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Testing a Model of Bacterial Vaginosis among Black Women

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by

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Dedication

I would like to dedicate this dissertation to my family. My husband Omar has been supportive emotionally and financially. Without his encouragement, I would have given up a long time ago. Since an early age my parents stressed the value of an education and without that I would never have considered this degree. Most importantly, I dedicate this to Shabazz and Genesis for being good for Daddy when Mommy had to go to study for hours at a time.

I would also like to thank each of my sister friends for their love and support. Each of you has given me encouragement in your own special ways. I am blessed to have such wonderful women in my life.

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Abstract

Bacterial vaginosis (BV) is an imbalance of vaginal flora which has been associated with increased risks of numerous gynecologic and obstetric morbidities including increased risk of acquisition of HIV from an infected partner and increased risk of preterm delivery. Black race has been consistently identified as a risk factor for BV. Black women also suffer from significant disparities in most of the morbidities also associated with BV when compared to women of others ethnicities and races. Traditional predictors of BV such as douching practices and sexual behaviors do not fully account for the racial disparities in BV prevalence. Researchers have begun to explore the potential relationship between stress and BV. Also, perceived racism has been identified as a potential stressor contributing to the health outcomes of Black women.

The purpose of this study was to test a predictive model of bacterial vaginosis among Black women. The Allostatic Load Model was the theoretical framework. Participants (N=94) completed a self-administered questionnaire and interview including measures of perceived stress, perceived racism, behavioral responses to stress and specific behavioral responses to racism along with traditional predictors of BV. Measurement scales included the Cohen Perceived Stress Scale, the John Henryism Scale of Active Coping, the Everyday Perceived Racial Discrimination Index, the Experiences of Discrimination Scale and the Telephone Administered Perceived Racism Scale (TPRS)

which included the experiences of racism personal, experiences of racism for blacks in general, concern for children and three behavioral responses to racism subscales. Bacterial vaginosis was diagnosed utilizing a self-collected vaginal swab which was analyzed utilizing the BVBlue point of care testing kit.

Twenty percent (N=19) women screened positive for bacterial vaginosis. Douching, sexual activity in the last three month and education were significantly associated with bacterial vaginosis. Age, income, hormonal contraceptive use and condom use were not associated with bacterial vaginosis. Neither perceived stress nor perceived racism was associated with BV. After completion of logistic regression analysis, only education continued to be a significant predictor of bacterial vaginosis.

The lack of an association between BV and the main study variables may have been related to the young age of the sample or the low rates of high perceived stress and high perceived racism. Perceived stress was positively associated with perceived racism and behavioral responses to stress. This association is likely a reflection of the stressful nature of perceived racism. Further research is needed to better understand how the stressful nature of racism and behavioral responses to stressors may influence health outcomes and if interventions can be utilized to promote adaptive behavioral responses.

Chapter One: Introduction

Statement of the Problem

Bacterial vaginosis (BV) is a clinical condition often referred to as an overgrowth of normal bacteria in the vagina. It is the most common vaginal infection among women of reproductive age. As opposed to other infections which are characterized by the presence of a single pathogen, BV is characterized by a diminished concentration of hydrogen peroxide producing lactobacillus and an abundance of gardnerella vaginalis as well as several different anaerobic gram negative bacteria such as Prevotella, Porphyromonas, Bacteroides, Peptostretococcus, Mycoplasma hominis, Ureaplasma urealyticum and/or Mobiloncus (G. B. Hill, 1993). This imbalance in vaginal flora may lead to the characteristic symptoms of homogenous, malodorous vaginal discharge but over half of those with BV report no symptoms (Amsel et al., 1983; Klebanoff et al., 2004).

The overall prevalence of BV varies greatly depending on the population. Estimates range from 4% among asymptomatic college students to 60% among women attending a sexually transmitted disease clinic (Mead, 1993). Among reproductive age women in the general population the estimated prevalence varies from 10% to 25% (Nansel et al., 2006). Race and ethnicity, education, income

and age are significant correlates of BV (Allsworth & Peipert, 2007). Prevalence of BV is consistently two to three times higher among Black women as compared to White women (Culhane, Rauh, McCollum, Elo, & Hogan, 2002; Culhane et al., 2001; Nansel et al., 2006). Black women are also at greater risk of many of the complications associated with BV.

Although BV was once considered a relatively benign or nuisance condition, it has been linked to significant gynecologic and obstetric morbidity. BV has been associated with pelvic inflammatory disease, gynecologic post-operative infection, Human Papillomavirus and HIV infection (Lin et al., 1999; R. Ness et al., 2005; Taha et al., 1998; Watts et al., 2005). BV in pregnancy has been associated with first trimester and early second trimester miscarriage, amniotic fluid infection, preterm premature rupture of membranes, preterm delivery, neonatal sepsis and postpartum endometritis (Goldenberg et al., 1998; Jacobsson, Pernevi, Chidekel, & Platz-Christensen, 2002; Leitich et al., 2003; Oakeshott et al., 2002; Ralph, Rutherford, & Wilson, 1999). Given the evidence of racial disparities in prevalence of BV and incidence of significant health consequences, further investigation of these relationships is indicated.

There is a growing body of evidence that suggests psychosocial and physiologic stress may be a significant contributor to health outcomes. Recent studies have found an association between chronic stress and prevalence of BV in both pregnant and non-pregnant populations (Culhane et al., 2002; Culhane et al., 2001; Nansel et al., 2006). The relationship of chronic stress to poor health

outcomes may be mediated by stress induced immune function changes leading to increased susceptibility to bacterial vaginosis.

Scientists have long been interested in the influence of stress on immune function and susceptibility to infectious illness. Both human and animal studies have provided strong evidence that chronic activation of the stress response leads to immune changes sufficient enough to influence health outcomes (Padgett & Glaser, 2003). The "stress response" involves perceptions of stress which lead to alterations in the autonomic and neuroendocrine systems. The alterations have the ability to influence immune function and therefore may predispose individuals to a variety of diseases (Kemeny & Schedlowski, 2007). Variations in the immune response of the vaginal mucosa are demonstrated in certain subgroups of women with BV (Cauci, Monte, Driussi, Lanzafame, & Quadrifoglio, 1998). The variations may help explain why some women develop complications of BV and others do not.

Certain groups of women may be more likely to experience excess exposure to chronic stressors. Black women face stressors within their lives that involve communities, relationships, economics, politics and discrimination, which may cause wear and tear on their systems (Lu & Halfon, 2003). It is this wear and tear that may contribute to adverse health outcomes. It has been postulated that the cumulative effect of stressors is physical deterioration which may prematurely age Black women and contribute to poor health outcomes (Geronimus, 1996). Perceived racism is a form of chronic stress that has been associated with increased risk of preterm delivery. Since Black women are more

likely to experience perceived racism, it can be hypothesized that this chronic stressor may help to explain the racial disparity in BV prevalence. To date, no studies have been published which investigate the relationship between perceived racism and BV.

Theoretical Framework

The allostatic load model provides a theoretical basis for investigating the relationship between chronic stressors and health outcomes. Allostasis is the term for the stress response, the body's attempts to maintain stability through any change and how the body provides energy to cope with any challenge. The body's many systems (cardiovascular, metabolic, immune and central nervous systems) help to maintain stability within the body by responding to internal and external stressors. These complex processes occur within the context of mediating factors such as an individual's environmental stressors, personal life experiences, genetic risk factors and developmental stage, and behavioral responses (McEwen & Seeman, 2004; McEwen & Stellar, 1993). Each of these factors influence whether an external event or condition leads to improved health, no change or worsening health conditions.

Need for Study

Bacterial Vaginosis is associated with several forms of reproductive health problems. Although BV is the most prevalent vaginal infection among reproductive age women, it has not been well understood. Recent research linking BV to such significant public health problems as HIV infection and preterm delivery, has spurred interest in understanding the potential causes of BV.

Chronic stress is a potential cause of immune dysfunction which may influence the incidence of BV. Racial disparities in both the incidence of BV and associated health outcomes necessitate further exploration of the potential causes of BV among Black women. Although several behavioral and demographic risk factors have been identified, they do not fully account for the racial disparity in BV incidence. Perceived racism is an example of a chronic stressor more common for Black versus White women and has been associated with other poor health outcomes. None of the studies of chronic stress and bacterial vaginosis have included the variable perceived racism.

Study Aims and Research Questions

The purpose of this study was to test a model of perceived racism, perceived stress, behavioral as well as individual factors and bacterial vaginosis among Black women. The utilization of these factors is based on the allostatic load model but testing the full model is beyond the scope of this study. The proposed model is depicted in Figure 1. The inclusion of perceived racism is aimed at addressing the concern that only using general measures of stress are not accounting for stressors unique to the experience of Black's living in the United States.

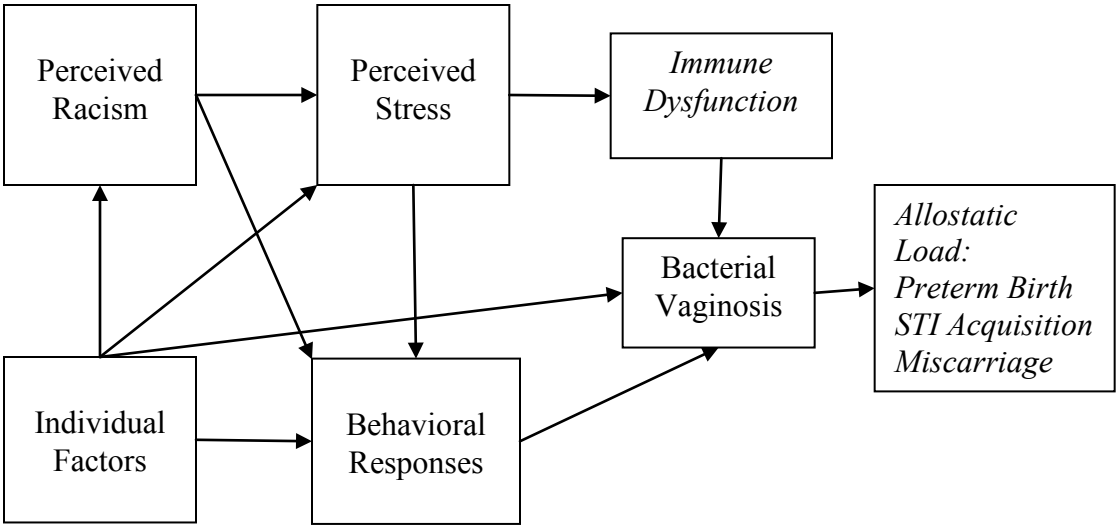

Figure 1: Conceptual Model of BV among Black women. Factors in italics not measured in this study.

Study Aim 1: To determine if perceived racism and behavioral responses to racism are associated with bacterial vaginosis after adjusting for potential confounders.

Study Aim 2: To determine if perceived stress and behavioral responses to stress are associated with bacterial vaginosis are adjusting for potential confounders.

Study Aim 3: To determine which individual factors are associated with bacterial vaginosis.

The research questions and hypotheses listed below were developed based on the study aims and guided this research study.

Research Question 1: Which individual factors are associated with bacterial vaginosis?

RQ1 Hypothesis 1: Hormonal contraceptive use will have a negative relationship with bacterial vaginosis.

RQ1 Hypothesis 2: Sexual activity will have a positive relationship with bacterial vaginosis.

RQ1 Hypothesis 3: Douching will have a positive relationship with bacterial vaginosis

RQ1 Hypothesis 4: Cigarette smoking will have a positive relationship with bacterial vaginosis.

Research Question 2: Is bacterial vaginosis associated with perceived racism and perceived stress after adjusting for potential confounders?

RQ2 Hypothesis 1: Perceived racism will have a positive relationship with perceived stress.

RQ2 Hypothesis 2: Perceived racism and perceived stress will have a positive association to bacterial vaginosis.

Research Question 3: Is bacterial vaginosis associated with perceived stress and behavioral responses to stress after adjusting for potential confounders?

RQ3 Hypothesis 1: Perceived stress will have a positive relationship with behavioral responses to stress.

RQ3 Hypothesis 2: Bacterial vaginosis will be associated with perceived stress and behavioral responses to stress.

Research Question 4: Is bacterial vaginosis associated with perceived racism and behavioral responses to racism after adjusting for potential confounders?

RQ4 Hypothesis 1: Perceived racism will have a positive relationship to behavioral responses to racism.

RQ4 Hypothesis 2: Bacterial vaginosis will be associated with perceived racism and behavioral responses to racism.

Chapter 2: Review of Relevant Literature

This chapter will include a review of the relevant literature. BV prevalence, risk factors and associated morbidities will be discussed. An in depth explanation of the pathogenesis of BV is provided with special focus on the influence of vaginal immune parameters. Research supporting an altered immune hypothesis helps to explain how immune suppressing factors such as chronic stress can influence bacterial vaginosis. This information is presented within the theoretical framework of the allostatic load model. Traditional risk factors for BV include douching and varying sexual behaviors. Recent studies have provided conflicting evidence of perceived stress as a contributing factor. The relationship of stress to health outcomes has been found to be moderated by coping factors such as behavioral responses to stress. This relationship is supported in both the scientific studies and the theoretical framework of the allostatic load model. Research is reviewed to support the inclusion of perceived racism as a factor with the model of BV among Black women.

Bacterial Vaginosis Overview

Vaginal Micro-Environment. Bacterial Vaginosis is commonly described as an overgrowth of normal vaginal flora. It would be better described as an imbalance of vaginal bacteria. Vaginal environmental factors, such as

immune parameters, influence the balance of microbes present which in turn influence the vaginal environment. Challenges in defining normal microflora also influence our understanding of bacterial vaginosis.

Pathogenic Flora. Historically a diagnosis of nonspecific vaginitis was given to women whose symptoms of increased malodorous vaginal discharge could not be attributed to Candida or trichomonas (Hill, 1993). A single pathogen named haemophilus vaginalis was then thought to be the sole cause of this nonspecific vaginitis (Gardner & Dukes, 1955). Haemophilus vaginalis is known today as gardnerella vaginalis. It has been identified in nearly all women with symptoms of BV but it has also been identified in 40 to 50% of asymptomatic women (Holst, 1990; Spiegel, Amsel, Eschenbach, Schoenknecht, & Holmes, 1980; Spiegel et al., 1983). Modern microbiologic technologies have allowed for the identification of multiple anaerobic species associated with BV including Prevotella, Porphyromonas, Bacteroides, Peptostretococcus, Mycoplasma hominis, Ureaplasma urealyticum and/or Mobiloncus (Hill, 1993). The combination of gardnerella vaginalis, anaerobic bacteria and/or mycoplasma hominis may interact to form a microbial foundation and the "pathologic core" for BV (Thorsen et al., 1998).

Evidence also suggests that in BV the protective mucus layer is replaced by a dense biofilm of bacteria on the vaginal epithelial surface. Swidinski et al. (2005) found this dense biofilm was adherent to at least 50% of the vaginal epithelial surface in 90% of the vaginal biopsies of reproductive aged women with BV, as compared to only 10% of asymptomatic controls ($p < 0.001$) whose

biofilms were loose, unstructured, likely non-adherent and consisting of mostly lactobacillus. In bacterial vaginosis, the dense biofilm consisted of up to 90% Gardnerella and up to 40% Atopobium in most specimens. Very few lactobacilli (1-5%) were detected. This study also suggests that the bacterial adhesion to epithelial cells typical of clue cells is caused by this dense biofilm. Desquamation of these biofilm covered epithelial cells leads to the appearance of clue cells on wet prep.

