm birth when they consumed certain dried fruits and onion type vegetables in one study (Myhre et al., 2013). In addition, consuming at least five servings per day of fruits and vegetables less than once a week predicted 31% higher risk of preterm birth (Smith et al., 2015). Conversely, other studies do not support this association (Soto et al., 2015; J. Wang et al., 2017).

The immune system is charged with recognition of host or foreign substances and responding to rid the body of foreign substances, such as viruses or bacteria. Certain types of lymphocytes called T helper cells (Th cells) produce signaling molecules that either increase or decrease the body’s response to invasion. There are two primary types of cells that predominate Th-1 and Th-2. Type Th-1 cells are primarily responsible for intracellular immunity and produce pro-inflammatory substances such as Interleukin 1 (IL-1), interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α). Type Th-2 is responsible for extracellular immunity and produce more anti-inflammatory substances such as IL 4, IL-5, IL-6 and IL-10. These signaling molecules are numerous and complex so for the purposes of this paper the term cytokine will be used to refer to them. During pregnancy the body must protect the foreign fetus from attack by the immune system, with primarily Th-2 associated immunity.

Recent studies have examined the link between dietary intake of fruits and vegetables and inflammatory markers. Two recent reviews highlighted the ability of diets high in fruit and vegetable intake on the expression of markers of inflammation
The reviewed studies supported that an inverse relationship exists between pro-inflammatory biomarkers and fruit and vegetable intake. While a “Western” type diet that is meat based has a positive relationship with inflammation state (Barbaresko et al., 2013).

**Purpose and Specific Aims**

This purpose of this study is to examine the relationships among maternal dietary intake of fruits and vegetables, trimester specific cytokine expression in early and mid-pregnancy, and birth outcomes. The specific aims are:

**Aim 1:** To determine whether fruit and/or vegetable intake during early and mid-pregnancy predicted first or second trimester specific cytokine expression, controlling for sociodemographic and clinical characteristics.

**Aim 2:** To determine whether fruit and vegetable intake during early and mid-pregnancy predicted preterm birth or gestational hypertension, controlling for sociodemographic and clinical risk factors.

**Methods**

The data for this secondary analysis are from a multi-center prospective trial of women with a healthy singleton gestation (K. Ashford et al., 2018). The purpose of the parent study was to evaluate the differences in maternal trimester specific serum and cervicovaginal cytokine expression between women who delivered at term and those who had preterm births. Data were collected during all three trimesters of pregnancy and postpartum. Only first trimester (8-13 weeks) and second trimester (18-23 weeks) cytokine and dietary data were used for this study since inflammatory state changes closer to delivery (K. B. Ashford et al., 2018; Bowen, Chamley, Keelan, et al., 2002). Sociodemographic characteristics, clinical data, and biologic specimens were collected.
during prenatal visits during the trimesters specified. Clinical and delivery data were obtained from the medical record.

**Sample/setting**

In the parent study, women were enrolled from three prenatal clinics in northern, central and western Kentucky from 2009-2013. Pregnant women 18 years or older with singleton gestation were included. Exclusion criteria included: pre-existing diabetes, autoimmune disease, heart disease, current illicit drug use, HIV, and bacterial vaginosis or sexually transmitted infection. Women with complete first trimester data for serum cytokines, dietary survey data, age, race and birth outcome were retained for the current study (N=180).

**Measures**

*Sociodemographic and Clinical Characteristics*

Participants reported sociodemographic variables that included age, race, income, marital status and education. Maternal health behaviors of interest included maternal smoking status, pre-pregnant weight, BMI and gestational weight gain. Weight and height were obtained from the medical record and used to calculate BMI. Pre-pregnant weight was by self-report when available, first recorded weight during pregnancy was used instead if this was not available. Last recorded maternal weight was obtained from medical record. Maternal smoking status was determined by first trimester urine cotinine level. Maternal risk factors were included if they could affect any of the birth outcomes of interest or inflammation. History of a prior preterm birth was determined via self-report.
Birth outcomes

Preterm birth was defined as gestational age at delivery of less than 37 completed weeks gestation and was obtained from the medical record. Hypertensive disorders of pregnancy with available data for this study included pregnancy induced gestational hypertension and preeclampsia, but due to insufficient cases preeclampsia was not included. Gestational hypertension diagnosis required at least two high blood pressure readings (systolic 140 or higher; diastolic 90 or higher) at least four hours apart after 20 weeks gestation, and diagnoses were obtained from the prenatal medical record.

Nutrition

Maternal nutrition variables collected and available for analysis were fruit and vegetable intake. First and 2nd trimester dietary intake of fruits or vegetables were measured to evaluate maternal nutrition in early pregnancy. Participants self-reported the number of servings daily of each food based on standard serving sizes. The number of servings per day of each food were used in analyses, as well as total fruits and vegetables combined. Dietary adequacy for fruit and vegetable intake was determined based on U.S. Department of Agriculture 2015-2020 guidelines (2015). Fruit intake recommendation was met if the participant consumed at least four servings daily. Vegetable intake was adequate if at least two and a half servings per day were eaten.

