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Sara Mitchell Edwards April 6, 2018

Brain-Gut Axis and Its Influence on Gestational Weight Gain

By

Sara Mitchell Edwards
Doctor of Philosophy

Nursing

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Brain-Gut Axis and Its Influence on Gestational Weight Gain

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An abstract of

A dissertation submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

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Abstract

Brain-Gut Axis and Its Influence on Gestational Weight Gain

By Sara Mitchell Edwards

Background: Excessive gestational weight gain (GWG) is a significant predictor of adverse obstetric outcomes, postpartum weight retention and lifelong health risks for the woman and infant. In this study I investigated the role of an understudied, but potentially important, biological contributor to maternal obesity and gestational weight gain among AA women: the maternal gut microbiome.

Purpose: The purpose of this dissertation was to

- Describe the relationship between maternal gut microbiome composition and interval and total gestational weight gain in AA women.
- Explore the possible influence of caloric intake, physical activity, and measures of chronic stress on the association between maternal gut microbiome composition and interval and total gestational weight gain in AA women.

Sample and Design: This was a prospective, longitudinal study of 27 pregnant women enrolled in a larger study investigating biobehavioral determinants of the microbiome and preterm birth in AA women (1R01NR014800). In this sub-study (1 F31 NR015722-01A1), the participants were enrolled for one additional study visit. Analyses included means, descriptive statistics, ANOVA testing and linear regression modeling to predict the key outcome variables of weight and *Firmicutes* to *Bacteroidetes* ratio change during the pregnancy.

Results: The difference in the change in ratio of FTB showed a negative correlation with the ratio at the first time point ($r = -.98$, $p < .001$). Also, the category of weight gain at mid-gestation was associated with change in FTB ratio ($f = 3.48$, $p = .05$), with significant difference in FTB ratio between the inadequate versus excessive gainers. A linear regression model examining the variables of FTB at 1 and ACE-HD explained 25 percent of the variance in the initial weight (Adjusted R square = .25, $F(2, 26) = 5.33$, $p = .01$).

Conclusion: Although interval and total GWG clearly impact obstetric outcomes, and although in non-pregnant populations the gut microbiome and weight gain are closely linked, little research to date has explored the association between the gut microbiome during pregnancy and interval or total GWG. Given the potential to modify the gut microbiome, a better understanding of its contribution to a healthy pregnancy is essential.

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Finally, I humbly present this work to the glory of God, and to His Son, our Lord and Savior, Jesus Christ.

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Chapter 1: Introduction to the Study: *Brain-Gut Axis and Its Influence on Gestational Weight Gain*

Gestational Weight Gain and Maternal and Neonatal Outcomes

Nearly two-thirds of US women of childbearing age are overweight or obese and nearly half, once pregnant, gain excess gestational weight (*Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009). In 2009, the Institute of Medicine (IOM) published recommendations for trimester-specific rate and total gestational weight gain (GWG) by pre-pregnancy body mass index (BMI) (*Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009). The most significant change from the original 1990 guidelines was a more restrictive weight gain recommendation for pre-pregnant obese women of only 11-20 pounds (compared to "at least 15 pounds" in the original guideline) (Krukowski, Bursac, McGehee, & West, 2013; *Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009). This more restrictive recommendation presents a particular challenge for African-American (AA) women, who are more likely than women of other races to enter pregnancy overweight or obese (Bowers et al., 2013; Brooten, Youngblut, Golembeski, Magnus, & Hannan, 2012; Gould Rothberg, Magriples, Kershaw, Rising, & Ickovics, 2011; Headen, Davis, Mujahid, & Abrams, 2012; Krukowski et al., 2013; Marshall, Guild, Cheng, Caughey, & Halloran, 2014; Misra, Hobel, & Sing, 2010; Paul, Graham, & Olson, 2013; Wise, Palmer, Heffner, & Rosenberg, 2010).

Among women in all BMI categories, only half of pregnant women achieve weight gain within the recommended range [1]. Women who start their pregnancies overweight are six times more likely than those of healthy weight to gain more than recommended [1]. As BMI increases, serum folate levels decrease, directly contributing to an increased incidence of fetal neural tube and other birth defects (Bodnar et al., 2011; Bodnar, Siega-Riz, Simhan, Himes, & Abrams, 2010; Davies, Maxwell, McLeod, Gagnon, Basso, Bos, Delisle, Farine, Hudon, Menticoglou, Mundle, Murphy-Kaulbeck, Ouellet, Pressey,

Roggensack, Leduc, Ballerman, Biringer, Duperron, Jones, Lee, Shepherd, & Wilson, 2010). Excessive GWG and postpartum weight retention also are important predictors of lifelong obesity and are associated with obstetric complications including fetal demise, preeclampsia, obstructive sleep apnea, thrombosis, gestational diabetes and cesarean births (Bodnar et al., 2010). With the exception of gestational diabetes, all these complications are more common among AA women, contributing to AAs having the highest national rates of maternal, fetal and neonatal morbidity and mortality (Bodnar et al., 2011; Bodnar et al., 2010; Davies, Maxwell, McLeod, Gagnon, Basso, Bos, Delisle, Farine, Hudon, Menticoglou, Mundle, Murphy-Kaulbeck, Ouellet, Pressey, Roggensack, Leduc, Ballerman, Biringer, Duperron, Jones, Lee, Shepherd, & Wilson, 2010; de Jongh, Paul, Hoffman, & Locke, 2013; Di Benedetto et al., 2012; Dominguez, 2011; Einerson, Huffman, Istwan, Rhea, & Joy, 2011; Facco, 2011; Gibson, Waters, & Catalano, 2012; Giurgescu, McFarlin, Lomax, Craddock, & Albrecht, 2011; Headen et al., 2012). Weight retention remains a problem for a large proportion of women even at a year postpartum (Amorim Adegboye & Linne, 2013; Biesmans, Franck, Ceulemans, Jacquemyn, & Van Bogaert, 2013; Gould Rothberg et al., 2011). Sixty percent of women retain 10-20 pounds at six months postpartum, regardless of pre-pregnant BMI (Biesmans et al., 2013; Boghossian, Yeung, Lipsky, Poon, & Albert, 2013; Choi, Fukuoka, & Lee, 2013; Lipsky, Strawderman, & Olson, 2012). If women retain weight, their subsequent pregnancies are subject to both increasing BMI and age, exacerbating obstetrical risks (Nehring, Schmoll, Beyerlein, Hauner, & von Kries, 2011; Okun et al., 2011; Ruchat & Mottola, 2012). The more stringent 2009 IOM guidelines, coupled with the obesity epidemic that most significantly burdens AA women of childbearing age, demands scientific scrutiny of the underlying mechanisms that contribute to excessive GWG in this population.