"Normal" Flora. Although the normal vaginal microflora is not fully understood, the "healthy" or asymptomatic vagina of a reproductive age woman is most often characterized by a predominance of certain Lactobacillus species. As is always the case, exceptions do exist and some women are able to maintain a normal vaginal ecosystem despite lower levels of Lactobacillus species (Hillier, 1998; Marrazzo et al., 2002).

Lactobacilli are involved in maintaining a healthy vaginal ecosystem by multiple pathways including the production of lactic acid, hydrogen peroxide and bacteriocins (Aroutcheva, Simoes, Shott, & Faro, 2001). These organic acids work synergistically to protect against pathogenic bacteria including those most often associated with BV (Vitali et al., 2007). Bacterial vaginosis is characterized by a decreased prevalence of hydrogen peroxide producing lactobacillus species (Hill, 1993; Vitali et al., 2007). It has been estimated that 75 to 90% of lactobacilli in most healthy vaginas produce hydrogen peroxide but only 5% do so in the BV affected vagina (Beigi, Wiesenfeld, Hillier, Straw, & Krohn, 2005; Eschenbach et al., 1989). Interestingly, not all species of Lactobacillus have a

health promoting effect. Some species do not produce bacteriocins or hydrogen peroxide (Donders, 2007).

Although most healthy vaginal ecosystems are dominated by lactobacillus species, multiple other bacteria are found in smaller quantities including those often associated with BV (Hill et al., 2005; Larsen & Monif, 2001; Vitali et al., 2007). In a sample of 144 North American White and Black women who were asymptomatic and without any evidence of vaginal infection on exam, Zhou et al. (2007) found that nearly one third of the Black women and 7% of White women sampled had vaginal ecosystems dominated by species other than lactobacillus. Our understanding of the normal vaginal ecosystem is as yet evolving and will be instrumental in defining alterations of normal such as Bacterial Vaginosis. It is possible that what constitutes normal may vary by race, region, cultural practices and other factors yet to be determined.

Vaginal Immunity. Bacterial Vaginosis is also characterized by the lack of an inflammatory response as evidenced by the relative absence of vaginal fluid leukocytes. Most women with BV are asymptomatic because inflammatory signs are usually absent. Erythema and irritation associated with infections such as Candida and Trichomonias provide evidence that the vagina is capable of mounting an inflammatory response. BV is therefore referred to as "vaginosis" and not "vaginitis". The lack of an inflammatory response may be related to vaginal immune processes (Cauci, 2004). Several researchers have postulated a microbial/host interaction which may influence risk for health consequences such as preterm delivery and HIV acquisition (Romero at el, 2004).

Immune function of the female reproductive tract is complicated by a need to resist bacterial colonization in the uterus and fallopian tubes yet accept sperm and a growing fetus (Wira & Fahey, 2004). Inability of the body to resist pathogenic bacterial colonization or accept sperm and a growing fetus can compromise a woman's reproductive health. In order to accomplish these goals, the local response of the innate and adaptive immune systems must function properly.

The innate immune system is considered the first line of defense against pathogens. It provides a rapid and non-specific response to the presence of foreign molecules such as bacterial endotoxin and lipopolysaccharide (Beutler, 2004). Once a vaginal pathogen is identified by innate immune function, pro-inflammatory cytokines are released and the acquired immune system is activated. The acquired immune system may take longer to become functional but it provides an antigen specific cell-mediated and antigen-mediated immunity (Witkin, Linhares, & Giraldo, 2007).

Vaginal epithelial cells contain toll-like receptors which are a component of innate immunity (Qualye, 2002). Eleven toll-like receptors have been identified, each of which is specific to varying viral and bacterial molecules (Janssens & Beyaert, 2003). Toll-like receptors help to coordinate a response specific to the molecules detected and are central to the regulation of adaptive immunity (Underhill & Ozinsky, 2002). It has been hypothesized that BV may develop when the activation of toll like receptors are inhibited (Witkin, Linhares, Giraldo et al., 2007)

Heat shock proteins are necessary for all forms of life. The inducible 70-kDa heat shock protein (hsp-70) is an antimicrobial component of the innate immune system which is present in the vagina (Giraldo et al., 1999). It is considered a part of a danger signal response which occurs in response to bacterial infection (Davies et al., 2006). Hsp-70 is released in response to physiologic stressors such as infection, inflammation, and extreme temperatures. It binds to Toll-like receptors and activates an immune response to fight pathogens (Campisi, Leem, & Fleshner, 2003). Vaginal hsp-70 has been associated with a down regulation of the pro-inflammatory immune response to bacterial vaginosis among pregnant women (Genc et al., 2005). A decrease in hsp-70 may predispose women to the negative consequences of BV.

Gardnerella vaginalis has commonly been associated with acute bacterial vaginosis. G. vaginalis hemolysin (Gvh) is a hemolytic toxin produced by G. vaginalis and is one potential virulence factor for BV-related bacteria (Cauci et al., 1996). A specific immunoglobulin A response against Gvh was found in 60% of women clinically diagnosed with BV (Cauci et al., 1996). Women with and without the local immune response were indistinguishable by clinical diagnostic criteria. Sialidase activity has been correlated with this local immune response. Women with BV and no immunoglobulin A response to Gvh have higher levels of sialidase activity than women with BV and an immunoglobulin A response to Gvh (Cauci, Driussi et al., 1998). Severity of the degradation of the IgA response is correlated with sialidase activity level (Cauci, Monte et al., 1998). Sialidase activity may therefore be a virulence factor not only because of its influence on

mucin but it may also mediate the immune response against other virulence factors (Gvh).

One key characteristic of Bacterial Vaginosis is the lack of neutrophils detected by microscopy. Despite this lack of inflammatory response, several proinflammatory cytokines have been associated with BV. Interleukin-1β and interleukin-1 receptor agonist polymorphisms have been associated with BV (Cauci et al., 2007). Interleukin-1β is a master cytokine which plays a key role in mediating vaginal innate immunity (Cauci, 2004). It has been associated with several inflammatory factors such as interleukin-8 and neutrophils (Cauci, Guaschino et al., 2003). In a sample of 200 pregnant women with BV, interleukin-1β was positively correlated with sialidase and prolidase ($p<.001$) (Cauci, Culhane, Di Santolo, & McCollum, 2008). It was also negatively correlated with the IL-8/IL-1β ratio. Sialidase was also negatively correlated with vaginal neutrophils. Although the high concentrations of IL-1β in BV positive women seem to be associated with sialidase and prolidase, these enzymes may also inhibit the usual proinflammatory response to IL-1β. This may explain the lack of neutrophils and reflects a possible impaired immunity.

Several researchers have hypothesized that alterations in vaginal immunity predispose women to complications of Bacterial Vaginosis (Cauci, 2004; Romero, Chaiworapongsa, Kuivaniemi, & Tromp, 2004; Witkin, Linhares, Giraldo et al., 2007). Although the diagnosis of BV is based on clinical symptoms, the cause of BV may be a host/microbe interaction. BV has been referred to as a microbial/mucosal immunity disorder (Romero et al., 2004). The host immune

function may influence bacterial colonization (Cauci, 2004). It has been hypothesized that some women may have either a hypo-vaginal or hyper-vaginal immune/inflammatory response (Simhan et al., 2003). Although the cause of BV is not fully understood, there is growing evidence to support an altered immunity hypothesis.

Prevalence. Bacterial Vaginosis (BV) is the most common vaginal infection among reproductive age women. Reported prevalence of this condition varies on the population studied. One of the first studies to report the prevalence of bacterial vaginosis (then known as haemophilus vaginalis vaginitis) included a sample of 579 primarily White sexually active women in a private gynecologic practice (Garnder & Dukes, 1955). The prevalence of BV was 13.3%. Of 500 women attending a sexually transmitted infections clinic, 23% of all women were diagnosed with BV and 33% of symptomatic women were diagnosed with BV (Thomason et al., 1988).

Since BV is not a reportable diagnosis, limited data are available on the general population prevalence rates of this condition. Women aged 14 to 49 years participating in the National Health and Nutrition Survey (NHANES) 2001-2004 were asked to submit a self-collected vaginal swab for diagnosis of bacterial vaginosis by Gram stain. Of 3,739 participants 29.2% (95% confidence interval 27.2% – 31.3%) were diagnosed with BV (Koumans et al., 2007). Half of these women were asymptomatic. The prevalence of BV may depend on the setting, population and method of diagnosis.

Associated Factors. Numerous demographic and behavioral factors are associated with rates of BV. Black women consistently have higher rates of BV as compared to Non-Hispanic White women. Hormonal contraceptives and condom use are negatively associated with BV while increased number of sexual partners, douching, cigarette smoking and stress may have a positive association with BV.

Race/Ethnicity. Almost every study that reports race as a variable, reports an increased prevalence of BV by 2 to 3 fold among Black women as compared to White women (Cherpes et al., 2008; Culhane et al., 2002; Holzman et al., 2001; Royce et al., 1999). NHANES data revealed the Non-Hispanic Black odds ratio for BV was 3.13 (95% CI 2.58-3.8), less than a high school education was associated with an odds ratio of 1.47 (95% CI 1.13-1.92), and a household income below the federal poverty level was associated with an odds ratio of 1.43 (95% CI 1.17 -1.74) (Koumans et al, 2007). This disparity persists after controlling for income, education, sexually transmitted infection history and douching practices.

Hormonal Contraceptives. Hormonal contraceptives have been consistently associated with a decreased risk of BV (Baeten et al., 2001; Riggs et al., 2007; Smart, Singal, & Mindel, 2004). Although most studies evaluating the relationship of contraceptive use and BV were cross sectional, a recent longitudinal study including 3,077 reproductive age women, examined whether hormonal contraceptive use was associated with the diagnosis of BV over the course of one year (Riggs et al., 2007). A lower BV prevalence was associated with the use of oral contraceptive pills (OR 0.76 CI 0.63-0.90) and hormonal

injection or implant (OR 0.64; CI 0.53-0.76). The physiologic mechanisms explaining these relationships have not been fully elucidated but several possibilities exist. It is speculated that estrogen may increase lactic acid which is known to help maintain normal vaginal flora (Shoubnikova, Hellberg, Nilsson, & Mardh, 1997). Injectable hormonal contraceptives (Depo Provera) may decrease the risk of abnormal pH (Brabin et al., 2005). Vaginal pH is known to influence the vaginal microflora. Estradiol and progesterone also function to regulate the innate and adaptive immunity of the female reproductive tract (Beagley & Gockel, 2003).

Sexual Behaviors. Bacterial vaginosis has been associated with frequency of intercourse, having an increase number of sexual partners and participating in noncoital receptive sexual behaviors (Koumans et al., 2007; Usher-Pines et al., 2009). The association of BV to sexual behaviors is well documented but there is not enough evidence to suggest that BV functions as a sexually transmitted infection (Verstraelen et al., 2010). The diagnosis of BV is quite rare prior to menarche (Swidsinski et al, 2010). Several studies have documented the occurrence of BV in virginal adolescents (Bump & Beusching, 1998; Vaca et al., 2010; Yen et al., 2003). This provides evidence that sexual activity is not a pre-requisite for BV. The high recurrence rate despite patient and partner treatment (Colli et al, 1997; Larsson et al., 2005; Moi et al., 1989; Vutyavich, Pongsuthirak, Vannareumol, Ruangsri & Luangsook, 1993) contradicts the male to female transmission theory.

Consistent condom use may also decrease the risk of acquisition and recurrence of BV among women at high risk for sexually transmitted diseases (Hutchinson et al., 2007). The findings in regards to the protective benefits of condom use have varied. Several cross sectional studies have found the consistent use of condoms to be protective (Calzolari, Masciangelo, Milite, & Verteramo, 2000; Shoubnikova et al., 1997; Smart, Singal & Mindel, 2004) A few studies have also found little or no protective benefit to condom use (Ahmed et al., 2001; Bradshaw et al., 2005; Schwebke, Desmond, & Oh, 2004). Hutchinson and associates (2007) used case cross over analyses of incident and recurrent periods of BV to assess the association between condom use and vaginal flora changes. Assessments of vaginal flora were made at baseline, 6, 12, 24 and 36 months. Number of sex partners, new partners, use of spermicide, recent douching and use of hormonal contraceptives were used in the final model. In the final model, consistent condom use was associated with a 45% decreased risk of BV when compared to no condom use. Condom use may help to maintain normal flora among sexually active women. The physiologic reason is not yet known but it has been speculated that semen may contain some as yet unknown factor which contributes to the transition from normal vaginal flora to BV (Smart, Singal & Mindel, 2004).

Douching. Vaginal douching has also been associated with BV. The cause and effect relationship between douching and BV remains unclear. The relationship between BV, douching and sexual behaviors are often difficult to disentangle. Frequency and/or timing of douching (after menses) may be more

predictive of BV (Schwebke, Desmond, & Oh, 2004; Zhang et al., 2004). Reason for douching may also be a significant factor. Many women report abnormal vaginal symptoms as a reason for douching (Ness et al., 2002). In this case BV may be the reason for douching and not the consequence of douching.

Cigarette Smoking. The strongest non-sexual non-demographic factor related to BV is cigarette smoking. Studies using multivariate logistic regression models have found cigarette smoking to be an independent predictor of BV (Cherpes et al., 2008; Evans et al., 2007). The risk of BV increases as the number of cigarettes smoked per day increases (Smart, Singal & Mindel, 2004). Cigarette smoking in pregnancy has been associated with an increase in anti-inflammatory cervical cytokines (Simhan, Caritis, Hillier, & Krohn, 2005). Although the reason for the relationship between smoking and BV is as yet unknown, it was hypothesized that this increase in anti-inflammatory cytokines creates an environment of immune hypo-responsiveness which may increase risk for complications such as preterm delivery. Cigarette smoking has also been associated with decreased estradiol levels (Wilson, Lee, Balen, & Rutherford, 2007). A rise in estradiol has been associated with a decrease in abnormal vaginal flora. It is also well established that in the general population smoking increases with increased stress. It has been suggested that smoking among Black women may related to an exposure to multiple sources of daily stress including but not limited to perceived racism (Webb & Carey, 2008). Perceived racism has been correlated with cigarette use but has been found to be mediated at least in part by stress (Guthrie, Young, Williams, Boyd, & Kintner, 2002).

Associated Conditions. Bacterial Vaginosis has been associated with multiple gynecologic morbidities including increased risk of sexually transmitted infections, HIV diagnosis, pelvic inflammatory disease and post-operative endometritis (Lin et al., 1999; Ness et al., 2004; Schwebke, 2003). BV has also been associated with obstetric complications including spontaneous miscarriage, preterm delivery of low birth weight infants, preterm premature rupture of membranes, chorioamnionitis and postpartum endometritis (Goldenberg et al., 2005; Hillier et al., 1995; McGregor & French, 2000; Sobel, 2005). It is the association with these reproductive health morbidities which has led to an increased interest in this condition once thought of as merely a nuisance.

Bacterial Vaginosis has been consistently associated with preterm premature rupture of membranes and preterm delivery of low birth weight infants. A meta-analysis of 18 studies and 22,232 pregnant patients revealed a greater than two-fold increased risk of preterm delivery for women diagnosed with BV. Studies that screened for BV prior to 16 or 20 weeks gestation revealed even greater risk for preterm delivery (OR 7.55 95% CI 1.80 – 31.65; OR 4.20 CI 1.99-49.34) (Leitich et al., 2003). Goldenberg, Hauth and Andrews (2000) propose that BV is actually a marker for intrauterine infection which can occur early in pregnancy or be present at conception. It may remain asymptomatic and undetected for several months eventually leading to preterm delivery. This may explain why currently available treatments for BV used during pregnancy have not been found to decrease the risk of preterm delivery.