Cytokines

First and second trimester cytokine values were used as they were least likely to be affected by increasingly pro-inflammatory state that occurs closer to delivery. Inflammation was measured through inflammatory cytokines present in maternal serum and cervicovaginal secretions. Specimens were collected from both maternal serum and
cervicovaginal secretions. Maternal clotted blood specimens were centrifuged for 10 minutes and the serum divide into aliquots to be stored at -80°C until analysis. Samples of cervicovaginal fluid were obtained using an Aware Messenger (Calypte) swab by sweeping the cervix for 30 seconds, then sweeping the vaginal vault/posterior fornix 360 degrees with removal. The swab was placed in the proprietary container, pressed against the inner wall to ensure maximum fluid seepage into the buffer fluid and the cap secured. All samples were immediately refrigerated and transported to the laboratory within six hours. They were stored at -20°C at least 24 hours. The samples were thawed for processing and then centrifuged, split into aliquots and stored at -80°C. A multiplex Beadlyte assay (MPXHCYTO-60K-06) was used to measure IL-1α, IL-1β, IL-6, IL-8, IL-10 and TNF-α on a Luminex IS-100 (Austin, TX) according to manufacturer’s recommendations. Singleplex assays were used for CRP (Millipore, Billerica, MA) and MMP-8 (R&D Systems, Minneapolis, MN). All cytokine data were generated using Milliplex Analyst Software. Log transformations were used on raw cytokine values as the latter are not normally distributed.
Health behaviors and Indicators

Maternal smoking status was determined based on first trimester urine cotinine using the NicAlert® urine assay. The test strip was submerged for 20 seconds in 20-30 ml of urine. The measurement was obtained after development and result recorded. Values range from 0-6, with a level of 3 or more used to identify smokers. Prepregnant weight was used to determine body mass index. Either first recorded maternal weight or patient self-reported pre-pregnant weight was used for pre-pregnant weight. Gestational weight gain was calculated from as the difference between pre-pregnant weight and last recorded weight prior to delivery. Body mass index was calculated using the Centers for Disease Control and Prevention (CDC) formula as weight in kilograms divided by the square of height in meters. Weight status based on BMI was characterized using the CDC definitions for underweight, normal weight, overweight and obese women.

Procedure

Approval for the original study was obtained from Institutional Review Board at each study site. After recruitment and informed consent, the participants completed questionnaires at their prenatal clinic. Survey data was obtained using Survey Monkey on I-Pads or on a paper survey, if requested. This was done during regular prenatal visits. The data was de-identified prior to entry into the secure database. A research nurse reviewed data entry from paper surveys for accuracy and completeness. When possible, missing data were entered using information from the medical record. Specimen collection for cytokine analysis was performed at each prenatal visit.

Data Analysis

Descriptive analysis, including frequency distributions and means and standard deviations, was used to summarize sociodemographic variables. Prior to analysis, all
cytokine values were natural log transformed to adjust for lack of normal distribution in the unadjusted values. Geometric means and standard deviations were used to summarize these outcomes at each trimester. Specific Aim 1 was tested using linear regression to evaluate whether first and second trimester intake of fruits and vegetables predicted early and mid-pregnancy cytokine expression, controlling for personal characteristics. Aim 2 was tested using linear regression to evaluate whether meeting dietary recommendations for fruit and or vegetable intake during pregnancy predict birth outcome. Aim 2 also required use of logistic regression to evaluate whether number of servings of fruits and vegetables predict adverse pregnancy outcome. Variance inflation factors did not exceed 1.6 for any of the included variables, suggesting multicollinearity did not distort regression estimates. Data analysis was conducted using SPSS 24; an alpha level of .05 was used for inferential testing.