Interval Gain: Most studies of pregnant women have considered pre-pregnant weight and total gestational weight gain, ignoring interval gain. A common finding was that while risks for poor obstetric outcomes were greatest among women in the highest BMI categories, this was especially so if excessive weight was gained (Althuizen, van Poppel, Seidell, & van Mechelen, 2009; Blomberg, 2011; Bodnar et al., 2010; Davies, Maxwell, McLeod, Gagnon, Basso, Bos, Delisle, Farine, Hudon, Menticoglou, Mundle, Murphy-Kaulbeck, Ouellet, Pressey, Roggensack, Leduc, Ballerman, Biringer, Duperron, Jones, Lee, Shepherd, Wilson, et al., 2010; R. R. Davis, Hofferth, & Shenassa, 2014; Facco, 2011; Fontaine, Hellerstedt, Dayman, Wall, & Sherwood, 2012; McClure, Catov, Ness, & Bodnar, 2013). Timing of GWG is, however, relevant to the risk of poor obstetric outcomes and obesity in the mother and child (Davenport, Ruchat, Giroux, Sopper, & Mottola, 2013; Durie, Thornburg, & Glantz, 2011; Fontaine et al., 2012; "Share with women. Weight gain during pregnancy," 2010). IOM guidelines state all women are to gain 1-4.5 pounds in the first trimester, with weekly gestational weight gain thereafter based on pre-pregnant BMI (*Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009). Inadequate weight gain in the first 20 weeks', in all BMI categories, increases the risk of preterm birth and fetal growth restriction (Beydoun, Tamim, Lincoln, Dooley, & Beydoun, 2011; R. R. Davis, Hofferth, & author reply, 2012; Headen et al., 2012; Hinkle, Sharma, & Dietz, 2010), whereas excessive GWG during the first (but not the second 20 weeks gestation) increases the risk of excessive neonatal body fat, a precursor to childhood obesity (Davenport et al., 2013).

Total Gain: Pre-pregnant weight is usually gleaned from maternal self-report at the first prenatal visit and is known to be reliable (Brunner Huber, 2007; Lederman & Paxton, 1998; Park, Sappenfield, Bish, Bensyl, et al., 2011). Prepregnant weight is reflective of the health of the mother, which directly affects ovulation, conception, placental and fetal development, and maternal health throughout pregnancy (Alavi,

Haley, Chow, & McDonald, 2013; Blomberg, 2011; Bodnar et al., 2011; Bogaerts et al., 2013; Brooten et al., 2012; Davies, Maxwell, McLeod, Gagnon, Basso, Bos, Delisle, Farine, Hudon, Menticoglou, Mundle, Murphy-Kaulbeck, Ouellet, Pressey, Roggensack, Leduc, Ballerman, Biringer, Duperron, Jones, Lee, Shepherd, & Wilson, 2010; E. M. Davis, Stange, & Horwitz, 2012; de Jongh et al., 2013; Di Benedetto et al., 2012; Hinkle et al., 2010; Lebby, Tan, & Brown, 2010; Leslie, Gibson, & Hankey, 2013; Li et al., 2013; Okun, Roberts, Marsland, & Hall, 2009; *Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009; Wong, de Souza, Kendall, Emam, & Jenkins, 2006; Yu et al., 2013). Preconception weight reduction as well as diet improvements and other modifiable self-care behaviors before or during the early weeks of pregnancy can reduce or eliminate risks from obesity and promote maternal health, as well as placental and embryonic development. Unfortunately, preconception health care is rarely sought, especially among AA women, so this opportunity is usually missed (Oza-Frank, Gilson, Keim, Lynch, & Klebanoff, 2014). If a normal BMI is not attained preconception, an elevated BMI confers many health risks to the mother and infant (Bodnar et al., 2010; Bowers et al., 2013; Davies, Maxwell, McLeod, Gagnon, Basso, Bos, Delisle, Farine, Hudon, Menticoglou, Mundle, Murphy-Kaulbeck, Ouellet, Pressey, Roggensack, Leduc, Ballerman, Biringer, Duperron, Jones, Lee, Shepherd, & Wilson, 2010; E. M. Davis et al., 2012; de Jongh et al., 2013; Dominguez, 2011; Gaillard et al., 2013; Halloran, Wall, Guild, & Caughey, 2011; Hinkle et al., 2010; Jang, Jo, & Lee, 2011; Kominiarek et al., 2013; Liu et al., 2014; O'Dwyer et al., 2013; Ouzounian et al., 2011; H. M. Salihu, 2011; "Share with women. Weight gain during pregnancy," 2010; Simas et al., 2011; Simas et al., 2012; Sui, Turnbull, & Dodd, 2013).

Gestational Weight Gain in AA Women

The prevalence of self-reported overweight/obese status is 58.5% among all childbearing age US women, yet is a striking 80% among AA women of childbearing

