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The Influence of Diet Quality on Vaginal Microbiota Composition in Pregnant Women

by

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Dissertation Proposal submitted to the Faculty of the
Daniel K. Inouye Graduate School of Nursing Graduate Program
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In partial fulfillment of the requirements for the degree of
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DEDICATION

This work is dedicated to my grandmother, Dolas Glover, who inspired me to persevere adversity with courage, faith, and hard work. To my aunt Kathy, uncle Mike, and uncle Kenny for always believing in me and giving me their unconditional love and support.

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To my mom, Linda C. Glover (1949 – 2012) who showed me the meaning of determination and, finally, to my dad, James G. Young (1947 - 2006) who taught me to hold my head up high no matter what life throws at me.

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Tonya Y. White

[Month Day, Year of BOR meeting, check
thesis and dissertation manual for dates]

ABSTRACT

The Influence of Diet Quality on Vaginal Microbiota Composition in Pregnant Women

Tonya Y. White, Doctor of Philosophy, 2021

Dissertation directed by William H. Catherino, Professor and Chair-Research Division,
Department of Obstetrics and Gynecology

Molecular bacterial vaginosis (BV), a microbial dysbiosis, contributes to adverse pregnancy outcomes and affects 30% of reproductive age women. It is unclear whether diet shapes the vaginal microbiota. This is important knowledge because diet is readily modifiable and may be leveraged to reduce adverse pregnancy outcomes. This study sought to fill the gap on whether diet influences the vaginal microbiota, thus BV, by examining the association between longitudinal diet quality and molecular BV. A subsample of 55 women from a large federally funded project (BEAM; NR014826) were included. No significant association between diet and molecular BV were observed. Gestation length, age, and presence of molecular BV at 6 – months gestation were noted to be significant predictors of molecular BV. This study substantiated findings that molecular BV contributes to early birth and obesity may be protective of BV. Diet does not play a notable role in shaping the vaginal microbiota.

Keywords: Diet, women's health, vaginal microbiota, pregnancy, bacterial vaginosis

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CHAPTER 1: INTRODUCTION

The purpose of this study was to investigate the effect of diet on the composition of the vaginal microbiota during pregnancy. The study presented here represents an original analysis of longitudinal data that were collected as part of a large federally funded prospective cohort project, titled *Birth, Eating and the Microbiota* (BEAM project: NR014826). This study titled, “The Influence of Diet Quality on Vaginal Microbiota Composition in Pregnant Women” is novel because it is the first to examine the longitudinal influence of diet on vaginal microbiota of pregnant women, specifically *Lactobacillus* spp. and the microbes associated with bacterial vaginosis, using community class type to compare the two groups. This chapter provides an overview of the background of the problem, followed by the study purpose, questions, aims, approach, significance, and assumptions. Finally, this chapter concludes with an outline of the chapters that follow.

BACKGROUND OF THE PROBLEM

The human body is host to trillions of bacteria and their genetic material, known as the human microbiome, which weigh roughly 1-3% of body mass and represent ten times more microorganisms (bacteria, fungi, protozoa, and viruses), than human cells in the body (92, 96). These bacteria live on and within the surfaces of the human body that are exposed to the external environment, and include: the skin, oral cavity, respiratory, gastrointestinal, genital and urinary tracts (55). These microbial communities directly and indirectly influence host health. The relationship between the microbiome and their

human host is symbiotic and there is mounting evidence to support their functional role in host immunity, pathogen resistance, digestion, and vitamin synthesis (98, 141).

Vaginal Microbiota and Dysbiosis

The vaginal microbiota refers to all of the microbes inhabiting the vagina (78). The vaginal microbial communities of healthy reproductive age women are typically dominated by one of several *Lactobacillus* species (102). Under the influence of estrogen, vaginal epithelial cells produce glycogen that promotes the colonization of *Lactobacillus* spp. (3). During pregnancy, increased levels of progesterone and estrogen (130) appreciably influence the structure and composition of the vaginal microbiota through creating an environment where *Lactobacillus* spp. thrive and, consequently, suppresses the growth of opportunistic and facultative organisms creating an environment that is less diverse than that of non-pregnant women (28, 42, 111).

The acidic environment provided by the commensal *Lactobacillus* spp. followed by the highly viscous mucus coating the vaginal epithelial surface and the phage that sits on it provide defense against opportunistic or pathogenic organisms. Reproductive age women are most commonly colonized with *L. crispatus*, *L. jensenii*, *L. iners*, or *L. gasseri* (102). *Lactobacilli* inhibit colonization of pathogenic and opportunistic organisms through one of several mechanisms with varying degrees of effectiveness depending on the species, these include: (1) a competitive exclusion mechanism of competing for binding sites and nutrients, (2) production of lactic acid that acidifies the vaginal niche, (3) production of hydrogen peroxide and other bacteriocin compounds, and (4) modulation of the host immune response (24). Combined, these mechanisms prevent the likelihood of pathogens gaining dominance in the vaginal space (3).

The commensal microbial communities in the vagina play a significant role in women's reproductive health (1, 20). Disturbances in the vaginal microbial communities brought on by an overgrowth of microbes associated with bacterial vaginosis (BV) increase women's susceptibility to disease states that are not observed in women with certain *Lactobacillus* dominated compositions (121). *Lactobacillus* dominated compositions of the vaginal microbiota, including *L. crispatus*, *L. gasseri*, and *L. jensenii* species, are associated with vaginal health in reproductive aged women because they produce lactic acid that acidifies the vaginal space and prevents overgrowth of pathogenic and opportunistic organisms, such as those associated with BV (48, 97, 102). *L. iners* does not produce the same lactic acid associated with the aforementioned species, but it appears to play an important role in restoration and maintenance of the vaginal niche through upregulation of host immunity (45, 75). The majority of reproductive age women are colonized with *Lactobacilli* (102).

Vaginal dysbiosis occurs when the usual *Lactobacilli* present on the vaginal epithelium are disrupted and there is concomitant overgrowth of anaerobic and facultative bacteria resulting in a rise in vaginal pH (72). BV is a dysbiotic condition characterized by a shift in the vaginal microbiota from the optimal *Lactobacillus*-dominated composition to a polymicrobial composition dominated by anaerobic and facultative bacteria and a relative absence of *Lactobacillus* spp. (82).

Bacterial Vaginosis

Typically, BV is considered a clinical diagnosis that manifest with symptoms such as a thin, homogenous discharge and malodor (19). However, not all women present with clinical symptoms. In fact, upwards of 80% of women with BV are subclinical or

asymptomatic (19, 63, 68, 101). Research suggests that women with BV associated microbiota are at significantly greater risk of adverse reproductive health outcomes such as doubling the risk of preterm birth and quadrupling the risk of contracting human immunodeficiency virus (HIV) even in the absence of clinical symptoms (6, 57, 61, 143). This is clinically important because women with BV are at greater risk for adverse health outcomes but may go unrecognized.

The prevalence of BV varies widely based on race and geography, but it is estimated to affect approximately 30% of reproductive age women in the United States and is one of the most common gynecological concerns prompting women to seek medical treatment (19, 38, 95, 128). For reasons that are unclear, the incidence of BV is significantly higher among African American (38, 45, 102) and Hispanic women compared to non-Hispanic Caucasian and Asian women (76, 102). Given that BV is a significant predictor of preterm birth it may play a role in the higher preterm birth rates observed in African American and Hispanic populations (76, 100). Although not the focus of the current study, BV may also disproportionately affect female service members as one study found that more than 80% of 841 military women surveyed reported they experienced vaginal symptoms in austere environments and over half reported that their symptoms interfered with their duties (113, 114). BV has been shown to be a significant predictor of repeated chlamydia and gonorrhea infections in service women (11, 12). The health risk associated with asymptomatic BV going unrecognized or untreated are substantial both in terms of health outcomes and financial impact (19, 31, 68, 119).

The financial impact of BV is estimated to be \$5B annually, but the economic burden may be as high as \$15B due to the secondary costs of treating BV-associated

complications, such as preterm birth and HIV (95). BV is also difficult to treat because antibiotic therapies are largely ineffective with high recurrence rates within months of treatment (19, 94). Given the large financial impact and the negative reproductive health outcomes, identifying BV in both clinical and subclinical women is essential to improving women's health outcomes and advancing the science on the condition.

There are several ways in which BV is diagnosed. The first is the Amsel criteria commonly used in the healthcare setting because it is a quick and inexpensive method of making the diagnosis based on clinical symptoms and the presence of clue cells (4). The second method is Nugent scoring which relies on a Gram stain analysis of the number of *Lactobacilli* present in vaginal samples (90). The Nugent scoring method has historically been used in the research setting but is being replaced by sequencing technologies, namely 16S ribosomal RNA (16S rRNA) as these sequencing technologies become less cost prohibitive. The advantage of 16S rRNA sequencing is that it provides a quantified, detailed analysis of all the vaginal microbiota and the identification of BV associated bacteria present in the vagina even in subclinical women, coined “molecular BV” (82, 138).

Molecular BV is a polymicrobial composition that represents an abundance of certain anaerobic organisms such as *Gardnerella vaginalis*, *Atopobium* spp., *Prevotella* spp., *Megasphaera* spp., *Sneathia* spp., *Leptotrichia* spp, and other BV Associated Bacteria (BVAB; BVAB-1, BVAB-2, BVAB-3 of the *Clostridiales* Order) (93).

Considering BV from a molecular standpoint is critical to advancing our understanding of the etiology, diagnosis, and influences that contribute to this dysbiotic condition (82). The method of classifying the vaginal microbiota into community state and class types based

on the predominant *Lactobacillus* spp. or polymicrobial composition has changed how the vaginal microbiota are studied and understood, particularly in the study of BV.

Vaginal Microbiota Community Types

Five Community State Types (CST) were identified in a landmark study of 396 healthy, non-pregnant, ethnically diverse, reproductive age women (102). *Lactobacillus* spp. dominated four of the CSTs: *L. crispatus* (CST I), *L. gasseri* (CST II), *L. iners* (CST III), and *L. jensenii* (CST V) and the other (CST IV) is a polymicrobial composition of facultative and obligate anaerobic bacteria characterized by low or absent *Lactobacilli* CST IV (103).

Vaginal microbial CSTs are not static, but rather dynamic ecosystems that are influenced by host factors, such as the menstrual cycle, sexual activity, pregnancy, and perhaps diet (45). The variance in the stability of the CSTs over time were observed to be mostly temporary in nature and would return to the ‘ground,’ or typical, state due to homeostatic forces (45). To examine the temporal and spatial dynamics of the vaginal microbiota, the authors created profiles of the CSTs (I-V) using time series data to designate a series of community states into one community class, either the *Lactobacillus*-dominated classes (designated the *L* class) or the diverse class (designated the *D* class) (45) to reflect how community composition changed over time (45, 102). The *L* class includes *L. crispatus*, *L. gasseri*, *L. jensenii*, and *L. iners* dominated class types. The *D* class represents the diverse polymicrobial composition with low or absent *Lactobacillus* spp. Situating the longitudinal variance of CSTs into classes based on the CST that a woman is in most of the time made it possible to examine transitions in the vaginal community types over time (45). In recognition of their longstanding association

with adverse health outcomes even in the absence of clinical symptoms, this study will focus on the polymicrobial composition of class *D*, or *molecular BV*.

Dietary Factors Associated with BV

Diet is a key determinant in the structure and composition of the gut microbiota (56), thus it is plausible that particular food components are essential in shaping the vaginal microbiota (24, 87, 120, 126, 129). Knowledge about the interaction between dietary intake and the composition of the vaginal microbiota could inform nutritional approaches aimed at reshaping the vaginal microbiota to one that will promote more favorable bacterial communities.

STATEMENT OF PROBLEM

BV increases a woman's risk for preterm birth and other adverse pregnancy outcomes (30, 66, 68, 119). Therapeutic dietary strategies targeted toward promoting healthy microbiome are recognized as adjunct or standalone therapies for disease states (26, 32, 53) and health maintenance (83, 105) and may be useful in fostering stability in the vaginal microbiota and promoting healthy pregnancy. However, it is unclear if and to what extent diet influences the vaginal microbiota composition in pregnancy. Few studies have examined the influence of diet on the prevalence of molecular BV and those that exist used cross-sectional designs and heavily relied on Nugent scores to diagnose BV (87, 120, 129). Inconsistencies in the literature in defining BV, the heavy reliance on Nugent scores, and the use of cross-sectional designs coupled with a paucity of evidence provide a limited scope of evidence to suggest that diet may play a role in the occurrence (87, 120, 129). To address these gaps, this novel study used longitudinally collected molecular and diet data to evaluate the relationship between diet quality based on the

Healthy Eating Index-2015 (HEI-2015) scores and associated vaginal microbial community class types as the unit of comparison.

PURPOSE OF STUDY

The purpose of this study was to examine the relationship between diet and the community class types (*L* class or *D* class) of the vaginal microbiota of pregnant women. To achieve this purpose the diet and molecular data from vaginal samples of pregnant women collected as part of the BEAM project were analyzed to determine whether diet (measured as HEI-2015 scores) correlated with changes in the composition of the vaginal microbiota (measured by transitions in the microbial community class type, *L* or *D*) over time.

SPECIFIC AIMS

Specific Aim 1

Assess the influence of diet on the vaginal communities among the cohort controlling for race and body mass index (BMI) (see Figure 1).

1a Binary logistic regression was conducted using the aggregate HEI-2015 *total* diet quality scores (the sum of the *adequacy* and *moderation* components yield the aggregate *total* score) and the two groups of women the ‘molecular BV group’ (those who have diverse class *D* [molecular BV] and the ‘non BV group’ (those who were dominated by *Lactobacillus* spp. class *L*) to determine if there was an association between diet (independent variable) and vaginal microbial community class type (dependent variable).

Hypothesis: The higher the HEI-2015 *total* diet quality scores (*adequacy* plus *moderation*) the lower the odds of molecular BV. Conversely, the

lower the *total* diet quality scores the higher the odds of molecular BV (class *D*).

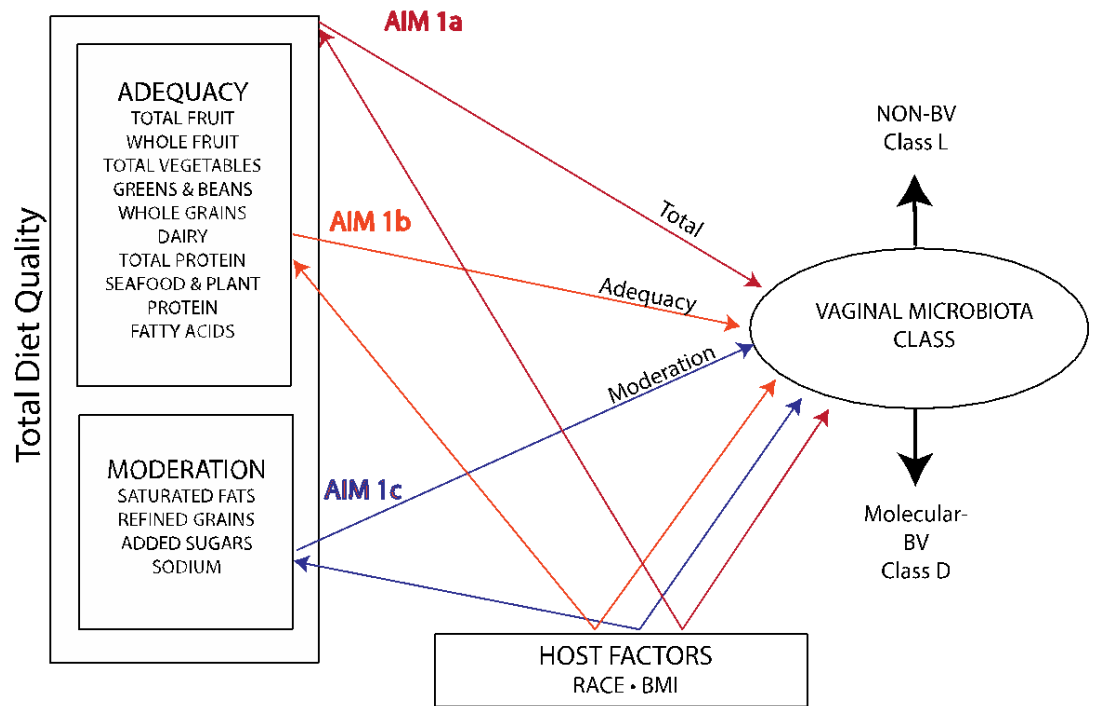
1b Controlling for differences based on race and BMI, binary logistic regression was performed to determine the association between diet quality using the individual *adequacy* component scores and the community class assignments, classified as the ‘non-BV’ group (class *L*) or the ‘molecular BV group’ (class *D*).

Hypothesis: The higher the *adequacy* diet scores the lower the odds of molecular BV (class *D*) and the lower the *adequacy* diet scores the higher the odds of molecular BV (class *D*).

1c Controlling for differences based on race and BMI, binary logistic regression modeling was performed to determine the association between diet quality using the individual *moderation* component scores and the community class assignments, classified as the ‘non-BV’ group (class *L*) or the ‘molecular BV group’ (class *D*).

Hypothesis: The higher the *moderation* scores the lower the odds of occurrence of molecular BV (class *D*) and the lower the *moderation* scores the higher the odds of molecular BV.

Figure 1. Specific Aim 1



Specific Aim 2

Analyzed the relationship between a diet high in consumption of total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein, seafood and plant protein, and fatty acids (high HEI-2015 *adequacy* component scores), or a diet high in consumption of saturated fats, refined grains, added sugars, and sodium (low HEI-2015 *moderation* component scores) and the community class type using longitudinal diet and community class assignment data in mixed effects logistic regression (see Figure 2).

2a Controlling for differences based on race and BMI, the HEI-2015 *adequacy*

scores were used to predict variance in community class assignment (class *L*)

and molecular BV class assignment (class *D*) in the cohort at three intervals of

participation during the study (Time 1 [T_1] --- 6 months, Time 2 [T_2] --- 7

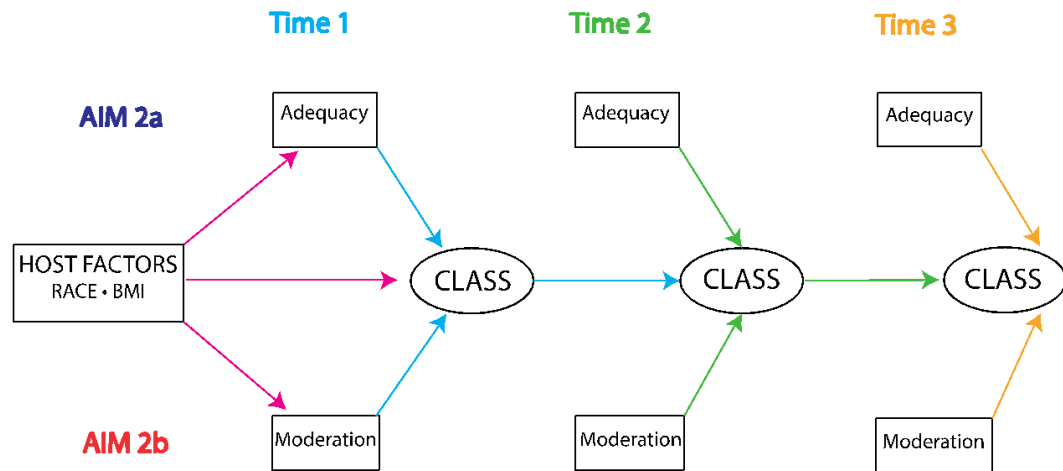
months, and Time 3 [T₃]*—*8 months gestation). The *adequacy* scores for each of the three respective timepoints were evaluated along with the longitudinal microbial data at the respective timepoint to determine if diet scores predicted variance in community class assignment over time.

Hypothesis: Lower HEI-2015 *adequacy* scores will be associated with increased odds of molecular BV (class *D*) at respective timepoints (6-, 7-, & 8-months gestation). Conversely, higher HEI-2015 *adequacy* diet quality scores will have decreased occurrence of molecular BV at respective timepoints.

2b Controlling for differences based on race and BMI, HEI-2015 *moderation* scores were used to predict variance in *Lactobacillus*-dominated community class assignment (class *L*) and molecular BV community class assignment (class *D*) in the cohort at the three intervals of participation during the study (6-, 7-, & 8-months gestation). The *moderation* scores for each of the three respective time points were evaluated along with the longitudinal microbial data at the respective timepoint to determine if diet scores predicted variance in community class assignment over time.