Bacterial Vaginosis is known to increase risk of acquisition of HIV and other sexually transmitted infections (Cohen, 1998). Cross sectional studies of HIV infected and uninfected women in Thailand, Uganda and the United States have found a positive correlation between HIV infection and BV diagnosis (Cohen et al., 1995; Sewankambo et al., 1997; Warren et al., 2001). Prospective longitudinal studies of both pregnant and non-pregnant women have found that a decrease in vaginal lactobacilli and an increase in abnormal flora consistent with BV are associated with an increased risk of acquisition of HIV infection (Martin et al., 1999; Taha et al., 1998). BV has also been associated with increased risk of Gonorrhea, Chlamydia, Human Papilloma Virus and Herpes Simplex Virus Type 2 (Cherpes, Meyn, Krohn, Lurie, & Hillier, 2003; Watts et al., 2005; Wiesenfeld, Hillier, Krohn, Landers, & Sweet, 2003).

Symptoms. Symptoms typically reported by women diagnosed with Bacterial Vaginosis include an increased, thin, malodorous vaginal discharge (Amsel et al., 1983). Clinically, BV is diagnosed by the presence of three out of four of the following criteria: a milky homogenous vaginal discharge, the presence of an amine (fishy) odor when 10% potassium hydroxide is added to the discharge, a vaginal pH of greater than 4.5, and the presence of clue cells in the vaginal discharge (Amsel et al., 1983). Clue cells occur when bacteria adhere to the edges of epithelial cells and blur the edges. In a review of Bacterial Vaginosis, Speigel (2002) describes the current state of knowledge in regards to the pathogenesis of BV. Initiating the cascade is the lysis of endogenous

lactobacilli. The reasons for the lysis are still unknown. The physiologic causes of classic BV symptoms as described by Speigel (2002) can be found in Table 1.

Table 1: Clinical Diagnostic Factors for Bacterial Vaginosis and Associated Physiologic Causes

Clinical Diagnostic Factors	Physiologic Cause
pH>4.5	The lysis of lactobacilli leads to decrease in hydrogen peroxide
Amine odor	Decreased hydrogen peroxide leads to an increase in mobiloncus
Homogenous vaginal Discharge	Decreased hydrogen peroxide leads to increased gardnerella production which stimulates sialidase production which causes mucin degradation
Clue cells	Sialidase causes the cleavage of GvH IgA which causes bacterial adhesion

Mucus secreted by the cervix forms a protective barrier by coating vaginal and cervical epithelial cells (Olmsted, Meyn, Rohan, & Hillier, 2003). Mucus provides protection via multiple pathways including the presence of mucin. Mucins control the viscosity of the mucus which in turn can physically clear the vagina of microbes (Wiggins, Hicks, Soothill, Millar, & Corfield, 2001). This is considered the first line of defense against vaginal microbes. Mucin degrading enzymes include sialidases, glycosidases, proteases and sulphatases. Sialidase breaks down glycoproteins and glycolipids exposing a carbon skeleton. This breakdown process in turn provides a source of energy for bacteria and also permits adhesion to epithelial cells (Wiggins et al., 2001). Several mucin degrading enzymes have been correlated with BV associated vaginal bacteria (Cauci, Monte et al., 1998; Olmsted et al., 2003).

Diagnosis. Commonly used methods of clinical diagnosis for Bacterial Vaginosis include Amsel's and/or Nugent's criteria. Amsel's criteria as mentioned previously, is based on the presence of three out of four parameters: milky homogenous vaginal discharge, a positive whiff test, presences of clue cells on a saline wet prep and vaginal pH of >4.5 (Amsel et al., 1983). Nugent's criteria are a standard method for the interpretation of a gram stain and have improved the reliability of BV diagnosis. A point system was devised based on the quantity of lactobacilli (large Gram positive rods), Gardnerella and Prevotella (small Gram positive rods and Gram negative rods) and Mobiluncus (curved, Gram variable rods) (Nugent, Krohn, & Hillier, 1991). Gram stain is most commonly used by researchers and has been considered the gold standard in BV diagnosis. The reliability of both of these methods may vary depending on the experience and skill of the individual making the assessment.

A variety of other tests have also become available for diagnosis of BV in clinical practice. The Affirm VP III is a DNA probe based on high concentrations of G. vaginalis (Becton Dickinson, Sparks, Maryland). The Affirm compares well to gram stain with 89.5% sensitivity and 97.1% specificity (Witt, Peticevic, Kaufmann, Gregor & Kiss, 2002). The FemExam test cards are a set of two cards. One care screens for pH and amines and the other screens for the presence of prolineamniopeptidase (Quidel, San Diego, California). The sensitivity and specificity for the use of both cards as compared to gram stain are 91% and 61.5% respectively (West et al., 2007). BVBlue is a point of care test which detects sialidase activity (BVBlue, Gryphus Diagnostics, LLC, Knoxville TN). Sialidase

is an enzyme secreted by anaerobic bacteria which seem to be associated with the pathogenesis of BV (Cauci, Driussi, et al., 1998; Cauci, Monte, et al 1998). The sensitivity and specificity for BVBlue when compared to gram stain was 91.7% and 97.8% respectively (Myzuik, Romanowski & Johnson, 2003). Therefore, this test provided an acceptable and effective means of screening for BV which is available within 10 minutes. Sialidase is the most widely studied enzyme and most highly correlated with BV. It is thought to be part of the cascade of changes causing BV and may also predispose to complications of BV.

Treatment. Currently, treatment is recommended for symptomatic relief but may also reduce risk for acquiring most sexually transmitted infections. First line therapy for non-pregnant women includes either oral metronidazole 500mg twice daily for seven days, metronidazole gel 0.75% inserted intravaginally once daily for 5 days or clindamycin cream 2% applied intravaginally at bedtime for 7 days (CDC, 2010). Two oral tinidazole regimens and an oral clindamycin regimen are listed by the CDC as suitable alternative regimens.

A Cochrane Database Systematic Review of antimicrobial therapies for the treatment of bacterial vaginosis included 24 trials with 4,422 participants (Oduyenbo, Anorlu, & Ogunsola, 2009). In six trials including clindamycin and metronidazole regimens, rates of treatment failure were equivalent regardless of route of administration at both 1 week (combined RR: 1.01, 95% CI: 0.69 to 1.46) and 1 month (combined RR: 0.91, 95% CI: 0.7 to 1.18). In four trials vaginal clindamycin was compared to oral metronidazole. Vaginal clindamycin had the

fewest side effects. Oral metronidazole was more likely to cause a metallic taste, nausea and vomiting.

Other antibiotic regimens have been studied with varying results. Tinidazole has the most consistent results comparable to standard treatment. Both a 1gram and 2 gram regimen were found to be significantly better than placebo (Livengood, Ferris, Wiesenfeld et al., 2007) and equivalent to oral metronidazole (Schwebke & Desmond, 2011). Ofloxacin and erythromycin have not been found to be effective against BV in double blinded randomized controlled trials (Wathne, Holst, Hovelius et al., 1993; Covino, Black, Cummings et al., 1993). The addition of azithromycin to metronidazole did not improve cure rate (Schwebke & Desmond, 2007).

Due to the potential side effects and increasing development of resistance, non- antibiotic regimens have been explored. Triple sulfonamide cream and single hydrogen peroxide douching have not been found to effective when compared to standard therapies (Chaithongwongwatthana, Limpongsanurak, Sitthi-Amorn, 2003; McCormack, Covino, Thomason et al., 2001). Lactic acid suppositories have been found to be equivalent to placebo (Boeke, Dekker, van Ejik et al., 1993). A double blinded placebo controlled trial of acetic acid gel was stopped early with only 15 participants per group due to ineffectiveness (Holley, Richter, Varner, et al., 2004).

Due to the predominance of lactobacilli in healthy vaginal microflora, several investigators have begun to evaluate the effectiveness of varying probiotic strains of lactobacilli in treatment of bacterial vaginosis. Probiotics are

microorganisms which, when given in sufficient quantities, offer health benefits to the host (Senok, Ismaeel & Botta, 2005). Probiotics are being studied as alternative treatment and adjunct therapy to standard treatment. Results of these studies have been mixed. This literature suffers from being underpowered and lacking in double blinded randomized controlled trials. A systematic review of randomized controlled trials using probiotics for the treatment of bacterial vaginosis using any diagnostic criteria found four published studies (Senok, Vestraelen, Temmerment & Botta, 2009). Each study evaluated a different application of probiotic (oral, vaginal capsules, vaginal tampons). Although two studies found a beneficial effect on physician reported resolution of symptoms, the other two did not find any benefit (Erickson, Carlsson, Forsum, & Larsson, 2005; Anukam, Osazuwa, Ahonkhai, et al., 2006; Anukun, Osazuwa, Oseme et a., 2006; Parent, Bossens, Bayot et al., 1996). See Table 2 for a complete review of the studies.

Table 2: Randomized Controlled Trials of Probiotic Use for the Treatment of Bacterial Vaginosis

Author & Year	Sample Size	Treatment Regimen	Results
Anukum, Osazuwa, Ahonkhai et al., 2006	125 non pregnant premenopausal Women	Oral metronidazole 500mg twice daily for seven days plus either oral *L.rhamnosus GR-1 and L. reuteri RC-14* ($1x10^9$ CFU per capsule) twice daily for 30 days or identical looking placebo	88% of women in probiotic arm vs 40% in placebo arm were BV negative at 30 days follow up (OR 0.09; 95% CI 0.03 to 0.026)
Anukum, Osazuwa, Oseme et al., 2006	40 non pregnant premenopausal women	Intravaginal probiotic capsule containing *L.rhamnosus GR-1 and L. reuteri RC-14* ($1x10^9$ CFU per capsule) nightly for 5 nights or 0.75% metronidazole gel twice daily for 5 days	64.7% of women in probiotic arm vs 33.3% of women in metronidazole arm were BV negative on day 30 (OR 0.27; 95% CI 0.07 to 1.10)
Eriksson et al., 2005	187 non pregnant menstruating women	Vaginal clindamycin ovules 100mg once daily for 3 days. During the following menstruation they were given either tampons impregnated with *L. casei var rhamnosus, L. gasseri and L. fermentum* ($1x10^8$ per tampon) or placebo tampons	69.4% of women in probiotic arm vs 73.3% of women in the placebo arm were BV negative after second menstruation (OR 1.21; 95% CI 0.67 to 2.19)
Parent et al., 1996	32 premenopausal women	Vaginal tablets containing *L. acidophilus* ($1x10^9$ CFU) and estriol 0.03mg or placebo for 6 nights	87.5% of women in probiotic arm vs 14.2% in the placebo arm were BV at 28 day follow up (OR 0.02; 95% CI 0.00 to 0.47)

There is not enough evidence to suggest the use of probiotics for the treatment of bacterial vaginosis. There is some evidence that certain probiotic lactobaccili strains may offer some benefit. Large double blinded randomized controlled trials with standard methodologies are required to evaluate the effectiveness of probiotic use.

Review of Literature: Stress

Stress and Immune Function. A meta-analysis of psychological stress and human immune function identified over 300 studies that have addressed this topic (Segerstrom & Miller, 2004). Overall these studies have provided sufficient evidence to support the claim that psychological stress influences immune responses. Acute stressors were referred to as time limited laboratory challenges (i.e. public speaking). They were found to be associated with an increase in innate immunity as evidenced by an increase in natural killer cells and a down regulation of certain adaptive immune functions. This supports the claim the acute stressors lead to a protective immune response. Chronic stressors referred to demands which were pervasive throughout the individual's life. They lead to changes in identity and social roles. Chronic stressors are stable with no perceived end. Many of the studies involving chronic stressors included caregivers of a person with dementia, or individuals living with a disability or unemployment. Chronic stress consistently had a negative effect on both innate and adaptive immunity. Chronic stress in humans has been linked to upper respiratory infection, poor wound healing, decreased response to vaccinations and progression of HIV infection (Cohen, 2005; Cole & Kemeny, 2001; Marucha &

England, 2007; Sloan, Collado-Hidalgo, & Cole, 2007). This supports the claim that chronic stress leads to immuno-suppression.

The influence of stress on health outcomes such as presence and progression of infection is likely mediated by alterations in behaviors and physiologic mechanisms. The neuroendocrine system including the hypothalamic pituitary adrenal (HPA) and the sympathetic adrenal medullary axes stimulate innate and adaptive immune system changes (Kemeny & Schedlowski, 2007). The hypothalamus receives information from the environment and in turn controls secretion of hormones from the pituitary and adrenal glands. One example of a stress hormone is corticotropin releasing hormone (CRH) which is secreted by the hypothalmus and stimulates adrenocorticotrophic hormone (ACTH). ACTH stimulates the secretion of glucocorticoid hormones from the adrenal gland. Glucocorticoid hormones help to regulate cytokine production. This HPA axis is known to be a stress responsive system and therefore one mechanism by which stress can influence immune function changes (Padgett & Glaser, 2003).

It is generally understood that bacterial vaginosis is related to specific vaginal immunity and not systemic immune function (Cauci, 2004). There is some evidence that the female reproductive tract is influenced by stress induced changes in neuroendocrine systems. The HPA axis regulates the female reproductive system (Kalantaridou, Makrigiannakis, Zoumakis, & Chrousos, 2004). CRH stimulates gonadotrophic releasing hormone which regulates secretion of leutinizing hormone and follicle stimulating hormone. This in turn regulates secretion of estradiol and progesterone by the ovary. In response to

stress, the HPA axis exhibits an inhibitory effect which can in turn lead to the "hypothalamic" amenorrhea. This helps to explain menstrual irregularities experienced by women during times of emotional and physical stress.

Stress induced changes in the HPA axis may also regulate immunity via control of estradiol and progesterone. Various components of innate and adaptive immunity which are expressed in the female reproductive tract are influenced by female sex hormones (Beagley & Gockel, 2003). As mentioned previously, hormonal contraceptives are also known to influence vaginal ecology. Estrogen is thought to influence lactic acid production and injectable progesterone may decrease the risk of abnormal pH (Brabin et al., 2005; Shoubnikova et al., 1997). Both factors are known to influence the vaginal ecology. Given this information the primary mediators related to BV may include traditional stress hormones as well as female sex hormones.

Stress and Bacterial Vaginosis. As it has been demonstrated that stress may influence both systemic and vaginal immune function and bacterial vaginosis is thought to be an immune inflammatory disorder, it is not surprising that investigators have begun to explore a potential relationship between the two. The majority of studies involving the relationship between stress and bacterial vaginosis have been conducted among pregnant women. Although the results have been conflicting, these studies have varied significantly in their measures of stress, sample sizes and ethnicity of the sample (Table 3).