age, by far the highest of all races (Ogden, Carroll, Kit, & Flegal, 2014). Given that the strongest predictor of excessive GWG is prepregnant BMI, and that 68% of AA women gain excessively, research is beginning to focus upon contributors to racial differences in pre-pregnant weight and inappropriate GWG (Flegal, Carroll, Kit, Ogden, & Pmid, 2012; Krukowski et al., 2013). There is a known strong relationship between GWG and birth weight (Bowers et al., 2013; Park, Sappenfield, Bish, Salihu, et al., 2011; Siega-Riz, Deierlein, & Stuebe, 2010). However, a single large population-based study in South Carolina additionally examined the interrelationship of pre-pregnant BMI, GWG and birth weight *by race* (Hunt, Alanis, Johnson, Mayorga, & Korte, 2013), finding: the *prevalence of obesity* in Non-Hispanic Blacks (38.6 %) to be substantially higher than Non-Hispanic Whites (24%); and the *prevalence of adequate* (24.2% vs. 27.1%) and *inadequate* (34.8% vs. 24.2%) *GWG* for Non-Hispanic Blacks and Whites, respectively, also differed (Hunt et al., 2013). *This unusual phenomenon of higher prevalence of inadequate GWG among AA, despite a higher incidence of pre-pregnancy overweight or obese,* was a confirmation of other smaller studies (Alhusen, Gross, Hayat, Woods, & Sharps, 2012; Brooten et al., 2012; Dailey, 2009; Park, Sappenfield, Bish, Salihu, et al., 2011; H. Salihu et al., 2010; H. M. Salihu, 2011; Savitz, Stein, Siega-Riz, & Herring, 2011) and is poorly understood (Dailey, 2009; Hunt et al., 2013; Savitz et al., 2011) but may reflect the complex socio- and bio-behavioral differences between the groups. Although infants of mothers with inadequate compared to normal GWG are at increased risk of growth restriction, stillbirth, seizures, prolonged hospitalizations and neonatal death (Fontaine et al., 2012; Simas et al., 2012; Stotland, Cheng, Hopkins, & Caughey, 2006), the focus of most research has been the prevalence of excessive GWG (41%) in this population (Dailey, 2009; Hunt et al., 2013; H. Salihu et al., 2010; H. M. Salihu, 2011). Given that the rates of pre-pregnant overweight/obesity *combined with* excessive weight gain by race are so high among AA women, these problems are magnified and health outcomes

even more dire. (Einerson et al., 2011; Gaillard et al., 2013). Term infants of non-diabetic mothers with excessive vs. normal GWG have lower Apgar scores and higher risk of seizures and macrosomia regardless of maternal prepregnant BMI (Simas et al., 2012; Stotland et al., 2006). AA women and infants also suffer the highest rates of maternal and infant morbidity and mortality by race in the United States, often stemming from weight-related conditions such as hypertensive disorders, Type 2 diabetes, embolism, and medically-induced preterm birth (R. R. Davis et al., 2012; Flegal, Kit, Orpana, Graubard, & Pmid, 2013; Gage, Fang, O'Neill, & DiRienzo, 2010; Magann, Doherty, Sandlin, Chauhan, & Morrison, 2013; Ramsay et al., 2002). Interestingly, a large retrospective cohort study in a regional center in Delaware that examined the effects of pre-pregnancy obesity, race/ethnicity and prematurity found the AA women were the only racial group where there was not an association of prematurity and obesity. The authors suggested that a possible etiology could be that childbearing-age AA women tend to have the lowest trunk fat mass and total fat mass for a given BMI when compared to other races (de Jongh et al., 2013; Rahman, Temple, Breitkopf, & Berenson, 2009).

A qualitative study of the cultural norms which exist among low-income pregnant AA women toward GWG revealed a general acceptance of larger body size and a belief that gaining more weight in pregnancy is necessary for a “healthy baby” (Groth, Morrison-Beedy, & Meng, 2012). The 26 study participants interviewed within the first half of pregnancy in this report, also attributed postpartum weight retention to genetics and older age, rather than poor quality diet and sedentary lifestyles (Groth et al., 2012). Another study of 33 pregnant and postpartum AA women also found a common misperception of suitable gestational weight gain was that it was due to the lack of discussion or incorrect advice by the care provider (Goodrich, Cregger, Wilcox, & Liu, 2013). The negative health consequences of obesity and excessive weight gain for

mother and baby are not commonly known and thus could serve as an educational topic during prenatal care visits with care providers (Goodrich et al., 2013; Groth & Morrison-Beedy, 2013; Groth et al., 2012).

The Brain-Gut Axis

Biobehavioral factors associated with both excessive and inadequate GWG are essential to identify in order to inform intervention studies, but remain poorly understood. In this study we investigated the role of an understudied, but potentially important, biological contributor to maternal obesity and GWG among AA women: the maternal gut microbiome. Increasing evidence supports that the gut microbiome greatly influences the bidirectional signaling between the gastrointestinal tract and the brain by integrating central nervous system function with endocrine and inflammatory pathways, leading to the coining of the term “brain-gut microbiota axis” (Karlsson, Tremaroli, Nielsen, & Backhed, 2013; Khanna & Tosh, 2014; Zhao, 2013). More simply called the “brain-gut axis”, it is regulated at the neural, hormonal, and immunologic levels, and perturbations of these systems are linked with alterations in the stress response, behaviors, and obesity (Karlsson et al., 2013; Zhao, 2013). Another key mechanism of the brain-gut axis is through the stress response that increases gut permeability, allowing passage of microbiota across the intestinal mucosa, affecting immune and neuronal cells of the enteric nervous system (Huurre et al., 2008; Karlsson et al., 2013; Manco, Putignani, & Bottazzo, 2010; Yang, Keshavarzian, & Rose, 2013). In mice, the spread of distinct strain-specific microbiota increases anxiety-like behavior and weight, yet is reversed with antibiotic therapy or probiotic treatment (Gareau, 2014; Rodrigues, Sousa, Johnson-Henry, Sherman, & Gareau, 2012; Yellon, Ebner, & Elovitz, 2009). Similar clinical responses are beginning to be seen among healthy human subjects (Angelakis, Merhej, & Raoult, 2013; Gareau, 2014; Manco, 2012; Nitert et al., 2013; Norris, Molina, & Gewirtz, 2013).

Study Aims

As AA women have been underrepresented in human microbiome studies, little is known about the intra-racial variations in the gut microbiome and the brain-gut axis. Even less is known about factors that influence the gut microbiome across BMI categories, the clinical significance of microbiome changes in pregnancy, and whether maternal behavior profiles are associated with microbiome composition linked with gestational weight gain (Gregory, 2011; Koren et al., 2012; Zhao, 2013). We hypothesize that an underlying mechanism that contributes to within-race variability in gestational weight gain patterns is the structure and dynamics of the gut microbiome at the onset and during pregnancy and, further, that the brain-gut axis is influenced by differential exposure to biobehavioral factors identified as important contributors to weight gain: diet, physical activity, sleep and social-emotional status (Althuizen et al., 2009; Bodnar et al., 2010; Daemers, Wijnen, van Limbeek, Bude, & de Vries, 2013; "Share with women. Weight gain during pregnancy," 2010). To test our hypotheses, we enrolled a socioeconomically diverse cohort of pregnant AA women to accomplish these Aims:

Aim 1: Describe the relationship between maternal gut microbiome composition and interval and total gestational weight gain in AA women.