Hypothesis: Lower HEI-2015 *moderation* scores will be associated with increased odds of molecular BV (class *D*) at the respective timepoints (6-, 7-, & 8-months gestation). Conversely, higher HEI-2015 *moderation* diet quality scores will have decreased odds of molecular BV at respective timepoints.

Figure 2. Specific Aim 2



RESEARCH QUESTIONS

- Do HEI-2015 diet quality scores predict alterations in the frequency of BV associated microbial community class type (molecular BV)?
- Are the differences in the HEI-2015 diet quality scores associated with vaginal microbial community class types (*L* class and *D* class)?
 - Are there significant differences in the HEI-2015 *total* diet scores between women in the non-BV group (*Lactobacillus* dominated community classes) compared to women in the molecular BV associated community class (*D*)?
 - Are there significant differences in the HEI-2015 *adequacy* or *moderation* scores between women in the non-BV group (*Lactobacillus* dominated community classes) compared to women in the molecular BV associated community class (*D*)?

- o If there are significant differences in the vaginal community class types based on HEI-2015 *total*, *adequacy*, or *moderation* diet scores, do these differences still exist after controlling for race and BMI?

APPROACH

This study was a prospective, original analysis of the influence of diet on the vaginal microbiota using data that were collected over the course of the second and third trimesters of pregnancy (NR014826). The BEAM project recruited a non-probability sample of primigravid women with singleton pregnancies who were aged 18 to 34 and between 12 to 22 weeks' gestation from prenatal clinics in the Baltimore metropolitan area. This study focused on a subsample of 55 women from that cohort who had complete dietary intake and vaginal microbiota data. The parent project received human subjects' approval from the Institutional Review Board (IRB) at University of Maryland, Baltimore and all women in the study consented to participate prior to enrollment. The research protocol was submitted for review to the Uniformed Services University IRB and approval granted for the use of these data for the specified research activities.

SIGNIFICANCE OF STUDY

The significance of this study is that it provides novel information about the influence of diet on the vaginal microbiota during pregnancy. Understanding the role diet plays in shaping the vaginal microbiota is critical because diet is readily modifiable and may be leveraged as an alternate or adjunct therapy in the treatment of BV toward improving women's health outcomes. These findings serve as a baseline for future inquiry on the longitudinal influence of diet on molecular BV.

ASSUMPTIONS

The two main assumptions in this study are a) diet influences the composition of the gut microbiota that, in turn, shapes the composition of vaginal microbiota either systemically or proximally by way of the perineum, and b) diet scores correlate with diet quality.

LIMITATIONS

The parent project recruited a non-probability sample of pregnant women residing in one major metropolitan area that resulted in an over-representation of African American women; thus, a limitation of this study is the findings may not be generalizable to a broader population. Another limitation of this study was that women self-selected the photos they chose to submit for analysis of dietary scoring. This may have resulted in an over- or under-representation of their actual diet. However, the photo-based dietary data collection method used in the parent project may have an advantage over the recall bias inherent in paper-based data collection methods (86).

DELIMITATIONS

This research study was conducted using data from a larger parent project that examined the influence of diet on preterm birth with a cohort of 66 pregnant women. The scope of this study was examining the influence of diet on the abundance of *Lactobacillus* spp. or BV associated community class types, thus was limited to a subset of 55 women who had complete dietary and vaginal microbiota data.

DEFINITION OF TERMS

- *Bacterial Vaginosis*—BV is characterized by a shift in the vaginal microbiota from a *Lactobacillus* dominated state to a polymicrobial community of facultative and obligate anaerobic bacteria and low or absent *Lactobacillus* spp (72).
- *Community State Type*—The term CST is used to denote a microbial community with similar microbial phylotype. In the case of vaginal microbiota, CSTs define the dominate *Lactobacillus* spp. (CST I-III, V) or a polymicrobial composition (CST IV) (102).
- *Community Class Type*—Defined as a profile of CSTs composed from time series data of a set of CSTs that are clustered into five classes of longitudinal dynamics labeled as *Lactobacillus* class (L) [*L. crispatus*, *L. gasseri*, *L. iners*, *L. Jensenii*] or diverse class D (45).
- *Dysbiosis*—Dysbiosis is an imbalance in the vaginal microbial communities that is represented by a shift in the relative abundance of health-associated microbes to an abundance of disease-associated microbes (72).
- *Healthy Eating Index 2015 Scores*— Healthy Eating Index Scores are based on recommendations from the Dietary Guidance for Americans. There are two components: *adequacy* and *moderation* (47).
 - *Adequacy components* are considered the mainstay of the diet and include fruits, vegetables, greens and beans, whole grains, dairy,

proteins, and fatty acids). High adequacy scores are representative of a high-quality diet (47).

- o *Moderation components* are intended to be consumed in small quantities to maintain health. Moderation components include refined grains, sodium, added sugars, and saturated fats. High intake of moderation components are associated with a low-quality diet (47).
- *Host*—The human host shares in a mutualistic relationship with commensal bacteria whereby the host supplies the habitat and nourishment for microbial ecosystems and the microorganisms provide the host with essential nutrients, pathogen protection, and immunomodulatory function (55).
- *Microbiome*—The term microbiome is used to denote all of the microbes including their genetic material on and within the body including on the skin, in the oral cavity, respiratory, gastrointestinal, genital and urinary tracts (55).
- *Microbiota*—references the microorganisms themselves. Although at times the terms *microbiome* and *microbiota* are used interchangeably (55).
- *Molecular BV*—is a term coined to represent the use of molecular techniques to identify the presence of “non-optimal” polymicrobial bacterial communities with low or absent *Lactobacillus* spp. (82)

CONCLUSIONS

Vaginal microbiota influences women's reproductive health outcomes. Dysbiosis of the vaginal microbiota, such as BV, have been associated with increased susceptibility to disease. There is a need to understand how diet influences changes in the vaginal microbiota because antibiotics alone are largely ineffective in the treatment of BV for many women, placing them at increased risk for preterm birth and STIs including HIV. Until now, research about the role of diet in the treatment of BV or as an adjunct therapy, were lacking. This research study contributes to the knowledge and begins to fill the gap related to the influence of diet on the vaginal microbiota during pregnancy over time. This study provides novel information about the influence of diet on the vaginal microbiota during pregnancy that help inform health care providers' clinical practice and update strategies for patient education.

In the chapters that follow, Chapter two is a literature review on diet and its relationship to BV. Gaps in the literature related to the influence of diet on BV were addressed as well as how the current study fills the gap in the literature. Chapter three includes the study methodology that details the research plan used to conduct this study. Chapter four is a summary of the analysis and results. Finally, Chapter five concludes with a discussion of how this study is situated in the current body of literature and future directions and implications of the findings.

CHAPTER 2: LITERATURE REVIEW

INTRODUCTION

The adverse effects of bacterial vaginosis (BV) in reproductive age women are well documented in the literature, yet little has changed in the way of treatment over the past 70 years or so because the etiology of BV is still not well understood. BV affects approximately one in three reproductive age women (19). It is a dysbiotic condition characterized by a shift in the vaginal microbiota from the optimal *Lactobacillus*-dominated composition to a polymicrobial composition dominated by anaerobic and facultative bacteria and a relative absence of *Lactobacillus* spp (102). BV is difficult to treat because antibiotic therapies are largely ineffective with high recurrence rates within months of treatment (18, 94, 133). Alternatives and adjunct therapies with probiotics are currently being explored with mixed results (70, 107, 142). Importantly, these studies suggest the possibility that diet may influence the vaginal microbiome by way of the gut microbiome, though the precise mechanism is unclear.

The purpose of this review is to present the current evidence on the relationship between diet (total diet, micro- and macro-nutrients), the vaginal microbiota, and molecular BV. Molecular BV is defined as a vaginal microbiota characterized as a polymicrobial community with low or absent *Lactobacillus* spp. through the use of culture-independent methods of identification (82). The significance of molecular BV lies in that these women have BV but are asymptomatic and therefore are at increased risk for adverse sequelae because they go untreated.

This review is a synthesis of the current literature on the influence of diet on BV associated vaginal dysbiosis. PubMed, Cumulative Index to Nursing and Allied Health

(CINAHL), and Google Scholar were searched for peer-reviewed literature related to the influence of diet on culture-dependent and culture-independent forms of BV using the search terms ‘vaginal microbiota’ OR ‘vaginal microbiome’ AND ‘bacterial vaginosis’ ‘vaginal dysbiosis’ AND ‘diet’ OR ‘nutrients’ OR ‘macronutrients’ OR ‘micronutrients’ AND ‘reproductive age women’ OR ‘pregnant women’. The search was limited to human studies of reproductive age women and articles published in the English language. Articles were excluded if they used season as a proxy for vitamin D, focused exclusively on adolescences, or defined BV by self-report.

This chapter is organized as follows: first, background information is provided on vaginal microbiota, dysbiosis associated with bacterial vaginosis, and factors that influence BV. Next, the results of the literature search are organized by micronutrients, macronutrients, and total diet. This chapter concludes with the gaps in the current literature and how this research study will begin to fill this gap.

BACKGROUND

Vaginal Microbiota

The vaginal microbiota refers to all of the different microbes inhabiting the vagina (78). The vaginal microbial communities of healthy reproductive age women are typically dominated by one of several *Lactobacillus* species (102). Under the influence of estrogen, vaginal epithelial cells produce glycogen that promotes the colonization of *Lactobacillus* spp. (121). The acidic environment provided by the commensal *Lactobacillus* spp. provide defense against opportunistic and pathogenic organisms. Reproductive age women are most commonly colonized either *L. crispatus*, *L. jensenii*, *L. iners*, or *L. gasseri* (102). *Lactobacilli* inhibit colonization of pathogenic and

opportunistic organisms through one of several mechanisms with varying degrees of effectiveness depending on the species, these include: (1) a competitive exclusion mechanism of competing for binding sites and nutrients (2) production of lactic acid that acidifies the vaginal niche, (3) production of hydrogen peroxide and other bacteriocin compounds, and (4) modulation of the host immune system (121). Combined, these mechanisms prevent the likelihood of pathogens gaining dominance in the vaginal space.

Although *Lactobacilli* are considered the optimal vaginal microbiota, there are important differences in the level of protection certain species provide. For example, *L. crispatus* has consistently been associated with a lower risk of adverse reproductive outcomes whereas *L. iners* is more easily displaced and therefore more transient in nature (16, 74). It does not provide the same level of lactic acid/pH lowering properties, but it does upregulate the host immune system (38, 91). Longitudinal studies have shown that composition of the vaginal microbiota is highly varied and dynamic; in some women it shifts rapidly from a *Lactobacillus* spp. dominated to one with low abundance of *Lactobacillus* spp. while in others the composition remains relatively stable (45).

Culture-independent methods provide a census of the microbes present in the vagina that cannot otherwise be identified. Using culture-independent methods such as 16S ribosomal RNA (16S rRNA) sequencing technologies, recognizable patterns in the vaginal microbiota of asymptomatic, non-pregnant reproductive age women were clustered according to composition and abundance and classified into one of five community state types (CSTs): CST I dominated by *L. crispatus*, CST II dominated by *L. gasseri*, CST III dominated by *L. iners*, CST V dominated by *L. jensenii*, and CST IV by a non-dominated, polymicrobial composition (102). This revolutionary observation has

changed how the vaginal microbiota are studied and understood. This classification method not only provides a standardized measure for communicating findings, but it has also challenged conventional thinking about what constitutes “normal” vaginal microbiota (45, 72, 103). This depth of taxonomic understanding of the vaginal microbial communities is essential to begin to understand the relationship between the host, the vaginal microbiota, and its role in reproductive health and disease.

Dysbiosis Associated with Bacterial Vaginosis

Vaginal dysbiosis occurs when the usual *Lactobacilli* present on the vaginal epithelium are disrupted and there is concomitant overgrowth of anaerobic and facultative bacteria. This is occasionally accompanied with a rise in vaginal pH, a thin, homogenous discharge, and malodor. The BV polymicrobial state type results from an increase in the abundance of certain anerobic organisms such as *Gardnerella vaginalis*, *Atopobium* spp., *Prevotella* spp., *Megasphaera* spp., *Sneathia* spp., *Leptotrichia* spp, and other BV Associated Bacteria (BVAB; BVAB-1, BVAB-2, BVAB-3 of the *Clostridiales* Order) (122).

BV is the most common gynecological problem affecting approximately 30% of women, but as many as 80% of those women are asymptomatic (Nugent-BV or molecular-BV) (57, 101). As stated earlier, asymptomatic BV is a significant problem because these women are at greater risk for adverse health outcomes but go unrecognized (82). Dysbiotic vaginal microbiota, like those associated with BV, are strongly linked with adverse health outcomes including an increased risk of sexually transmitted infections (STIs), such as human immunodeficiency virus (HIV), human papilloma virus (HPV), and herpes simplex virus (HSV) (7, 84, 123).

BV is typically diagnosed in one of three ways: Amsel criteria, Nugent score, or, in the case of molecular BV, through sequencing technologies (82). In the clinical setting however, BV is diagnosed with a medical history, a vaginal exam, and Amsel's criteria that includes a microscopic examination for the detection of clue cells, vaginal pH > 4.5, characteristic fishy odor noted with a positive whiff test (5). A positive diagnosis is made if three of the following criteria are satisfied: a vaginal pH > 4.5, a positive whiff test, thin grey or white discharge, and/or the presence of clue cells. This type of BV is referred to as Amsel-BV (82). This technique is readily used because it is a quick and inexpensive way to assess and treat BV in the clinical setting. However, this technique has been criticized for being too subjective (9, 117)

In the research setting, the Nugent score has historically been used to diagnose clinical and subclinical BV. This is known as Nugent-BV (82). The Nugent score is a Gram-staining method used to evaluate the abundance of bacterial morphotypes that is dependent on the researcher's skill to accurately interpret the microorganisms. This method is limited in two ways. First, there is researcher subjectivity that can introduce bias (146). Secondly, the Nugent scoring method lacks the specificity to differentiate between the types of *Lactobacilli* present. Therefore, as sequencing technologies become more available and less expensive, the Nugent score is expected to be replaced with more specific sequencing methodologies (82).

Traditional cultivation techniques, described as culture-dependent methods, have been useful in identifying many of the bacteria that reside in the human vagina. However, advances in sequencing technologies, referred to as culture-independent methods, have vastly improved the detection and identification of known and previously unidentified

bacteria without the need for culture. The discovery of *L. iners* through culture-independent methods is a prime example of how sequencing technologies are moving the science forward to help clinicians and scientists better understand BV (75). The methods for determining the bacteria present in the vagina have direct implications for diagnosis and research on the pathogenesis of BV. This type of BV is deemed molecular-BV (82). Sequencing technologies are helping to advance understanding of which vaginal microbial communities place women at greater risk for disease.

The vaginal microbial community is a dynamic ecosystem that is influenced by host factors. Ethnicity, obesity, and pregnancy are widely studied areas in vaginal microbiota research because of recognized differences in populations and the profound impact it has on health outcomes in both clinical and subclinical BV (17, 37, 38, 112).

For reasons that are not clear, ethnic differences have been observed in pregnant and non-pregnant reproductive age women (38, 45, 73, 102). Women of African descent and Hispanic women have a higher predisposition to be colonized with a diverse polymicrobial mixture of anaerobic and facultative bacteria (45, 102). Given the tendency of women of Hispanic and African descent to have a polymicrobial composition, asymptomatic vaginal dysbiosis may not necessarily be an “unhealthy” state, but rather a risk factor for adverse reproductive health outcomes that needs to be considered in research and clinical settings (72, 82).

Obesity may be another confounding variable in the study of the vaginal microbiota because associations between BV and obesity have been observed (17, 87, 109, 139). However, there are inconsistencies between studies as to whether obesity is a

risk factor for BV (37, 80). Still other studies found that obesity had a protective effect in the incidence and prevalence of BV (71, 92). These inconsistencies warrant further examination and may be related to host factor differences between the samples.

Pregnancy is another factor that has an effect on the vaginal microbial composition. For instance, during pregnancy the vaginal microbiota are more stable because of the higher hormone levels (111). As pregnancy progresses, *L. crispatus* is noted to increase in abundance while *L. iners* decreases (147). However, BV is still prevalent in approximately 25% of pregnant women (19). This is clinically important because ascending bacteria from the lower genital tract are thought to be a primary source of infection in pregnancy contributing to preterm labor/birth (110).

The exact pathogenesis of BV remains unknown but the vaginal microbiota of symptomatic and asymptomatic women have a distinct polymicrobial composition with low or absent *Lactobacillus* present (133). Therapeutic regimens that target the causal pathogens have been used, but with very little effect. Metronidazole and clindamycin are the current antibiotics of choice for the treatment of BV because they have been shown to successfully treat the BV associated pathogens, however they also kill the *Lactobacilli*. Therefore, in some women treatment is only temporary (18, 94). Reoccurrence of BV happens in upwards of 60% of women afflicted with BV associated microbial structures (133).

In addition to antibiotic treatment, oral probiotics and prebiotics have been shown to restore *Lactobacillus* spp. in the vagina though the results are inconsistent (70, 108). These studies suggest that there is a path for gastrointestinal microbiota to directly (proximity) or indirectly (systemically) alter the vaginal microbiota to influence BV

treatment outcomes (24). Based on emerging evidence, diet may play an important role in modulating the structure and composition for promoting and maintaining the health of the vaginal microbiota.

DIET FACTORS ASSOCIATED WITH BV

Diet may shape the composition and diversity of the vaginal microbiota by indirectly influencing the vaginal milieu that ultimately determines the type of microbes that thrive in the vagina (87, 116). Although the mechanisms of actions are unclear, studies have reported associations between diet, altered immunity, and vaginal microbial composition (10).

The literature search on the diet factors associated with BV resulted in a total of 15 peer-reviewed publications. Only five of the 15 were less than five years old (15, 109, 120, 129, 131). Twelve of the publications addressed the relationship between specific micronutrients and BV (13, 15, 22, 33, 44, 51, 109, 125, 127, 129, 131, 132) and three focused on macronutrients (87, 120, 126). Only 10 of the 15 studies were conducted on US populations (13, 33, 44, 51, 87, 120, 126, 127, 129, 132). Two used a cohort from a weekly iron and folate supplementation trial (15, 109), two used a cohort from the Hormonal Contraceptive Longitudinal study (120), One study used the National Health and Nutritional Examination Survey (NHANES) cohort (51), and two studies used a cohort from the Longitudinal Study of Vaginal Flora (87, 126).

Although several of the studies were longitudinal designs, diet was only examined at one time point (120, 126, 129). Assessment of diet by the Food Frequency Questionnaire (FFQ) has been shown to be a reliable, valid method of diet assessment but the weakness in this method is that it relies on diet recall and can be extremely time

consuming. This was pointed out in one study that had stopped collecting diet data on subjects once they reached power due to the time and resource constraints (87).

BV was determined by different criteria in the literature. BV was defined by Amsel's criteria for inclusion in two studies (44, 132), Nugent scores in nine studies (13, 22, 33, 51, 87, 125–127, 131), and sequenced data was used in four studies (15, 120, 129). Limitations in the current body of literature were differences in defining BV, cross-sectional diet assessment, and recall bias.

Another limitation in the current literature is the cross-sectional analysis of the composition of the vaginal microbiota. Cross-sectional studies of BV are of limited value in that the vaginal microbiota of reproductive age women is dynamic, thus longitudinal studies provide better insight into the role diet may play in influencing BV associated dysbiosis or eubiosis.

Micronutrients

A total of 12 micronutrient articles were identified, two examined a combination of vitamins A, C, E, or β -carotene (22, 127), seven focused on the relationship between vitamin D and BV (13, 33, 43, 52, 125, 131, 132) two examined the relationship between iron and BV (15, 109), and one found a link between low intake of betaine and BV (129). The role of micronutrients is important in immune function, epithelial integrity, and promoting healthy vaginal microbiota (59). Vitamin A promotes vaginal mucosal integrity because of its effect on epithelial cell and host immune system (140, 145). Vitamin A also plays a role in the stability of the vaginal microbiome.