Table 3: Bacterial Vaginosis and Stress: A Review of the Literature

Authors & Year	Stress Measures	Sample	Results
Culhane et al., 2001	Perceived Stress Scale (PSS) 14 item version	454 pregnant women	After adjusting for race, vaginal douching, number of lifetime sexual partners and use of illicit drugs, chronic stress remained significant and independent. Moderate to high stress vs. low stress, OR= 2.3, 95% CI: 1.2-4.3; high stress vs. low stress, OR= 2.2, 95% CI: 1.1 – 4.2.
Ruiz et al., 2001	PSS 10 item version and serum cortisol	78 pregnant women	No association between perceived stress and BV. A significant increase in cortisol was noted in women during second and third trimester ($p < .01$ to $< .05$) with genitourinary infections (BV and/or chlamydia).
Culhane et al., 2002	PSS 14 item housing, material hardship and neighborhood safety	2,304 ethnically diverse pregnant women	After adjustment for race, marital status, age, smoking, douching, age of sexual debut, number of sexual partners and receipt of oral sex, perceived stress was independently associated with BV (OR= 1.3, 95%: CI 1.0-1.6).
Harville et al., 2005	Global Assessment of Recent Stress	411 Black Women	Women with high level of stress were more likely to have BV but the results did not reach statistical significance.
Nansel et al., 2006	PSS 10 item version	3,614 women followed with quarterly assessment for 1 year	BV Incidence: Adjusted for age, race, income, douching, sexual activity, number of recent partners and occurrence of a new sex partner, perceived stress was independently associated with BV incidence (OR = 1.29, 95% CI: 1.12-1.48). BV Prevalence: Adjusted for age, race, income, douching, sexual activity, number of recent partners and use of hormonal contraceptives, perceived stress remained independently associated with BV prevalence (OR=1.10, 95% CI: 1.01-1.20). Case cross over analysis: matched OR = 2.05, 95% CI: 1.15-3.66.

Table 3: Continued

Authors & Year	Stress Measures	Sample	Results
Harville et al., 2007	Inventories: PSS, Speilberger State Trait Anxiety Inventory, Sarason Life Experiences Survey	897 pregnant women	After adjustment for age, race and income, state anxiety (OR= 2.3, 95%: CI 0.8-2.5), perceived stress (OR= 1.4, 95% CI: 0.8-2.5) and life experiences (OR=1.3, 95% CI 0.7-2.4) were not associated with BV.
Paul et al., 2008	PSS 14 item Stressful Life Events scale	328 parous (previously not currently pregnant) women	After adjustment for income, marital status, STI history and current smoker status, stressful life events score was independently predictive of BV among White women (OR=1.13, 95% CI: 1.02–1.26) and Black women (OR: 1.11, 95% CI: 1.04-1.1.9).
Uscher-Pines et al., 2009	PSS 4 item	1,886 urban pregnant women	After adjustment for age, race, number of sexual partners, marital status, insurance and smoking, perceived stress was not associated with BV in the first trimester (OR = 1.01, 95% CI: 0.98-1.05).

All of these studies found consistently higher rates of BV among Black women. One particular study sought to examine if differences in chronic stress could help to explain racial/ethnic differences in BV among pregnant women (Culhane et al., 2002). This cross sectional study included 2,304 ethnically diverse pregnant women. Stress was measured at the individual and neighborhood levels. Individual measures of stress included perceived stress, housing quality, interpersonal conflict, material hardship and neighborhood danger. Neighborhood stress was assessed through the use of aggravated assault rates and homeless shelter intake data. Individual and community level stressors

varied by race. Black women reported threats to personal safety at more than double the rate of White women. Black women were also more than twice as likely to live in a neighborhood with a higher than average assault rate. After adjustment for demographic and behavioral risk factors, perceived stress was independently associated with BV (OR 1.3 95% CI 1.0-1.6). Black women may be exposed to multiple sources of stress and therefore more predisposed to suffer from stress related illness.

Racism as a Stressor. In an effort to understand certain racial and ethnic health disparities, stressors unique to people of color have been postulated as a possible cause. Considering the history of racial discrimination in the U.S., it has been hypothesized that racism may have a significant impact on the health of minority populations in this country. Recently, racism has begun to be considered within the stress model. The impact of stress may be related to the perception of an act as racist (Clark, Anderson, Clark and William, 1999). This idea certainly fits the allostatic load model which posits that the perception of stress may influence health outcomes either directly by stimulating physiologic responses or indirectly by stimulating behavioral responses to cope with the stress and thereby influencing physiologic responses.

Health disparities. Blacks in the United States suffer disproportionately in regards to morbidity, mortality, injury and disability (Centers for Disease Control & Prevention, 2005). Although overall life expectancy in the United States has risen over the last 50 years, Black Americans will live on average five

years less than White Americans (Kung, Hoyert, Xu, & Murphy, 2008). Much of this can be attributed to greater rates of hypertension, stroke, diabetes and obesity among Black Americans (Mays, Cochran, & Barnes, 2007).

Significant racial disparities also exist among reproductive health outcomes including sexually transmitted disease infection, human immunodeficiency virus diagnosis and pregnancy outcomes such as preterm delivery and low birth weight. Overall women are more likely to experience long term consequences of sexually transmitted infections. Women diagnosed with sexually transmitted infections are at greater risk of pelvic inflammatory disease, infertility and pelvic pain (Centers for Disease Control & Prevention, 2008). Black women in the United States are plagued by reproductive health outcomes substantially worse than their White counterparts.

In 2004, human immunodeficiency virus infection was the leading cause of death for Black women ages 30 to 34 years (Centers for Disease Control & Prevention, 2007) . HIV/AIDS diagnoses from 2001-2004 were highest among Black women (Centers for Disease Control & Prevention, 2007). The rate of new diagnoses for Black women was 20 times greater than for White women (67 versus 3.2 per 100,000). Of the reportable bacterial sexually transmitted infections, Black women are more likely to be diagnosed with gonorrhea, chlamydia and primary or secondary syphilis. Black women are diagnosed with chlamydia at seven times the rate, gonorrhea at 14 times the rate and syphilis at 16 times the rate of White women (Centers for Disease Control & Prevention , 2008). Interestingly, a recent national survey of 18 to 26 year olds revealed that

high risk sexual and drug behaviors do not explain the racial disparities in STD and HIV diagnosis (Hallfors, Iritani, Miller, & Bauer, 2007). A combination of environmental, contextual and institutional factors may contribute to this disparity. The authors suggested that the increased likelihood of Black young adults to choose a partner from within their race but with higher risk behaviors may contribute to this racial disparity in STD/HIV diagnosis. The disproportionate rate of incarceration of Black males relative to White males was also presented as a possible contributor (Hallfors et al., 2007).

The infant mortality rate for Blacks is twice that of Whites in the United States. Much of this disparity between Black and White infant mortality rates can be attributed to the higher rates of low birth weight and preterm delivery among Black mothers and infants. Preterm delivery and low birth weight are the leading causes of infant mortality second only to congenital anomalies (Mathews & MacDorman, 2007). In 2003, the percentage of preterm delivery among Black women was 17.7% as compared to 11.4% among White women (Mathews & MacDorman, 2007). An even greater disparity exists in the percent of infants born of low birth weight (less than 2500 gm) which ranges from 7.0% among White women to 13.5% among Black women (Mathews & MacDorman, 2007).

Accounting for disparities. Historically, poor health outcomes among Black's were attributed to biological inferiority. The history of Black American's supposed innate susceptibility to poor health outcomes can be traced back to Pre-Civil War arguments to support slavery (Krieger, 1987). The idea of racial inferiority was utilized to support the need for the free labor provided by slaves.

So called "scientific evidence" of a biological explanation for the difference between Black and White Americans was tainted by political needs to support slavery (Krieger, 1987).

It might be argued then that these disparities are due to a genetic predisposition. Data regarding pregnancy outcomes seems to contradict this presumption. If the racial disparities in pregnancy outcomes were due to genetic differences between the races, one would expect to notice higher rates of preterm delivery and low birth weight among all Black women. Despite their typically lower socioeconomic status, Black women who immigrate to the United States from Africa have lower rates of preterm delivery and low birth weight than U.S. born Black women (David & Collins, 1997).

Race, instead of being considered a biological variable, should instead be understood as a socially constructed concept. As described by Joseph Graves (2004) in "*The Race Myth,"* humans are genetically more alike than not. Based on what is known about the human genome, 67% of our chromosomes are fixed and do not vary from person to person. Any two people can share 86% of the remaining 33% of traits. This means that any two people from any two places in the world can be genotypically 95.38% the same. Individuals may be genetically more similar to someone of another race than they are to someone of their own race. Based on this genetic similarity, race cannot be considered a biological concept, instead it is a social concept based on physical characteristics (Graves, 2004).

If race is not a biological category but instead a socially constructed one, are there social differences unique to Blacks as compared to Whites? Blacks in the United States have been oppressed since they were brought to this country as slaves. Racist policies, actions and ideologies were used to support slavery, segregation, and limited or partial citizenship. Although racism is no longer legally sanctioned as it was up until the Civil Rights Movement of the 1960's, Blacks may still experience racial discrimination in their day to day lives. Racism is a potential chronic stressor which may contribute to racial disparities in health outcomes. The continuation of poor health outcomes experienced by Blacks in the United States may be related to multiple factors including cultural and genetic differences as well as social inequalities such as unequal access to health care, employment and educational opportunities (Mays et al., 2007). Recent years have seen a rise in interest in the potential role of racism on negative health outcomes (Clark, Anderson, Clark, & Williams, 1999; Geronimus, 1996; Krieger, 1999; Mays et al., 2007; Paradies, 2006; Peters & Peters, 2006; Taylor et al., 2007; Williams, 1999)

In a review of 138 quantitative studies on self-reported racism and health outcomes, Paradies (2006) found 14 studies included self-reported stress and self-reported racism. Numerous other studies included mental health outcomes usually associated with stress such as anxiety, depression and psychological distress. Positive associations were found in 72% of the studies with 28% finding no association and only one study finding a negative association. Overall,

perceived racism has been found to increase the risk of negative mental health outcomes including perceived stress.

Behavioral Responses to Stress. Behavioral responses to perceived stress may be considered adaptive or maladaptive (Clark et al., 1999). Within the allostatic load model adaptive response would stimulate physiologic responses which in turn promote adaptation instead of allostatic load. Maladaptive responses would promote negative health outcomes and therefore allostatic load. Some behavioral responses are clearly considered maladaptive. An example of this would be cigarette smoking. There is no evidence that smoking would ever improve health outcomes.

Whether a behavioral response is considered adaptive or maladaptive may vary depending on the chronicity of the stressor and the individual differences. An example of this in the literature can be found when referring to John Henryism. John Henryism is a type of high effort coping in response to psychosocial stressors. This was named after the folk hero of the mid to late 1800's. He was an incredibly strong, skilled and uneducated black man working to chisel rock to create tunnel for the railroad. According to the legend he battled against a steam powered hammer and won. Immediately following the battle he died of exhaustion. John Henryism has been defined conceptually as a sense that one can meet environmental demands through hard work and determination (James, 1987). Overall, it would seem that a sense of control could be associated with positive health outcomes but this is not always the case. Studies have

provided conflicting results in regards to the use of this coping response and the influence on cardiovascular health outcomes. Use of this coping response may not be the same for men as it is for women, and may also vary by socioeconomic status (Bonham, Sellers, & Neighbors, 2004; Dressler, Bindon, & Neggers, 1998; Merritt, Bennett, Williams, Sollers, & Thayer, 2004).

Few studies have explored the coping responses of individuals experiencing the chronic stress of perceived racism. A recent study explored the experiences of racism among a group of Black childbearing women (Nuru-Jeter et al., 2009). The women's responses to racism were mostly active describing expression of emotion and/or demonstration of action. Some women described more passive responses in which they suppressed their feelings or ignored the situation. It is unclear which of these approaches is the healthiest. On one hand a passive response may lead to pent up anger or emotions which later explode, on the other hand an active response may lead to a confrontation which is difficult to handle. This exploratory study actually confirms findings from an earlier study which found that 41% of Black female participants reported experiencing strong active emotional responses to racism but 16% reported passive emotional responses such as powerlessness (Vines et al., 2006). Further work is needed to better understand the relationship between particular responses to racism and health outcomes.

Theoretical Framework: Allostatic Load

Multiple stress theories have attempted to describe the relationship between stress and health outcomes. One of the challenges with theoretical

frameworks involving stress is the difficulty in simultaneously combining biological, psychological and social factors (Carlson & Chanberlain, 2005). The Allostatic Load model attempts to explain how biology, environment and behavior interact to influence health (McEwen & Stellar, 1993).

One of the difficulties with stress research is the ambiguity involved with the term stress. In this model, stress is used to refer to an occurrence which is perceived as threatening and therefore stimulates a response (McEwen & Wingfield, 2003). The assessment of an event as threatening may be influenced by individual differences including but not limited to genetics, lifetime experiences, and developmental stage (McEwen & Seeman, 1999). The perception of stress may directly influence physiologic responses or it may stimulate behavioral responses (i.e. smoking, drinking and eating) which may in turn influence physiologic responses (McEwen, 1998).

This theoretical framework provides a basis for the physiologic mechanisms behind stress and health interactions. Rather than using the general term of stress, this framework builds on the constructs of allostasis and allostatic load. Allostasis refers to the body's ability to maintain stability through change (Sterling & Eyer, 1988). If homeostasis is the process of maintaining stability for systems which are essential for life (i.e. maintenance of body temperature) then allostasis refers to the changes required to maintain those systems (McEwen & Wingfield, 2003). The physiologic responses required to maintain stability during a challenge can be maintained for a limited time but prolonged or repetitive occurrences can cause damage to the body referred to as allostatic load (McEwen,

1998). Allostatic load is influenced by behavioral and physiologic responses to perceived stress as well as genetic, developmental and experiential differences (Figure 2) (McEwen & Seeman, 1999). Differences in each of these factors may help to explain individual variations in health outcomes.

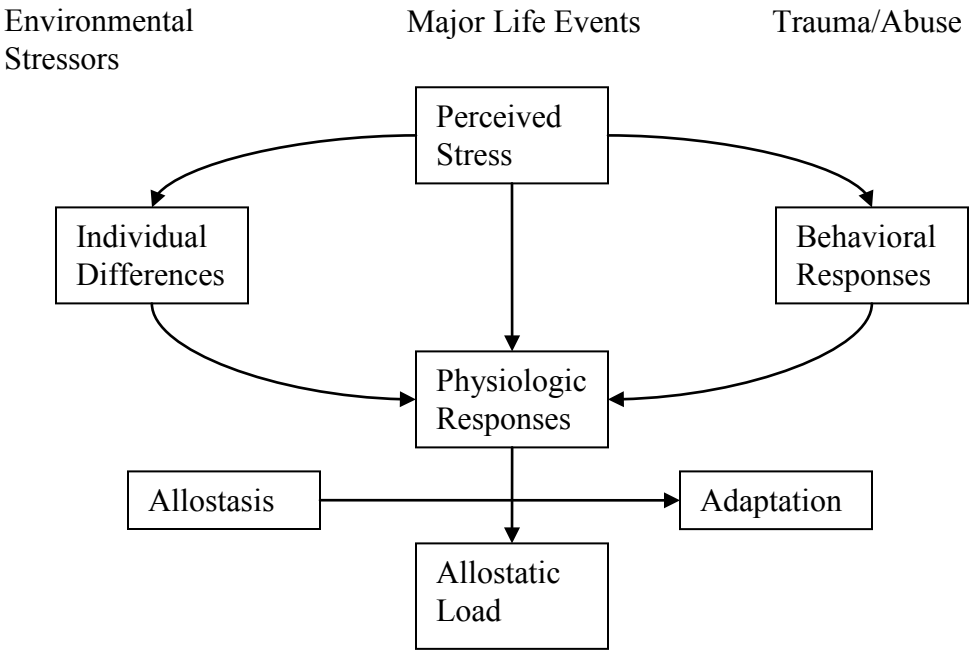

Figure 2: The Stress Response and Development of Allostatic Load (McEwen, 1998)

Several studies have begun to explore the physiologic mediators of the stress response. The primary mediators of allostatic responses include but are not limited to hormones of the hypothalamic-pituitary-adrenal axis, catecholamines and cytokines (McEwen & Wingfield, 2003). Much of the data available on measures of allostatic load come from the MacArthur Studies of Successful Aging, which operationalized the concept as a combination of measures relevant to disease risk and related to primary mediators (Seeman, McEwen, Singer, Albert, & Rowe, 1997). For example, waist-hip ratio was utilized as a measure

because it was thought of as a measure of adipose tissue deposition which is influenced by glucocorticoid activity (Seeman et al., 2004). Glucocorticoids are considered primary mediators. As we continue to explore how basic physiologic responses may influence health outcomes, the list of primary mediators will likely grow. Primary mediators related to bacterial vaginosis and associated complications may include measures of general immune function as well as specific vaginal immune function.