Results

Participant characteristics

The mean age of the 180 participants was 27.0 years (SD=5.4). The majority had less than $50,000 per year household income (52.8%), completed at least high school (90.6%), were white (81.7%) and were non-smokers (79.4%). Most were married or cohabitating (81.1%) and were pregnant for the first time (64.1%). More than half were overweight or obese prior to pregnancy (52.9%). More than half gained in excess of Institute of Medicine recommendations during pregnancy (55.7%). The incidence of preterm birth was 16.7% in this sample, with 8.9% developing gestational hypertension, and 4.2% with preeclampsia (Table 4.1).
### Table 4.1 Sociodemographic and clinical characteristics of sample (N=180)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (± SD) or n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>27.01 (±5.39)</td>
</tr>
<tr>
<td><strong>Education:</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; High school</td>
<td>17 (9.4%)</td>
</tr>
<tr>
<td>At least high school</td>
<td>163 (90.6%)</td>
</tr>
<tr>
<td><strong>Total household income:</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;$25,000/year</td>
<td>53 (30.1%)</td>
</tr>
<tr>
<td>$25,000-49,999/year</td>
<td>40 (22.7%)</td>
</tr>
<tr>
<td>$50,000 or more/year</td>
<td>83 (47.2%)</td>
</tr>
<tr>
<td><strong>Race:</strong></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>147 (81.7%)</td>
</tr>
<tr>
<td>Non-white</td>
<td>40 (18.3%)</td>
</tr>
<tr>
<td><strong>Marital status:</strong></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>16 (8.9%)</td>
</tr>
<tr>
<td>Dating</td>
<td>15 (8.3%)</td>
</tr>
<tr>
<td>Living with partner</td>
<td>33 (18.3%)</td>
</tr>
<tr>
<td>Married</td>
<td>113 (62.8%)</td>
</tr>
<tr>
<td>Separated</td>
<td>3 (1.7%)</td>
</tr>
<tr>
<td><strong>Smoking status:</strong></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>37 (20.6%)</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>143 (79.4%)</td>
</tr>
<tr>
<td><strong>Gravida:</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>107 (64.1%)</td>
</tr>
<tr>
<td>2</td>
<td>21 (12.6%)</td>
</tr>
<tr>
<td>3</td>
<td>23 (13.6%)</td>
</tr>
<tr>
<td>4 or more</td>
<td>16 (9.6%)</td>
</tr>
<tr>
<td><strong>First trimester intake:</strong></td>
<td></td>
</tr>
<tr>
<td>Fruit servings/day</td>
<td>2.4±1.2</td>
</tr>
<tr>
<td>Vegetable servings/day</td>
<td>2.3±1.2</td>
</tr>
<tr>
<td><strong>Second trimester intake:</strong></td>
<td></td>
</tr>
<tr>
<td>Fruit servings/day</td>
<td>2.5±1.2</td>
</tr>
<tr>
<td>Vegetable servings/day</td>
<td>2.4±1.1</td>
</tr>
<tr>
<td><strong>Intake met:</strong></td>
<td></td>
</tr>
<tr>
<td>First trimester fruit</td>
<td>32 (17.8%)</td>
</tr>
<tr>
<td>First trimester vegetable</td>
<td>5 (2.8%)</td>
</tr>
<tr>
<td>Second trimester fruit</td>
<td>27 (17.6%)</td>
</tr>
<tr>
<td>Second trimester vegetable</td>
<td>72 (46.8%)</td>
</tr>
<tr>
<td><strong>Pre-pregnancy BMI:</strong></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>Normal weight</td>
<td>83 (46.6%)</td>
</tr>
<tr>
<td>Overweight</td>
<td>51 (28.7%)</td>
</tr>
<tr>
<td>Obese</td>
<td>43 (24.2%)</td>
</tr>
</tbody>
</table>
Table 4.1 (Continued) Sociodemographic and clinical characteristics of sample (N=180)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (± SD) or n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational weight gain:</td>
<td></td>
</tr>
<tr>
<td>Less than recommended</td>
<td>30 (18%)</td>
</tr>
<tr>
<td>Adequate</td>
<td>44 (26.3%)</td>
</tr>
<tr>
<td>More than recommended</td>
<td>93 (55.7%)</td>
</tr>
<tr>
<td>Preterm birth</td>
<td>30 (16.7%)</td>
</tr>
<tr>
<td>Hypertensive disorders:</td>
<td></td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>22 (13.1%)</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>7 (4.2%)</td>
</tr>
</tbody>
</table>

Frequency and percent (%) were used for categorical values, mean and standard deviation (SD) for continuous variables.
For most participants, dietary intake was below recommended amounts for fruits and vegetables in both first and second trimester. Mean intake was below 2.5 servings daily (SD≤1.2) for both fruits and vegetables during both first and second trimester. First trimester fruit intake recommendations were met by 17.8% of participants, while only 2.8% consumed the recommended amounts of vegetables. Second trimester intake of fruits was similar with 17.6% reporting adequate intake. Vegetable intake in the second trimester was adequate for nearly half of participants (46.8%).

The geometric means and standard deviations for serum and cervicovaginal cytokines are shown in Table 4.2. The mean values were generally stable from first to second trimester as expected earlier in pregnancy. Serum values were smaller than cervicovaginal for IL-1α, IL-1β, IL-6 and IL-8 while the reverse is true for IL-10, CRP, MMP-8 and TNF-α.
Table 4.2 Geometric means and standard deviations of first and second trimester cytokines (pg/mL)

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Serum</th>
<th>First trimester</th>
<th>Second trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>1.70 (6.81)</td>
<td>1.55 (6.33)</td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.98 (5.87)</td>
<td>0.94 (5.98)</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>2.31 (5.07)</td>
<td>2.38 (5.51)</td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>6.09 (2.89)</td>
<td>5.65 (2.95)</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>7.39 (4.24)</td>
<td>8.40 (4.00)</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>32.49 (2.97)</td>
<td>35.65 (3.00)</td>
<td></td>
</tr>
<tr>
<td>MMP-8</td>
<td>12793.60 (2.76)</td>
<td>15875.00 (2.61)</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>6.13 (3.45)</td>
<td>7.20 (3.40)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cervicovaginal</th>
<th>First trimester</th>
<th>Second trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>500.95 (3.78)</td>
<td>650.67 (4.71)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5.61 (9.56)</td>
<td>6.76 (9.42)</td>
</tr>
<tr>
<td>IL-6</td>
<td>3.46 (5.06)</td>
<td>3.73 (4.71)</td>
</tr>
<tr>
<td>IL-8</td>
<td>1544.88 (3.81)</td>
<td>1756.36 (5.92)</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.60 (2.56)</td>
<td>1.10 (3.46)</td>
</tr>
<tr>
<td>CRP</td>
<td>3.88 (14.13)</td>
<td>2.60 (13.11)</td>
</tr>
<tr>
<td>MMP-8</td>
<td>76596.00 (4.78)</td>
<td>99927.48 (5.89)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.87 (3.06)</td>
<td>0.78 (2.94)</td>
</tr>
</tbody>
</table>