Vitamins A, C, E, β -Carotene

In a parent study that examined the effect of weekly vitamin A or β -carotene supplementation on maternal mortality among pregnant and postpartum women in rural Bangladesh, a cross-sectional substudy was conducted to determine the prevalence and incidence of BV across three separate timepoints: at baseline, at 32 weeks' gestation, and at three months postpartum (22). In this study, BV was defined as a Nugent score of 7 to 10. Symptomatic BV was defined as women with a Nugent score of 7 to 10 and reporting abnormal vaginal discharge for which women were offered treatment with metronidazole, but many did not complete (22). Less than 1% of women were considered to have symptomatic BV. At baseline, the prevalence of BV was 5.6% in the placebo group, 7.9% in the vitamin A group, and 9% in the β -carotene group. After supplementation with vitamin A and β -carotene, the authors found a significant reduction in the odds of BV by 30 to 40% at 32 weeks' gestation and at three months postpartum, but not in the third trimester when compared to the placebo groups (22).

There were several limitations with this study. First, there were significant differences between the placebo, Vitamin A, and β -carotene groups in terms of BV incidence and prevalence, sociodemographic characteristics, and study adherence. Secondly, very few women were considered to have symptomatic BV, therefore, CST IV may represent their "normal," CST for which vitamin supplementation may be too narrow a scope to investigate the effect of nutrients on the vaginal microbiota composition. It is possible that overall diet quality may have played a role in the incidence and prevalence of BV considering differences in sociodemographic confounders, but total diet was not assessed. Finally, the study was underpowered (<30%)

to adequately determine the effect of supplementation on the incidence and prevalence of BV.

The association of micronutrients and vaginal infections was also examined in a large US-based cohort of HIV infected and at-risk women (127). This was a cross-sectional substudy of the HIV Epidemiology Research Study, known as HERS. The authors analyzed 16 micronutrients to determine if there was an association with various vaginal types of infections (trichomoniasis, BV, *Candida*, HPV, abnormal cervical cytology). The authors found that higher risk of BV was associated with lower serum concentrations of vitamins A, C, E and β -carotene. BV was defined as a Nugent score of 7 to 10 regardless of symptoms. In absence of micronutrient reference standards, reference distributions were determined by comparing the HERS cohort aged 20 to 57 to a NHANES female cohort aged 16 to 60. The vaginal exam and non-fasting serologies were collected at different time points within a two-week span. This study found a significantly higher prevalence of BV in HIV-infected women which is not surprising given that BV could have been the precipitating factor (127).

BV has been associated with an increased risk of HIV infections in women by as much as two to four times (6, 23, 46, 54). Also, over half the women in both the HIV infected and HIV uninfected groups were African American, thus, for a large portion of these women, a polymicrobial composition may be their “normal” (37). Another major limitation of this study is their assessment of nutritional/micronutrient status. The authors report that women were asked about their diet and supplement intake, but that information was not included in this publication or analysis (22). The authors assert that the HERS cohort was below the national average when compared against the NHANES

group (1976-1980 and 1988-1994) but given generational changes in dietary practices and the socioeconomic status of the HERS study cohort the NHANES sample may have biased the point of reference. Lastly, vaginal samples and serum samples were collected at different times as much as two weeks apart. The vaginal microbiota structure and composition are dynamic and can be influenced by hormonal changes and menses, and so can some nutritional indices such as iron (35). These confounders were also not considered in the study which weakens the findings.

Iron

Two studies examine cohort data from a trial conducted on the safety of weekly iron and folic acid supplementation in reproductive age women (15, 109). The first study examined the influence of iron supplementation on the risk of lower genital tract infections. The authors did not observe differences in the incidence of BV among women receiving iron supplements, but did detect differences at baseline in iron-deficit women who were more likely to have *Lactobacilli*-dominated microbiota (15). The second study examined the association of vaginal infections to nutritional indices and iron biomarkers (109). These authors observed higher vaginal lactoferrin levels among women with *L. iners* – dominated and polymicrobial community state types and a > 6-fold increase in BV (109). Findings from these studies may not be generalizable for several reasons, first, the samples were of primarily adolescents with mean ages less than 18 years old. Secondly, participants were from a malaria-ridden environment that may have influenced their iron status. There was also a significant loss to follow up in the parent project.

Vitamin D

Vitamin D, active form 1 α , 25-dihydroxyvitamin D3 [1,25(OH)₂D3], is a key immune regulator in both innate and adaptive immune responses (27). It also plays a key role in cell proliferation (124, 136). The immunostimulatory properties of vitamin D include stimulation of monocyte proliferation, increased production of interleukin IL-1, and other signaling pathways to enhance anti-microbial activities (115). Vitamin D mediated host defense is triggered through the production of the antimicrobial protein cathelicidin which activates monocyte and macrophage activity. Cathelicidin is an essential bactericidal peptide. Given the significant role vitamin D plays in immune regulation, it is not surprising that a larger body of literature on micronutrients is dedicated to examining the relationship between vitamin D and BV.

The majority, but not all, of the studies concluded that a deficiency in vitamin D was associated with an increase in susceptibility to BV (13, 33, 44, 51). For example, among pregnant and non-pregnant women where BV was defined by Nugent scores of 7 to 10 and vitamin D was determined by serum concentrations, vitamin D deficiency was observed to be significantly associated with BV (13, 33, 51). This relationship was also examined in a pregnant cohort of HIV positive and HIV negative women where BV, diagnosed by Amsel criteria, was noted to be significantly associated with serum vitamin D levels less than 20 among HIV positive women only (44). However, the relationship between vitamin D deficiency and BV was refuted in a study of Zimbabwean women where the incidence and prevalence of BV (Nugent scores 7 to 10) was not associated with serum vitamin D levels below 30 (131).

Vitamin D has also been examined in the context of treatment and prevention of BV. In a study of asymptomatic, vitamin D deficient (defined as serum $< 75\text{nmol/l}$), reproductive age, Iranian women, oral supplementation with vitamin D was shown to significantly improve cure rates of BV (diagnosed by Nugent scores) compared to a placebo group (OR 10.08, $p < 0.001$) (125). This was not the case in another study that examined the effect of high-dose vitamin D for prevention or recurrence, where vitamin D was not observed to improve BV at any time point (132).

The limitation with examining and interpreting results of specific micronutrients are twofold. First, racial differences in vitamin D levels have been observed in that approximately 40% of African American women have shown to be vitamin D deficient, thus skewing the results. The other argument is that micronutrients interact, therefore examining one specific micronutrient may be too narrow a focus to determine the influence on BV.

Vitamin D studies have suggested that there may be a relationship between vitamin D status and BV, however the results have been inconclusive. Vitamin D studies are considered in this review but are of unclear significance because a large portion of African American women (42%) have insufficient vitamin D (14) and are more likely to have CST IV (102), thus the results of these studies are confounded.

Macronutrients

Only three studies were identified that examined the relationship of macronutrients and BV. The first is a study that conducted a cross-sectional analysis of diet data obtained from the Block 98 Food Frequency Questionnaire and compared it against Nugent scores for 1521 non-pregnant women to determine if diet influenced the

incidence of BV (defined as scores of 7 to 10) (87). The prevalence of BV in this cohort was approximately 42% with 15% characterized as having severe BV (Nugent scores $\geq$ 9). The authors found a significant increase in BV among women who consumed high fat diets and that total fat and monounsaturated fat were significantly associated with severe BV. Diets high in protein, folate, vitamin E, and calcium had an inverse relationship with severe BV (87).

Using that same cohort, the glycemic index (GI), mean daily glycemic load (GL), Healthy Eating Index (HEI) and Naturally Nutrient Rich (NNR) scores were examined to determine if there was a relationship with the incidence of BV. They found that HEI values >70 were associated with a significant reduction in BV, NNR was negatively associated with BV, and GL was positively associated with BV (87, 126).

Another study, using data obtained from a Block Brief 2000 FFQ, found that low fiber intake was associated with a high risk of molecular BV (120). Specifically, there was an observed trend in women whose fiber intake came from beans and grains having lower molecular BV. Given the limited perspective of the influence of micronutrients and isolated macronutrients, there is a need to dial out to provide a more complete assessment of the influence of diet on the composition of the VMB (120).

Total Diet

Only one study was found that examined the relationship between total diet in relation to BV among primarily African American (86%), non-pregnant, reproductive age women (87). In this study diet was measured at a single point in time (at the second visit) using the Block 98 FFQ and BV was assessed at the second and third visits measured as Nugent scores $\geq$ 7 with severe BV as scores $\geq$ 9. The calorie consumption, fat, and

protein intake were significantly different among African American women compared to Caucasian women and fiber intake was two-thirds below the recommended daily allowance for all participants.

In this study, micronutrients were not associated with the incidence of BV (87). After adjusting for sociodemographic factors, BMI, birth control and alcohol use, fat intake was significantly associated with BV (OR = 1.5, 95% CI = 1.1 – 2.4). Moreover, total fat, saturated fat, and monounsaturated fat were significantly associated with severe BV (87).

CONCLUSION

While great strides have been made in the science pertaining to optimizing the health of the vaginal microbiota of reproductive age women, the current findings are confusing and lack clarity in terms of clinical application, therefore, there is still a need for continued study of this topic. The studies presented here, when taken together, suggest that diet may modulate changes in the composition, stability, and diversity of the vaginal microbiota. However, the body of literature on whether diet quality influences the composition of the vaginal microbiota or if it is associated with or predictive of BV is extremely limited. Given that BV is strongly associated with adverse pregnancy outcomes, it is vital to understand the influence of diet on the vaginal microbiota during pregnancy. Knowledge on the role diet plays in shaping the vaginal microbiota is critical because diet is readily modifiable and may be leveraged as an alternative or adjunct therapy in the treatment of BV. In order to fully understand the impact of diet on the vaginal microbiota longitudinal studies are necessary. This study is the first to use longitudinal collected molecular and diet data to examine the association between diet

quality and the vaginal community class type related to diet quality. This study investigated the relationship between diet and the community class type of the vaginal microbiota longitudinally to determine if there are differences in the composition of vaginal microbiota among pregnant women based on their diet quality scores.

CHAPTER 3: METHODS

INTRODUCTION

The purpose of this study was to examine whether diet quality influenced changes in *Lactobacilli* present in the vaginal microbiota of participants over time. This novel study was designed to address a knowledge gap on the longitudinal influence of diet on the molecular composition of the vaginal microbiota. This study focused on bacterial vaginosis (BV) associated microbes (class *D*) in recognition of their longstanding association with adverse health outcomes and *Lactobacillus*-dominated community class types (class *L*) that generally are accepted as representing optimal type vaginal microbiota.

The longitudinally collected sequenced vaginal microbiota and diet quality data from a cohort of pregnant women who participated in the BEAM project (NR014826) were used to address the research questions and aims of this study. Sequenced vaginal data and Healthy Eating Index 2015 (HEI-2015) diet quality scores were examined to determine the effect on community class types. This chapter presents the methodology used to accomplish the specific aims and answer the research questions to achieve the purpose of this study. First, details of the research design, study sample, and data source are presented. Next, measures, study variables, data preparation and data analysis are discussed. Finally, this chapter concludes with ethical considerations, limitations, and a brief summary of this chapter and an introduction to the chapters that follow.

RESEARCH DESIGN

This study was an original analysis that used data collected as part of a longitudinal federally funded parent project, known as the BEAM project (NR014826).

The BEAM project prospectively investigated whether diet influenced the vaginal microbiota and preterm birth in a cohort of pregnant women from 2014 to 2018. Data collection included, but was not limited to, weekly vaginal swabs that were self-collected by study participants at home from recruitment at 12 to 22 weeks' gestation up through birth. In the parent project, the FoodFoto™ system, an image-based method was used to collect diet data that were converted to food codes by trained turkers. Details of the FoodFoto™ system are published elsewhere (105).

This study was designed to attempt to fill a gap in knowledge on the longitudinal influence of diet on the vaginal microbiota of pregnant women using community class type to compare the non-BV and molecular BV groups. Prior to this study, the body of literature had not addressed longitudinal influence of diet on changes in the vaginal microbiota from a molecular BV perspective. This left a major gap in knowledge because the vaginal microbiota is dynamic and cyclic in nature, thus cross-sectional evaluations do not provide sufficient information about the changes that occur at the molecular level over time in relation to diet quality. This study provides baseline knowledge to begin to fill that gap.

RESEARCH QUESTIONS

Restated from Chapter one, the research questions guiding this study are:

- Do HEI-2015 diet quality scores predict alterations in the frequency of BV associated microbial community class type (molecular BV)?
- Are the differences in diet quality associated with vaginal microbial community classes (class *L* and class *D*)?

- o Are there significant differences in the HEI-2015 *total* diet scores between women in the non-BV group (*Lactobacillus* dominated community class *L*) compared to women in the molecular BV associated community class (*D*)?
- o Are there significant differences in the HEI-2015 *adequacy* or *moderation* scores between women in the non-BV group (*Lactobacillus* dominated community class *L*) compared to women in the molecular BV associated community class (*D*)?
- o If there are significant differences in the vaginal community class types (*L* or *D*) based on HEI-2015 *total*, *adequacy*, or *moderation* diet scores, do these differences still exist after controlling for race and BMI?

SAMPLE

The BEAM participants were comprised of a nonprobability sample of primigravid women who had been recruited from two major medical centers providing obstetric care in the Baltimore area. Data were collected weekly from enrollment through delivery. Participants were between the ages of 18-34 and had a singleton fetus. From a total of 66 women who had vaginal samples sequenced as part of the BEAM project, a subset of 55 participants from the BEAM project comprised the sample of this study.

Inclusion Criteria

Participants were included in the analysis if they had both sequenced vaginal data and HEI-2015 diet quality scores. Gestational months 6 through 8 had the most complete

diet and vaginal microbiota data. Differences in recruitment and delivery timeframes resulted in insufficient data for the overall sample at gestational months 5, 9 and 10. Participants were excluded from the analysis if they had more than one missing data point at the gestational months of interest (6, 7 or 8). The majority of the included sample had complete diet and vaginal microbial data for the gestational months of interest, thus missing values were presumed missing at random and were ignored for the purpose of analysis.

DATA SOURCES

Demographic data and HEI-2015 diet quality score data were extracted directly from the parent project dataset. The vaginal microbial data were converted to community class types for analysis in this study by a person trained and qualified in the use of VALENCIA (VAGinal community state type Nearest Centroid classifier). Further details of data sources and preparation are presented below.

Dietary Intake Data

The parent project collected nutritional data using FoodFoto™ an image-based diet data collection system operated through iPhone technology (105). FoodFoto™ captured photographs of participants' foods at the time of consumption. The specifics of the food data collection are described elsewhere (105). Trained analysts assigned food codes from the U.S. Department of Agriculture's Food Patterns Equivalents Database (FPED) that represented the content in the photo (134). Images that contained a combination of foods types (i.e., spaghetti with meatballs) were separated into its component parts and each assigned a respective food code. Diet quality estimation were

derived from food codes assigned to the FoodFoto images. Serving sizes of each food item was estimated to determine density. Within the parent project, coding reliability between analysts for diet density and diet quality were performed on a random sample of 20% of the images and interclass correlation coefficients between analysts were 0.96 and 0.92, indicating good interrater reliability.

The next step in the parent project was to convert the FPED food codes into HEI-2015 *total* and component (*adequacy* and *moderation*) scores using the publicly accessible SAS code available through the National Cancer Institute (134). The HEI-2015 *total* score represents the sum of the component (*adequacy* and *moderation*) scores, with a possible maximum score of 100. The HEI-2015 score increases as consumption of *adequacy* components increases, conversely the HEI-2015 score decreases as consumption of *moderation* components increases. Diet quality is more fully described below.

Vaginal Data

Vaginal microbiota data were collected in the parent project in the form of weekly vaginal swabs that were self-collected by participants (BEAM project: NR014826).

Following DNA extraction using validated protocols, the hypervariable region V4 of the bacterial 16S rRNA gene was targeted. Characterization of the composition of vaginal microbiota were performed using 16S rRNA gene amplicon sequencing.

MEASURES

Diet Quality

In this study, diet quality was defined by the degree to which participants' food choices aligned with the Dietary Guidelines for Americans (DGA) recommendations based on their respective HEI-2015 scores. The HEI-2015 score is both a valid and reliable measure of diet adherence to the DGA key recommendations (60, 104) that has been in use since 1995 (49). The HEI-2015 *total* score ranges from 0 to 100 and is comprised of 13 food categories that combine to make up the *adequacy* and *moderation* component scores (see Table 1) (64). The higher the *total* score, the higher the diet quality is presumed to be because higher scores represent compliance with the DGA's dietary recommendations.

The *adequacy* component score includes nine of the food categories (total fruits [5 points], whole fruits [5 points], total vegetables [5 points], greens and beans [5 points], whole grains [10 points], dairy [10 points], total protein foods [5 points], seafood and plant proteins [5 points], fatty acids [10 points]) and makes up 60% of the total score (64). The *moderation* component score includes four of the food categories (saturated fats [10 points], sodium [10 points], added sugars [10 points], and refined grains [10 points]) and makes up 40% of the total score (64). The assigned score is based on cup, ounce, or teaspoon equivalent serving sizes per 1000 kcals (135). The *adequacy* score increases up to the maximum possible score as the consumption of each of the nine food categories increases. Conversely, the *moderation* score decreases if the consumption of each of the four food categories exceeds the recommended daily allowance and the score of zero is possible with excess intake of the *moderation* components. The maximum 100 points is achieved when the daily recommended amount of each food category is met.

Table 1: HEI-2015 Components and Scoring Standards from the USDA's Center for Policy and Promotion (65, 137).

Component	Max	Standard for Maximum Score	Standard for Minimum Score
HEI-2015 ¹	Points		of Zero
Adequacy:			
Total Fruit ²	5	≥ 0.8 cup equivalent/1000 kcal	No Fruit
Whole Fruit ³	5	≥ 0.4 cup equivalent/1000 kcal	No Whole Fruit
Total Vegetables ⁴	5	≥ 1.1 cup equivalent/1000 kcal	No Vegetables
Greens and Beans ⁴	5	≥ 0.2 cup equivalent/1000 kcal	No Dark Green Vegetables or Beans or Peas
Whole Grains	10	≥ 1.5 -ounce equivalent/1000 kcal	No Whole Grains
Dairy ⁵	10	≥ 1.3 cup equivalent/1000 kcal	No Dairy
Total Protein Foods ⁶	5	≥ 2.5 -ounce equivalent/1000 kcal	No Protein Foods
Seafood and Plant Proteins ^{7,8}	5	≥ 0.8 -ounce equivalent/1000 kcal	No Seafood or Plant Proteins
Fatty Acids ⁹	10	$(\text{PUFAs} + \text{MUFAs})/\text{SFAs} > 2.5$	$(\text{PUFAs} + \text{MUFAs})/\text{SFAs} \leq 1.2$
Moderation:			
Refined Grains	10	≤ 1.8 -ounce equivalents/1000 kcal	≥ 4.3 -ounce equivalent/1000 kcal
Sodium	10	≤ 1.1 g/1000 kcal	≥ 2.0 g per 1000 kcal
Added Sugar	10	≤ 6.5 % of energy	≥ 26 % of energy

Saturated Fats	10	$\leq 8\%$ of energy	$\geq 16\%$ of energy
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Note: Source: Adapted from: Krebs-Smith, et al. ¹ Intakes between the minimum and maximum standards are scored proportionately. ² Includes fruit juice. ³ Includes all forms except juice. ⁴ Includes any beans and peas not counted as Total Protein Foods. ⁵ Includes all milk products, such as fluid milk, yogurt, and cheese, and fortified soy beverages. ⁶ Beans and peas are included here (and not with vegetables). ⁷ Includes seafood, nuts, seeds, soy products (other than beverages) as well as beans and peas counted as Total Protein Foods. ⁸ Ratio of polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) to saturated fatty acids (SFAs). ⁹

Vaginal Microbiota Data

For the purpose of this study, the vaginal microbiota was defined by community class type. The vaginal microbiota sequenced data from the parent project was used to determine the community class type of participants. A trained and qualified analyst used VALENCIA to identify the overall community class type for each participant included in the subsample for this study. VALENCIA is a tool that was designed and validated to comprehensively assess taxonomic profiles of reproductive age women and classify them according to their similarity to a set of reference centroids (40). In this analysis, taxa were retained if they had at least 1000 reads.

Gestational months were classified into 4-week intervals starting at 17 weeks' gestation through birth as follows: month 5 = weeks 17 – 20, month 6 = weeks 21 – 24, month 7 = weeks 25 – 28, month 8 = weeks 29 – 32, and month 9 = weeks 33 – 36. The community class types are dichotomized into 'non-BV' (*Lactobacillus*-dominated class types, class *L*) and 'molecular BV' (community class type *D*) groups. Participants were categorized into the non-BV or molecular BV groups based on the VALENCIA community class type classification. For the overall classification, women who maintained *Lactobacillus* throughout the study were classified as the non-BV group (*L* class) and women that had a polymicrobial community class (*D* class) or transitioned in

and out of *D* class over the course of the study were classified as the molecular BV group.