This model provides a basis for our understanding of the potential pathways by which perceived racism, stress, and behavioral responses to stress may be related to bacterial vaginosis. Stress may affect risk of BV through immune system down regulation or through behavioral responses such as smoking, active coping or other behavioral responses. Perceived Racism may be an example of an environmental stressor more common among Black women and therefore may add to their overall perceived stress. Bacterial Vaginosis, once thought of as a nuisance factor, is likely a reflection of multiple immune responses which seems to predispose women to numerous morbidities. Within this framework the multiple gynecologic and obstetric complications associated with bacterial vaginosis would be an example of allostatic load.

Chapter Summary

Bacterial vaginosis is no longer considered a benign nuisance problem. It has been associated with significant reproductive health morbidities and therefore has found the attention of numerous research scientists and clinicians. Although not completely understood numerous physiologic pathways contribute to the

occurrence and virulence of BV. Research has begun to provide evidence that BV may be a microbial/mucosal immunity disorder (Romero et al., 2004). Vaginal immune function is sensitive to stress related immune changes. These changes may predispose women to bacterial vaginosis. The allostatic load model of stress explains how perceived stress may influence health outcomes. The literature reviewed suggests that the stress responsive physiologic changes which predispose to BV would fit this theoretical model.

Bacterial vaginosis is considerably more prevalent among Black women. These women are also at significantly greater risk of developing complications associated with BV such as sexually transmitted diseases, pelvic inflammatory disease and preterm delivery. Racial disparities in BV prevalence cannot be fully accounted for by risk factors such as douching or sexual behavior characteristics. Recent studies suggest that chronic stress may account for some of the unexplained variance. These studies may be limited by the measures of stress utilized. It has been suggested that Black women may experience unique stressors such as perceived racism. Perceived racism has been associated with health outcomes such as hypertension and preterm delivery which are also thought to be stress responsive outcomes.

The allostatic load model indicates that behavioral responses to perceived stress may influence physiologic responses and therefore health outcomes. Little is known about how black women respond to perceived racism and which responses would be adaptive. Cigarette smoking could be considered a maladaptive behavior which has been found to increase risk of BV. Coping

responses are known to be potential mediators of the stress and health outcome relationship. The adaptivity of coping responses may vary by type of stressor experienced. The influence of coping with racism on stress may depend on the level of personal coping resources the individual has available to draw upon.

Based on the above review of literature, the proposed relationship between perceived racism, behavioral responses, perceived stress, and BV is depicted in Figure 1. All of the variables included in this study are depicted in Figure 3. This model is theoretically based on the allostatic load model. Perceived racism is proposed to have an indirect relationship with BV. This relationship is hypothesized to be mediated by perceived stress. Individual differences and behavioral responses will also be taken into account.

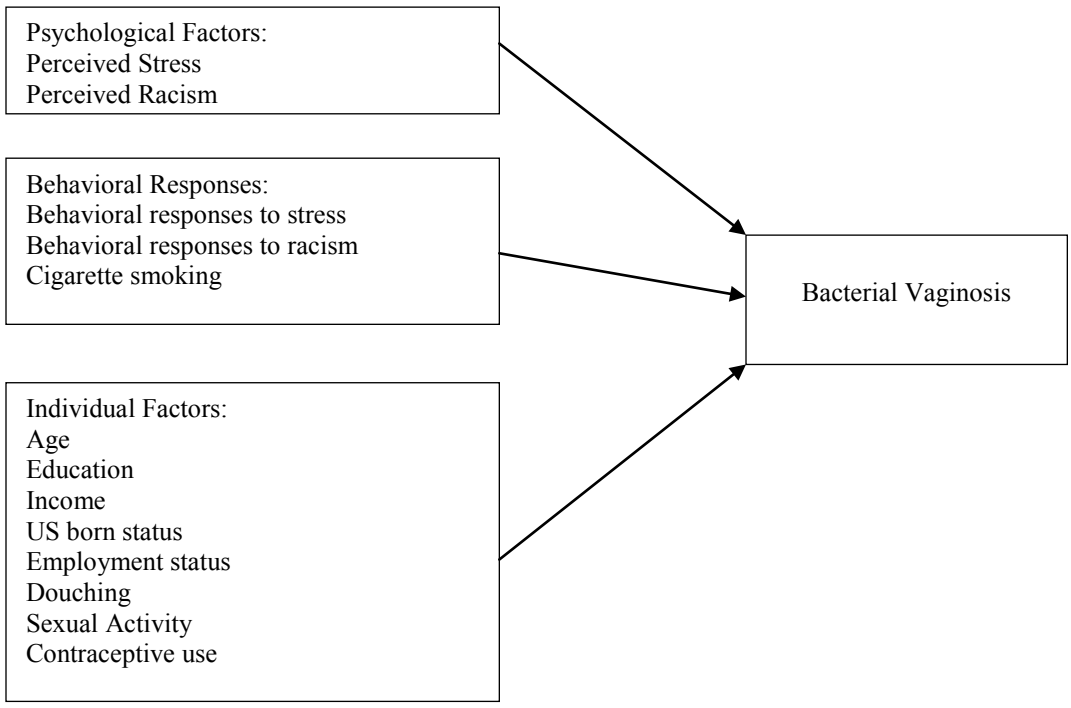

Figure 3: Input and Output Study Variables

Chapter Three: Methods

This chapter describes the methods used to explore the relationship between perceived racism, behavioral responses to racism, perceived stress, behavioral responses to stress and bacterial vaginosis among Black women. The sample selection and recruitment procedures will be discussed. A discussion of the methodology including instrumentation will be reviewed and will be followed by the data analysis plan.

Study Design

The study was a cross sectional design. Women were asked to present for a single study visit lasting about 45 minutes. During the visit they completed a self-administered questionnaire, self-collection of a vaginal swab and an interview questionnaire (See Figure 4). After the interview the swab was developed and the results were shared with the participant.

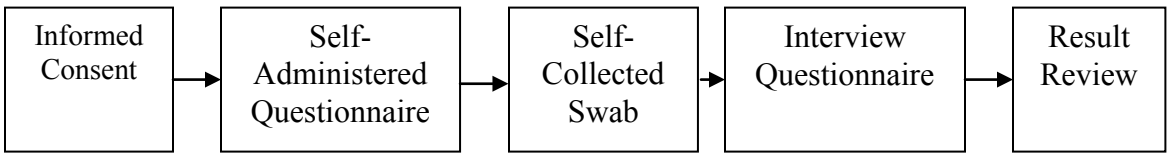

Figure 4: Procedures for Data Collection

This is the first study this researcher is aware of to explore a relationship between perceived racism and BV. As this model of predictors of Bacterial Vaginosis among Black women is in the early stages of development, the cross

sectional design is an appropriate choice. Although this design does not permit the determination of cause and effect, this is implied based on the theoretical framework for the model.

Setting

The research study took place in the medical clinic of a large metropolitan university. For convenience, participants were offered the choice of two similar clinics located in different parts of the city. One clinic was located on the university campus and the other was located in the downtown area of the city. Both clinics offered complementary patient parking. The study visit took place within a clinic exam room. This provided a private place to complete questionnaires and a privacy curtain which was drawn closed during the self-collection of the vaginal swab.

Sample

A convenience sample of women who self-identified as Black or African American was recruited from the university area. The plan for data analysis originally included use of structural equation modeling to test the model as proposed in Figure 1. A sample size of 200 is often adequate for medium sized models such as the proposed model (Boomsma, 1983). According to MacCallum, Browne and Sugawara (1996) a minimum sample size of 187 is required to achieve a power of 0.80 with 60 degrees of freedom. Given this information a sample size of 200 was sought. A total sample of 94 was obtained and therefore the plan for data analysis was revised to logistic regression analysis.

Recruitment Strategies

Given that in the Spring semester of 2010 the University of South Florida had 3,265 Black female students enrolled, the researcher determined that the local university population of students, staff, faculty and medical clinic patients would provide a sufficient population from which to recruit (University of South Florida, 2010). Women were also recruited from the community at large through participation in local community health fairs. Recruitment began through a mass emailing of recruitment flyers to the USF Health student and staff list serves (Appendix A). Fliers were also emailed to Black student organization presidents or chapter chairs. Due to the continuous nature of this study, recruitment fliers were emailed on average twice per semester. Hard copies of the fliers were also posted in the USF clinic areas of gynecology, pediatrics and general internal medicine. After the first three months of recruitment, permission was obtained to offer a $20 gift card as incentive for participation.

Over the course of 14 months, 128 women contacted the researcher by phone or email to inquire about the study. Each woman was screened by the researcher for inclusion criteria (Appendix 2). Ninety-four of the 128 (73.4%) women participated in the study. Table 4 lists the reasons individuals did not participate. Individuals indicating lack of incentive as a reason for not participating were re-contacted after the incentive was approved. None of those individuals responded after the second contact. As the study only required one visit, no participants were lost to follow up.

Table 4: *Individuals Did NOT Participate in Study (N=34)*

Reason	Number of People
No reply to researchers follow up email or phone call	10
Scheduling conflicts	6
No reason given	6
No incentive offered	3
Distance too far	2
Pregnant	2
Taking antibiotics	2
No show	1
Sick	1
Autoimmune disorder	1

Selection Method

Once a potential participant established initial contact, the study requirements and potential burden were described to them. If the individuals indicated interest, they were screened for inclusion and exclusion criteria. Inclusion criteria included being a self-described Black woman, who was able to speak and read English and was between 18 and 35 years old. Women were excluded if they were pregnant or within eight weeks of giving birth, had been on antibiotics or immunosuppressive medication within the last month or had ever been diagnosed with an autoimmune disorder (i.e. HIV, lupus, rheumatoid arthritis). Anyone within eight weeks of giving birth or on a short course of antibiotics was given the opportunity to schedule a visit for a later date. See Appendix B for study screening tool.

After an individual expressed interest and met the inclusion/exclusion criteria, the approved University of South Florida Human Subjects Institutional Review Board consent form was reviewed with them. They were given an opportunity to review it individually and given an opportunity to ask questions.

After the researcher was assured the potential participant understood what was required, the consent form was signed.

Measures

Self-Administered Questionnaire. The self-administered questionnaire was completed after the informed consent form was signed. The questionnaire included the Experiences of Discrimination Scale, Everyday Perceived Racial Discrimination Index, and the Perceived Stress Scale (Appendix C). Also included were measures of contraceptive use, douching, sexual activity and cigarette use (Appendix D).

Experiences of Discrimination Scale. The Experiences of Discrimination (EOD) Scale was originally designed for use in the Coronary Artery Risk Development in Young Adults (CARDIA) study. The validation study included a sample of 200 Black and Latino working adults aged 25 to 64 years (Krieger, Smith, Naishadham, Hartman and Barbeau, 2005). Cronbach's alpha coefficient was 0.74 and test retest reliability coefficient was 0.70. Structural equation modeling also demonstrated that the EOD had the highest correlation (r=0.79) to an underlying discrimination factor as compared to single item measures of perceived racism. The instrument was designed to assess experiences of discrimination in six well known situations such as "at school", "at work" and "from the police or in the courts". Participants are asked how often in the past five years they have been treated unfairly or discriminated against because of their race. Responses include never, rarely, some of the time or most of the time.

Responses are summed to provide a total number of domains of discriminatory experiences.

Everyday Perceived Racial Discrimination Index. The Everyday Perceived Racial Discrimination Index (EPRDI) was originally designed for use in the 1995 Detroit Area Study (Williams, Yu, Jackson & Anderson, 1997). This 10 item measure was designed to assess both chronic and daily exposure to discrimination. It inquires about the individuals' perception of other people's behavior towards them because of their race, skin color or ethnicity. It includes items such as being treated with less respect, receiving poorer service in restaurants or stores, people acting as if they think the participant is not intelligent or people acting afraid of the participant. Item response scores are summed to provide a total perceived racism score. Higher scores reflect higher levels of perceived racism. Cronbach's alpha coefficients were reported as .88 in a racially diverse community based sample (Williams et al., 1997). The scale asks the participant to indicate how often they experience varying types of discrimination in their daily life due to their race or ethnicity. The original response scale was a 4 point Likert scale ranging from 1 (often) to 4 (never). This scale was modified to a 7 point Likert scale ranging from 1 (never) to 7 (very often) and including a midpoint of 4 (sometimes). The inclusion of a midpoint allows the participant to express neutrality. An increase in the number of options to seven improves reliability and validity without overburdening the participant with an excessive number of choices (Lozano, Garcia-Cueto & Muniz, 2008).

Perceived Stress Scale. Stress was measured using Cohen's Perceived Stress Scale (PSS), which is a 14 item self-report measure of chronic stress (Appendix C). The scale was standardized on a community sample of 466 college students. The Cronbach's alpha reliability reported ranged from .84 to .86 and test retest reliability was .85 (Cohen, Karmack & Mermelstein, 1983). Participants are asked about how they felt or thought a particular way. Seven items are reverse scored (items 4,5,6,7,9,10 and 13). This measure has been widely used in a variety of populations and is considered an effective means of assessing global stress.

Contraceptives. Study participants were asked about current use of hormonal contraceptives known to decreased the risk of bacterial vaginosis including oral contraceptives (birth control pills), injectable contraceptives ("The shot" or "DepoProvera"), transdermal contraceptives ("the patch" or "Ortho Evra", or the contraceptive vaginal ring ("The ring" or "Nuva Ring").

Douching. Douching was defined as the introduction of water or a solution inside of the vagina. Douching was first defined. The study participant was asked if they had ever douched. If they answered yes, they were asked the average number of times they douche per month.

Sexual Activity. Participants were asked about sexual activity in the past three months. If they had been sexually active, they were asked about number of partners and frequency of condom use.

Cigarette Smoking. Questions regarding cigarette smoking were designed by the researcher for the purposes of this study. Women were asked if they had

smoked cigarettes at all in the last three months. If so, they were asked how many cigarettes per day they smoked on average.

Self-Collected Swab: Bacterial Vaginosis. BV Blue is a point of care test available to screen for bacterial vaginosis. The OSOM BV BLUE Test is an enzyme activity test for use in the detection of vaginal fluid specimens for sialidase activity, an enzyme produced by bacterial pathogens such as *Gardnerella vaginalis*, *Bacteroides* spp., *Prevotella* spp., and *Mobiluncus* spp. The OSOM BV BLUE Test is designed to provide a clear, simple indication of elevated sialidase activity in patient's vaginal fluid samples. The generation of a blue or green color indicates a positive test result; a yellow color indicates a negative test result. Gram Stain has often been considered the gold standard in diagnosis of BV but because of the time and expertise required it is not used as a point of care test. The sensitivity and specificity for BV Blue when compared to gram stain was 91.7% and 97.8% respectively (Myzuik, Romanowski & Johnson, 2003). Therefore, this test provided an acceptable and effective means of screening for BV which is available within 10 minutes.