Regression analyses

Predictors of Cytokines

Multiple linear regression was used for these analyses while controlling for four variables known to affect inflammation: smoking status, age, race and obesity. Meeting daily recommendations for vegetable intake for first trimester predicted higher first trimester log-transformed serum CRP ($\beta=.20$, $p=.011$), IL-1α ($\beta=.24$, $p=.003$), IL-6 ($\beta=.20$, $p=.014$), and TNFα ($\beta=.19$, $p=.021$), and lower log-transformed cervicovaginal IL-6 ($\beta=-.24$, $p=.006$). Meeting recommended fruit intake in the first trimester was associated with higher log-transformed serum IL-1β ($\beta=.16$, $p=.045$) and lower log-transformed...
cervicovaginal IL-1α ($b=-.24$, $p=.006$) (Table 4.3). The number of fruit or vegetable servings did not predict trimester specific cytokine expression, nor did any of the second trimester intakes.
<table>
<thead>
<tr>
<th>Cytokine Source</th>
<th>Independent Variables</th>
<th>lnCRP sβ (p value)</th>
<th>lnIL-1α sβ (p value)</th>
<th>lnIL-1β sβ (p value)</th>
<th>lnIL-6 sβ (p value)</th>
<th>lnTNF-α sβ (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum:</td>
<td>Age</td>
<td>.06 (.442)</td>
<td>.14 (.073)</td>
<td>.10 (.213)</td>
<td>.12 (.122)</td>
<td>.10 (.175)</td>
</tr>
<tr>
<td>First trimester</td>
<td>Pre-Pregnant obesity</td>
<td>.21 (.005)</td>
<td>-.02 (.772)</td>
<td>.06 (.452)</td>
<td>.11 (.142)</td>
<td>-.02 (.842)</td>
</tr>
<tr>
<td></td>
<td>White Race</td>
<td>-.09 (.228)</td>
<td>.13 (.086)</td>
<td>.10 (.179)</td>
<td>.07 (.382)</td>
<td>.16 (.035)</td>
</tr>
<tr>
<td></td>
<td>Smoking</td>
<td>.16 (.029)</td>
<td>-.08 (.263)</td>
<td>-.15 (.058)</td>
<td>-.12 (.121)</td>
<td>-.02 (.786)</td>
</tr>
<tr>
<td></td>
<td>Fruit intake met</td>
<td>-.02 (.353)</td>
<td>-.14 (.076)</td>
<td>.16 (.045)</td>
<td>-.11 (.155)</td>
<td>-.15 (.055)</td>
</tr>
<tr>
<td></td>
<td>Vegetable intake met</td>
<td>.20 (.011)</td>
<td>.24 (.003)</td>
<td>.11 (.169)</td>
<td>.20 (.014)</td>
<td>.19 (.021)</td>
</tr>
<tr>
<td></td>
<td>Model R² (p-value)</td>
<td>.13 (.001)</td>
<td>.11 (.003)</td>
<td>.08 (.030)</td>
<td>.09 (.018)</td>
<td>.08 (.020)</td>
</tr>
<tr>
<td>Cervicovaginal fluid:</td>
<td>Age</td>
<td>-.14 (.095)</td>
<td>-</td>
<td>-.19 (.021)</td>
<td>-.26 (.003)</td>
<td></td>
</tr>
<tr>
<td>First trimester</td>
<td>Pre-pregnant obesity</td>
<td>.04 (.627)</td>
<td>-.06 (.481)</td>
<td>-</td>
<td>-.01 (.881)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White Race</td>
<td>.03 (.755)</td>
<td>-</td>
<td>.18 (.026)</td>
<td>.07 (.372)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Smoking</td>
<td>.09 (.255)</td>
<td>-</td>
<td>-.01 (.955)</td>
<td>.07 (.432)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fruit intake met</td>
<td>-.24 (.006)</td>
<td>-</td>
<td>-.07 (.429)</td>
<td>-.04 (.611)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vegetable intake met</td>
<td>.13 (.152)</td>
<td>-</td>
<td>-.24 (.006)</td>
<td>-.11 (.224)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Model R² (p-value)</td>
<td>.09 (.031)</td>
<td>-</td>
<td>.12 (.005)</td>
<td>.08 (.046)</td>
<td></td>
</tr>
</tbody>
</table>
Predictors of Birth Outcomes

Logistic regression was used to assess these relationships while controlling for known risks or predictors of each outcome such as age, race, and smoking status. Eating more vegetable servings in the first trimester predicted lower odds of PTB (OR=.44; p=.007). Higher vegetable intake in the second trimester predicted higher odds of gestational hypertension (OR 2.19, p=.030). Prior history of preterm birth (n=47) is a known predictor of preterm birth and was included in all models predicting preterm birth. The adequacy of gestational weight gain based on Institute of Medicine recommendations was also a significant predictor of preterm birth for both first trimester models, as well as the model using the met category for fruit or vegetable intake (p<.05 for all). Fruit intake was not a significant predictor of PTB in either trimester of interest. (Table 4.4).
Table 4.4 Regression models and corresponding odds ratios for birth outcomes ($N=180$)