Aim 2: Explore the possible influence of caloric intake, physical activity, and measures of chronic stress on the association between maternal gut microbiome composition and interval and total gestational weight gain in AA women.

Methods

This study, *Brain-Gut Axis and Its Influence on Gestational Weight Gain*, 1 F31 NR015722-01A1, involves a subset of women participating in a larger 5-year parent study (*Biobehavioral Influences on the Microbiome and Preterm Birth*, 1RO1 NR

014800, “Parent Study”) with on-going recruitment of a socioeconomically (SES) diverse cohort of up to 540 pregnant AA women. In the Parent Study, women are recruited during the 1st trimester (8-14 weeks) and followed through delivery with data collected at 3 times; twice via in-person contact during prenatal appointments (8-14 & 24-30 weeks), and once via medical records review post-delivery. In the ongoing Parent Study, swab samples are collected to determine the composition of the oral, vaginal, and gut microbiome, and blood samples for micronutrient status and biological indicators of stress and inflammation. Questionnaires are administered to assess demographics, multiple measures of stress and mood, experiences of racism and discrimination, and dietary intake. Medical record abstraction is ascertained for weight and height at the first and subsequent prenatal visits, as well as complications and outcomes of the pregnancy.

For this sub-study an additional (3rd) prenatal patient encounter visit with data collection was added for a subset of 27 women who consented to participate during the period of February 2015 and November 2015. This visit occurred between 35-41 weeks’ gestation and involved a 3rd rectal swab to determine late pregnancy gut microbiome and to repeat completion of some of the questionnaires on stress, mood, and diet and add data on activity. Data from the Parent Study relevant to the specific aims of the proposed study, as well as the data collected at this added visit have been used in analyses. (See **Figure 1**.)

Human Subjects Research: Involvement and Characteristics

This study met Federal Regulations defining minimal risk: “minimal risk means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.” [§46.102].

This study involved research with a vulnerable population, namely, pregnant women; as such, the federal regulations governing human subjects research indicate that special consideration of protections be given, depending upon the expected risks and benefits to the pregnant woman and her fetus/neonate, the age of the pregnant women, the study time-frame, and the expected viability of the neonate [§45 CFR 46(Subpart B)]. Considering these requirements and that this research was non-interventional (and, specifically, did not involve in any experimental medications, devices, or procedures), did involve adult pregnant women 18-35 years of age in a minimal risk study [45 CFR 45.102.i] that involved follow-up of the pregnant woman at two points during the pregnancy and acquisition of her delivery outcome data (with no follow-up of the woman or her infant beyond the delivery hospitalization) [45 CFR 46.204.d], this study involved two consent procedures: 1. Informed consent of the adult pregnant woman herself at the time of Parent Study enrollment (8-14 weeks' gestation) for participation in the study for herself through the end of the pregnancy (the delivery hospitalization) and 2. Informed consent for this cohort sub-study involved the selection of pregnant African American women without obstetrical or medical problems or complications who have completed the first and second study visits (between 8-14 and 24-30 weeks' gestation) for the Parent Study and who give separate informed consent to undergo a 3rd data collection visit between 35-41 weeks' gestation. No intervention strategies were delivered or evaluated as part of this research Parent or sub-study, and the usual standard of obstetrical care was not altered in any way for the women who participated in this research**.**

Sources of Research Material

We obtained research data from the following sources (collected through informed consent involving the parent study as well as the proposed study at the data

collection time points): (1) questionnaires and interviews with subjects; (2) medical chart review; (3) rectal swabs.

1. Questionnaire and interview data included sociodemographic profiles, recent health diagnoses and symptoms, recent health and hygiene behaviors, recent use of alcohol/tobacco/drugs, average daily kilocalorie intake, as well as data concerning stressors and depression. In all cases, standardized and validated questionnaire and interview instruments were employed by research coordinators who had been trained in their proper administration.

2. Medical Chart review ascertained parameters related to the prenatal (development of complications, infections, and any treatments administered, results of routine laboratory studies) and intrapartum course, as well as the outcome of the pregnancy (type of delivery, induction or other procedures, liveborn or stillborn, birth weight, gestational age, size for gestational age).

3. Rectal swab samples were used to extract microbial DNA for 16S sequencing for characterization of the gut microbiome. Self-swabs were done in a manner consistent with existing protocols that show the validity and safety of patients performing these swabs for themselves, including during pregnancy. Biohazards were always handled using standard universal precautions protocols with patient and researchers using gloves. Transportation of the samples was done in a mobio tube, in a sealed laboratory plastic bag marked “Biohazard” while kept on ice (later safely discarded in the laboratory sink) until the sample was placed in proper storage freezer. The labeled samples were marked as a biohazard in the -80 degree F freezer.

Protection against Risk/Potential Risks

Some of the questions in the surveys could be stressful to subjects, but were deemed no more stressful than a clinical interview. We explained to each woman from the beginning of the interview that she did not have to answer any question that makes her uncomfortable. Furthermore, the research team members administering the study instruments were well-trained to recognize signs of psychological distress and followed a protocol of contacting the woman's primary care provider, or other supportive provider within Grady Health System, should the woman score ≥10 on the Edinburgh Depression Scale or show signs of distress from any of the other question.

There was no recognized risk to the collection of rectal swab samples – whether by self-collection or via provider collection. Self-collection procedures involving swabs of these sites have been found to be valid, safe, and acceptable to women, including during pregnancy from the vaginal and rectal sites. Likewise, there is no recognized risk to subjects for the collection of their hair samples as a very small amount of hair is taken.

We recognize that breach of confidentiality is a potential risk of this study; however, the investigators will follow strict guidelines (see *Protection from Risks,* below) to protect the confidentiality of all participants.

Confidentiality

All research investigators and staff were fully trained, and required to maintain training, in human research protections, including the most up-to-date strategies for maintaining the confidentiality of research data. Second, all data are protected from anyone who does not have a staff position in the study. To that end, all participants were immediately be assigned study ID numbers upon entry into the study, and all data collected from then on were labeled only with the ID number. Only the PI, Project Coordinator, and interviewers had access to the password-protected computer file matching ID numbers with names. Third, the raw data was kept in locked filing cabinets,

accessible only to the PI, Project Coordinator, and interviewers. Fourth, all data collection at the home visits and during the delivery admission were done on password-protected and encrypted tablet computers with REDCap software, which is a software that allows for the encrypted transfer of data to the Emory server over the internet in a HIPAA-compliant, encrypted manner. Fifth, the Emory server is password-protected, encrypted, and backed up to ensure data confidentiality and integrity. Finally, laboratory samples were maintained in locked areas. An ID number was used to identify these samples, and access was limited to project staff under the supervision of the investigator. Data was kept locked away separately from identifying information, and no one had access to the data or the identifying information other than the Investigators directly involved. All data has been obtained specifically for research purposes.