Women were instructed on how to obtain a self-collected swab from the lower third of the vagina. Self-collected swabs have been found to be acceptable and effective means of collecting vaginal secretion for bacterial vaginosis screening (Strauss, Euker, Savitz, & Thorp, 2005) while at the same time allowing women privacy from the health care provider.

Interview. After the self-collection of the vaginal swab, a structured interview was completed. This interview included collection of demographic data, completion of each of the subscales of the Telephone Administered Perceived Racism scale and the John Henryism Scale of Active Coping.

Demographics. Age, marital status, highest level of education completed, annual family income, US birth status and current employment status were assessed through the use of questions designed by the researcher for use in this study (Appendix E).

Telephone Administered Perceived Racism Scale. The Telephone Administered Perceived Racism Scale (TPRS) was designed for epidemiologic studies often conducted over the phone and was tested in a sample of African American women (Vines et al., 2001). The 10 item Experiences of Racism Personal (Cronbach alpha=.88), 10 item Experiences of Racism Group (Cronbach alpha=.82) and 8 item Concern for Children's Future (Cronbach alpha=.90) subscales were used to measure perceived racism (Vines et al., 2001). This measure was administered by the researcher in person during the one study visit.

Behavioral responses were measured using the behavioral responses to racism subscales of the Telephone Administered Perceived Racism Scale (TPRS). This subscale includes passive behaviors (6 items) such as accepting or ignoring it (Cronbach's alpha= 0.68), external active behaviors (2 items) such as working harder to prove them wrong (Cronbach's alpha= .77) and internal active behaviors (2 items) such as prayer (Cronbach's alpha=.85).

The TPRS also includes a list of behaviors used to "cope with racism" including exercising, overeating, drinking alcohol, smoking cigarettes and others. Each item was evaluated individually for its relationship with Perceived Racism and Perceived Stress.

John Henryism Scale of Active Coping. Behavioral response to stress was measured using the John Henryism Scale of Active Coping (JHAC). John Henryism is a concept involving an individual's tendency to behaviorally respond to psychosocial stressors in a high effort and active manner (James, Keenan, Strogaz, Browning & Garrett, 1992). The 12 items on this scale are used to assess: mental and physical energy, focused goal oriented determination, and a strong work ethic (James, Strogatz, Wing & Ramsey, 1987). Sample items from this scale include: "Once I make up my mind to do something, I stay with it until the job is completely done" and "When things don't go the way I want them to, that just makes me work even harder." Respondents were instructed to indicate if the item is true or false and then asked if they thought it was somewhat or completely true or false. Completely true corresponds to a score of 5 and completely false corresponds to a score of 1. The total score is obtained from summing the individual responses and can vary from 12 to 60. The Cronbach's alpha reported in a sample of 452 black women was .77 (James et al. 1992).

End of Visit Procedures

Once the self-administered questionnaire was reviewed for completion, the woman was informed of her BV result. A woman screening positive for BV

was given a letter indicating she had participated in the study and screened positive for Bacterial Vaginosis. She was then referred to her primary gynecologic provider for further assessment and treatment. Participants were given a $20 gift card to Wal-Mart, thanked for their participation and were offered a flyer to share with other potential participants.

Data Management

All data were gathered on paper questionnaires. All of the forms were labeled with a unique identifier. Only the informed consent forms contain the first and last names of participants. All of the forms were stored in a locked cabinet in the researcher's office throughout the duration of the study. Data were entered into an EXCEL spreadsheet saved on a password protected computer. Only the unique identifiers were entered onto the spreadsheet.

Data Analysis

The initial plan was to utilize structural equation modeling to test the proposed model of Bacterial Vaginosis among Black women. Structural equation modeling is a statistical approach which is used to test a series of hypothesized relationships between observed and latent variables (Kaplan, 2000). Observed variables are directly measured while latent variables are unobserved but implied through the covariances among the indicators as in factor analysis. The covariances, parameter estimates and tests of model fit are very sensitive to sample size and therefore Structural Equation Modeling is considered a large

sample technique (Ullman, 2001). A sample size of 200 is often adequate for medium sized models such as the proposed model (Boomsma, 1983). As the final sample size was 94 the plan for data analysis was revised. Given the available sample size, the predictive nature of the model and the predicted intercorrelation of the variables, a decision was made to utilize logistic regression analysis to predict bacterial vaginosis from the model factors.

SPSS 19.0 was utilized for statistical analyses. SPSS FREQUENCIES was used to check for accuracy of input, missing data, distributions and univariate outliers. There were no missing data. The Mahalanobis distance is one measure of multivariate outliers. It is a measure of the distance of a case from the central scores of the other cases (Tabacknick & Fidel, 2007). The Mahalanobis distance can be evaluated using a χ^2 distribution with the degrees of freedom equal to the number of variables and a conservative probability estimate of <.001. The assumption of linearity is an important one given that regression analyses are based on correlations. The Pearson's r is a measure of the linear relationship between variables and therefore does not capture significant non-linear relationships (Tabachnik & Fidel, 2007). Homoscedasticity refers to the variance of one continuous variable being the same at all levels of another continuous variable (Tabachnik & Fidel, 2007). This is reflected as an oval shape in the scatterplots between the two variables. SPSS SCATTERPLOT was utilized to produce bivariate scatterplots for review.

Mutlicolinearity and singularity refer to problems that occur when variables are too highly correlated. Mutlicolinearity refers to variables that are

correlated ≥ .90 and singularity refers to variables that are redundant (Tabachnik & Fidel, 2007). It was anticipated that the three original measures of perceived racism would be mutlicolinear but they did not correlate at ≥ .90. It is likely that each may contribute something unique to the assessment of perceived racism.

Research Question 1: Which individual factors are associated with Bacterial Vaginosis?

Bivariate statistics were calculated for each of the demographic, sexual and behavioral variables to bacterial vaginosis. A chi-square was calculated to determine the relationship between each discrete independent variable such as smoking, douching, marital status, employment status, sexual activity, hormonal contraceptive use, US birth status, and bacterial vaginosis. Bivariate correlation (Pearson *r)* is a measure of the size and direction of the linear relationship between two variables (two continuous or one continuous and one dichotomous). Correlations were calculated for continuous variables such as age, income, number of sexual partners, frequency of douching, frequency of condom use, years in the United States and bacterial vaginosis. Individual factors with a correlation ≥ 0.20 or a chi square with a p value <0.05 were included in any subsequent multivariate analysis calling for the inclusion of individual factors. Logistic regression of bacterial vaginosis onto individual factors was conducted. The chi square was evaluated to test the significance of the full model and the Wald criteria were evaluated to test the significance of the individual predictors.

Research Question 2: Is bacterial vaginosis associated with perceived racism and perceived stress after adjusting for individual factors?

RQ2 Hypothesis 1: Perceived stress will be associated with perceived racism.

Bivariate correlations were calculated to determine the relationship between perceived stress (PSS), the multiple measures of perceived racism and bacterial vaginosis. Measures of perceived racism and perceived stress with correlations coefficients of ≥ 0.20 were entered into a standard multiple regression analysis to determine if perceived stress is associated with perceived racism and individual factors.

RQ2 Hypothesis 2: Individuals with higher perceived racism and perceived stress will have an increased risk of bacterial vaginosis.

Bivariate correlations were assessed to determine if perceived racism and perceived stress were associated with bacterial vaginosis. As none of these measures had a correlation to bacterial vaginosis of ≥ 0.20 no further multivariate analysis was conducted.

Research Question 3: Is bacterial vaginosis associated with perceived stress and behavioral responses to stress after adjusting for individual factors?

RQ3 Hypothesis 1: Perceived stress will have a positive relationship with behavioral responses to stress.

The bivariate correlation was calculated to determine the relationship between perceived stress and behavioral responses to stress. As the correlation was ≥ 0.20 a standard multiple regression analysis was conducted for behavioral responses to stress on perceived stress and individual factors.

RQ3 Hypothesis 2: Bacterial vaginosis will be associated with perceived stress and behavioral responses to stress.

Bivariate correlations were assessed to determine entry into the model. As neither perceived stress nor behavioral responses to stress met criteria for entry into the model no further multivariate analysis was conducted.

Research Question 4: Is bacterial vaginosis associated with perceived racism and behavioral responses to racism after adjusting for potential confounders?

RQ4 Hypothesis 1: Perceived racism will have a positive relationship to behavioral responses to racism.

The bivariate correlation was calculated to determine the relationship between perceived racism and behavioral responses to racism. Only the behavioral response to racism with a correlation ≥ 0.20 was entered into the standard multiple regression analysis of behavioral responses to racism onto racism and individual factors.

RQ4 Hypothesis 2: Bacterial vaginosis will be associated with perceived racism and behavioral responses to racism.

As neither perceived racism nor any of the behavioral responses to racism had a correlation to bacterial vaginosis ≥ 0.20, no further multivariate analysis was conducted.

Chapter Summary

Chapter three summarized the study design and methodology. This study used a cross sectional design and included a sample of 94 reproductive aged Black women. The study variables included demographic variables such as age, marital status and education, traditional covariates of bacterial vaginosis such as contraceptive use, sexual activity, douching and smoking, as well as psychosocial variables such as perceived racism, behavioral responses to racism, perceived stress and behavioral responses to stress. The original plan was to utilize structural equation modeling to test a predictive model of bacterial vaginosis among Black reproductive aged women. Based on sample size restrictions the plan was revised and instead multivariate logistic and linear regression analyses were utilized to test the predicted model relationships.

Chapter four will offer descriptive statistics from our study sample. It will also present the results of the bivariate and multivariate analyses.

Chapter Four: Results

Sample Description

The average age of the study participants was 25.9 years, with an age range from 18 to 34.9 years (SD=4.5). The most common marital status was single, never married (n=56, 59.6%). A quarter of women were married (n=24) and nearly 10% (n=9) were living with their partner. Most women were currently employed (n=76, 80.9%). Of those not currently employed, 13 (72%) were currently enrolled as students. Over half of participants had at least a bachelor's degree (n= 49, 52.2%) and 38.3% (n=36) had some college or an associate's degree. Table 5 contains complete study participant demographics.

Table 5: Demographic, Behavioral and Psychosocial Characteristics of Women Screened for Bacterial Vaginosis

Variable	Total (N=94)	BV – (N=75)	BV + (N=19)	p value
Age (mean years ± SD)	25.9 ±4.5	25.6±4.5	26.1±4.8	0.67
Marital Status				
Single/not living with Partner	61(64.9)	49(65.3)	12(63.2)	0.85
Education				
Less than 12th grade	3(3.2)	0 (0)	3(15.8)	0.002
12 grade or GED	6(6.4)	4(5.3)	2(10.5)	
Some College or Associates	36(38.3)	28(35.3)	8(42.1)	
College	17(18.1)	13(17.3)	4(21.1)	
Some Graduate School	17(18.1)	16(21.3)	1(5.3)	
Graduate Degree	15(16)	14(18.6)	1(5.3)	
Income				
Less than $15,000	9(9.6)	8(10.7)	1(5.3)	0.71
$15,000 to $29,999	19(20.2)	14(18.7)	5(26.3)	
$30,000 to $44,999	31(33.0)	25(33.3)	6(31.6)	
$45,000 to $59,999	13(13.8)	12(16.0)	1(5.3)	
$60,000 or more	22(23.4)	16(21.3)	6(31.6)	
Employed	76 (80.9)	60(80.0)	16(84.2)	0.67
US born	76 (80.9)	59(78.7)	17(89.5)	0.29
Behavioral variables				
Ever Douched (Yes)	27(28.7)	18(24.0)	9(47.4)	0.04
Sexually Active in last 3 Months	67(71.2)	48(64.0)	19(100)	0.002
Condom Use (n=67) sexually active women)				
Never	39 (58.2)	28(58.3)	11(57.9)	0.41
Rarely	5 (7.4)	2(4.2)	3(15.8)	
Some of the time	2 (3.0)	2(4.2)	0(0)	
Most of the time	8 (12.0)	4(8.3)	4(21.1)	
Every time	13 (19.4)	12(25.0)	1(5.3)	
Cigarette Use in prior 3 Months	3(3.2)	1(1.3)	2(10.5)	ND
Psychosocial Variables				
Perceived Stress				
PSS Mean Score ± SD	23.8±6.4	23.9	23.1	0.62
Low Stress [no (%)]	20(21.3)	16(21.3)	4(21.1)	
Medium Stress	72(76.6)	57(76)	15(78.9)	

Table 5: Continued

Variable	Total (N=94)	BV – (N=75)	BV + (N=19)	p value
High Stress	2(2.1)	2(2.7)	0(0)	
Perceived Racism				
EOD Mean ±SD	10.6 ±3.6	10.5±2.9	11.2±5.5	0.41
Low EOD	26(27.7)	19(25.3)	7(36.8)	
Medium EOD	66(70.2)	55(73.3)	11(57.9)	
High EOD	2(2.1)	1(1.3)	1(5.3)	
EPRDI Mean ±SD	26.8 ±9.7	26.5±9.2	27.9±11.7	0.56
Low EPRDI	61(64.9)	48(64.0)	13(68.4)	
Medium EPRDI	31(33.0)	27(36.0)	4(21.1)	
High EPRDI	2(2.1)	0(0)	2(10.5)	
Experiences of Racism Group	27.9±4.6	27.9±4.4	28.2±5.8	.76
Low	7(7.4)	5(6.6)	2(10.5)	
Medium	55(58.5)	46(61.3)	9(47.4)	
High	32(34.1)	24(32)	8(42.1)	
Experiences of Racism Personal	20.1±5.7	19.9±5.3	20.8±7.0	.51
Low	53(56.3)	44(58.7)	9(47.4)	
Medium	37(39.4)	29(38.7)	8(42.1)	
High	4(4.3)	2(2.6)	2(10.5)	
Concern of Children (Mean±SD)	21.6±6.9	21.2±6.7	22.9±7.7	.35
Low	22(23.4)	18(24)	4(21.1)	
Medium	33(35.1)	28(37.3)	5(26.3)	
High	39(41.5)	29(38.7)	10(34.5)	

Predicted covariates for bacterial vaginosis measured included cigarette smoking, douching, sexual activity, hormonal contraceptive use and condom use. Only three women reported smoking cigarettes in the past three months. Most women had never douched (n=67, 71.3%). Of those who had ever douched 40.7% (n=11) did so monthly. The majority of women (n=67, 71.3%) had been sexually active in the past three months. Of those who had been sexually active,

six (9%) had more than one partner. Oral contraceptive pills were the most popular method of hormonal contraception (n=30, 31.9%) and in the last three months condoms were never used by 58.2% of women who had been sexually active.

Women born outside of the United States made up 19.1% (n=18) of the sample. They reported living in the US between 1 and 22 years with a mean of 13.8 years. There was no significant difference between US and foreign born women in BV, perceived stress, perceived racism, or active coping. There were also no significant correlations between number of years in the US and any of the main study variables (Table 6).

Table 6: Test of Differences and Correlations Between Birth Status/Years in the United States and Bacterial Vaginosis, Perceived Stress, Perceived Racism and Active Coping.