<table>
<thead>
<tr>
<th>Birth Outcome</th>
<th>Independent variables</th>
<th>First trimester</th>
<th>Second trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intake Met OR (p value)</td>
<td># servings OR (p value)</td>
<td>Intake Met OR (p value)</td>
</tr>
<tr>
<td>Preterm Birth</td>
<td>Age</td>
<td>.97(.517)</td>
<td>.98(.660)</td>
</tr>
<tr>
<td></td>
<td>White Race</td>
<td>1.29(.671)</td>
<td>.914(.884)</td>
</tr>
<tr>
<td></td>
<td>Smoking</td>
<td>.80(.697)</td>
<td>1.17(.788)</td>
</tr>
<tr>
<td></td>
<td>History of PTB</td>
<td>3.74(.011)</td>
<td>5.84(.002)</td>
</tr>
<tr>
<td></td>
<td>IOM weight gain</td>
<td>.46(.011)</td>
<td>.44(.007)</td>
</tr>
<tr>
<td></td>
<td>Fruit intake</td>
<td>.74(.683)</td>
<td>1.15(.538)</td>
</tr>
<tr>
<td></td>
<td>Vegetable intake</td>
<td>.00(.999)</td>
<td>-.830(.007)</td>
</tr>
<tr>
<td></td>
<td>Model R² (p value)</td>
<td>.21(.004)</td>
<td>.29(&lt;.001)</td>
</tr>
<tr>
<td>Gestational Hypertension</td>
<td>Age</td>
<td>.95(.437)</td>
<td>.95(.419)</td>
</tr>
<tr>
<td></td>
<td>White Race</td>
<td>.00(.998)</td>
<td>.00(.998)</td>
</tr>
<tr>
<td></td>
<td>Smoking</td>
<td>.40(.413)</td>
<td>.36(.363)</td>
</tr>
<tr>
<td></td>
<td>IOM weight gain</td>
<td>6.40(.061)</td>
<td>6.19(.064)</td>
</tr>
<tr>
<td></td>
<td>Fruit Intake</td>
<td>1.18(.852)</td>
<td>1.08(.978)</td>
</tr>
<tr>
<td></td>
<td>Vegetable Intake</td>
<td>1.27(.864)</td>
<td>1.18(.536)</td>
</tr>
<tr>
<td></td>
<td>Model R² (p value)</td>
<td>.20(.024)</td>
<td>.21(.020)</td>
</tr>
</tbody>
</table>
Discussion

Consistent with published data, only small numbers of pregnant women in this study met recommended fruit and vegetable intakes (17.8% and 2.8% respectively in first trimester). In 2015, 10.7% of adults in Kentucky met recommended fruit intake, while only 7% consumed enough vegetables (Lee-Kwan et al., 2017).

Maternal serum and cervicovaginal levels of inflammatory cytokines was affected by maternal intake of vegetables, and to a lesser extent fruit. Vegetable intake of at least 2.5 servings per day had a positive relationship with proinflammatory serum CRP, IL-1α, IL-6, and TNF-α in this study. Fruit intake increased serum inflammatory IL-1β and decreased cervicovaginal IL-1α. There are no published studies examining the impact of fruit and vegetable intake on inflammation among those with healthy pregnancy or including cervicovaginal cytokines. The current study findings contrast with prior studies using fetal membrane and myometrial explants which expressed lower concentrations of inflammatory cytokines TNF-α, IL-1β, and IL-6 when exposed to a citrus extract (Morwood & Lappas, 2014). Inflammatory status among women with gestational diabetes was not affected by fruit or vegetable intake (Asemi, Samimi, Tabassi, Sabihi, & Esmaillzadeh, 2013). Among overweight women, a diet high in fruits and vegetables did lower expression of IL-6 and IL-1β according to Yeon, Kim, and Sung (2012a). A recent published review and meta-analysis also contrasted with my findings with fruit or vegetable intake promoting lower serum CRP and TNF-α levels in adults, but it did not include pregnant women (Hosseini et al., 2018). Possible explanations for contradictory findings could be the type of fruit or vegetable ingested. Some fruits and vegetables, such as berries and dark green leafy vegetables, contain more bioactive compounds called
phytochemicals which provide more anti-inflammatory benefits (Yu-Jie et al., 2015). Intakes of known proinflammatory foods such as of sugar, processed foods and red meat characteristic of “Western” diets promote inflammation (Barbaresko et al., 2013) and could have affected cytokine secretion as well. During pregnancy, women are instructed to limit fish consumption to 12 ounces weekly due to concerns of methylmercury contamination (Gynecologists, 2015). In 2016 Americans consumed an average of 2.7 ounces per week of fish, which is one-third the recommended amount (Kantor, 2016). Fish and shellfish are high in anti-inflammatory omega-3 fatty acids. The type of fruits and vegetables consumed, and other dietary intake is unknown in this population and could also account for the differences in findings among pregnant women.

Dietary intake of vegetables during the first trimester of pregnancy resulted in 56% lower risk of PTB for every serving of vegetables controlling for history of PTB as well as other known risks factors. Prior findings supported the ability of some vegetables and dried fruit to reduce risk of preterm birth (Myhre et al., 2013), while infrequent consumption of fruits and vegetables increased the risk (Smith et al., 2015). Dietary patterns that emphasize intake of fruits and vegetables reduce risk of preterm birth, while those that emphasize meats and fatty foods promote increased risk of preterm birth overall (Chia et al., 2016; Englund-Ogge et al., 2014; C. L. Martin, Sotres-Alvarez, & Siega-Riz, 2015; Mikkelsen et al., 2008; Rasmussen, Maslova, Halldorsson, & Olsen, 2014).