Data and Safety Monitoring

Data and safety monitoring was conducted by the Dual-Principal Investigators of the parent study, E. Corwin and A. Dunlop, and the PI of this sub-study, S. Edwards, appropriate reporting to the Emory University Institutional Review Board was completed. While none occurred, the PI's and study staff were vigilant for any health related concerns or adverse events that could have occurred as a result of or during this study. The PI was available to evaluate any comments or suggestions provided by the research staff and/or study volunteers and to follow up on any adverse events reported. As this research studies did not involve an intervention, and did not constitute a clinical trial, a Data Safety Monitoring Committee was not required.

Study Sample and Sites

In the Parent Study, pregnant AA women were recruited among 18 to 40 year-old women presenting to prenatal care clinics of Emory Midtown and Grady Hospitals, which provide prenatal care to ~3,000 women annually. Given that SES is a determinant of the stress, nutrition, and health behaviors under study, the diversity across these hospitals

provided us with sufficient variation in the biobehavioral factors of interest to probe their impact on the brain-gut axis and gestational weight gain using method of insurance (Medicaid versus Private, employer) as a proxy for SES.

Recruitment

For the Parent Study, AA women who present to Emory and Grady prenatal clinics between 8-14 weeks' (determined by standard criteria based upon last menstrual period and/or first trimester ultrasound), and who meet baseline eligibility criteria (see below) are offered a "Dear New-Mom-to-Be" pamphlet describing the study and asked by the clinic nurse if they are interested in speaking with a research coordinator present at the site to learn more; the coordinator meets with those who express interest to verify eligibility, fully describe the study, and complete the consent process. Consenting women were offered $30 for completing study procedures at the first encounter and an additional $30 at the second encounter, $60 total.

Study Eligibility

Inclusion criteria for the Parent Study are: 1) AA race (via self-report). By limiting participation to AAs, this study will identify intra-race risk variation in the gut microbiome and 2) Singleton pregnancy between 8-14 weeks' gestation (verified by clinical record). 3) Ability to comprehend written and spoken English. 4) Age 18 to 40 years (inclusive). 5) Parity zero to four (verified by prenatal record), to limit factors known to impact weight (*e.g.,* number of prior births). 6) No chronic medical conditions or chronic medications (verified by prenatal record), as these may impact gut microbiome and stress.

Selection of Participants for the Sub-Study

During the 24-30 week visit, Parent Study participants were asked by the research coordinator or the PI of this proposed study (Edwards) if they would be willing to return for an additional data collection visit during their 35th-41st week. If so, arrangements were made to meet during a scheduled prenatal clinic visit at one time

between the 35th to 41st weeks. At that visit, the PI (Edwards) reviewed with the subject a new Informed Consent and, once signed, the woman completed the demographic, stress, activity and mood questionnaires, and collected a rectal swab for determination of late-pregnancy gut microbiome composition. Subjects were given an additional $20 for this extra sub-study visit, for a total of $80 for women completing all three encounters.

Data Collection

Collection of data for the Parent Study and the sub-study combined occurred at four time points (prenatal visits between 8-14, 24-30, and 35-41 weeks and post-delivery medical chart review), however, in the sub-study, and for this dissertation, only data from the 1st and 3rd visits and the medical chart review were utilized (**Table 1**): Data collection occurred prospectively for the entire cohort and was conducted by an experienced research team, trained in all aspects of the protocol and in the ethical conduction of human subjects' research. All biologic samples were collected and processed using universal precautions and methods consistent with the Human Microbiome Project.

Data Management

Questionnaire and clinical data was entered into computer tablets via REDCap management software. For ready access and analyses, data was stored in five databases, which contained a unique ID for each subject within each record so that data could be easily linked across the parent and substudy-merged databases. The first database contained the sociodemographic and questionnaire data; the second contained clinical medical record and clinical microbiology data; the third contained biomeasures of stress data; the fourth contained nutrient measures; and the fifth contained 16S rRNA sequencing pertaining to the gut microbiome.

Data Analyses

For the analysis of the data in this proposed study, only the first trimester, the midpoint gestational weight gain and third trimester data were included, along with the medical chart abstraction. The addition of the third trimester data point was important given that total and interval GWG were being assessed and most women deliver at or near term. Initial data exploration included: 1) determining the distributions of variables and assessing the need for data transformations, 2) insuring assumptions of statistical analyses are satisfied, 3) identifying co-linearity and outliers that require further investigation. For categorical variables, we checked for sparse cells and regrouped categories as needed. In building predictive models, we checked linearity assumptions for continuous predictors and considered higher-order terms if needed. For missing data, we performed sensitivity analysis of the impact of missing data. We proceeded to analyze the data using the following approach for the hypothesis to examine the relationship between maternal gut microbiome composition during pregnancy and interval and total GWG.

Limitations

As this study is exploratory, hypothesis testing is not the primary focus of the statistical analysis. Rather we analyzed data with the specific intent of establishing population parameters (e.g., effects sizes) for the variables being investigated. Further, given the high cost and extensive time required for microbiome analysis, the budget limits and time constraints of the number of subjects and time points to be used in the analysis, the yield of subjects with all data was 27, less than the original target of 80. Thus, the small sample size limits statistical power. The benefit of the within-race analysis can be also seen as a limitation in terms of generalizability to other races. Objective measures of physical activity and sleep would have enhanced the design but were seen as unduly burdensome for the participants and cost-prohibitive.

Summary

The purpose of this prospective, longitudinal study was to first describe the relationship between the maternal gut microbiome composition, in this case represented by *Firmicutes* and *Bacteroidetes* ratio, and interval and total gestational weight gain in AA women. The second aim was to explore the possible influence of dietary intake, physical activity, and measures of chronic stress on the association between maternal gut microbiome composition and interval and total gestational weight gain in AA women. The aims have been analyzed and are presented in Chapters 2, 3 and 4 of this dissertation. Chapter 2, "The Maternal Gut Microbiome in Pregnancy" is published in the American Journal of Maternal Child Nursing (Edwards, Cunningham, Dunlop, & Corwin, 2017) and Chapters 3 and 4 will be submitted for publication in order to disseminate the study findings. Chapter 5 summarizes the findings and presents implications for future research and translation to practice. Ultimately, we anticipate that the information gathered from this study will guide interventions to reduce gestational health disparities experienced by AA women. In so doing, this study will address three of the four NINR research priority areas: Promoting Health/Preventing Disease, Improving Quality of Life, and Eliminating Health Disparities.