STUDY VARIABLES

Dependent Variable

The primary dependent variable for this study was the vaginal microbiota community class types: *Lactobacillus*-dominated class types (class *L*) or the diverse polymicrobial community class type (class *D*).

Independent Variable

The primary independent variable for this study was diet quality defined as HEI-2015 *total*, *adequacy*, and *moderation* scores.

Demographics/Confounding Variables

Demographic data were extracted from the parent project dataset and converted to binary variables due to the demographic characteristics and limited size of the sample. Age and gestational date at delivery were retained as interval data. African Americans comprised the majority of the sample, thus were coded as 1 and all others were coded as 2. Body mass index (BMI) was converted to obese ($\text{BMI} \geq 30$) = 1 and non-obese ($\text{BMI} < 30$) = 2. Education was coded as university degree = 1 and all others (less than university degree attainment) = 2. Income was coded as high income $\geq \$75\text{K}$ = 1 and all others ($< \$75\text{K}$) = 2. Employment was coded as unemployed = 1 and all others (students and employed) = 2. Finally, marital status was coded as single = 1 and all others (married or with domestic partner) = 2.

DATA ANALYSIS

Data Preparation

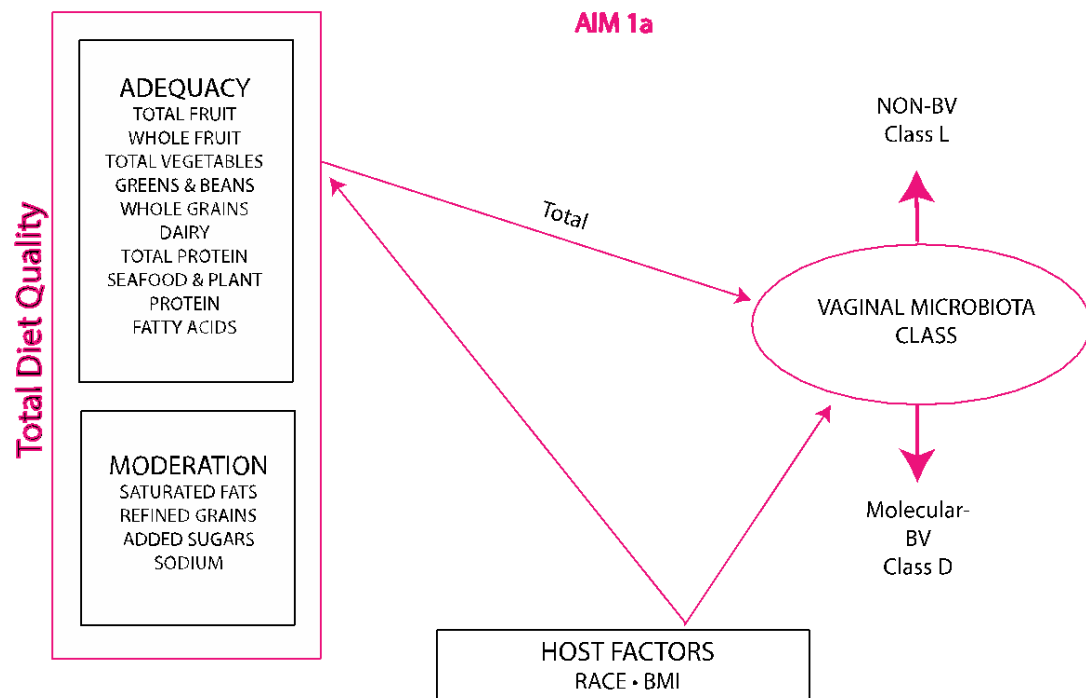
Before analysis, the data set was examined for accuracy and completeness. To answer the research questions, data were merged and cleaned. *Total*, *adequacy* and *moderation* scores were reviewed for accuracy. An investigator journal was maintained to notate all data merging and coding. After merging and cleaning, data were reviewed for accuracy and inconsistencies. Data were further examined by generating frequencies of nominal and ordinal data and means and standard deviations of HEI-2015 scores. All statistical analyses were performed in SPSS, version 27 at a 5% level of significance and a 95% confidence interval.

Descriptive statistics (mean, median, standard deviation and range) were ran to determine the sample characteristics and the *total* and component (*adequacy* and *moderation*) HEI-2015 diet quality scores. Sociodemographic characteristics (race and BMI) and vaginal microbiota composition based on community class type (class *L* or class *D*) were examined in relation to the *total* and component (*adequacy* and *moderation*) HEI-2015 scores using adjusted mixed effect logistic regression. These data are presented in a table format using frequencies, means, and standard deviations in Chapter four.

Specific Aim 1

The goal of specific aim 1 was to assess the influence of diet quality on the vaginal communities among a subsample of women controlling for race (African American or not) and BMI (BMI over 30 or not) and to answer the question: *are the differences in diet quality associated with vaginal microbial community classes (class L and class D)?* To achieve this aim, logistic regression was conducted modelling the aggregate HEI-2015 *total* diet quality scores (obtained by summing *adequacy* and *moderation* components to yield the aggregate *total* score) and the two groups of women (those who have molecular BV type polymicrobial structures [diverse class D] grouped as the ‘molecular BV group’ and those who are dominated by *Lactobacillus* spp. [class L] grouped as the ‘non BV group’) to determine if there is an association between diet (independent variable) and vaginal microbial community classes (dependent variable; see Figure 3).

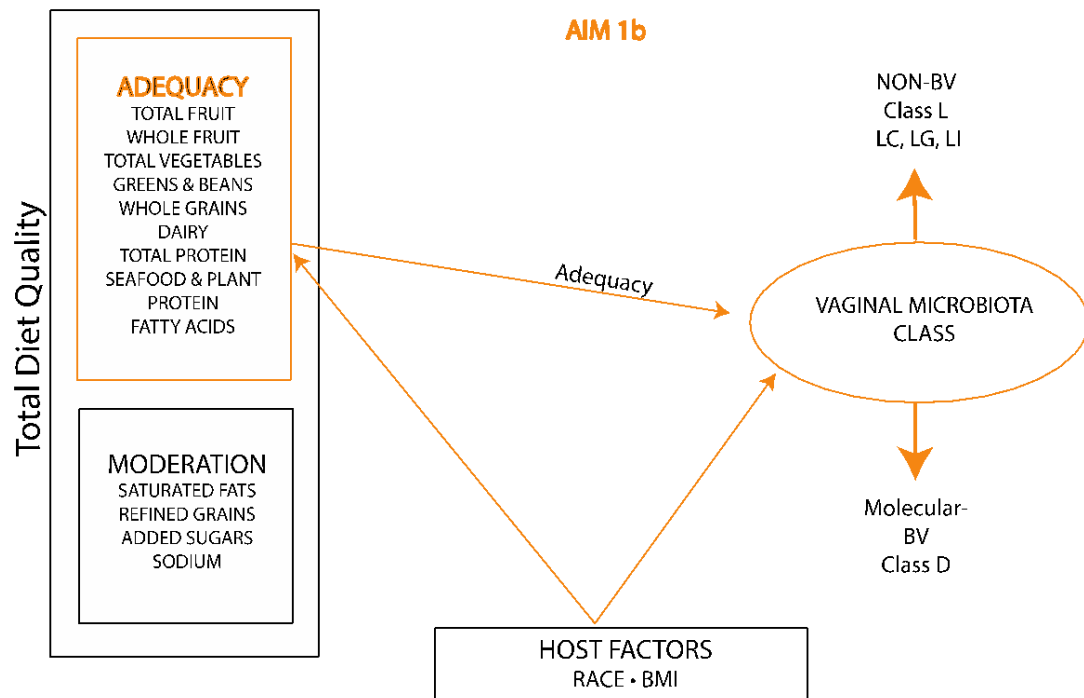
Figure 3. Specific Aim 1a



The composition of the vaginal microbiota was first characterized using VALENCIA to identify participants that have primarily *Lactobacillus*-dominated community class types (*L*) or the diverse polymicrobial community class type (*D*). Binary logistic regression was performed to evaluate the association of diet quality on the incidence of molecular BV. The vaginal microbiota community class types were dichotomized into a ‘non-BV group’ and a ‘molecular BV group.’ The polymicrobial community class type *D* was designated as the outcome variable (= 1) because this is suggestive of a molecular BV class that predisposes women to greater health risks and the factor attempting to be predicted. The *Lactobacillus*-dominated community class types (*L*) served as the reference case (= 0). Next, the microbial composition (‘molecular BV group’ and ‘non-BV group’) and diet quality (HEI-2015 *total*, *moderation*, and *adequacy* scores) were modelled using logistic regression to determine the probability and odds of the association between diet quality and molecular BV.

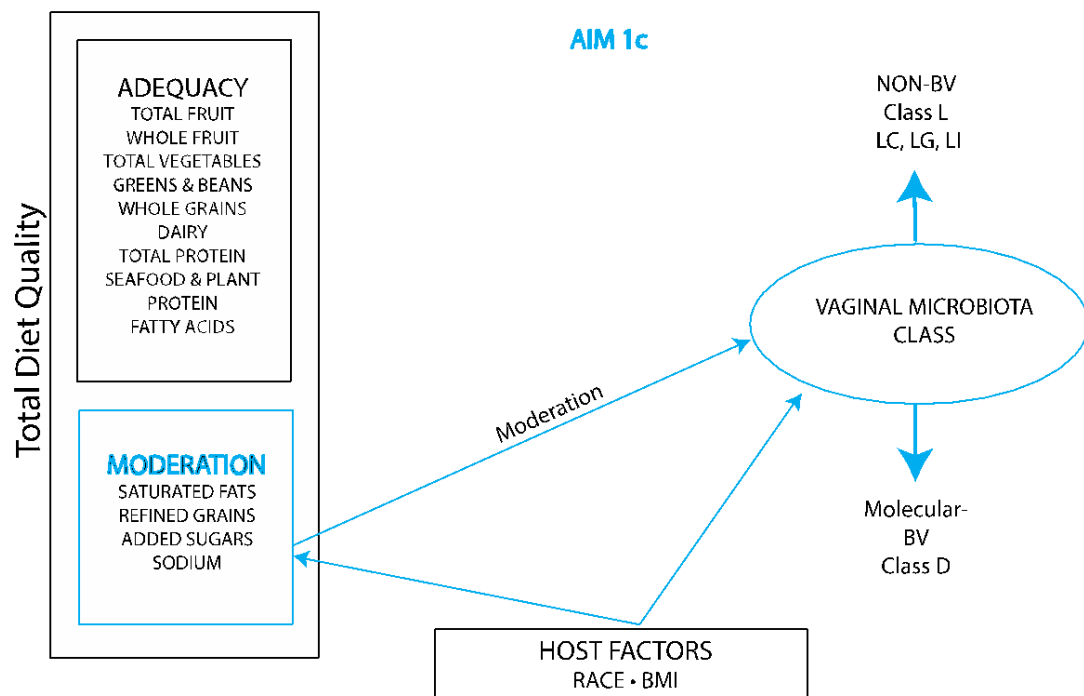
The second part of aim 1 was to examine the *adequacy* component scores controlling for differences based on race (African American or not) and obesity (BMI over 30 or not), thus logistic regression modeling was performed to determine the association between diet quality using the individual *adequacy* component scores and the community class assignments (‘non-BV’ group and ‘molecular BV’ groups; see Figure 4).

Figure 4. Specific Aim 1b



Finally, the third part of specific aim 1 was to examine the *moderation* component scores controlling for differences based on race (African American or not) and obesity (BMI over 30 or not) using the individual *moderation* component scores and the community class assignments ('non-BV' group and 'molecular BV' groups; see Figure 5).

Figure 5. Specific Aim 1c



The associations between diet quality and community class types were examined by modelling each community class type (class *L* or class *D*) with the predictor variables (individual *adequacy* and *moderation* component scores) and confounding variables (race and BMI) in adjusted mixed effects logistic regression models. The characteristic estimates are presented in chapter four as odds ratios that represent the probability of molecular BV based on diet quality after controlling for individual characteristics.

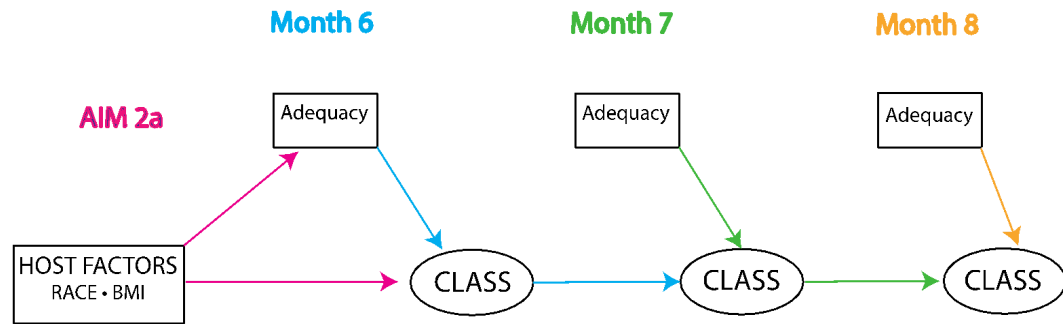
The expected findings were 1) women with higher HEI-2015 *total* diet quality scores (the sum of *adequacy* and *moderation* components) would more likely be associated with *Lactobacillus* dominated classes (*L*) in the non-BV group and women with lower *total* diet quality scores would more likely be associated with the molecular BV group (class *D*); and, 2) that low HEI-2015 *adequacy* and low *moderation* scores would be negatively associated with increased odds of occurrence of molecular BV class *D* and women with high HEI-2015 *adequacy* and high *moderation* diet quality scores would be positively associated with increased odds of occurrence of *Lactobacillus* dominated community classes, class *L*.

Specific Aim 2

The goal of specific aim 2 was to analyze the relationship between a diet high in consumption of total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein, seafood and plant protein, and fatty acids (high HEI-2015 *adequacy* component scores), or a diet high in consumption of saturated fats, refined grains, added sugars, and sodium (low HEI-2015 *moderation* component scores) and the community class type using longitudinal diet and community class assignment data in adjusted multivariate mixed modeling. This aim was designed to answer the research question: *do HEI-2015 diet quality scores predict alterations in the frequency of BV associated microbial community class type?* Controlling for differences based on race (African American or not) and obesity (BMI over 30 or not), the HEI-2015 *adequacy* component scores were used to predict variance in community class assignment (class *L*) and BV associated class assignment (class *D*) in the cohort at three intervals of participation

during the study (Time 1 [T₁]*—*6-month, Time 2 [T₂]*—*7-month, and Time 3 [T₃]*—*8-month gestations; see Figure 6).

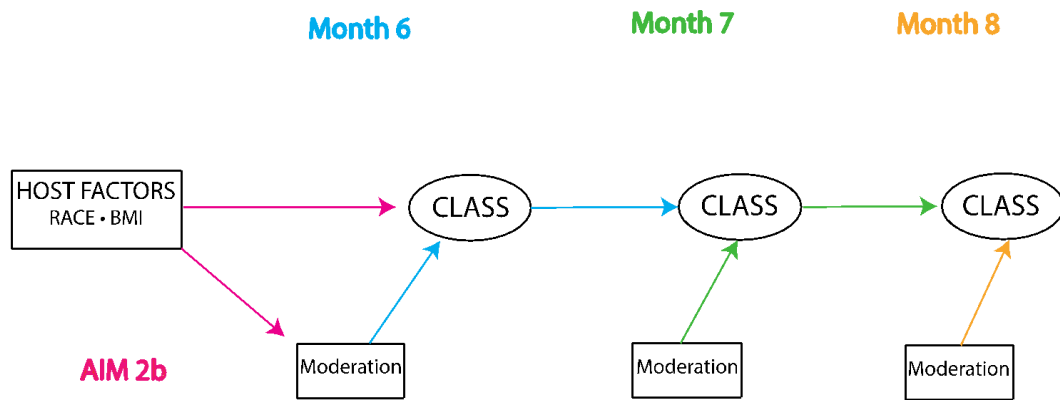
Figure 6. Specific Aim 2a



The *adequacy* component scores for each gestational month 5 through 10 were entered as the respective monthly *adequacy* scores. VALENCIA generated microbial profiles for three separate one-month point estimates were computed to determine the most common community class type for each woman. Logistic regression was performed using the variables of microbial composition (‘molecular BV group’ and ‘non-BV group’) and diet quality (HEI-2015 *total*, *moderation*, and *adequacy* scores). The ‘non-BV group’ was set to = 0 and the ‘molecular BV group’ was set to = 1. These subsets (‘non-BV group’ and ‘molecular BV group’), were used to compare the three time points (6 month-, 7 month-, and 8 month- gestations).

The second part of aim 2 was to examine the association of HEI-2015 *moderation* component scores, controlling for differences based on race (African American or not) and obesity (BMI over 30 or not), to predict variance in *Lactobacillus*-dominated community class assignments (class *L*) and BV associated community class assignment (*D*) in the cohort at the three-time intervals of participation during the study (6 month-, 7 month-, and 8 month- gestations; see Figure 7).

Figure 7. Specific Aim 2b



The *moderation* component scores for each gestational month 5 through 10 were entered as the respective monthly *moderation* scores and the VALENCIA profiles that represent the respective microbial data point estimates as the most common community class type for each woman. The binary outcome variable (‘molecular BV group’ and ‘non-BV group’) and diet quality scores (‘*adequacy*,’ and ‘*moderation*’ components) were assessed at 6 month-, 7 month-, and 8 month – gestations independently. Logistic regression analyses were performed to predict the odds of individual factors for molecular BV (class *D*). Predictor variables along with confounding variables (race and

obesity) were entered into adjusted mixed effect models for 6 month-, 7 month-, and 8 month- gestations.

The expected findings from these analyses were: 1) low HEI-2015 *adequacy* scores would be negatively associated with increased occurrence of molecular BV (class D) at respective timepoints (6 month-, 7 month-, and 8 month- gestation). Conversely, women with high HEI-2015 *adequacy* diet quality scores would be positively associated with increased occurrence of *Lactobacillus* dominated class (*L*) at respective timepoints (6 month-, 7 month-, and 8 month- gestations); and 2) that low HEI-2015 *moderation* scores would be associated with increased occurrence of molecular BV (class D) at respective timepoints (6 month-, 7 month-, and 8 month- gestations). Conversely, women with high HEI-2015 *moderation* diet quality scores would be associated with increased occurrence of *Lactobacillus* dominated community class assignment (*L*) at respective timepoints (6 month-, 7 month-, and 8 month- gestations). The characteristic estimates are presented as odds ratios in Chapter four.

HUMAN SUBJECTS' PROTECTION

The parent project received human subjects' approval from the Institutional Review Board (IRB) at the University of Maryland, Baltimore and pre-enrollment consent was obtained from all participants. The BEAM research protocol was submitted to the Uniformed Services University (USU) IRB for review. This study did not require a specific modification since the work is within the scope of the original protocol. USU IRB approval was granted for the use of these data for the specified research activities.

LIMITATIONS

There are several potential limitations with this study. The primary limitation was sample size. Given the small sample size, possible confounders were collapsed into binary variables limiting the statistical analyses that could be performed. Another limitation was that the majority of the sample were African Americans, thus between group differences were not possible to determine. Generalizability of findings are limited due to the over-representation of one racial group. Additionally, this sample was comprised of pregnant women and may not be generalizable to non-pregnant women. Finally, the dietary and vaginal data collection methods could be considered limitations. First, participants self-selected which food photos to submit for analysis, however this limitation is likely to be similar to self-report biases on food questionnaires. Secondly, women self-collected vaginal samples. Although women were trained on the proper technique to self-collect mid-vaginal swabs, it is possible that the technique was not accurately performed.

CONCLUSION

The vaginal microbiota community class type profiles and the HEI-2015 diet scores of women were analyzed to determine if there is an association between diet quality and molecular BV. This study's key innovation lies in the advanced analysis of vaginal microbial class profiling and a diet quality assessment method that does not rely on recall. This is the first known study to examine the association of longitudinal diet quality on molecular vaginal community class profiles. This study is relevant to understanding how the diet influences the vaginal microbiota. Knowledge generated from

this study has important implications for nursing practice because diet is readily modifiable and may be leveraged as adjunct therapy in the treatment of BV. The results of this study will be presented in the next chapter followed by the discussion and relevance of the findings.