Eating more vegetables in the second trimester predicted higher risk of gestational hypertension in this study (OR 2.09, p=.039). Fruit intake did not affect risk of gestational hypertension. These findings contrast with other studies that reported lower risk of preeclampsia or gestational hypertension with higher intakes of plant based foods.
(Brantsaeter et al., 2007; Endeshaw et al., 2015; Timmermans et al., 2011; Torjusen et al., 2014; Vieira et al., 2017). Again, the type of vegetables were unknown for this study and could have affected findings.

In this study, mothers with inadequate gestational weight gain were more likely to deliver preterm than those with adequate or excess gains. This is consistent with prior findings that inadequate gains increase the risk of delivering preterm, whereas excess gains was associated with lower risk of preterm birth (Enomoto et al., 2016; N. Li et al., 2013).

Both preterm birth and hypertensive disorders of pregnancy have an inflammatory component (Aggarwal et al., 2019; Harmon et al., 2016; Harper et al., 2013; Lyon et al., 2010). Multiple systematic reviews and meta analyses support an inverse relationship with diets high in fruit or vegetable content and markers of inflammation in both health and disease states as well as a positive relationship between diets high in meats and processed foods (Barbaresko et al., 2013; Casas et al., 2014; Griffiths et al., 2016; Hosseini et al., 2018; Yeon et al., 2012a). Low intake of fruits and vegetables among Kentuckians may explain the increased incidence of adverse gestational outcomes. The “Western” diet consumed by many Americans is high in red meat, sugars and processed foods, and low in fruits, vegetables and whole grains which may also contribute to adverse gestational outcomes due to a pro-inflammatory diet. Though obesity and gestational weight gain were not related to adverse outcomes in this sample, they are linked to adverse gestational outcomes overall. According to recent statistics, two out of every three women is overweight or obese in the United States (Diseases, 2017). The balance between pro- and anti-inflammatory mediators essential to healthy pregnancy may be altered due to obesity...
state as well as dietary factors and result in adverse gestational outcomes such as preterm birth and hypertensive disorders of pregnancy.

**Strengths**

This study has several strengths. It presents several firsts for this topic. It is the first to evaluate the effect of fruit or vegetable intake on markers of inflammation. It is also the first study to use cytokine data from multiple trimesters in relation to outcomes. Finally, it is the first study to evaluate the impact of diet on cervicovaginal cytokines. The relationship between vegetable intake in the first trimester and lower risk of preterm birth was a novel finding, as was the second trimester intake of vegetables and higher gestational hypertension risk.

**Limitations**

Limitations for this study exist and include the use of secondary data for analysis, which constrained availability of all data relevant to research goals. Given the 3-month lookback window, the dietary data may have been inaccurate due to incomplete recollection, and report of fruit and vegetable consumption may have been inflated due to social desirability of higher intake. Another limitation is the relatively small sample size and limited instances of some adverse gestational outcomes. Finally, the availability of only fruit and vegetable in general prevented analysis of the quality of the fruits and vegetables consumed, as all are not equal in nutritional or anti-inflammatory value.

**Conclusions**

In conclusion, maternal inflammatory markers are related to fruit and vegetable intake and birth outcomes. These findings indicate the need for further research to investigate the impact of fruits and vegetables alone and in the context of overall dietary
intake on both inflammatory state and birth outcomes. Longitudinal studies that include larger, more representative samples are needed to either confirm or refute these promising findings. In addition, including more of the variables suggested by the food environment and birth outcomes model such as overall diet, exercise and more complete risk factors would provide a more comprehensive view how maternal diet is impacted by environment, as well as how diet affects biological factors and birth outcomes.
CHAPTER FIVE: Discussion and Conclusions

The overall purpose of this dissertation was to examine relationships between maternal nutrition during pregnancy, maternal inflammatory state during pregnancy and adverse perinatal outcomes. Three chapters were included with the individual purposes of: 1) to review and evaluate the current evidence on the relationship between maternal omega-3 fatty acid intake and inflammation in pregnancy; 2) to evaluate the current state of the science on the impact of maternal dietary consumption of fruits and vegetables on the incidence of preterm birth, gestational diabetes, preeclampsia, small for gestational age, gestational weight gain and measures of inflammation or oxidative stress in pregnancy; and 3) to examine the relationships between maternal intake of fruits and vegetables, trimester specific cytokine expression in early and mid-pregnancy and birth outcomes.

Synthesis of Findings and Implications

The first study was a critical review of literature examining the impact of maternal intake of omega-3 fatty acids in pregnancy on inflammation in pregnancy. Seven randomized controlled trials published between 2008-2016 met inclusion criteria and were included in the full review. Biomarkers of inflammation in both maternal and fetal tissues were affected by supplementation in five of seven studies. Maternal serum CRP decreased over time in those receiving the supplement, while this pro-inflammatory cytokine increased over time for those who did not receive treatment. Both IL-1 and IFN-γ were lower at delivery for those treated. Maternal adipose tissue levels of IL-6, IL-8 and TNF-α were lower in those supplemented as well. Both cord and placental levels of several cytokines were lower in those who were supplemented with omega-3 fatty acids.