Figure 1.
Conceptual Framework of Study

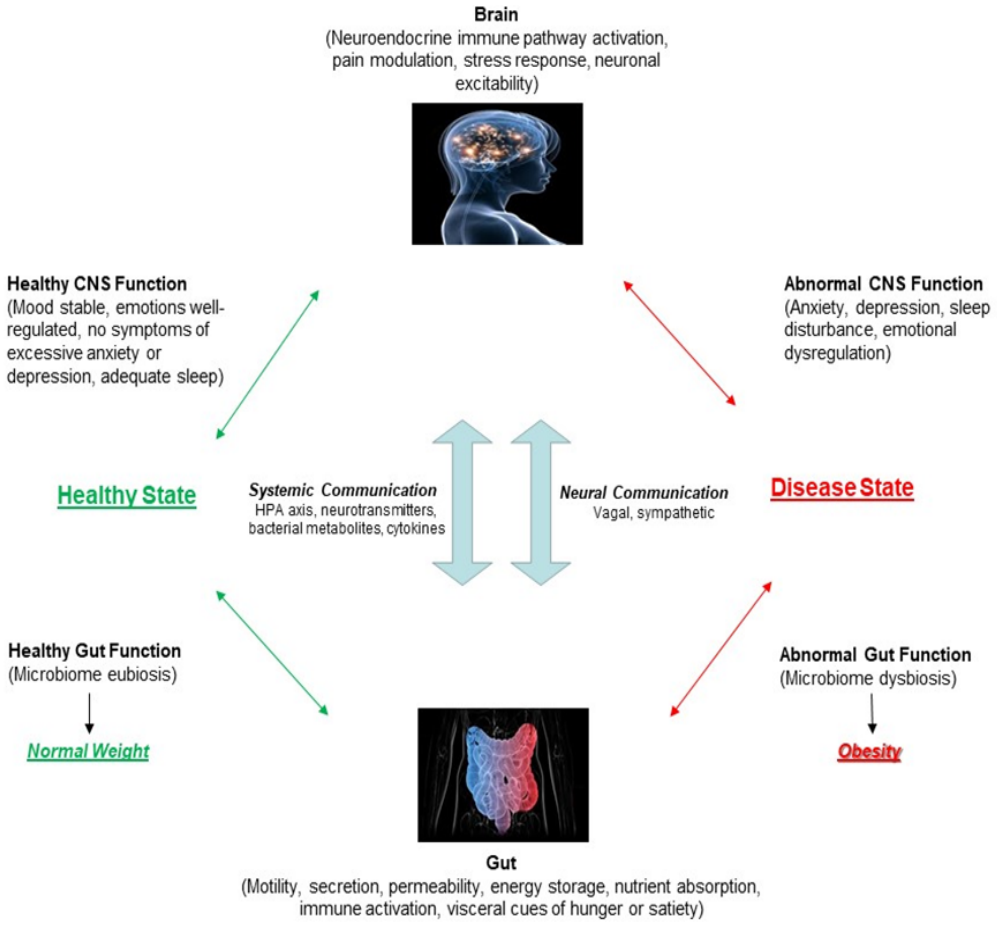

Table 1.
Overview of Data Collection for this Study

Items Collected for Entire Cohort	**Type of Assay/Analysis**	**Purpose**
Rectal swab	Gut microbiome 16S rRNA gene sequencing	Characterize the microbiome
Sociodemographic questionnaire (1st time point only)	Family size and household income (for determination of poverty/income ratio), marital status, insurance status	For adjustment in statistical models
Stressor exposure questionnaires	Adverse Childhood Experiences-Household Dysfunction Questionnaire (ACE-HD), Childhood Trauma Questionnaire (CTQ-SF)	Measure acute and chronic stressors for impact on microbiome
Health surveys	Illnesses, diagnoses, medications, Pregnancy Physical Activity Questionnaire (PPAQ)	Measure health & behavior for impact on microbiome and energy consumption
Modified Block-Bodnar Food Frequency Questionnaire	Intake of kilocalories per day	Measure of diet quality by average daily caloric intake for impact on microbiome
Medical record abstraction	Measured weight, height, and gestational age at first and subsequent prenatal care visit; health history, prenatal Infections, complications, and treatments	Calculation of total and interval gestational weight gain and BMI; Measure health & treatments for impact on microbiome
4th Time Point: After delivery		
Medical record abstraction	Measured weight and gestational age at last prenatal visit; Birth outcome (gestational age, birth weight, size-for-age), Delivery type & method, Complications	Calculation of total and interval gestational weight gain and BMI; Determination of birth and pregnancy outcomes.

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Chapter 2: The Maternal Gut Microbiome during Pregnancy

The Gut Microbiome

The 100 trillion bacteria that reside in the human intestinal tract, referred to as the gut microbiota, are essential to human metabolism through nutrient processing and development of the immune system (Power, O'Toole, Stanton, Ross, & Fitzgerald, 2014) (see Table 1 for definitions of terms used in this article). It is estimated that the genes contained within the human microbiota, known as the microbiome, are 150-fold greater than those contained within the host human genome (Power et al., 2014). The development of the human gut microbiome begins before birth and proceeds in a systematic manner, affected by factors including maternal oral microbiome, mode of delivery, maternal and infant diet, and environmental exposures (Brown et al., 2013). Development of the microbiome occurs simultaneously with and plays a key role in neurologic system maturation (Borre et al., 2014). Significant early-life disruptions of the microbiome, such as through major illness or exposure to high dose, broad-spectrum antibiotics, can lead to chronic disease or mental disorders later in life (Borre et al., 2014).