CHAPTER 4: RESULTS

INTRODUCTION

The purpose of this study was to examine the effect of diet on the composition of the vaginal microbiota during pregnancy. To achieve that goal, community class types were used to estimate the composition of the vaginal microbiota to compare the changes in the groups over time. The data used for this analysis were collected as part of a larger project that sought to examine the influence of diet on the vaginal microbiome and the relationship between preterm birth, known as the *Birth, Eating and the Microbiota* (BEAM; NR014826) project. The research question guiding this study was, do HEI-2015 diet quality scores predict alterations in the frequency of molecular BV? Of specific interest was whether there were differences in the HEI-2015 diet quality scores associated with vaginal microbial community class types (*L* class and *D* class). Furthermore, are there significant differences in the HEI-2015 *total*, *adequacy* or *moderation* diet scores between women in the non-BV group (*Lactobacillus* dominated community classes) compared to women in the molecular BV associated community class (*D*)? And, finally, if there are significant differences in the vaginal community class types based on HEI-2015 *total*, *adequacy*, or *moderation* diet scores, do these differences still exist after controlling for race (African American or not) and obesity (body mass index (BMI) ≥ 30)?

This study had two specific aims. Specific aim 1 was a cross-sectional approach to assess the influence of diet quality on the vaginal communities in a subset of women while controlling for race (African American or not) and obesity (BMI ≥ 30 or not) to determine if there are differences in diet quality (HEI-2015 *total*, *adequacy*, and

moderation scores) associated with the vaginal microbial community classes (molecular BV and non-BV groups). The hypothesis was that low *moderation* scores would be associated with molecular BV and high *total* and *adequacy* scores would not. To achieve this aim, logistic regression was conducted for each of the HEI-2015 components (*total*, *adequacy*, and *moderation*) modelling the aggregate diet quality scores and the two groups of women, the non-BV group and the molecular BV group, to determine if there is an association between diet (independent variable [IV]) and the vaginal microbial community class type (dependent variable [DV]).

The second aim was a longitudinal approach to analyze the relationship of HEI-2015 *adequacy* and *moderation* diet quality scores and molecular BV using diet and community class assignment data at 6-, 7-, and 8-month gestations in adjusted multivariate mixed models controlling for race (African American or not) and obesity (BMI ≥ 30 or not). The hypotheses for specific aim 2 were that women with high HEI-2015 *adequacy* or high *moderation* scores would have decreased odds of molecular BV at the respective timepoints; and women with low HEI-2015 *adequacy* or low *moderation* scores would have increased odds of molecular BV at respective timepoints.

In the remainder of this chapter analysis procedures and results are presented. Results are provided by the specific aims and the questions they address. Demographic characteristics of the sample are described, and key findings are highlighted.

DESCRIPTIVE DATA

Demographic Data

The overall sample was comprised of 55 pregnant women ages 18 to 34 ($\bar{x} = 25.6$, $SD = 4.9$). Gestation at delivery ranged from 34 – 41 weeks ($\bar{x} = 38.6$ weeks, $SD = 1.5$ weeks). BMI ranged from 17.9 – 60 ($\bar{x} = 28.7$, $SD = 8.4$), 40% ($n = 22$) fell into the obese category with a BMI over 30 and 60% ($n = 33$) had a BMI under 30. The majority of participants were African American ($n = 39$, 70.9%). Twenty participants had a university degree (36.4%) and 11 (20%) had an annual household income of greater than \$75K. Roughly 27% ($n = 15$) were unemployed and over half (54.5%) were single. Demographic characteristics of the sample are provided (see Table 2).

Table 2. Sociodemographic Characteristics

<i>n</i> = 55	Range	$\bar{x}$ (<i>SD</i>)	
Age	18 - 34	25.6 (4.9)	
Weeks' gestation at Delivery	34 - 41	38.6 (1.5)	
Body Mass Index	17.9 - 60	28.7 (8.4)	
		<i>f</i>	%
Obesity	BMI $\geq$ 30	22	40
	BMI < 30	33	60
Race	African American	39	70.9
	Non-African American	16	29.1
Education	University degree	20	36.4
	Non-university degree	34	63.6
	$\geq$ 75K	11	20
Household Income	< 75K	42	76.4
Employment Status	Missing	2	3.6
	Unemployed	15	27.3
	Employed/Student	40	72.7
Marital Status	Single	30	54.5
	Married/with partner	25	45.5

Vaginal Data

In gestational month 5 (recruitment), $n = 41$, 27 (65.9%) women were categorized as non-BV and the other 14 (34.1%) as molecular BV. Month 6, $n = 54$, had 30 (55.6%) in the non-BV group and 24 (44.4%) in the molecular BV group. Month 7, $n = 52$, there was 37 (71.2%) in the non-BV group and 15 (28.8%) in the molecular BV group. Month 8, $n = 52$, 39 women (75%) fell into the non-BV group and 13 (25%) into the molecular BV group. In month 9, $n = 41$, 32 (78%) were in the non-BV group and 9 (22 %) were in the molecular BV group. Lastly, out of a total of 55 women, 31 (56.4%) maintained *Lactobacillus*-dominated community class types (non-BV) over the course of participation in the study and 24 (43.6%) either transitioned into and out of molecular BV (17 transition cases) or maintained a polymicrobial composition throughout the study. For the cross-sectional analyses, all 55 participants were included. In the longitudinal analyses, only gestational months 6, 7, and 8 were included because they had the most complete data. Participants were excluded from the longitudinal analyses if they had missing data points which resulted in a reduction of 2 participants in some analyses. Gestational months and overall aggregate community class types are presented (see Table 3).

Table 3. Community Class Types by Gestational Month and Overall

	<i>n</i>	Non-BV group <i>f</i> (%)	Molecular BV <i>f</i> (%)
Month 5	41	27 (65.9%)	14 (34.1%)
Month 6	54	30 (55.6%)	24 (44.4%)
Month 7	52	37 (71.2%)	15 (28.8%)
Month 8	52	39 (75%)	13 (25%)
Month 9	41	32 (78%)	9 (22%)
Overall	55	31 (56.4%)	24 (43.6%)

Diet Data

The monthly and overall mean ($\bar{x}$) HEI-2015 *total*, *adequacy*, and *moderation* diet scores with standard deviations (*SD*) and ranges are provided (see Table 4). In month 5, *n* = 52, HEI-2015 *total* scores ranged from 18.7 – 70.7 ($\bar{x}$ = 40.8, *SD* = 12.4), *adequacy* scores ranged from 5.1 – 48.4 ($\bar{x}$ = 26.2, *SD* = 9.4), and *moderation* scores ranged from 3.3 – 27.9 ($\bar{x}$ = 14.6, *SD* = 6.2). Month 6, *n* = 55, HEI-2015 *total* scores ranged from 23 – 68 ($\bar{x}$ = 41.7, *SD* = 12.3), *adequacy* scores ranged from 11.3 – 53.8 ($\bar{x}$ = 29.5, *SD* = 8.5), and *moderation* scores ranged from 0 – 28.6 ($\bar{x}$ = 12.2, *SD* = 6.6). In month 7, *n* = 53, HEI-2015 *total* scores ranged from 11.2 – 72.4 ($\bar{x}$ = 39.9, *SD* = 11.5), *adequacy* scores ranged from 10.7 – 49.9 ($\bar{x}$ = 27.7, *SD* = 7.6), and *moderation* scores ranged from 0 – 30

($\bar{x} = 12.1$, $SD = 6.9$). In month 8, $n = 53$, HEI-2015 *total* scores ranged from 10 – 68.9 ($\bar{x} = 39.2$, $SD = 12.9$), *adequacy* scores ranged from 7.2 – 43.8 ($\bar{x} = 27$, $SD = 8.1$), and *moderation* scores ranged from 0 – 30.5 ($\bar{x} = 14.3$, $SD = 7.2$). Month 9, $n = 45$, HEI-2015 *total* scores ranged from 13.2 – 66.1 ($\bar{x} = 40.3$, $SD = 11.7$), *adequacy* scores ranged from 8.4 – 43 ($\bar{x} = 26.6$, $SD = 8.7$), and *moderation* scores ranged from 0.42 – 26.4 ($\bar{x} = 13.6$, $SD = 6.7$). Finally, the overall HEI-2015 *total* scores ranged from 24.1 – 68.8 ($\bar{x} = 40.1$, $SD = 9.6$), overall *adequacy* scores ranged from 17.2 – 41.5 ($\bar{x} = 27.1$, $SD = 6.8$), and overall *moderation* scores ranged from 7 – 27.6 ($\bar{x} = 13.4$, $SD = 4.5$).

Table 4. HEI-2015 Diet Scores by Gestational Month and Overall

HEI-2015 Scores	<i>n</i>	<i>Total</i> Scores		<i>Adequacy</i> Scores		<i>Moderation</i> Scores	
		Range	$\bar{x}$ (<i>SD</i>)	Range	$\bar{x}$ (<i>SD</i>)	Range	$\bar{x}$ (<i>SD</i>)
Month 5	52	18.7 - 70.7	40.8 (12.4)	5.1 - 48.4	26.2 (9.4)	3.3 - 27.9	14.6 (6.2)
Month 6	55	23 - 68	41.7 (12.3)	11.3 - 53.8	29.5 (8.5)	0 - 28.6	12.2 (6.6)
Month 7	53	11.2 - 72.4	39.9 (11.5)	10.7 - 49.9	27.7 (7.6)	0 - 30	12.1 (6.9)
Month 8	53	10 - 68.9	39.2 (12.9)	7.2 - 43.8	27 (8.1)	0 - 30.5	14.3 (7.2)
Month 9	45	13.2 - 66.1	40.3 (11.7)	8.4 - 43	26.6 (8.7)	0.42 - 26.4	13.6 (6.7)
Overall	55	24.1 - 68.8	40.1 (9.6)	17.2 - 41.5	27.1 (6.8)	7 - 27.6	13.4 (4.5)

ANALYSES

All data were analyzed using SPSS (version 27) with an $\alpha = 0.5$ and a confidence interval (CI) set at 95%. The first analyses were to examine whether community class type varied by personal characteristics. Tests of associations were computed using chi square (χ^2) test statistic for nominal variables and independent t -test for continuous variables to compare the percentages associated with each predictor variable between the non-BV group and the molecular BV group. The χ^2 did not show significant associations between molecular BV and race (African American or not), employment status (unemployed or not), or obesity (BMI ≥ 30 or not). This may be a consequence of small sample size. However, statistically significant associations were observed. Women who were single ($p = 0.033$) tended to have higher rates of molecular BV and women who had a high household income greater than \$75K ($p = 0.015$), and/or a university degree ($p = 0.035$) tended to have lower rates of molecular BV. When necessary the Fisher's Exact test was reported (high income > \$75K).

Using independent t -test, statistically significant associations were observed with age ($p = 0.003$), as age was inversely correlated with the likelihood of BV and with gestational week at delivery ($p = 0.031$), meaning younger women were more likely to have molecular BV and as gestational age decreased the likelihood of molecular BV increased. The mean overall HEI *total*, *adequacy* and *moderation* diet quality scores were not significantly different between the non-BV group (*L* class) and the molecular BV group (*D* class). Equal variances were assumed based on nonsignificant Levene's test. For regression analyses, variables with p values of less than 0.1 were included in the models. The results of the tests of associations are presented (see Table 5).

Table 5. Tests of Associations

Characteristic		Non-BV <i>f</i> (%)	Molecular BV <i>f</i> (%)	<i>p</i>
Single	Yes	13 (43.3%)	17 (56.7%)	0.033*
	No	18 (72%)	7 (28%)	
African American	Yes	19 (48.7%)	20 (51.3%)	0.074
	No	12 (75%)	4 (25%)	
Income $\geq$ \$75K	Yes	10 (90.9%)	1 (9.1%)	0.015*
	No	20 (47.6%)	22 (52.4%)	
Unemployed	Yes	9 (60%)	6 (40%)	0.739
	No	22 (55%)	18 (45%)	
University Degree	Yes	15 (75%)	5 (25%)	0.035*
	No	16 (45.7%)	19 (54.3%)	
Obesity	Yes	13 (59.1%)	9 (40.9%)	0.739
	No	18 (54.5%)	15 (45.5%)	
$\bar{x}$ (SD)		Non-BV <i>f</i> (%)	Molecular BV <i>f</i> (%)	<i>p</i>

	<i>n</i> = 31	<i>n</i> = 24	
Age	27.2 (5)	23.4 (3.39)	0.003*
Weeks' gestation at delivery	39 (1.3)	38 (1.7)	0.031*
Overall HEI 2015 <i>Total</i> Score	41.6 (10.6)	38.1 (8.1)	0.183
Overall HEI 2015 <i>Adequacy</i> Score	28 (7.5)	25.9 (5.5)	0.263
Overall HEI 2015 <i>Moderation</i> Score	13.7 (4.8)	13 (4.1)	0.559

Next, a univariate analysis was conducted to examine the relationships between diet scores and individual characteristics to determine the direction of influence the factors had on the risk of molecular BV (see Table 6). Diet quality scores were not significant but there was a marginal trend toward higher scores reducing the odds of molecular BV. With a larger sample a difference may have been detected because income and education are drivers of diet quality. Race (African American or not) and obesity (BMI $\geq$ 30 or not) were also not significant, however African American participants (71% of the sample) were 3 times more likely to have molecular BV compared to non-African American women.

Obesity showed a slight trend toward reducing the odds of molecular BV. Associations with unemployment were not statistically significant. However, being single (marital status [single or not], $p = 0.036$) significantly increased the odds of molecular BV and household income ($\geq$ \$75K or not, $p = 0.028$), education level (university degree or not, $p = 0.024$), age ($p = 0.006$), and gestational weeks at delivery ($p = 0.04$) showed a significant reduction in risk of molecular BV. Based on these results, having a household

income of > \$75K reduces the risk of molecular BV by almost 10% and having a university degree reduces the risk by almost 80%. Additionally, the odds of molecular BV increase by 17% for every 1 year younger in age and the odds of molecular BV increased 35% for every 1 week decrease in gestational length.

Table 6. Univariate Analysis of Variables

<i>n</i> = 55	β	<i>SE</i>	Wald	<i>df</i>	<i>p</i>	OR	95% CI	
							Lower	Upper
<i>Total</i> scores	-0.041	0.031	1.758	1	0.185	0.96	0.904	1.02
<i>Adequacy</i> scores	-0.048	0.042	1.269	1	0.26	0.953	0.877	1.036
<i>Moderation</i> scores	-0.037	0.063	0.353	1	0.552	0.963	0.852	1.09
African American	1.15	0.66	3.033	1	0.082	3.158	0.866	11.519
Obesity	-0.185	0.557	0.111	1	0.739	0.831	0.279	2.475
Unemployment	0.205	0.615	0.111	1	0.739	1.227	0.367	4.1
Single	1.213	0.578	4.401	1	0.036*	3.363	1.083	10.441
Income > \$75K	-2.398	1.093	4.81	1	0.028*	0.091	0.011	0.775
University degree	-1.501	0.667	5.062	1	0.024*	0.223	0.06	0.824
age	-0.18	0.065	7.615	1	0.006*	0.835	0.734	0.949
Gestational week at delivery	-0.419	0.204	4.239	1	0.04*	0.658	0.441	0.98

SPECIFIC AIMS

Specific Aim 1

The purpose of the first aim was to investigate the influence of diet quality on the vaginal microbiota using the overall aggregate community class type among the cohort controlling for race (African American or not) and obesity ($\text{BMI} \geq 30$ or not). Binary logistic regression was conducted modelling the aggregate HEI-2015 *total*, *adequacy*, and *moderation* diet quality scores independently with the two groups of women classified as either the ‘non-BV group’ or the ‘molecular BV group’ to determine if there was an association between diet quality scores (IV) and the vaginal microbial community class (DV). There were three separate analyses to this aim.

Specific Aim 1a

The first part of aim 1 was to examine the influence of the HEI-2015 *total* diet quality scores on the vaginal community class type controlling for differences based on race (African American or not) and obesity ($\text{BMI} \geq 30$ or not).

Model 1

To address specific aim 1a, a binary logistic regression analysis was performed ($n = 55$) to investigate if the HEI-2015 *total* diet quality scores, race (African American or not), and obesity ($\text{BMI} \geq 30$) predict whether a woman will have molecular BV (class *D*). The outcome of interest was molecular BV. The possible predictor variables were HEI-2015 *total* diet quality score, race, and obesity. The Hosmer-Lemeshow was not significant ($p = 0.735$) indicating the model fit the data. The model resulted in the IVs HEI-2015 *total* diet quality scores, race, and obesity not being significant, thus not contributing to molecular BV (see Table 7).

Table 7. Aim 1a, Model 1

$n = 55$	β	SE	Wald	df	p	OR	95% CI	
							Lower	Upper
<i>Total</i> diet score	-0.026	0.04	0.416	1	0.519	0.974	0.901	1.054
Obesity	-0.738	0.632	1.365	1	0.243	0.478	0.139	1.649
African Am.	1.127	0.822	1.881	1	0.17	3.086	0.617	15.444

Model 2

Factors observed to have a significant association ($p \leq 0.1$) with community class type (see Table 5) were included in the binary logistic regression model to investigate if the HEI-2015 *total* diet quality scores predict whether a woman will have molecular BV (class *D*) when adjusting for race (African American or not), marital status (single or married/with partner), household income ($> \$75K$ or not), university degree (university degree or not), age (in years), and/or delivery gestation (in weeks). The Hosmer-Lemeshow goodness of fit test was not significant ($p = 0.105$) indicating the model fit the data.

In this model, age approached significance ($p = 0.052$) and gestational age was significant ($p = 0.021$). Denoting a trend of a 17% increase in the odds of molecular BV for every 1-year younger in age and 48% increased odds of molecular BV for every 1-week earlier the birth (gestational age at delivery). This analysis was followed up by a forward and backward stepwise regression. The forward stepwise regression model resulted in age and gestation at delivery significantly contributing to molecular BV.

According to these results, as age ($p = 0.006$) decreased by 1 year the risk of molecular BV was increased by 28% and for every 1 week earlier the delivery (gestational age, $p = 0.032$) the odds of molecular BV was observed to be 40% higher. Gestational length at delivery and age were again significant in the backward stepwise matrix. The contribution of age to the odds of molecular BV went from non-significant to significant between regression matrixes that may be a result of being underpowered (see Table 8).

Table 8. Aim 1a, Model 2.

<i>n</i> = 53	β	<i>SE</i>	Wald	$\frac{d}{f}$	<i>p</i>	OR	95% CI	
							Lower	Upper
Total diet quality	-0.017	0.052	0.101	1	0.751	0.984	0.888	1.09
African American	-1.409	1.465	0.925	1	0.336	0.244	0.014	4.315
Single	0.883	0.901	0.96	1	0.327	2.419	0.413	14.148
Income > 75K	-2.449	1.434	2.919	1	0.088	0.086	0.005	1.434
University degree	0.73	1.161	0.396	1	0.529	2.076	0.213	20.222
Age	-0.182	0.093	3.782	1	0.052	0.834	0.695	1.001
Gestation	-0.659	0.285	5.358	1	0.021*	0.517	0.296	0.904
Forward Stepwise								
Age	-0.187	0.068	7.467	1	0.006*	0.83	0.726	0.949
Gestation at Del	-0.506	0.236	4.594	1	0.032*	0.603	0.379	0.958
Backward Stepwise								
Age	-0.187	0.068	7.467	1	0.006*	0.83	0.726	0.949

Gestation at Del	-0.506	0.236	4.594	1	0.032*	0.603	0.379	0.958
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To validate this analysis, a linear regression was done to assess for multicollinearity and to identify how these factors contribute to the HEI-2015 *total* score in the presence of molecular BV. No collinearity exists in these data based on the variance inflation factors (VIF) of < 4. The results of this analysis were that African American ($p = 0.009$) women tended to have a reduction in *total* diet quality scores by 7.6 points and obese ($p = 0.028$) women had a reduction in *total* scores by 5.2 points. Also, for every 1-year decrease in age ($p = 0.043$) participants had a decrease in their *total* diet scores by 0.53 points indicating that younger women had significantly lower diet scores. There was a non-significant reduction in *total* diet scores by 1.3 points in women with molecular BV. Gestational week at delivery did not contribute much to *total* diet score (see Table 9).