The available research supports the ability of omega-3 to affect inflammation in pregnancy. In addition, no adverse events were reported in any of the included studies.
However, the optimal time and duration of administration, as well as dosage were less clear. The American diet is deficient in omega-3 and contains excessive amounts of omega-6 fats promoting less than optimal ratios and promoting inflammation. Increasing intake of omega-3 via food or supplement helps balance this ratio. Americans consume only one-third the recommended amount of fish and shellfish (Kantor, 2016). In addition, pregnant women are limited or restricted in seafood intake due to worries about mercury contamination resulting in a diet woefully lacking in omega-3 fatty acids. This author feels there is enough evidence to support inclusion of omega-3 supplements in pregnancy in order to promote improved omega-6 to omega-6 ratios and reduce inflammation.

The second study was also a review of recent literature evaluating the impact of maternal fruit and vegetable consumption on adverse pregnancy outcomes. Nineteen studies published between 2004-2017 met inclusion criteria and were included in the review. Higher fruit and vegetable intake in pregnancy reduced the risk of preeclampsia and small for gestational age. The results were inconclusive for preterm birth and gestational diabetes. Maternal fruit and vegetable intake did not affect gestational weight gain. There were no publications evaluating the effect of maternal fruit and vegetable intake on inflammation or oxidative stress markers in pregnancy.

The significant finding of this review was the overall lack of available evidence on the impact of maternal fruit and vegetable intake on inflammation and birth outcomes. However, promoting improved intakes of fruits and vegetables to meet requirements is important as many Americans do not meet recommended intakes for these key foods (Lee-Kwan et al., 2017). Since the evidence that exists does support reduced risk of some adverse outcomes, counseling during antenatal visits should be provided throughout pregnancy.
The third study examined the relationships between maternal fruit and vegetable intake in the first two trimesters of pregnancy on cytokine expression during these trimesters and the risk for preterm birth and gestational hypertension. The specific aims were: 1) to examine whether fruit and/or vegetable intake during early and mid-pregnancy predict first or second trimester specific cytokine expression, controlling for sociodemographic and clinical characteristics; and 2) to assess whether fruit and vegetable intake during early and mid-pregnancy predicts preterm birth or gestational hypertension, controlling for sociodemographic and clinical risk factors. A secondary analysis of data was conducted and included 181 healthy women with singleton gestation and complete first trimester sociodemographic, cytokine and birth outcome data. First and second trimester serum and cervicovaginal cytokine data was used as the inflammatory state changes drastically closer to term.

Meeting recommended two and a half servings per day of vegetables in the first trimester predicted higher first trimester serum CRP, IL-6 and TNF-α, and lower first trimester cervicovaginal IL-6. Meeting recommended four servings per day of fruit in the first trimester predicted higher first trimester serum IL-1β and lower first trimester cervicovaginal IL-1α. There were no second trimester associations between intake and cytokines. First trimester number of vegetable servings reduced the odds of delivering preterm by 54% per serving. Second trimester vegetable intake increased risk of gestational hypertension by more than two-fold per serving.

There is limited research examining the impact of fruit and vegetable intake on inflammation and birth outcomes. This is the first study to examine the impact of fruit or vegetable intake on maternal serum or cervicovaginal cytokine expression in pregnancy.
overall. Findings from other studies on adults overall support lower cytokine expression with higher fruit and vegetable intake (Hosseini et al., 2018; Yeon et al., 2012a). Prior studies reported conflicting results on the impact of a diet high in fruits and vegetables on preterm birth. Some types of fruits and vegetables did reduce risk in one study (Myhre et al., 2013), while others did not report an effect (Soto et al., 2015; J. Wang et al., 2017). One study reported an increased risk of preterm birth for those who ate fruits and vegetables infrequently (Smith et al., 2015). However, these results are similar to prior findings that support reduced risk of hypertensive disorders of pregnancy from a diet high in plant based foods (Brantsaeter et al., 2009; Endeshaw et al., 2015; Timmermans et al., 2011; Vieira et al., 2017).

These findings provide evidence that dietary intake of omega-3 fatty acids and fruits and vegetables affects inflammation and some adverse birth outcomes, however future research is needed. Further study should include randomized controlled trials to determine the optimal dosage of omega-3 fatty acids DHA and EPA that promote optimal inflammatory balance during pregnancy. Additional longitudinal studies with larger sample sizes are needed to add the limited body of evidence on the effects of fruit and vegetable intake during pregnancy on gestational outcomes and inflammatory state during pregnancy. Future research should include the use of a framework to guide planning, design, and implementation in order to conduct studies that are comparable in design and allow generalizability of findings.

In addition to research, the findings of this dissertation support the need for evidence based clinical practice changes. Implications for practice include through evaluation of the maternal diet and encouraging optimal intakes based on current available
evidence, as many are at risk of inadequate dietary intakes of fruits, vegetables and omega-3 rich foods. Fish intake twice weekly is currently recommended and should be encouraged, specifically fish high in n-3 such as salmon. At least four servings daily of fruit and two and a half servings daily of vegetables should also be stressed to expectant mothers. Healthy diet during pregnancy may affect inflammation as well as reduce risk of preterm birth and gestational hypertension and should be incorporated into routine antenatal care and counseling.