Variable	Test by Birth Status	p value	Correlation Years in the US	p value
Bacterial Vaginosis	$\chi^2 = 1.14$	.29	-.28	.25
Perceived Stress (PSS)	t = -0.22	.83	.26	.30
Perceived Racism				
EPRDI	t = -1.24	.22	.11	.68
TPRS	t = 0.54	.59	.39	.11
Active Coping (JHAC)	t = 0.32	.75	.39	.11

Note: PSS=Perceived Stress Scale (Cohen et al., 1983); EPRDI=Everyday Perceived Racial Discrimination Index (Williams et al, 1997); TPRS=Telephone Adminstered Perceived Racism Scale (Vines et al., 2001); JHAC=John Henryism Active Coping Scale (James et al., 1992)

Psychological Factors

Each of the scales for perceived racism, perceived stress, behavioral responses to racism and active coping was a simple summation of the total items unless otherwise indicated. The individual items on the EOD Scale were totaled after any responses of not applicable were recoded from 6 to 0. The TPRS

contained three subscales. Each subscale was summed to obtain a total TPRS score. The Experiences of Racism Personal and Experiences of Racism Group subscales were a summation of 10 total items each. The Concern for Children subscale was a summation of 8 items. Each of the subscales were analyzed individually and summed to create a total TPRS score. The passive behavioral responses to racism scale required reverse scoring of item 1 and 7. As both these items asked about "speaking up to try and change things", the reverse scoring allowed it to reflect not speaking up to try and change things and therefore became a passive behavior. Table 7 presents the range, mean, standard deviation and cronbach alphas for each measure of perceived racism, perceived stress and the behavioral responses to stress and racism scales. All of the scales demonstrated at least acceptable internal consistency as reflected by cronbach alpha of ≥ 0.70 (Cronbach & Shavelson, 2004).

Most women scored medium to low on all measures of perceived stress and racism. Table 5 describes the distribution of scores for each measure of perceived stress and racism. Only 2% of women scored high on the Experiences of Discrimination and the Everyday Perceived Racial Discrimination Index and only 4% scored high on the Experiences of Racism Personal scale. Responses for the experiences of racism group and concern for children scale were higher with 34% and 41% scoring high respectively.

Table 7: Study Sample Descriptive Statistics for Measures of Perceived Racism, Perceived Stress and Behavioral Responses to Stress and Racism

Scale	Range	Mean	SD	Cronbach's Alpha
Experiences of Discrimination (EOD) Scale	2-27	10.6	3.6	.73
Everyday Perceived Racial Discrimination Index (EPRDI)	10-52	26.8	9.7	.86
Telephone Administered Perceived Racism Scale				
Experiences of Racism Personal	10-33	20.1	5.7	.82
Experiences of Racism Group	13-37	27.9	4.6	.83
Concern for Children	8-32	21.6	6.9	.90
Behavioral Responses to Racism				
Passive Behavior	10-21	14.1	2.5	.84
External Active Behavior	2-8	6.8	1.9	.86
Internal Active Behavior	2-8	7.1	1.6	.95
Perceived Stress Scale	2-38	23.8	6.4	.82
John Henryism Active Coping Scale	35-59	52.1	5.0	.70

Data Analysis by Research Question

Research Question 1: Which individual factors are associated with bacterial vaginosis?

RQ1 Hypothesis 1: Hormonal contraceptive use will have a negative relationship with bacterial vaginosis.

RQ1 Hypothesis 2: Sexual activity will have a positive relationship with bacterial vaginosis.

RQ1 Hypothesis 3: Douching will have a positive relationship with bacterial vaginosis

RQ1 Hypothesis 4: Cigarette smoking will have a positive relationship with bacterial vaginosis.

Nineteen of the 94 (20%) participants screened positive for bacterial vaginosis. Women did not vary significantly by age, marital status, or income. Relevant characteristics of study population are presented in Table 5. Education was negatively correlated with bacterial vaginosis (r=-.322, p=.002). In Table 5, it is demonstrated that study participants were distributed relatively evenly between Associate's degree or less and Bachelor's degree or higher. The rate of BV for women with an Associate's degree or less was twice that of women with a Bachelor's degree or higher. Age and income were not significantly associated with bacterial vaginosis. Due to low numbers of reported use of the depo provera injection (n=4), patch (n=2), ring (n=3), mirena (n=9), paraguard (n=0) and implanon (n=0), only oral contraceptive pills use (n=32) had sufficient observations to calculate a chi square. A significant relationship did exist between BV and douching as well as BV and sexual activity. As only 3 women reported smoking in the three months prior to the study a relationship was not able to be determined.

Education, douching and sexual activity were considered for entry to the logistic regression model. As none of the sexually inactive women screened positive for bacterial vaginosis, this variable did not meet the adequate expected frequency requirement of logistic regression. A direct logistic regression analysis

was conducted on bacterial vaginosis as the dependent variable and education and douching as the independent variables. A test of the full model with both predictors against a constant only model was statistically reliable, χ^2 (2, N = 94) = 12.56, p < .01, indicating the predictors, as a set, reliably predict bacterial vaginosis status. Nagelkerke R^2 of 0.20 indicates a small relationship between bacterial vaginosis and the predictors. The prediction success varied from 96% for BV negative women to 26.3% for BV positive women, for an overall success rate of 81.9%. Table 8 summarizes the logistic regression statistics. The Wald criterion demonstrated that that only education made a significant contribution to prediction (p < 0.01).

Table 8: Logistic Regression Analysis of Bacterial Vaginosis as a Function of Individual Factors

Variable	B	SE B	Wald	df	Exp(B)
Education	-0.67†	0.25	7.22	1	0.51
Douching	-0.80	0.56	2.02	1	0.45

Note: †= p<0.01

Research Question 2: Is bacterial vaginosis associated with perceived racism and perceived stress after adjusting for individual factors?

RQ2 Hypothesis 1: Perceived racism will have a positive relationship with perceived stress.

Correlations between each of the measures of perceived racism and perceived stress were evaluated. The everyday perceived racial discrimination index and the experiences of racism personal scale were positively correlated (r≥0.20) to perceived stress (Table 9).

A standard multiple regression analysis was conducted between perceived racism as the dependent variable and age and education as independent variables (Table 10). Based on their correlations to perceived stress the Everyday Perceived Racial Discrimination Index and the Experiences of Racism Personal scale were selected as the measures of perceived racism to be included in further analysis. Age and education were selected as the individual factors to be considered in this and subsequent analyses. This decision was based on the correlation coefficients ≥ 0.20 to perceived stress and/or perceived racism. Income had no correlation coefficients ≥ 0.20 and therefore was not included in further analyses. Prior to analysis data were reviewed for assumptions of multivariate analysis. No missing data, significant skewness or kurtosis were found for any measures of perceived racism or perceived stress. With the use of a $p<0.001$ criterion for Mahalanobis distance no multivariate outliers were found. Table 10 displays results of the multiple regression analysis. The R for regression of EPRDI was not significantly different than zero, $F(2, 91) = 2.30$, $p = 0.11$. The R for regression of Experiences of Racism Personal was significantly different than zero, $F(2, 94) = 4.34$, $p = 0.02$. Only education significantly contributed to Experiences of Racism Personal $sr^2 = 0.001$. Age and education combined contributed another 0.06 in shared variability. Altogether, 9% (7% adjusted) of the variability in both measures of perceived racism was predicted by knowing age and education.

Table 9: Correlations of Main Study Variables

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Bacterial Vaginosis	1	-.05	.09	.06	.03	.07	.10	-.04	-.16	.07	.08	-.05	-.32†	.04
2. PSS		1	.17	.23*	.17	.27†	. 03	.30†	.06	.18	.14	-.19	-.01	-.03
3. EOD			1	.45†	.41†	.51†	.21*	.04	.11	.02	.17	.21*	.19	-.03
4. EPDRI				1	.32†	.44†	-.00	.22*	.12	.18	.12	.13	.21*	.07
5. Experiences of Racism Group^					1	.72†	.34†	.15	.11	.10	-.11	.18	.28	.09
6. Experiences of Racism Personal^						1	.26*	.08	.17	.17	.19	.05	.29	.06
7. Concern for Children^							1	-.03	.09	.10	.14	.11	.00	.03
8. JHAC								1	.19	-.08	-.16	-.08	-.00	.01
9. Passive BRR#									1	-.07	.04	-.16	.08	.16
10. External Active BRR#										1	.11	.07	-.20	.05
11. Internal Active BRR#											1	.08	-.08	.05
12. Age												1	.28†	.30†
13. Education													1	.15
14. Income														1

Notes: PSS=Perceived Stress Scale (Cohen et al., 1983); EPRDI=Everyday Perceived Racial Discrimination Index (Williams et al., 1997); TPRS=Telephone Administered Perceived Racism Scale (Vines et al., 2001); JHAC=John Henryism Active Coping Scale (James et al., 1992); BRR=Behavioral Responses to Racism (Subscale of TPRS).

^ Refers to subscales included in TPRS Total. # Refers to subscales of TPRS not included in total score.

* p≤.05. † p≤.01

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Table 10: Standard Multiple Regression of Perceived Racism onto Age and Education.

Variable	B	SE B	B	sr^2	R^2	Adjusted R^2
EPRDI					.09	.07
Age	0.17	0.23	0.08			
Education	1.35	0.78	0.18			
Experiences of Racism Personal					.09	.07
Age	-0.05	0.13	-0.04			
Education	1.30†	0.45	0.30	.001		

Note: EPRDI= Everyday Perceived Racial Discrimination Index; † = $p < 0.01$

Perceived stress was regressed onto perceived racism (EPRDI and Experiences of Racism Personal), age and education (Table 11). R for regression was significantly different than zero, $F(4, 89) = 3.56$, $p = 0.01$. Only age contributed significantly to perceived stress ($sr^2 = 0.04$). All of the independent variables combined contributed another 0.06 in shared variability. Altogether, 14% (10% adjusted) of the variability in perceived stress was predicted by knowing age, education and scores of perceived racism.

Table 11: Standard multiple regression of perceived stress onto age, education and perceived racism.

Variable	B	SE B	B	sr^2	R^2	Adjusted R^2
Model					.14	.10
Age	-0.29*	0.15	-0.21	0.04		
Education	-0.24	0.52				
EPRDI	0.25	0.07				
Experiences of Racism Personal	0.11	0.13				

Note: EPRDI= Everyday Perceived Racial Discrimination Index; * = $p < 0.05$

RQ2 Hypothesis 2: Perceived racism and perceived stress will have a positive association to bacterial vaginosis.

Neither the measures of perceived racism nor perceived stress were associated with bacterial vaginosis (Table 9) therefore no further logistic regression analysis was conducted.

Research Question 3: Is bacterial vaginosis associated with perceived stress and behavioral responses to stress after adjusting for potential confounders?

RQ3 Hypothesis 1: Perceived stress will have a positive relationship with behavioral responses to stress.

Table 9 demonstrates correlations of perceived stress and behavioral responses to stress as measured with the John Henryism scale of Active Coping did have a significant positive correlation ($r=0.30$, $p<0.01$). Prior to analysis data were reviewed for assumptions of multivariate analysis. No missing data were found. The John Henryism scale of Active Coping (JHAC) did have significant skewness and kurtosis. The negative skewness and positive kurtosis means that the scores tended to be in the higher range. A square root transformation of the JHAC scale improved the skewness and kurtosis so that neither was significantly different from the normal curve. The square root transformation of the JHAC also improved the linearity and homoscedasticity of its bivariate scatterplots. Using $p<0.001$ criterion for Mahalanobis distance no multivariate outliers were found. Table 12 displays results of the multiple regression analysis. The R for regression of the square root transformation of the JHAC was significantly different than zero, $F (3, 90) = 3.45$, $p = 0.02$. Only perceived stress significantly contributed to the square root transformation of the JHAC $sr^2 = 0.10$. Altogether, 10% (7% adjusted) of the variability in the square root transformation of JHAC was predicted by knowing the score

of perceived stress. Age and education did not contribute to the prediction of JHAC.

Table 12: Standard Multiple Regression Analysis of Behavioral Responses to Stress onto Perceived Stress, Age and Education.

Variable	B	SE B	β	sr^2	R^2	Adjusted R^2
Model					0.10	0.07
Age	0.02	0.02	0.13			
Education	-0.00	0.07	-0.01			
Perceived Stress	0.04†	0.01	0.32	0.10		

Note: † = $p < 0.01$

RQ3 Hypothesis 2: Bacterial vaginosis will be associated with perceived stress and behavioral responses to stress.

Neither perceived stress nor behavioral responses to stress had correlations with bacterial vaginosis ≥ 0.20 (Table 9), therefore no further logistic regression analysis was conducted.

Research Question 4: Is bacterial vaginosis associated with perceived racism and behavioral responses to racism after adjusting for potential confounders?

RQ4 Hypothesis 1: Perceived racism will have a positive relationship to behavioral responses to racism.

Internal active behavioral responses to racism was positively correlated to experiences of racism personal ($r = 0.19$). As this nearly met criteria for further evaluation the decision was made to conduct a standard multiple regression analysis of internal active behavioral responses to racism onto experiences of racism personal, age and education (Table 13). R for regression was not significantly different than zero, $F(3, 90) = 1.67$, $p = 0.18$. Only experiences of racism personal contributed significantly to perceived stress ($sr^2 = 0.04$).

Table 13: Standard Multiple Regression Analysis of Internal Active Behavioral Responses to Racism onto Perceived Racism, Age and Education.

Variable	B	SE B	β	sr^2	R^2	Adjusted R^2
Model					0.05	0.02
Age	-0.02	0.04	-0.07			
Education	-0.12	0.14	-0.10			
Experiences of Racism Personal	0.06*	0.03	0.22	0.04		

Note: * = $p < 0.05$

RQ4 Hypothesis 2: Bacterial vaginosis will be associated with perceived racism and behavioral responses to racism.

None of the measures of perceived racism or the behavioral responses to racism had correlations with bacterial vaginosis of ≥ 0.20, therefore they did not meet criteria for further logistic regression analysis.

Chapter Summary

This chapter summarized the results of data analysis. Participant characteristics were reviewed including age, marital status, income, educational status, employment status and US birth status. Bivariate relationships between all study variables including demographic variables, traditional covariates for BV and the main study variables (perceived stress, perceived racism, behavioral responses to stress and racism) were presented. Multivariate analyses were conducted to test the predictive relationships of the study model (Figure 1).

Chapter 5 will include a discussion of the results presented in this chapter. The discussion will include how study results compare to previous research, the limitations this study and suggestions for further areas of research.

Chapter 5: Discussion

Discussion of Findings

The results of this study demonstrated that perceived stress and perceived racism were not associated with bacterial vaginosis. Of the 8 published studies including measures of psychosocial stress and bacterial vaginosis four had found a relationship between perceived stress and BV (Culhane et al., 2001; Culhane et al., 2002; Nansel et al., 2006; Harville et al., 2005). Of those studies only one was in a non- pregnant population (Nansel et al., 2006). It is possible that the experiences of perceived stress are more pronounced during pregnancy and therefore more likely to precipitate the physiologic stress response. One study conducted by Harville and associates (2005) with a sample of Black non-pregnant women noted that women with high level stress were more likely to have BV but the results did not reach statistical significance. Although all of the other published studies of BV and stress used the Perceived Stress Scale, this study used the Global Assessment of Recent Stress. Although most other studies have focused on perceived stress, one study found that stressful life events were associated with BV prevalence but perceived stress was not (Paul , Boutain, Manhart & Hitti, 2008). It is possible that life stressors may cause physiologic changes regardless of an individual's perception of stress. It is possible that there may be other measures of stress better suited

to assess this variable in this population.