The gut microbiome provides a broad range of functions. Most importantly, it collects indigestible particles from food and assimilates nutritional particles, such as vitamins and minerals (Mayer, Savidge, & Shulman, 2014). It acts with the liver to detoxify and excrete xenobiotics, ubiquitous harmful foreign chemicals present in the environment (Mayer, Savidge, et al., 2014). The host's systemic and intestinal immune systems depend greatly on the gut microbiome's function of epithelial cell renewal and management of intestinal integrity (Power et al., 2014). Without a healthy intestinal wall, bacteria migrate across the gut into the general circulation increasing systemic inflammation (Power et al., 2014). This migration occurs through a mechanism of

intestinal permeability sometimes referred to as “leaky gut” (Power et al., 2014). In a leaky gut, the junctions, composed of various proteins that link adjacent intestinal epithelial cells, are weak and fail to act as an integral barrier to migrating pathogens (Power et al., 2014). Increased intestinal permeability is hypothesized to be the mechanism by which a dysbiotic gut is linked with such low-grade inflammatory disorders as obesity and its associated conditions, insulin-resistance and Type 2 diabetes mellitus (Mokkala et al., 2016). High permeability also allows the leakage of bacteria themselves, or lipopolysaccharides (LPS) contained in the cell walls of gram-negative bacteria, thus inducing a systemic inflammatory condition called metabolic endotoxemia in the host (Cani, Osto, Geurts, & Everard, 2012). In pregnancy, this inflammatory state can lead to vascular dysfunction of the placental tissue with deleterious fetal effects such as growth restriction (Kashtanova et al., 2016; Kim, Romero, Chaemsaithong, & Kim, 2015).

In contrast, certain dietary nutrients, for example unsaturated fatty acids and whole grains, and certain strains of probiotics suggested to promote a healthy gut microbiome enhance intestinal integrity and reduce systemic inflammation (Griffin, 2015; Kashtanova et al., 2016). For example, short chain fatty acids (SCFA), such as acetate, propionate and butyrate, are byproducts of fiber fermentation called prebiotics and serve as an energy source to intestinal epithelial cells, enhancing the connections between the cells (David et al., 2014). Maternal serum SCFA levels may also influence metabolic changes seen in pregnancy: maternal weight gain, glucose metabolism and levels of various metabolic hormones (Koren et al., 2012; Priyadarshini et al., 2014). Studies of omega-3 long-chain polyunsaturated fatty acids (PUFAs) also suggest a similar protection of the intestinal wall through strengthening of cellular connections (Li et al., 2008). A recent randomized double-blind clinical trial examined the effect of 2000 mg omega-3 supplementation versus placebo for 25 weeks in overweight and obese

pregnant women [1]. While the gut microbiome was not evaluated, the primary finding of the study was a significant reduction in the plasma inflammatory marker CRP among the supplemented group. This could prove to be a strategy to reduce the low grade inflammation and higher obstetric risks suffered by overweight and obese women.

The Brain-Gut Axis

Animal and human studies support the existence of a significant bidirectional relationship between the gut and the brain, involving multiple neurologic and endocrine signaling systems (Mayer, Knight, Mazmanian, Cryan, & Tillisch, 2014). Normal central nervous system function relies on a healthy gut microbiome or "eubiosis" (Aitken & Gewirtz, 2013). The discovery of two-way signaling between the gut and brain has led to this system being coined, the "Brain-Gut Axis" (Foster & McVey Neufeld, 2013; Gareau, 2014). In a healthy individual, the autonomic nervous system (ANS), the enteric nervous system (ENS), the hypothalamic-pituitary-adrenal (HPA) axis, and the immune system work in synchrony bidirectionally between brain and gut (Cepeda, Katz, & Blacketer, 2016; Foster & McVey Neufeld, 2013). When there is a disruption of any of the components of this system, disease may manifest (Foster & McVey Neufeld, 2013; Gareau, 2014). The bidirectional nature of the brain-gut axis, magnified by gut inflammation, can impact the central nervous system, body weight, immune status and behavior (Cepeda et al., 2016) See **Figure 1**.

Hormonal Changes of the Gut Microbiome in Pregnancy

The prenatal period is marked by dramatic hormonal shifts with resultant unique inflammatory and immune changes that alter gut function and bacterial composition (Brantsaeter et al., 2011; Koren et al., 2012). If this response to a dynamic endocrine milieu is excessive, it may be associated with obstetric complications such as pre-eclampsia, preterm birth, and gestational diabetes (Koren et al., 2012). Some of these obstetric conditions are precursors of chronic diseases such as hypertension or diabetes

in both the mother and child (Koren et al., 2012). Estrogen and progesterone impact the composition of the gut microbiome through their effect on bacterial metabolism and growth and virulence of pathogenic bacteria (Mulak et al., 2014). An example of this hormonal effect is the susceptibility to *Listeria monocytogenes* infections in pregnancy partly due to elevated estrogen and progesterone levels, leading to more dire consequences, including preterm delivery or stillbirth (Garcia-Gomez, Gonzalez-Pedrajo, & Camacho-Arroyo, 2013).

Dramatic changes in ovarian hormones during the prenatal and postpartum periods affect gut contractility, transit, secretion, visceral sensitivity and immune function at multiple other sites, including the brain (Mayer, Savidge, et al., 2014). Up to 38% of pregnant women become constipated in pregnancy due to rising progesterone and reduced motilin hormone levels, which act to increase intestinal transit time (Shin, Toto, & Schey, 2015). Androgen, estrogen and progesterone receptors in the epithelium of the lower intestine also reduce anal sphincter tone (Shin et al., 2015). It is plausible that the hormone levels involved in altering transit time and sphincter tone are an adaptive response to allow greater nutrient and energy harvest, thus facilitating weight gain in pregnancy.