In conclusion, this dissertation adds to the current science through critical evaluation of existing literature and research. The current evidence supports the positive impact of omega-3 fatty acid on inflammation in pregnancy. The evidence also supports a positive impact on both preeclampsia and optimal fetal growth. The research findings provide support for fruit and vegetable intakes to reduce risk of preterm birth and gestational hypertension, as well as affect the inflammatory state during pregnancy.
APPENDIX A: Newcastle - Ottawa Quality Assessment Scale Case Control Studies

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

**Selection** (maximum 4 points)
1) Is the case definition adequate?
   a) yes, with independent validation*
   b) yes (record linkage or based on self-reports)
   c) no description

2) Representativeness of the cases
   a) consecutive or obviously representative series of cases*
   b) potential for selection biases or not stated

3) Selection of Controls
   a) community controls*
   b) hospital controls
   c) no description

4) Definition of Controls
   a) no history of disease (endpoint)*
   b) no description of source

**Comparability** (maximum 2 points)
1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for _______________ (Select the most important factor) *
   b) study controls for any additional factor*

**Exposure** (maximum 3 points)
1) Ascertainment of exposure
   a) secure record (e.g. surgical records) *
   b) structured interview where blind to case/control status*
   c) interview not blinded to case/control status
   d) written self-report or medical record only
   e) no description

2) Same method of ascertainment for cases and controls
   a) yes*
   b) no

3) Non-Response rate
   a) same rate for both groups*
   b) non-respondents described
   c) rate different and no designation
APPENDIX B: Newcastle - Ottawa Quality Assessment Scale Cohort Studies

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection (maximum 4 points)
1) Representativeness of the exposed cohort
   a) truly representative of the average ______________ (describe) in the community*
   b) somewhat representative of the average ____________ in the community*
   c) selected group of users e.g. nurses, volunteers
   d) no description of the derivation of the cohort

2) Selection of the non-exposed cohort
   a) drawn from the same community as the exposed cohort*
   b) drawn from a different source
   c) no description of the derivation of the non-exposed cohort

3) Ascertainment of exposure
   a) secure record (e.g. surgical records) *
   b) structured interview*
   c) written self-report
   d) no description

4) Demonstration that outcome of interest was not present at start of study
   a) yes*
   b) no

Comparability (maximum 2 points)
1) Comparability of cohorts on the basis of the design or analysis
   a) study controls for _____________ (select the most important factor) *
   b) study controls for any additional factor*

Outcome (maximum 3 points)
1) Assessment of outcome
   a) independent blind assessment*
   b) record linkage*
   c) self-report
   d) no description

2) Was follow-up long enough for outcomes to occur
   a) yes (select an adequate follow up period for outcome of interest) *
   b) no

3) Adequacy of follow up of cohorts
   a) complete follow up - all subjects accounted for*
   b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost)*
   c) follow up rate < ____ % (select an adequate %) and no description of those lost
   d) no statement
APPENDIX C: Newcastle-Ottawa Scale Adapted for Cross-Sectional Studies

**Selection:** (Maximum 5 stars)
1) Representativeness of the sample:
   a) Truly representative of the average in the target population. *(all subjects or random sampling)*
   b) Somewhat representative of the average in the target population. *(non-random sampling)*
   c) Selected group of users.
   d) No description of the sampling strategy.
2) Sample size:
   a) Justified and satisfactory. *
   b) Not justified.
3) Non-respondents:
   a) Comparability between respondents and non-respondents’ characteristics is established, and the response rate is satisfactory. *
   b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory.
   c) No description of the response rate or the characteristics of the responders and the non-responders.
4) Ascertainment of the exposure (risk factor):
   a) Validated measurement tool. **
   b) Non-validated measurement tool, but the tool is available or described.*
   c) No description of the measurement tool.

**Comparability:** (Maximum 2 stars)
1) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled.
   a) The study controls for the most important factor (select one). *
   b) The study control for any additional factor. *

**Outcome:** (Maximum 2 stars)
1) Assessment of the outcome:
   a) Independent blind assessment. *
   b) Record linkage * OR
   c) Self report*.
   d) No description.
2) Statistical test:
   a) The statistical test used to analyze the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value). *
   b) The statistical test is not appropriate, not described or incomplete.

This scale has been adapted from the Newcastle-Ottawa Quality Assessment Scale for cohort studies to perform a quality assessment of cross-sectional studies for the systematic review, “Are Healthcare Workers’ Intentions to Vaccinate Related to their Knowledge, Beliefs and Attitudes? A Systematic Review”.

I have not selected one factor that is the most important for comparability, because the variables are not the same in each study. Thus, the principal factor should be identified for each study.
REFERENCES


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https://www.cdc.gov/nchs/products/databriefs/db287.htm


diet and risk of preterm birth among Danish women: a prospective cohort study. 


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VITA

Lori Ogden, RN

Education

University of Kentucky  BSN  1999, December  Nursing

Professional Experience

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<td>Staff Nurse-Labor &amp; Delivery</td>
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<td>Staff Nurse-Call Center</td>
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<td>Staff Nurse-Labor and Delivery</td>
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<td>2008-2012</td>
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<td>Resource Nurse-Labor and Delivery</td>
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Awards and Honors

Summa Cum Laude BSN December, 1999

Teaching

08/2015-05/2016 University of Kentucky College of Nursing, Teaching Assistant
NUR310: Evidence Based Nursing

2006-2007 Instructor AWHONN Fetal Monitoring Course