Table 9. Contributing Factors to HEI-2015 *Total* Diet Quality Score

<i>Total</i> Score	Unstandardized Coefficients		Standardized Coefficients		<i>p</i>	Collinearity Statistics	
	β	<i>SE</i>	β	<i>t</i>		Tolerance	VIF
African American	-7.629	2.8	-0.363	-2.724	0.009*	0.637	1.569
Obesity	-5.165	2.276	-0.265	-2.269	0.028*	0.829	1.206
Age	0.534	0.258	0.27	2.074	0.043*	0.667	1.499
Gestation at delivery	-0.057	0.71	-0.009	-0.081	0.936	0.897	1.115
Molecular BV	-1.331	2.399	-0.07	-0.555	0.582	0.705	1.418

The results of these analyses do not support the hypothesis that higher HEI-2015 *total* diet quality scores lower the odds of molecular BV or that lower *total* diet quality scores increase the odds of molecular BV (class *D*).

Specific Aim 1b

The second part of aim 1 was to examine the influence of HEI-2015 *adequacy* component scores on the vaginal community class type controlling for differences based on race (African American or not) and obesity (BMI ≥ 30 or not)

Model 1

Binary logistic regression analysis was performed ($n = 55$) to investigate if the HEI-2015 *adequacy* diet quality scores, race, and obesity predict whether a woman will have molecular BV (class *D*). The outcome of interest was molecular BV. The possible predictor variables were HEI-2015 *adequacy* diet quality score, race, and obesity. The Hosmer-Lemeshow goodness of fit was not significant ($p = 0.680$) indicating the model fit the data. The model resulted in the IVs HEI-2015 *adequacy* diet quality scores, race, and obesity not being significant, thus not contributing to molecular BV. Forward and backward stepwise regression analyses were run sequentially adjusting for predictors, however, neither model sufficiently predicted the odds of having molecular BV (see Table 10).

Table 10. Aim 1b, Model 1

$n = 55$	β	SE	Wald	$\frac{d}{f}$	p	OR	95% CI	
							Lower	Upper
Adequacy score	-0.023	0.049	0.211	1	0.646	0.978	0.887	1.077
Obesity	-0.679	0.62	1.197	1	0.274	0.507	0.15	1.711
African Am.	1.264	0.778	2.636	1	0.104	3.539	0.77	16.28

Model 2

Community class type and associated factors with a p value less than 0.1 (see Table 5) were included in a binary logistic regression model to investigate if the HEI-2015 *adequacy* diet quality scores predict whether a woman will have molecular BV (class D) when adjusting for race (African American or not), marital status (single or married/with partner), household income ($> \$75K$ or not), university degree (university degree or not), age (in years), and delivery gestation (in weeks). The Hosmer-Lemeshow goodness of fit was not significant ($p = 0.333$) indicating the model fit the data. Gestational age at delivery was significant ($p = 0.022$) indicating that for every 1-week decrease in gestation there was an associated 48% increase in the odds of molecular BV. In a forward and backward stepwise regression model, both age and gestational week at delivery were found to significantly contribute to molecular BV. This model indicates that as age ($p = 0.006$) decreases by 1 year the odds of molecular BV increases by 17% and for every 1 week decrease in gestational age ($p = 0.032$) the risk of molecular BV goes up 40% (see Table 11).

Table 11. Aim 1b Model 2.

$n = 53$	β	SE	Wald	df	p	OR	95% CI	
							Lower	Upper
<i>Adequacy</i> score	-0.02	0.06 5	0.097	1	0.756	0.98	0.863	1.113

African American	-1.358	1.41 1	0.927	1	0.336	0.257	0.016	4.082
Single	0.903	0.90 3	1.001	1	0.317	2.467	0.421	14.475
Income > \$75K	-2.467	1.43 3	2.966	1	0.085	0.085	0.005	1.406
University degree	0.7	1.15 5	0.368	1	0.544	2.015	0.209	19.374
Age	-0.179	0.09 5	3.547	1	0.06	0.836	0.694	1.007
Gestation at Delivery	-0.656	0.28 6	5.284	1	0.022*	0.519	0.296	0.908
Forward Stepwise								
Age	-0.187	0.06 8	7.467	1	0.006*	0.83	0.726	0.949
Gestation at Delivery	-0.506	0.23 6	4.594	1	0.032*	0.603	0.379	0.958
Backward Stepwise								
Age	-0.187	0.06 8	7.467	1	0.006*	0.83	0.726	0.949
Gestation at Delivery	-0.506	0.23 6	4.594	1	0.032*	0.603	0.379	0.958

The condition of collinearity was satisfied based on a VIF of < 4. There were non-significant trends in reduced HEI-2015 *adequacy* diet scores by 4 points in African American women and 2.4 points in obese women. Age and gestational week at delivery were not significantly associated with *adequacy* scores. Furthermore, they did not contribute significantly to *adequacy* diet scores. Women with molecular BV trended toward slightly higher adequacy scores, though this was not statistically significant (see Table 12).

Table 12. Contributing Factors to HEI-2015 *Adequacy* Diet Quality Score

<i>Adequacy</i> Score	Unstandardized Coefficients		Standardized Coefficients		<i>p</i>	Collinearity Statistics	
	β	<i>SE</i>	β	<i>t</i>		Tolerance	VIF
African American	-4.079	2.213	-0.276	-1.843	0.071	0.637	1.569
Obesity	-2.358	1.798	-0.172	-1.311	0.196	0.829	1.206
Age	0.398	0.204	0.286	1.952	0.057	0.667	1.499
Gestation at delivery	0.055	0.561	0.012	0.098	0.922	0.897	1.115
Molecular BV	0.284	1.864	0.021	0.152	0.879	0.754	1.327

The results of this analysis do not support the hypothesis that higher HEI-2015 *adequacy* diet quality scores lower the odds of molecular BV or that lower *adequacy* diet quality scores increase the odds of molecular BV (class *D*).

Specific Aim 1c

Finally, the third part of specific aim 1 was to examine the influence of HEI-2015 *moderation* component scores on the vaginal community class type controlling for differences based on race (African American or not) and obesity (BMI ≥ 30 or not).

Model 1

A binary logistic regression analysis was performed ($n = 55$) to investigate if the HEI-2015 *moderation* diet quality scores, race, and obesity predict whether a woman will have molecular BV (class *D*). The outcome of interest was molecular BV. The possible predictor variables were HEI-2015 *moderation* diet quality score, race, and obesity. The Hosmer-Lemeshow goodness of fit was not significant ($p = 0.817$) indicating the model fit the data. The model showed that the variables HEI-2015 *moderation* diet quality scores, race, and obesity do not significantly contribute to molecular BV. Forward and backward stepwise regression analyses were run sequentially adjusting for predictors, however, neither model predicted the likelihood of having molecular BV (see Table 13).

Table 13. Aim 1c, Model 1.

$n = 55$	β	SE	Wald	df	p	OR	95% CI	
							Lower	Upper
<i>Moderation</i> score	0.012	0.078	0.023	1	0.878	1.012	0.868	1.18
Obesity	-0.634	0.63	1.014	1	0.314	0.53	0.154	1.822
African Am.	1.472	0.808	3.32	1	0.068	4.356	0.895	21.208

Model 2

Associated factors (with a $p < 0.1$) and community class type (see Table 5) were included in a binary logistic regression model to investigate if the HEI-2015 *moderation* diet quality scores, race, marital status, high income ($> \$75K$), university degree, age, and delivery gestation predict whether a woman will have molecular BV (class *D*). The Hosmer-Lemeshow goodness of fit test was not significant ($p = 0.271$) indicating the model fit the data. Age ($p = 0.042$) and gestational week at delivery ($p = 0.025$) were significant predictors of the odds of molecular BV. However, HEI-2015 *moderation* diet quality scores, marital status, household income ($> \$75K$), and university degree did not

significantly contribute to molecular BV. In this model, for every 1-year younger in age there was an increase in the odds of molecular BV by 17% and for every 1-week earlier the birth there was an associated 48% increase in the odds of molecular BV. Age ($p = .006$) and gestational age ($p = .032$) remained significant in both the forward and backward stepwise models (see Table 14).

Table 14. Aim 1c, Model 2

$n = 53$	β	SE	Wald	df	p	OR	95% CI	
							Lower	Upper
<i>Moderation score</i>	0.055	0.106	0.265	1	0.607	1.056	0.858	1.301
African Am.	-1.023	1.384	0.546	1	0.46	0.36	0.024	5.417
Single	0.89	0.9	0.978	1	0.323	2.435	0.417	14.203

Income > \$75K	-2.622	1.486	3.114	1	0.078	0.073	0.004	1.337
University degree	0.592	1.157	0.262	1	0.609	1.808	0.187	17.453
Age	-0.185	0.091	4.13	1	0.042*	0.831	0.695	0.993
Gestation at Delivery	-0.649	0.29	4.992	1	0.025*	0.523	0.296	0.923
Forward Stepwise								
Age	-0.187	0.068	7.467	1	0.006*	0.83	0.726	0.949
Gestation at Delivery	-0.506	0.236	4.594	1	0.032*	0.603	0.379	0.958
Backwards Stepwise								
Age	-0.187	0.068	7.467	1	0.006*	0.83	0.726	0.949
Gestation at Delivery	-0.506	0.236	4.594	1	0.032*	0.603	0.379	0.958

No multicollinearity was noted ($VIF < 4$). There were reductions in HEI-2015 *moderation* diet scores by 3.4 points in African American ($p = 0.024$) women and 2.2 points in obese ($p = 0.07$) women, but these were not significant. As age decreased by 1 year there was a decrease in *moderation* scores by .14 points and there was a trend toward lower moderation scores as gestational week at delivery increased, though neither were significant. There was also a trend of higher moderation scores in women with molecular BV (see Table 15).

Table 15. Contributing Factors to HEI-2015 *Moderation* Diet Quality Score

<i>Moderation</i> Score	Unstandardized Coefficients		Standardized Coefficients		p	Collinearity Statistics	
	β	SE	β	t		Tolerance	VIF
African American	-3.375	1.453	-0.346	-2.323	0.024*	0.637	1.569

Obesity	-2.189	1.181	-0.242	-1.853	0.07	0.829	1.20 6
Age	0.135	0.134	0.147	1.007	0.319	0.667	1.49 9
Gestation at delivery	-0.194	0.368	-0.066	-0.528	0.6	0.897	1.11 5
Molecular BV	0.269	1.224	0.03	0.219	0.827	0.754	1.32 7

The results of this analysis do not support the hypothesis that lower HEI-2015 *moderation* diet quality scores increase the odds of molecular BV or that higher *moderation* diet quality scores decrease the odds of molecular BV (class D).

Specific Aim 2

The purpose of the second aim was to analyze the relationship between a diet high in consumption of HEI-2015 *adequacy* components (total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein, seafood and plant protein, and fatty acids) and a diet high in consumption of HEI-2015 *moderation* components (saturated fats, refined grains, added sugars, and sodium) and the community class type using longitudinal diet and community class assignment data in adjusted generalized linear models GLM) with generalized estimating equations (GEE) at three different timepoints (6 –, 7 –, and 8 – months gestation).

Specific Aim 2a

The first part of aim 2 was to model HEI-2015 *adequacy* diet quality scores adjusting for race and obesity at 6 –, 7 –, and 8 – months gestation to investigate whether

this component of diet quality predicts variance in molecular BV in the cohort at the three-time intervals. The Exchangeable matrix was the best fit according to the Quasi Likelihood under Independence Model Criterion (QIC). Based on this model, HEI-2015 *adequacy* diet quality scores, race, and obesity do not significantly contribute to variations in molecular BV at 6 –, 7 –, and 8 – months gestation. However, women in their 6th month of pregnancy were ~2.2 times more likely ($p = 0.021$) to have molecular BV compared to month 8 (see Table 16).

Table 16. Aim 2a, Model 1

$n = 54$	β	SE	Wald	df	p	OR	95% CI	
							Lower	Upper
<i>Adequacy</i> score	0.004	0.026	0.018	1	0.893	1.004	0.954	1.056
African American	0.929	0.6026	2.379	1	0.123	2.533	0.778	8.254
Obesity	-0.532	0.5259	1.024	1	0.312	0.587	0.209	1.646
6 Month	0.797	0.3462	5.294	1	0.021 *	2.218	1.125	4.371
7 Month	0.056	0.249	0.05	1	0.822	1.058	0.649	1.723

The HEI-2015 *adequacy* scores were also adjusted for associated factors (see Table 5; $p < 0.1$) and community class type (class *L* – non-BV group and class *D* – molecular BV group) in another GLM using GEE to investigate whether HEI-2015 *adequacy* diet quality scores, marital status, high income (> \$75K), university degree, age, and gestation at delivery predict variations in molecular BV over the three timepoints. The Auto Regressive (AR1) matrix was the best fit based on the QIC. The HEI-2015 *adequacy* diet quality scores were not significantly associated with variations in molecular BV over the three timepoints. However, there were several other significant

associations observed. First, participants with a university degree ($p = 0.012$) were 7.8 times more likely to have molecular BV compared to those without a university degree. Second, gestational age (in weeks) at delivery ($p = 0.001$) was significantly associated with variations in BV in that for every 1 week decrease in gestation the odds of molecular BV were increased by 45%. Finally, the risk of molecular BV was 2.8 times higher at gestational month 6 ($p = 0.019$) compared to month 8. These results are based on the model with the best fit to the data, but it bears mentioning that university degree, gestational month 6, and gestational week at delivery were significant in all of the models with marital status (single, $p = 0.036$) being significant in the 2 – dependent model indicating that participants who were single were at a 4 times greater likelihood of having molecular BV compared to those who were married or had a domestic partner (see Table 17).

Table 17. Aim 2a, Model 2.

$n = 52$	β	SE	Wald	df	p	OR	95% CI	
							Lower	Upper
<i>Adequacy</i> score	0.004	0.0288	0.019	1	0.889	1.004	0.949	1.062

Single	1.339	0.6979	3.681	1	0.055	3.816	0.972	14.983
Income > \$75	-1.195	1.009	1.402	1	0.236	0.303	0.042	2.188
University Degree	2.062	0.8172	6.369	1	0.012*	7.864	1.585	39.012
Age	-0.109	0.0611	3.199	1	0.074	0.897	0.795	1.011
Gestation at Delivery	-0.598	0.173	11.97 1	1	0.001*	0.55	0.392	0.771
6 Month	1.035	0.4414	5.493	1	0.019*	2.814	1.185	6.684
7 Month	-0.06	0.3042	0.038	1	0.845	0.942	0.519	1.71

The results of this analysis do not support the hypothesis that lower HEI-2015 *adequacy* scores increase the odds of molecular BV or that higher *adequacy* scores decrease the odds of molecular BV (class *D*). However, with an α of 0.05, significant findings from this aim were that women at month 6 gestation had a 2.2 to 2.8 times greater odds of molecular BV than those at month 8. Another important finding is that women without molecular BV maintained their pregnancy significantly longer than those with molecular BV. In fact, the odds of molecular BV were 45% higher for every decrease in gestation at birth by 1 week. These associations may be better supported with a larger sample size.

Specific Aim 2b

The second part of aim 2 was to examine the association of HEI-2015 *moderation* diet quality scores, controlling for race (African American or not) and obesity (BMI $\geq$ 30

or not) to predict variance in molecular BV in the cohort at the three-time intervals of participation during the study (6 –, 7 –, and 8 – months gestation). The independent model was the best fit according to the QIC. This model did not show HEI-2015 *adequacy* diet quality scores, race, or obesity to be associated with variations in molecular BV at 6 –, 7 –, and 8 – months gestation. Month 6 ($p = 0.004$) remained significantly associated with molecular BV (2.6 times higher) compared to month 8 (see Table 18).

Table 18. Aim 2b, Model 1.

$n = 55$	β	SE	Wald	df	p	OR	95% CI	
							Lower	Upper
<i>Moderation</i> score	0.02	0.0324	0.39	1	0.532	1.02	0.958	1.087
African American	0.837	0.6405	1.706	1	0.191	2.308	0.658	8.1
Obesity	-0.498	0.5447	0.837	1	0.36	0.608	0.209	1.767
6 Month	0.947	0.3317	8.159	1	0.004 *	2.579	1.346	4.94
7 Month	0.24	0.2617	0.843	1	0.359	1.272	0.761	2.124

The HEI-2015 *moderation* scores were then modelled adjusting for associated factors (see Table 5; $p < 0.1$) and community class type to examine whether HEI-2015 *moderation* diet quality scores, marital status, high income (> \$75K), university degree, age, and gestation at delivery predict variations in molecular BV over the three timepoints. The independent model was the best fit based on the QIC.

The HEI-2015 *moderation* diet quality scores were not significantly associated with variations in molecular BV over the three timepoints. Similar to aim 2a, university

degree ($p = 0.006$), gestational week at delivery ($p = 0.000471$), and month 6 ($p = 0.004$) were significant predictors in variations of molecular BV at the three timepoints. In addition, age ($p = 0.042$) was also associated with variations in molecular BV. Gestational week at delivery showed a reduction in the odds of molecular BV by 46% for every 1 week the pregnancy continued, meaning molecular BV was significantly implicated in earlier delivery. Based on this model, women at 6 months gestation were at 3.4 times higher risk of molecular BV compared to month 8. The unique finding in this model is that for every 1-year younger in age the odds of molecular BV were increased by 12%. Although the independent model was the best fit for the data, in 3 of the 5 models' marital status (single) reached statistical significance indicating that single women had 3.8 to 7 times higher odds of molecular BV compared to those married or with domestic partner involvement (see Table 19).

Table 19. Aim 2b, Model 2.

$n = 53$	β	SE	Wald	df	p	OR	95% CI	
							Lower	Upper
Moderation score	0.03	0.038	0.603	1	0.437	1.03	0.956	1.11

Single	1.203	0.7744	2.413	1	0.12	3.33	0.73	15.19
Income > \$75K	-1.615	1.0212	2.501	1	0.114	0.199	0.027	1.472
University Degree	2.248	0.8236	7.452	1	0.006*	9.471	1.885	47.577
Age	-0.125	0.0616	4.146	1	0.042*	0.882	0.782	0.995
Gestation at Delivery	-0.615	0.1757	12.229	1	0.000*	0.541	0.383	0.763
6 Month	1.22	0.4288	8.101	1	0.004*	3.389	1.462	7.852
7 Month	0.215	0.3608	0.354	1	0.552	1.239	0.611	2.514

The results of this analysis do not support the hypothesis that lower HEI-2015 *moderation* scores increase the odds of molecular BV or that higher *moderation* scores decrease the odds of molecular BV. Findings from this aim were similar to those in aim 2a with the exception of age. The odds of molecular BV were higher in this model at month 6 (2.6 to 3.4 times) compared to the model with the HEI-2015 *adequacy* diet quality scores. Variations in molecular BV were significantly associated with gestational week at delivery, for every decrease in gestational age by 1 week there was a 45% increase in risk of molecular BV observed. University degree was also associated with a higher likelihood of having molecular BV. Age was the distinct finding in this model indicating that the younger the woman the higher her odds for having molecular BV.

RESEARCH QUESTIONS

Research question 1

Do HEI-2015 diet quality scores predict alterations in the frequency of molecular BV?

There were statistically significant differences observed in molecular BV at gestational month 6 compared to month 8, however HEI-2015 diet quality scores did not significantly contribute to the variance in molecular BV at 6 –, 7 –, and 8 – months gestation. There were differences in the diet scores between the two groups, however these differences did not reach significance and diminished after adjusting for the covariates of age, gestation at delivery, marital status, university degree, race, and high income. The first comparison had an $n = 51 - 53$, after adjustment for confounders the samples had an $n = 49 - 51$. The reduction in the sample size may have contributed to the change observed.

General linear model (GLM) repeated measures were used to examine the mean HEI-2015 *total*, *adequacy*, and *moderation* diet quality scores and community class type at 6 –, 7 –, and 8 – months gestation. For HEI-2015 *total* diet quality scores with a $n = 51$, the 6 months mean scores and standard deviations were 43.6 (12.3) in the non-BV group, 39.3 (11.1) in the molecular BV group, and 41.7 (11.9) overall. At 7 months, the scores were 40.7 (12.5) in the non-BV group, 37.9 (9.1) in the molecular BV group, and 39.5 (11.2) overall. Finally, in month 8 the scores were 40.7 (14.6) in the non-BV group, 37 (10.8) in the molecular BV group, and 39.1 (13.1) overall (see Table 20 and Figure 8). Sphericity was assumed based on Mauchly's test ($p = 0.051$), gestational month ($p = 0.144$) and interaction between gestational month and community class type ($p = 0.855$) were not significant. However, after adjusting for confounding variables ($n = 49$) there

was an interaction effect between age and gestational month ($p = 0.049$) indicating that there is a combined effect of age and gestational month that significantly contribute to the variance in molecular BV (see Table 21). In other words, younger women at earlier gestational ages have higher odds of molecular BV that is associated with diet quality compared to older women at later stages of pregnancy. There were no other significant interactions noted and the differences in variance diminished with adjusting for these factors (see Figure 9).