Several studies have found that women with the highest levels of stress were the most likely to have an increased risk of bacterial vaginosis. Harville and associates (2005) found that women in the highest third of the total stress score had a 45% higher prevalence of BV. Another study found that women in the highest quartile of stress had an OR 2.4 (CI 1.1-4.2) for BV (Culhane et al., 2001). Although the mean score of perceived stress reported is similar to that reported in other studies, only 2% of women had high levels of perceived stress scores. This may explain the lack of association between perceived stress and BV.

The lack of a relationship between perceived racism and BV may also be explained by the low rates of perceived racism. Only 2% of women scored high on the Experiences of Discrimination and the Everyday Perceived Racial Discrimination Index and only 4% scored high on the Experiences of Racism Personal scale. Responses for the experiences of racism group and concern for children scale were higher with 34% and 41% scoring high respectively. Both of these scales refer to an individual's concern or perception of others experiences with racism. Neither of these measures were associated with perceived stress and therefore it is possible that this does not illicit a physiologic stress response.

It is possible that the low rates of perceived stress and racism may have been due to the relatively young age of the sample (mean age = 25.9). Younger women may not have had as much opportunity in life to have many experiences of racism and general stress. Evidence suggests that the cumulative effect of stressors is physical deterioration which seems to prematurely age Black women and contribute to poor health outcomes

(Geronimus, Hicken, Keene & Bound, 2006). Although these experiences may prematurely age Black women in comparison to White women, younger Black women are likely to have less physical deterioration then older Black women.

Most studies report a BV prevalence of about 30% among Black women. The prevalence of BV in this study was 20%. It is possible that the lower prevalence of BV and the small number of women reporting high perceived stress and high perceived racism made detection of a relationship difficult.

Perceived racism and perceived stress did have the expected positive association; meaning as perceived racism increased so did levels of perceived stress. This supports the idea that perceived racism may function as a stressor for Black women. Perceived racism is quite pervasive, with most individuals from minority groups reporting the experience at some point in their lifetime (Brondolo, 2008). The level of stressfulness related to perceived racism may vary depending on numerous factors. Age, skin color, gender, current socioeconomic status, lifetime socioeconomic status, region of residence, country of origin and neighborhood crime may each be potential contributors. The frequency, duration, acuity of experiences and ability to cope with racism may also influence an individuals' ability to maintain physiologic balance (McEwen & Seeman, 2004).

Behavioral responses to stress and racism were associated with perceived stress and racism as anticipated but not with bacterial vaginosis. This lack of association may also be related to the relative lack of high scores on measures of perceived stress and racism. Only one other study has included a measure of behavioral responses to stress other than cigarette smoking. Harville and associates (2007) included the John Henryism

scale of Active Coping and found that it had an adjusted OR = 1.7 (CI 0.7-4.2). It may be that cigarette smoking is the behavioral response to stress that is likely to have the greatest influence of risk of BV. It is the only non-sexual and non-demographic factor most consistently associated with BV. This study was not able to determine a relationship to cigarette smoking due to the small number of women (3) reporting cigarette use in the past 3 months. It is possible that a larger sample size would have confirmed the finding of other studies.

Bivariate relationships between Bacterial Vaginosis and traditional covariates were assessed. Douching and sexual activity did have the predicted significant relationship, while hormonal contraceptive and condom use did not. The relationships between BV, douching and sexual activity are the most well documented in the literature. The effect of hormonal contraceptive use may be more difficult to disentangle, as some women use both condoms and hormonal contraceptives. The lack of a significant relationship between BV and condom use may be related to the small number (9) of sexually active women with more than one partner. Given that most sexually active women had only one partner in the prior three months, they would be considered relatively low risk for sexually transmitted infections. There is some evidence to suggest that women at high risk for sexually transmitted infections may benefit most from consistent condoms use (Hutchinson et al., 2007).

Limitations/Strengths

This study was limited based on the cross sectional design, small sample size, homogenous sample, and single measure of stress. The cross sectional design limits the ability to make causal inferences. The design was also a factor in the assessment of

perceived stress and racism. The Experiences of Discrimination scale asks about experiences in the past 5 years. Women may have limited recall over that length of time. Evidence suggests that individuals may only remember as little as 50% of an events crucial details after 5 years (Bradburn, Rips & Shevell 1987). Women with older experiences of discrimination may have had lower recall of such events.

Potential bias was minimized through two approaches. Each of the study instruments had previously demonstrated adequate internal validity in similar samples. Also, participants were not informed of the hypothesized relationships between the study variables. They were not given the results of the BVBlue test until the end of the study visit. Interviewer bias was also limited by delaying the development of the BVBlue test until the end of the study visit.

The small sample size was a limitation. A sample size of at least 200 would have permitted the use of structural equation modeling to model prevalence of bacterial vaginosis among Black women. A larger sample may have also provided the power to detect a relationship given the low frequency of high stress and racism in the current sample.

The multiple measures of perceived racism are a strength of the study. The experiences of discrimination scale included experiences of perceived experiences of institutional racism such as being treated unfairly because of race when trying to get a loan or when appearing in court. The experiences of racism personal scale and the everyday perceived racial discrimination index included experiences of interpersonal racism such as people acting as if they are afraid of you or not being given credit for their contributions. The concern for children scale and the experiences of racism general

scales each identify an individual's perception of how racism may influence others. Concern for others may be a significant factor in the stress of women as they tend to be more relationship focused then men. Additional characteristics of perceived racism that were not measured include level of acuity, number of experiences and timing of experiences.

Suggestions for Future Research

Stress is also multidimensional and challenging to measure. Measures have included daily hassles, major life events and global stress. Physiologic measures of stress have included systemic endocrine factors such as cytokines, cortisol and corticotropin releasing hormone. Specific vaginal immune parameters may be more relevant to risk of bacterial vaginosis and require further exploration. Measures of vaginal immune function such as anti - Gardnerella vaginalis hemolysin immunoglobulin A, vaginal heat shock protein-70, and interleukin 1β should be included in future studies evaluating a potential relationship between experiences of stress and risk of bacterial vaginosis.

Future studies should expand upon the multiple measures of racism included in this study. Harrell and associates (2000) have described multiple levels of racism including interpersonal, institutional and cultural. Racism may be present in many forms such as interpersonal interactions, institutional policies or cultural communications. All levels of racism have the potential to influence health status of individual as well as communities.

Behavioral responses to racism have a significant potential to mediate the racism-stress relationship. Further research is needed to explore the benefits and limitations of various strategies. Effectiveness of coping strategies may be based on multiple factors.

In a review of coping with racism literature, Brondolo and associates (2009) found that no studies have evaluated effectiveness of strategies based on the timing of the incident (before, during or after) or based on the practical versus emotional repercussions. Intervention based studies will be needed to test if strategies can be taught and if learned strategies can improve health status.

The allostatic load model includes multiple factors that are likely to influence the stress disease relationship. Complex statistical methods will be needed to study psychosocial and physiologic measures of stress, measures of varying levels of racism and their interaction with numerous other stressors. For example, an individual may experience varying degrees and type of racism based on socioeconomic status. Individuals living in or near poverty may have less access to quality education, transportation, health care, and employment opportunities. Disentangling the complex relationships will be challenging and will require large studies with diverse populations.

Conclusions

This study sought to test a model of bacterial vaginosis among Black reproductive aged women. This was the first study the author is aware of to include perceived racism as a potential predictor of bacterial vaginosis. The model as depicted did not explain the variance in bacterial vaginosis in this sample. This may have been explained by the very low rates of high stress and racism in the study sample. Studies with larger sample sizes or samples with higher stress levels may have different findings.

Bacterial vaginosis is the most common vaginal infection among reproductive aged women and may contribute to the racial disparities in numerous reproductive health outcomes. Our understanding of the microbial pathogens and immune dysfunction

associated with BV has significantly improved along with improve microbiological technologies. Despite this improved understanding, more research is needed to determine what factors precipitate the cascade of physiologic changes leading to BV.

Future studies should include a larger more diverse sample as well as physiologic measures of immune dysfunction. A larger sample will allow for detection of small relationships. A more diverse sample may demonstrate a greater variety in levels of stress and racism. Measures of vaginal immune dysfunction have should be studied in relation to individual level experiences of racism and stress.

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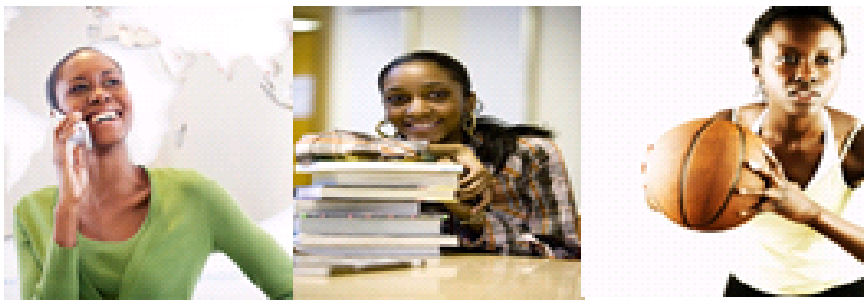
APPENDICES

Appendix A
Recruitment Flyer



The Tampa Black Women's Reproductive Health Study

Are you a Black woman between the ages of 18 and 35 years?

If so, you may be eligible to participate in a new research study.

The purpose of this study is to evaluate factors that may contribute to the reproductive health of Black women.

- **No physical exam required.**
- **Free reproductive health screening.**

The study involves only one study visit lasting about 45 minutes at the USF Health Medical Clinic or the USF Health South Tampa Center.

You may not be eligible if you have certain major medical conditions, are pregnant, or recently had a baby.

For more information, please contact Jessica Brumley @ (727) 804 2902 or jbrumley@health.usf.edu, Principal Investigator, USF College of Nursing.

Appendix B
Study Screening Tool

The Tampa BV Study
Screening Questionnaire (SQ)

1. How old are you? __ __ years

2. Which of the following would best describe you?

1 ☐ Black or African American
2 ☐ White or Caucasian
3 ☐ Hispanic or Latino
4 ☐ Asian or Pacific Islander
5 ☐ Native American
6 ☐ Other: _____

4. Are you currently pregnant?

1 ☐ Yes
2 ☐ No
3 ☐ Not sure

5. Have you delivered a baby in the last 8 weeks?

1 ☐ Yes
2 ☐ No

6. Have you ever been told that you have Lupus, Rheumatoid Arthritis, HIV/AIDS, or any other immune system disorder?

1 ☐ Yes
2 ☐ No

7. Have you taken or been given any medications in the last month?

1 ☐ Yes 2 ☐ No

7a. If yes, what medications have you taken?_____

_____

_____

Appendix C

Perceived Stress Scale

Instructions: The questions below ask you about your feelings and thoughts. In each case, you will be asked to indicate how often you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is don't try to count the number of times you have felt a certain way but rather make your best estimate. Please circle the best answer.

	Never	Almost never	Some-times	Fairly often	Very often
1. How often have you been upset because of something that happened unexpectedly?	1	2	3	4	5
2. How often have you felt that you were unable to control the important things in life?	1	2	3	4	5
3. How often have you felt nervous or "stressed"?	1	2	3	4	5
4. How often have you dealt successfully with irritating life hassles?	1	2	3	4	5
5. How often have you felt that you were effectively coping with important changes that were occurring in your life?	1	2	3	4	5
6. How often have you felt confident about your ability to handle personal problems?	1	2	3	4	5
7. How often have you felt that things were going my way?	1	2	3	4	5

	Never	Almost Never	Some-times	Fairly often	Very Often
8. How often have you found that you could not cope with all the things that you had to do?	1	2	3	4	5
9. How often have you been able to control irritations in your life?	1	2	3	4	5
10. How often have you felt that you were on top of things?	1	2	3	4	5
11. How often have you been angered because of things that happened that were outside of your control?	1	2	3	4	5
12. How often have you found yourself thinking about things that you have to accomplish?	1	2	3	4	5
13. How often have you been able to control the way you spend your time?	1	2	3	4	5
14. How often have you felt difficulties were piling up so high that you could not overcome them?	1	2	3	4	5

Appendix D

Measure of Covariates

These last few questions are about some of your personal habits. Some of the questions may make you feel uncomfortable or awkward. You may refuse to answer any questions but it would be most helpful if all questions are completed.

1. In the last three months have you smoked any cigarettes *(Circle your answer)*

Yes $_1$

No$_2$ (if no skip to question 3)

Refuse $_{99}$

2. In the last three months, on average how many cigarettes do you smoke a day ____ ____ (number of cigarettes)

1 pack =20 cigarettes

If less than 1 per day enter 1

If you refuse enter 99

3. Douching is the squirting of water or a solution into the vagina (couchy, pussy, nana).

Have you ever douched?

No_2 ---------- Skip to question number 6.

Yes_1 If yes continue ↓

4. On average how many times per month do you douche? _____

5. What are your reasons for douching? (Please circle all that apply).

To feel clean

Vaginal odor (bad smell)

Vaginal discharge

After sex

After my period (monthly flow)

Other *(fill in your answer)*_____

Refuse

6. Have you used any of these types of birth control in the last 3 months? *(Circle Yes or No for each)*

Birth Control Pills:……Yes or No

Depo Provera (The shot):…….....Yes or No

Ortho Evra (Birth Control Patch):……Yes or No

Nuva Ring (The ring):……. Yes or No

Mirena IUD……..Yes or No

Paraguard IUD (copper T)……Yes or No

Implanon (single rod)……...Yes or No

7. Have you had vaginal sex in the last 3 months? *(Circle your answer)*

Yes $_{1}$

No $_{2}$

Refuse $_{99}$

If you answered yes please continue. If you refused or answered no then you have reached the end. Thank you for agreeing to participate in our study. Please hand this back to the study nurse.

8. How many different people (partners) have you had sex with in the last 3 months? *(Write in the number of partners. If you refuse write in refuse)*

_____ number of partners

9. How often have you used condoms when you had sex in the last three months? *(Circle your answer)*

Never$_0$

Rarely$_1$

Some of the time$_2$

Most of the time$_3$

Every time$_4$

Appendix E

Demographics

A1. What is your birthdate? ____ ____/___ ___/___ ____

☐ Refused = 98

☐ Don't know = 99

A2. Where you born in the United States?

1☐ Yes 98☐ Refused

2☐ No 99☐ Don't know

A3. If no, how many years have you been living in the United States ☐☐ years

98☐ Refused

99☐ Don't know

A4. What was the highest grade of school you completed?

1☐ Less than 12th grade 5☐ Some graduate school

2☐ Grade 12 or GED 6☐ Graduate Degree

3☐ Some college/Associates 7☐ Other: _____

4☐ College Degree 98☐ Refused

A5. Do you have a job?

No …….0 ➔ A4. If no, what do you do for money? _____

Yes…….1 ➔ A4. If yes, what is or was your job?_____

Refused…….98

Don't Know……..99

A6. What is your marital status?

Never Married…….1

Married……2

Living with partner…….…….3

Divorced……..4

Separated……..5

Widowed…….6

Refused……..…….98

A7. What is your total family income before taxes for the last calendar year? Be sure to consider all sources of income such as second job, child support, social security, disability etc…

Less than $15,000 …..……...1

$15,000 to $29,999…….2

$30,000 to $44,999…….3

$45,000 to $59,000…….4

$60,000 or more…….5

Refused……..…..…….98