Table 20. HEI-2015 *Total* Scores by Gestational Age and Community Class

HEI-2015 <i>Total</i> Diet Score $\bar{x}$ (<i>SD</i>)	Non-BV $n = 29$	Molecular BV $n = 22$	Overall $n = 51$
Month 6	43.6 (12.3)	39.3 (11.1)	41.7 (11.9)
Month 7	40.7 (12.5)	37.9 (9.1)	39.5 (11.2)
Month 8	40.7 (14.6)	37 (10.8)	39.1 (13.1)

Figure 8. HEI-2015 *Total* Diet Quality Scores by Gestational Month

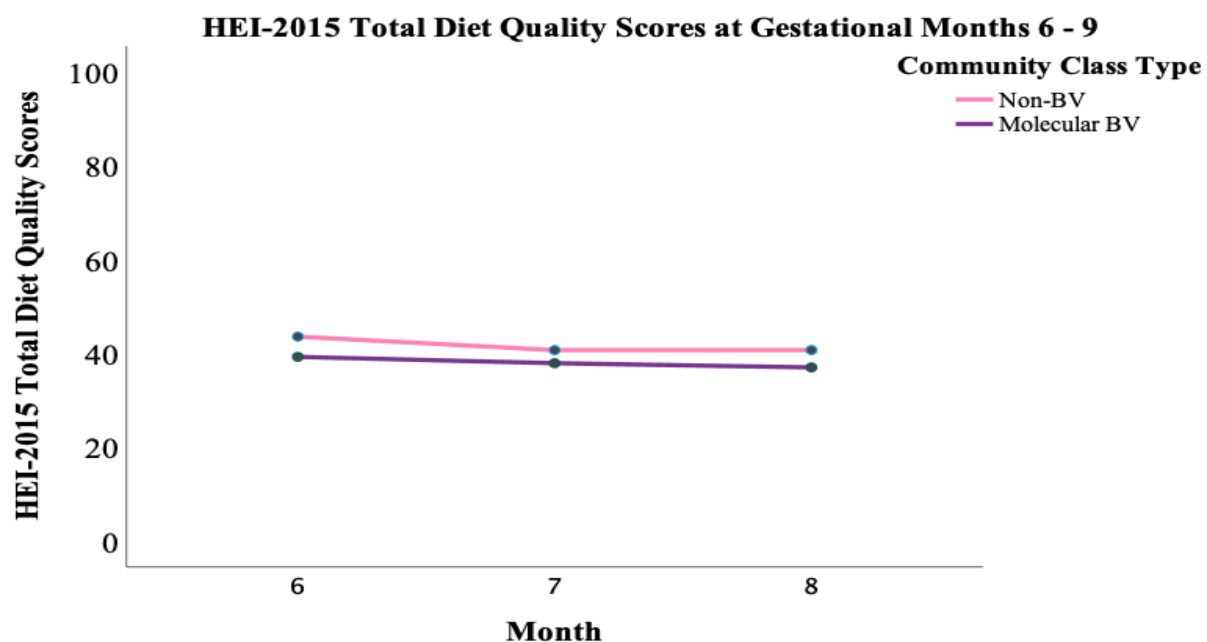


Figure 9. HEI-2015 *Total* Scores by Gestational Month Adjusting for Covariates

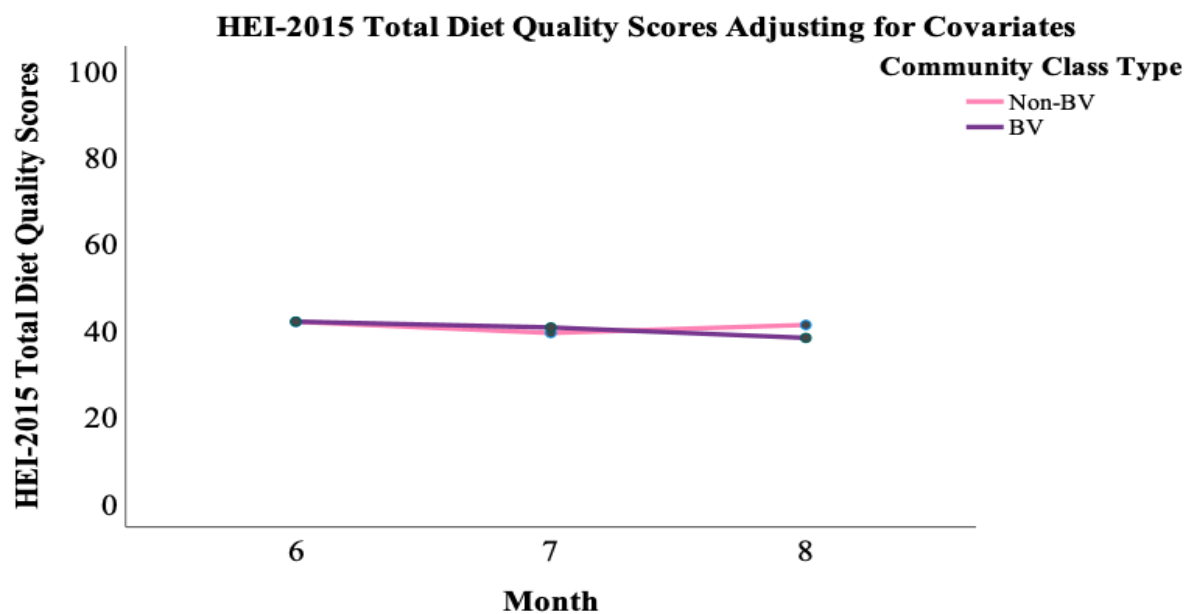


Table 21. HEI-2015 *Total* Score Interactions

HEI-2015 <i>Total</i>	<i>p</i>
Month	0.355
Month * Age	0.049*
Month * Gestation at delivery	0.257
Month * Single	0.924
Month * University degree	0.345
Month * African American	0.543
Month * High income	0.162
Month * Community class type	0.404

Next GLM repeated measures were used to examine the mean differences in HEI-2015 *adequacy* diet quality scores and community class at 6 –, 7 –, and 8 – months gestation ($n = 53$). At 6 months scores were 31 (7.7) in the non-BV group, 27.8 (8.9) in the molecular BV group, and 29.6 (8.4) overall. At 7 months, the scores were 28.4 (9) in the non-BV group, 26.9 (5.6) in the molecular BV group, and 27.7 (7.6) overall. Finally, in month 8 the scores were 27.5 (9) in the non-BV group, 26.4 (7.1) in the molecular BV group, and 27 (8.1) overall (see Table 22 and Figure 10). Sphericity was violated based on Mauchly's test ($p = 0.000$), thus Greenhouse-Geisser p -values are reported.

Gestational month ($p = 0.047$) was statistically significant but the interaction between gestational month and community class type was not significant ($p = 0.444$). Thus, even though the HEI-2015 *adequacy* scores are significantly different between gestational months, the interactive effect between diet quality and community class type is similar between the non-BV group and the molecular BV group. After controlling for associated factors ($n = 51$), there were no significant interaction effects observed (see Figure 11 and Table 23).

Table 22. HEI-2015 *Adequacy* Scores by Gestational Age and Community Class

HEI-2015 <i>Adequacy</i> Diet Score $\bar{x}$ (SD)	Non-BV $n = 29$	Molecular BV $n = 24$	Overall $n = 53$
Month 6	31 (7.7)	27.8 (8.9)	29.6 (8.4)
Month 7	28.4 (9)	26.9 (5.6)	27.7 (7.6)
Month 8	27.5 (9)	26.4 (7.1)	27 (8.1)

Figure 10. HEI-2015 *Adequacy* Diet Quality Scores by Gestational Month

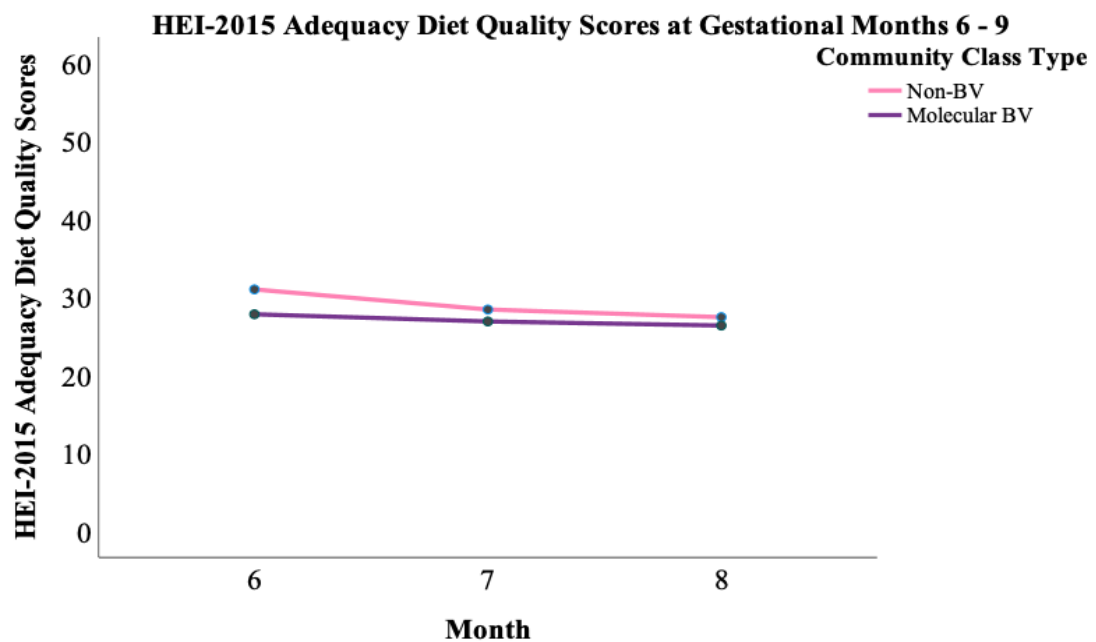


Figure 11. HEI-2015 *Adequacy* Scores by Gestational Month Adjusting for Covariates

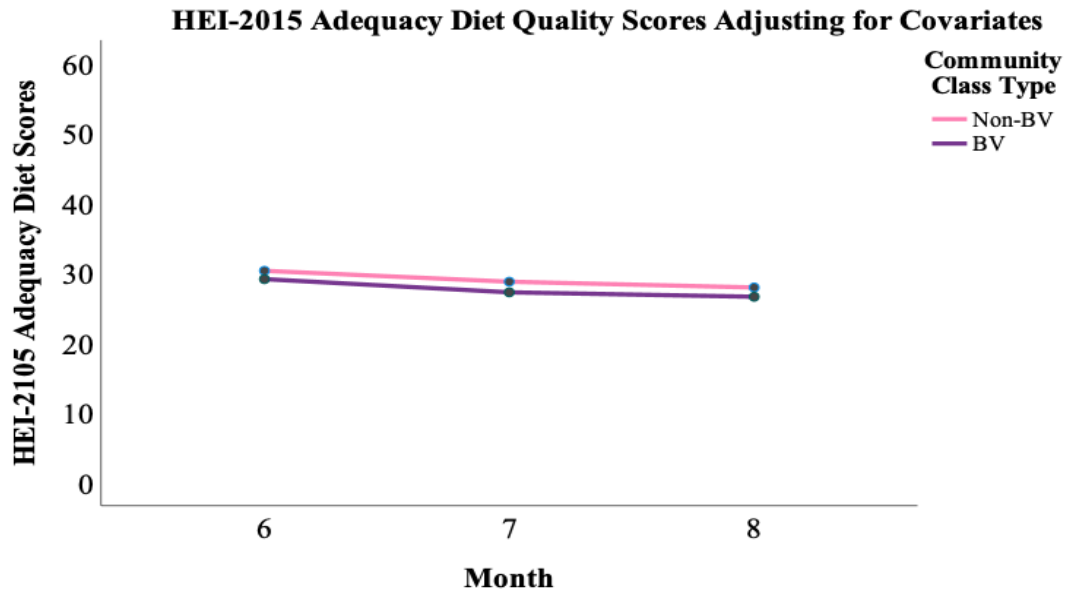


Table 23. HEI-2015 *Adequacy* Score Interactions

HEI-2015 <i>Adequacy</i>	<i>p</i>
Month	0.502
Month * Age	0.117
Month * Gestation at delivery	0.769
Month * Single	0.424
Month * University degree	0.755
Month * African American	0.329
Month * High income	0.449
Month * Community class type	0.969

The final GLM repeated measures was done to examine the mean differences in HEI-2015 *moderation* diet quality scores and community class, $n = 51$, at 6 –, 7 –, and 8 – months gestation. At 6 months scores were 12.6 (6.9) in the non-BV group, 10.7 (5.4)

in the molecular BV group, and 11.8 (6.3) overall. At 7 months, the scores were 12.4 (6.9) in the non-BV group, 10.9 (6.4) in the molecular BV group, and 11.8 (6.6) overall. Finally, in month 8 the scores were 13.8 (7.2) in the non-BV group, 15.1 (7.4) in the molecular BV group, and 14.4 (7.2) overall (see Table 24 and Figure 12). Sphericity was assumed based on Mauchly's test ($p = 0.208$). Gestational month ($p = 0.009^*$) was statistically significant but the interaction between gestational month and community class type was not significant ($p = 0.238$). This indicated that the HEI-2015 *moderation* scores were significantly different between gestational months, but that the effect of diet quality on community class type were similar between the non-BV and molecular BV groups. After controlling for associated factors ($n = 49$), there was a significant difference in HEI-2015 *moderation* scores at each month, but no interaction effects were observed (see Figure 13 and Table 25).

Table 24. HEI-2015 *Moderation* Scores by Gestational Age and Community Class

HEI-2015 <i>Moderation</i> Diet Score $\bar{x}$ (SD)	Non-BV $n = 29$	Molecular BV $n = 22$	Overall $n = 51$
Month 6	12.6 (6.9)	10.7 (5.4)	11.8 (6.3)
Month 7	12.4 (6.9)	10.9 (6.4)	11.8 (6.6)
Month 8	13.8 (7.2)	15.1 (7.4)	14.4 (7.2)

Figure 12. HEI-2015 *Moderation* Diet Quality Scores by Gestational Month

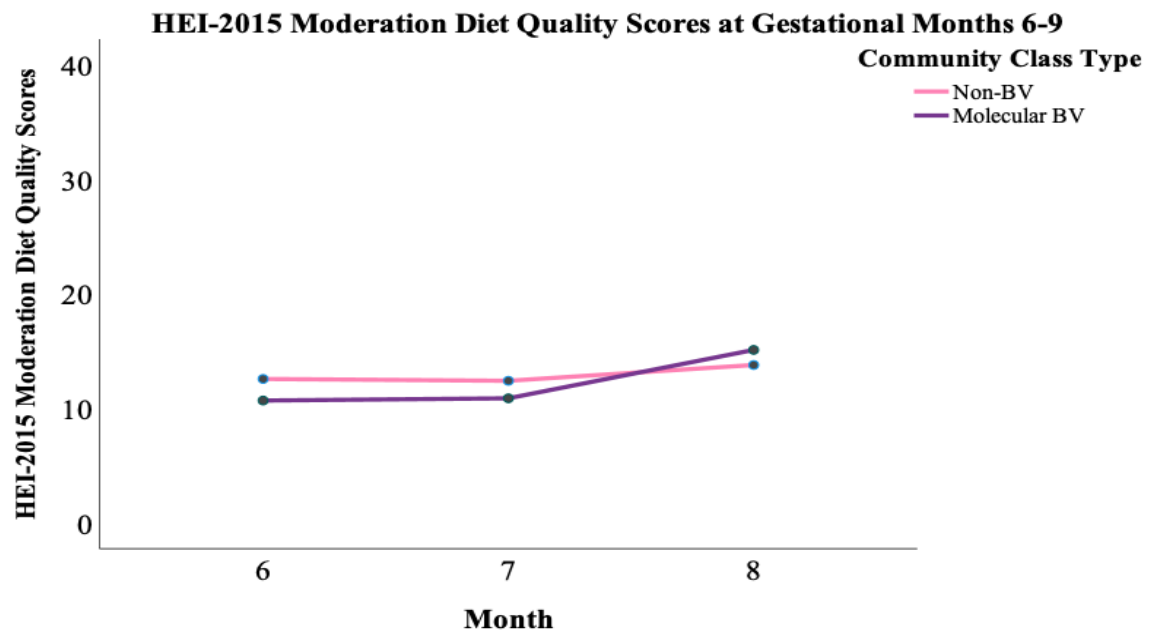


Figure 13. HEI-2015 *Moderation* Scores by Gestational Month Adjusting for Covariates

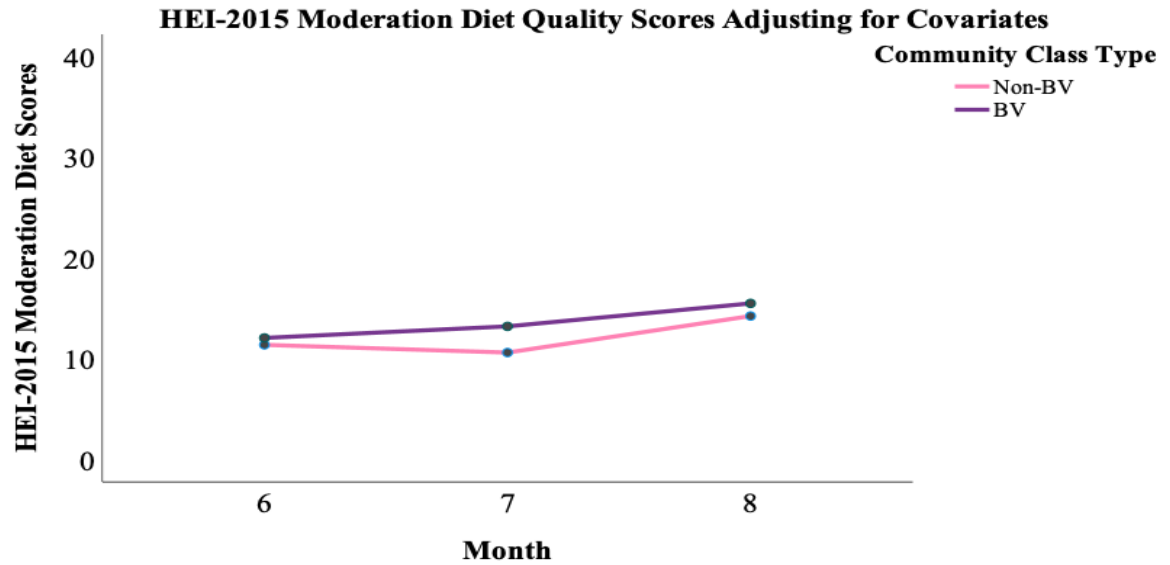


Table 25. HEI-2015 *Moderation* Score Interactions

HEI-2015 <i>Moderation</i>	<i>p</i>
Month	0.021*
Month * Age	0.183
Month * Gestation at delivery	0.037
Month * Single	0.210
Month * University degree	0.452
Month * African Am	0.430
Month * High income	0.654
Month * Community class	0.751

Research question 2

Are the differences in the HEI-2015 diet quality scores associated with vaginal microbial community class types (*L* class and *D* class)?

Visual representation of the data (see Figure 14) suggests there were differences in the overall median, interquartile range, and highest observed HEI-2015 *total*, *adequacy*, and *moderation* diet quality scores between the two groups with the molecular BV group having the lower scores. An independent *t*-test was conducted to determine if there were differences in the mean HEI-2015 *total*, *adequacy*, and *moderation* diet quality scores between women in the non-BV group compared to women in the molecular BV group. Based on an assumption of equal variances in the results of the *t*-test, the HEI-2015 *total*, *adequacy* and *moderation* diet quality scores did not differ significantly between women in the non-BV group compared to women in the molecular BV group (see Table 26).

Figure 14. Boxplots of 2015-HEI Diet Quality Scores by Community Class

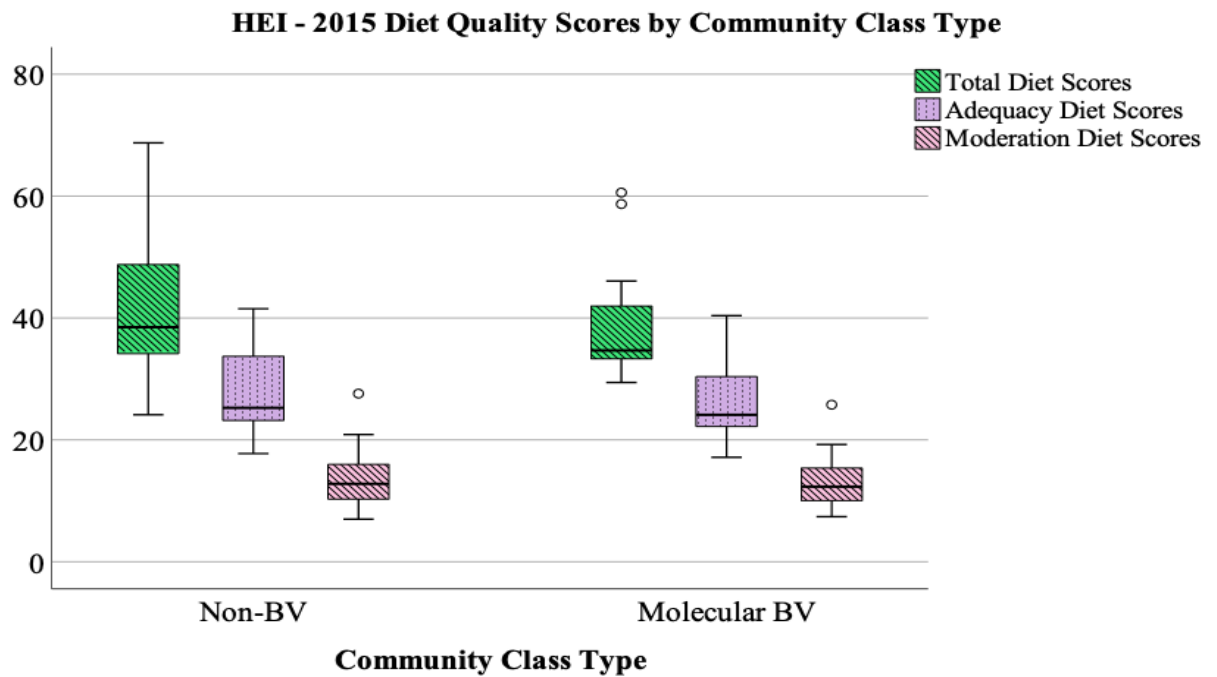


Table 26. HEI-2015 Diet Quality Scores by Community Class Type

Mean HEI-2015 Diet Quality Scores			
	$\bar{x}$ (SD)		
	<i>n</i> = 31	<i>n</i> = 24	
Component	Non-BV	Molecular BV	<i>p</i>
<i>Total</i>	41.6 (10.6)	38.1 (8.1)	0.086
<i>Adequacy</i>	28 (7.5)	25.9 (5.5)	0.061
<i>Moderation</i>	13.7 (4.8)	13 (4.1)	0.381

Research question 3

Are there significant differences in the HEI-2015 *total* diet scores between women in the non-BV group (*Lactobacillus* dominated community classes) compared to women in the molecular BV associated community class (*D*)?

The mean HEI-2015 *total* diet scores were higher in the non-BV group ($\bar{x} = 41.6$, $SD = 10.6$) compared to the BV group ($\bar{x} = 38.1$, $SD = 8.1$). However, based on the

results of an independent *t*-test (see Table 26), the HEI-2015 *total* diet score differences between the two groups did not reach statistical significance.

Research question 4

Are there significant differences in the HEI-2015 *adequacy* or *moderation* scores between women in the non-BV group (*Lactobacillus* dominated community classes) compared to women in the molecular BV associated community class (*D*)?

The independent *t*-test (see Table 26) results showed that the mean HEI-2015 *adequacy* diet scores were higher in the non-BV group ($\bar{x} = 28$, $SD = 7.5$) compared to the BV group ($\bar{x} = 25.9$, $SD = 5.5$). The HEI-2015 *moderation* diet scores were similar between the two groups. Neither the HEI-2015 *adequacy* or *moderation* diet quality scores were significantly different.

Research question 5

If there are significant differences in the vaginal community class types based on HEI-2015 *total*, *adequacy*, or *moderation* diet scores, do these differences still exist after controlling for race and BMI?

Based on the above *t*-test results (see Table 26), there were no significant differences between the two groups. This was confirmed by adding race (African American or not) and obesity ($BMI \geq 30$ or not) in a binary logistic regression model. In a small sample of 55 women, HEI-2015 diet quality scores did not reliably predict whether a person will have molecular BV or not in this cohort. However, there is a slight

trend toward *adequacy* scores reducing the odds of molecular BV and *moderation* scores increasing the odds of molecular BV (see Table 27).

Table 27. HEI-2015 Diet Quality Scores Controlling for Race and Obesity

<i>n</i> = 55	β	<i>SE</i>	Wald	<i>df</i>	<i>p</i>	OR	95% CI	
							Lower	Upper
<i>Adequacy</i> scores	-0.024	0.05	0.23	1	0.632	0.976	0.885	1.077
<i>Moderation</i> score	0.016	0.079	0.042	1	0.837	1.016	0.871	1.187
African Am.	1.328	0.846	2.463	1	0.117	3.774	0.719	19.821
Obesity	-0.656	0.631	1.08	1	0.299	0.519	0.151	1.788

SUMMARY OF RESULTS

Although the hypotheses were not supported regarding the effect of diet on the community class type, there were some important findings in this study that suggests that diet plays a role in pregnancy health. In the cross-sectional approach, adjusting for HEI-2015 diet quality scores, race (African American or not), and obesity (BMI $\geq$ 30 or not) there were no significant differences observed. However, from the longitudinal perspective, adjusting for these same factors, there were clear and significant differences in the risk of molecular BV at gestational month 6 compared to month 8. These statistically significant differences were also observed in both the HEI-2015 *adequacy* and *moderation* models after adjusting for age, marital status, education level, race, and household income.

The longitudinal analyses also suggests that diet and age are important factors that influence gestational age at delivery. The younger the woman, the greater her odds of having molecular BV. Molecular BV significantly increases the odds of delivering at an

earlier gestational age. Being single may also be a risk factor for having molecular BV, although this was not evident in all models. It is less clear, but nonetheless significant, what effect having a university degree has on the risk of molecular BV.

Overall, HEI-2015 *total*, *adequacy*, and *moderation* scores were slightly higher in the non-BV group compared to the molecular BV group, but these differences were not significant and did not remain after adjusting for associated factors. This may be a consequence of sample size. In the next chapter, interpretations and conclusions of these results will be discussed in the context of the current body of literature as well as implications for practice and future directions for research.

CHAPTER 5: DISCUSSION

Using longitudinally collected diet and vaginal microbiota data from pregnant women who participated in the BEAM project (NR014826), this study was designed to contribute to the understanding of the role diet plays in shaping the vaginal microbiota. The central research question guiding this inquiry was did diet quality, based on HEI-2015 scores, predict alterations in the frequency of molecular BV. Particularly, were differences in HEI-2015 diet quality scores associated with molecular BV? Additionally, what were the differences in the HEI-2015 *total*, *adequacy* or *moderation* diet scores between women who were molecular BV positive and those that were not and whether those differences still existed after controlling for race, obesity, age, gestational age at delivery, marital status, education, and household income.

This study had two specific aims. The first aim used a cross-sectional approach to assess the influence of diet quality on the vaginal communities to determine if differences in diet quality based on the HEI-2015 *total*, *adequacy*, and *moderation* scores were associated with molecular BV adjusting for race, obesity, age, gestational age at delivery, marital status, education, and household income. The second aim was a longitudinal approach that analyzed the relationship of HEI-2015 *adequacy* and *moderation* diet quality scores and molecular BV using diet data at 6-, 7-, and 8-month gestations in adjusted multivariate mixed models controlling for the confounding variables.

This chapter details the major findings, where these findings converge or deviate from current literature, and the contributions of this study to knowledge on the dietary influence on the vaginal microbiota and molecular BV. Finally, this chapter concludes

with the limitations and challenges of the study, areas for future research, and nursing implications.

INTERPRETATION OF FINDINGS

Diet Quality and Molecular BV

This is the first longitudinal study that has looked at the influence of diet on fluctuations in the vaginal microbiota and the recurrence of molecular BV that is significantly associated with adverse pregnancy outcomes. The sample size was small given the research question but that is not abnormal in omics studies of this type due to high cost of data collection and sample processing. Overall, diet quality scores could not reliably predict whether a person would have molecular BV. However, there was a marginal increase in the overall HEI-2015 *total*, *adequacy*, and *moderation* diet quality scores in women who did not have molecular BV, but this was not statistically significant. Conversely, in women that had molecular BV their overall HEI-2015 *total*, *adequacy*, and *moderation* scores were slightly lower. These differences were not significant and did not remain after adjusting for race and obesity in one model and race, marital status, household income, university degree, age, and gestational age at delivery in another model.

A separate longitudinal analysis was conducted to determine if the HEI-2015 *moderation* and *adequacy* scores increased fluctuation in the occurrence of episodes of molecular BV. These findings were not predictive in terms of identifying the effect diet has on molecular BV. Based on these results, it remains unclear whether women's intake

of foods originating in either the adequacy or moderation components reduce the occurrence of molecular BV.

It was surprising to find that the HEI-2015 diet quality scores did not contribute to molecular BV in this cohort given that other studies have shown that diets high in fiber (*adequacy* component) reduce the risk of molecular BV (120) and that diets high in fats (*moderation* component) increase the risk of molecular BV (87). It is highly likely that statistical significance was not met because of the longitudinal design, small sample size, and the inability to control for all contributing variables. While every effort was made to limit the number of confounding variables and to correct for the remaining variables, there were too many given the number of women in the cohort. This study found that diet had a negligible impact on molecular BV. Thus, it remains unclear if diet quality modulates the composition of the vaginal microbiota.

Gestational Age and Molecular BV

One critically important finding that is supported by the current literature was that women in the cohort who were positive for molecular BV had a shorter gestation (29, 41, 89). The data demonstrated that women at the sixth month of gestation were significantly more likely to have molecular BV compared to women in later pregnancy. This is highly clinically relevant because infants born prior to 37 weeks' gestation are considered premature and are subject to higher rates of morbidity and mortality including sepsis and respiratory distress syndrome (77).

Diet Quality, Molecular BV and Early Birth

Based on how these data were analyzed, this study did not show significant differences in HEI-2015 *adequacy* or *moderation* scores and the occurrence of molecular BV or that these diet components contributed significantly to gestational length. If the sample would have been bigger then perhaps differences would have been identified. Previous studies have detected trends related to diet quality and gestational length where diets high in adequacy components such as fiber, vegetables, fruits, quality proteins and greens trend toward reducing the risk of preterm birth (34, 79, 83). Conversely, the literature suggests that higher intake of less optimal foods found in the *moderation* components show trends in increased risk of preterm birth (21, 34, 83). There is a logical leap that if diet quality influences molecular BV and molecular BV contributes to early birth, which is well supported in the literature for over two decades (39, 62, 66), then diet quality would play a role in early birth. However, the link between diet quality, molecular BV, and early birth was not established in this study perhaps due to the limited sample size.

Another finding of consequence in this study was that after adjusting for race, marital status, household income, university degree, age, and diet scores, gestational age at delivery was 40 – 50% shorter across all analyses. The significance of this finding is the role sociodemographic characteristics have in influencing the quality of dietary intake among this cohort that may contribute to the occurrence of molecular BV. The findings from this study showed that sociodemographic characteristics influence molecular BV. This is an area of research that warrants further examination with a larger sample size.

African American and Molecular BV

African American women comprised 71% of the sample. In the univariate analysis, African American women were three times more likely to have molecular BV compared to non-African American women, however, this was not statistically significant. Even after adjusting for diet scores and obesity there was still a trend in odds of molecular BV being 3 – 4 times higher in African American women. This finding is comparable with current literature in that African American women, regardless of the presence or absence of BV symptoms, are 2 – 4 times more likely to have a diverse class of vaginal microbiota compared to non-Hispanic White women (2, 38, 72, 103). Interestingly, after adjusting for diet scores, income, education, marital status, age and delivery gestation, African American women trended toward a reduction in the odds of molecular BV. This aberrant finding deviates from the current literature likely because of the small sample size and the oversampling of African American women. Molecular BV remains a critical area of needed research in this population, particularly in light of the significant health and birth disparities among African American women.

Obesity and Molecular BV

Body mass index (BMI) in this cohort ranged from 17.9 – 60 with 40% ($n = 22$) falling into the obese category with a BMI over 30. In these women, the HEI-2015 *total*, *adequacy*, and *moderation* diet quality scores were moderating for molecular BV. Interestingly, contrary to some current literature, obesity had a protective effect after adjusting for diet quality and race (17, 63). This was also observed in two other studies of non-pregnant women in Kenya and Korea, but both were limited by control for confounding variables (71, 92). These findings suggest that it is diet quality or other

etiologic exposure, not obesity itself, that predisposes obese women to molecular BV.

This is a clinically important finding because obese women are at greater risk of adverse reproductive health outcomes (8). This finding supports additional research on this topic with an adequately powered sample.

Other Factors and Molecular BV

Age

The age range of this cohort was 18 – 34 years old with a mean of around 26 years old. Age was a contributing factor in overall diet quality scores. Younger women had significantly lower HEI-2015 total diet scores and a trend toward lower *adequacy* and *moderation* scores. In the longitudinal analysis, *adequacy* scores were not significantly associated with age and risk of molecular BV, but *moderation* scores were. In this study, age was also significantly associated with molecular BV in that affected women were roughly 23 ($SD = 3$) years old compared to the non-BV women who were around 27 ($SD = 5$) years old. The lower overall diet quality scores, the association with lower *moderation* scores over time, and the higher predisposition to molecular BV suggests that diet may have a moderating role in their odds of molecular BV. However, other confounding factors related to age, such as age at sexual debut, number of sexual partners, and sexual orientation may also be contributing factors (63).

Income and Education

In tests of associations, as expected, both income and education were significantly associated with a reduction in molecular BV. In this cohort, only 20 women (36%) had a university degree and 11 (20%) had an annual household income over \$75K. The literature supports a direct correlation between income and education and a reduction in the odds of molecular BV (2). In this study, adjusting for diet scores, race, marital status, household income, university degree, age, and gestational age at delivery, neither income nor education significantly predicted a reduction in molecular BV, although income trended toward a small reduction. However, surprisingly, women with university degrees were found to have a significant increase in the odds of molecular BV compared to women without a university degree. This association was no longer significant after correcting for gestational age suggesting that gestational age in this small group of women largely moderated this association. This finding is novel and likely due to an underpowered sample size.

Marital Status

Single women made up nearly 60% of this cohort. They were 3 – 4 times more likely to have molecular BV compared to married women or women with involved partners. This statistically significant association was consistent with the univariate analysis that showed single women had nearly four times greater odds of molecular BV than their non-single counterparts. In addition to the financial implications of not having an involved partner, there may be an increase in anxiety and psychosocial stress experiencing pregnancy/parenting alone. Anxiety and psychosocial stressors in pregnant women have been linked to alterations in the gut microbiome (50), increased incidence of

molecular BV (85), and adverse pregnancy outcomes (69). Also, the addition of a new or same-sex partner significantly increases the odds of molecular BV (36, 58, 99).

In general, the associations of sociodemographic predictors to molecular BV found in this study are consistent with others' findings, however the current body of literature on risk factors for BV are becoming dated. Given advances in sequencing technologies, there is an urgent need for a renewed focus in research on the risk factors associated with BV to fully understand this women's health issue.

IMPLICATIONS FOR PRACTICE

Findings from this study also have important implications for advanced nursing practice. First, this study supports previous research on the link between molecular BV and preterm birth. Intervention studies that attempted to demonstrate treatment of BV during pregnancy could prevent preterm birth were not successful (25, 67) because BV recurs relatively quickly (81). The findings of those studies infer that the treatment of BV itself needs to involve a systemic approach as opposed to treating the pathogens locally. Thus, practitioners should assess for modifiable factors related to molecular BV in pregnant women to prevent early birth. Secondly, younger women are at greater risk of molecular BV, thus providers should carefully assess for high-risk or contributing factors for molecular BV in this population.

IMPLICATIONS FOR RESEARCH

Clearly this study was underpowered. The significant association between molecular BV in women with university degrees was an unexpected finding that warrants additional research. Importantly, there is a need for additional research to understand the association between obesity and other etiologic exposures that contribute to a protective effect from molecular BV. There is also a pressing need for research on the contribution of sociodemographic characteristics to the incidence of molecular BV. Finally, the contributing factors for the significant finding of an increased risk of molecular BV at 6-months compared to 8 – months gestation found in this study are not clearly understood and require additional research as this is a high-risk time for early birth.

IMPLICATIONS FOR NURSING

This study emphasizes the importance of understanding the impact of modifiable factors on the vaginal microbiome. However, the microbiome is seldomly addressed in nursing curriculum (106). This is a critical shortfall because the microbiome moderates human physiology and the vaginal microbiome has a significant influence on women's health outcomes. Given that nurses play a large role in patient education it is essential that they understand the linkage between the microbiome starting at the undergraduate level. The findings from this study provide a basis for examining for high-risk behaviors and other risk factors for molecular BV particularly in younger, unmarried women. This study also provides support for the need to educate pregnant women on the factors associated with molecular BV to prevent early birth.

MILITARY RELEVANCE

This study was not conducted with a military population, but nevertheless it has important military relevance given that upwards of 80% of female service members experience vaginal symptoms related to BV in austere environments (113, 114). The findings from this study suggest that age and marital status may be contributing factors to the occurrence of molecular BV. This is particularly important when considering young recruits. However, age and marital status are not modifiable factors thus future exploration is needed to determine the modifiable risk factors of molecular BV in young, unmarried service members.

LIMITATIONS

In addition to being underpowered, this study had several other limitations to be considered. First, this study was designed based on the assumption that the gut microbiome influences the vaginal microbiome which may not be true. Secondly, the parent project recruited a non-probability sample of pregnant women residing in one major metropolitan area that resulted in an over-representation of African American women; thus, a limitation of this study is the findings may not be generalizable to a broader population. Third, women self-selected the photos they chose to submit for analysis of dietary scoring. This may have resulted in an over- or under-representation of their actual diet. However, the photo-based dietary data collection method used in the parent project may have an advantage over the recall bias inherent in paper-based data collection methods (86). Fourth, diet scores did not effectively isolate for prebiotic and probiotic foods such as yogurt and oats that women consumed and may have directly influenced their vaginal microbiome. Fifth, women self-collected vaginal samples.

Although women were trained on the proper technique to self-collect mid-vaginal swabs, it is possible that the technique was not accurately performed. However, women have been shown to reliably self-collect vaginal swabs comparable to providers (88). Finally, the inability to control for other confounding variables known to contribute to molecular BV given the number of variables already adjusted for in the models which may have been too many.

STRENGTHS

This was an original analysis that leveraged prospectively collected vaginal and diet data from a large federally funded study (BEAM, NR014826). Although underpowered, this study filled a needed gap in the literature and provides a baseline for much needed future inquiry in the realm of the vaginal microbiome. It also expands on the findings of the parent project that propels the mission of the National Institute of Nursing Research. This is the first known longitudinal study of the influence of diet on the molecular BV using community class types to compare the groups.

CONCLUSION

Molecular BV remains a significant health condition that affects a quarter of all pregnant women. It is implicated in adverse health outcomes such as preterm birth. Contrary to other studies, this study did not find a relationship between diet quality and molecular BV. However, differences may have been detected with an adequately powered sample. This study does substantiate that molecular BV contributes to earlier birth and the young women are at significantly higher risk of molecular BV. Unexpected findings, and likely due to an underpowered sample, where that university degrees increase the risk

of molecular BV and African American women have reduced risk of molecular BV when adjusting for diet scores, income, education, marital status, age and delivery gestation. An important finding in this study was that obesity was protective against molecular BV, this warrants further investigation. There is still further research needed to move the science of molecular BV forward.

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