Metabolic Changes of the Gut Microbiome in Pregnancy

The consequences of gut dysbiosis that lead to obesity and, likely, to excessive gestational weight gain, have only recently begun to be explored in human pregnancy (Angelakis, Merhej, & Raoult, 2013). Of particular interest is the recognition that the immune and metabolic changes that occur normally in pregnancy are actually comparable to metabolic syndrome outside of pregnancy (Chassaing & Gewirtz, 2014). With no differences in diet, including total energy intake, pregnant women have been found to gain greater adiposity with significantly higher leptin, insulin and insulin resistance measures, cholesterol, and glycated hemoglobin with each trimester of

pregnancy compared to their non-pregnant counterparts (Collado, Isolauri, Laitinen, & Salminen, 2008). The microbial diversity in the gut at the start of pregnancy appears to be similar to that of non-pregnant women (Santacruz et al., 2010). Yet, as the pregnancy advances, the abundance of gut bacteria associated with inflammatory states increases in nearly 70% of women (Santacruz et al., 2010). The greatest change in the gut microbiota occurs in the ratio of certain key bacteria (*Firmicutes: Bacteroidetes*), mimicking the higher levels of *Firmicutes* seen in obesity (Santacruz et al., 2010). Levels of proinflammatory cytokines (including IFN-y, IL-2, IL-6 and TNF-a) also rise in serum, adipose and placental tissue later in pregnancy and the mucosal surfaces throughout the gastrointestinal tract reflect a low-grade inflammatory state (Cani et al., 2012). Thus, while these same metabolic and immunologic changes in a man or non-pregnant woman would be considered abnormal and a sign of a disease state, in the context of a normal pregnancy, they appear to be required, improving energy storage in fat and providing for fetal growth and lactation (Cani et al., 2012; Chassaing & Gewirtz, 2014).

If, however, the mother gains excessive gestational weight or enters pregnancy obese, these metabolic changes may magnify the risk of gestational diabetes, fetal macrosomia, or preeclampsia (Josefson, Hoffmann, & Metzger, 2013). A recent study using Japanese macaques conversely suggested that a maternal high-fat diet, but not obesity, plays a significant role in structuring a dysbiotic gut microbiome of the primate fetus [2]. As shown in Table 2, additional relevant outcomes have been reported in recent clinical studies of the maternal gut microbiome and maternal and fetal health (Bisanz et al., 2014; Brantsaeter et al., 2011; Gomez-Arango et al., 2016; Laitinen, Poussa, Isolauri, & Pmid, 2009; Luoto, Laitinen, Nermes, & Isolauri, 2010; Mokkala et al., 2016).

Clinical Implications of the Gut Microbiome in Pregnancy

The gut microbiome and the brain-gut axis are key players in the critical prenatal period when the maternal and fetal microbiome are particularly sensitive and changes can impact fetal brain development (Borre et al., 2014; Mayer, Knight, et al., 2014). Negative interference with the brain-gut axis during these times are associated with a higher maternal and child risk for chronic intestinal disorders, conditions which affect weight and growth, and even neuropsychiatric disorders (Mayer, Knight, et al., 2014). Health care recommendations that promote the IOM guidelines for maternal weight gain by prepregnant BMI category, high quality diet and gut eubiosis should be considered in the delivery of prenatal care and in client education. Stress, infection and antibiotic use, especially during pregnancy, can lead to dysbiosis and may increase risk of neurodevelopmental disorders (Bilder et al., 2013; Vela et al., 2015). Appropriate type, duration and indication of prebiotic, probiotic and antibiotic use in the prevention and treatment of illness will become an important topic for discussion with clients in the future, since all act on the gut microbiome and may impact overall health and gestational weight gain (Griffin, 2015). There are no current clinical guidelines to facilitate these discussions and further research is needed.

Dietary and lifestyle patterns that impact the brain-gut axis also need to be assessed. Pre- and probiotic use and increasing dietary fiber and fermented foods (such as yogurt, kefir, kombucha, and miso) have been suggested to improve constipation and other gastrointestinal conditions commonly seen in pregnant women, while also promoting eubiosis of the gut (Griffin, 2015). Primary care and preconception evaluations should also include dietary assessment and counseling to reach a healthy prepregnant body mass index and optimize overall health (Egan et al., 2014).

Supplementation with specific strains of probiotics, such as *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus*, combined with dietary counseling has proven effective in improving glucose metabolism in healthy pregnant and lactating

women (Brantsaeter et al., 2011). Daily intake of milk-based dietary probiotics has been reported to reduce blood pressure in pregnancy as well as the incidence of preeclampsia among primiparous women (Brantsaeter et al., 2011; Gomez-Arango et al., 2016). While it is perhaps too early to adopt the use of probiotic supplements as an effective strategy for widespread use in pregnancy, encouraging probiotic milk and other food products may be worth consideration for women who might be at greater risk for gestational diabetes and preeclampsia.

Conclusion

Diet and lifestyle practices are modifiable factors that can affect the brain-gut axis, and ultimately, the long-term health of women and infants. The degree to which the gut microbiome contributes to the neurodevelopmental, immunological and intestinal health of the pregnant woman and her fetus is only now beginning to be measured. Health practices and novel therapies to promote eubiosis and treat dysbiosis of the maternal gut are still to be discovered but will likely prove fundamental in the care of women in pregnancy.

Clinical Implications

Nurses can promote gut health in pregnant patients by:

- Conducting a detailed dietary assessment, individualized education and counseling to follow a healthy low-fat diet that includes daily probiotic dairy or other cultured food products.
- Encouraging safe omega-3 polyunsaturated fatty acid dietary intake, such as through ground flax seeds, wild salmon and tuna (up to twice weekly) which may increase intestinal wall integrity, reduce inflammatory markers in serum and tissues and improve glucose metabolism.
- Discussing safe food handling practices to limit heightened risk of food-borne pathogen exposure

- Monitoring gestational weight gain that follows the IOM guidelines for weight gain during pregnancy to minimize the risk of metabolic and hypertensive disorders, especially in high-risk groups.

Table 1
Definition of Terms

TERM	DEFINITION
Antibiotic	A type of antimicrobial drug used in the prevention or treatment of bacterial infections; some also have antiprotozoal activity
Brain-Gut Axis (Or Gut-Microbiome-Brain Axis)	Bidirectional signaling between the gut microbes and the brain via immunological, endocrine, and neural pathways
Dysbiosis	An unhealthy imbalance in microbial composition in a part of the body
Eubiosis	A microbial composition that is balanced and associated with health in a part of the body
Microbial Diversity	The range of different kinds of unicellular organisms, bacteria, archaea, protists, and fungi in a particular environment
Microbiota	The microbial community that resides on or within a particular environment, including bacteria, archaea, protists, and fungi
Prebiotic	Selectively fermented dietary ingredients that result in specific changes in the consumption and/or activity of the gastrointestinal microbiota
Probiotic	Live microorganisms which when administered in adequate amounts confer a health effect on the host

Figure 1
The bidirectional communication of the brain-gut axis occurs via systemic and neural mechanisms. It contributes to a healthy state with gut eubiosis and normal weight or a disease state with gut dysbiosis and increased risk of obesity.

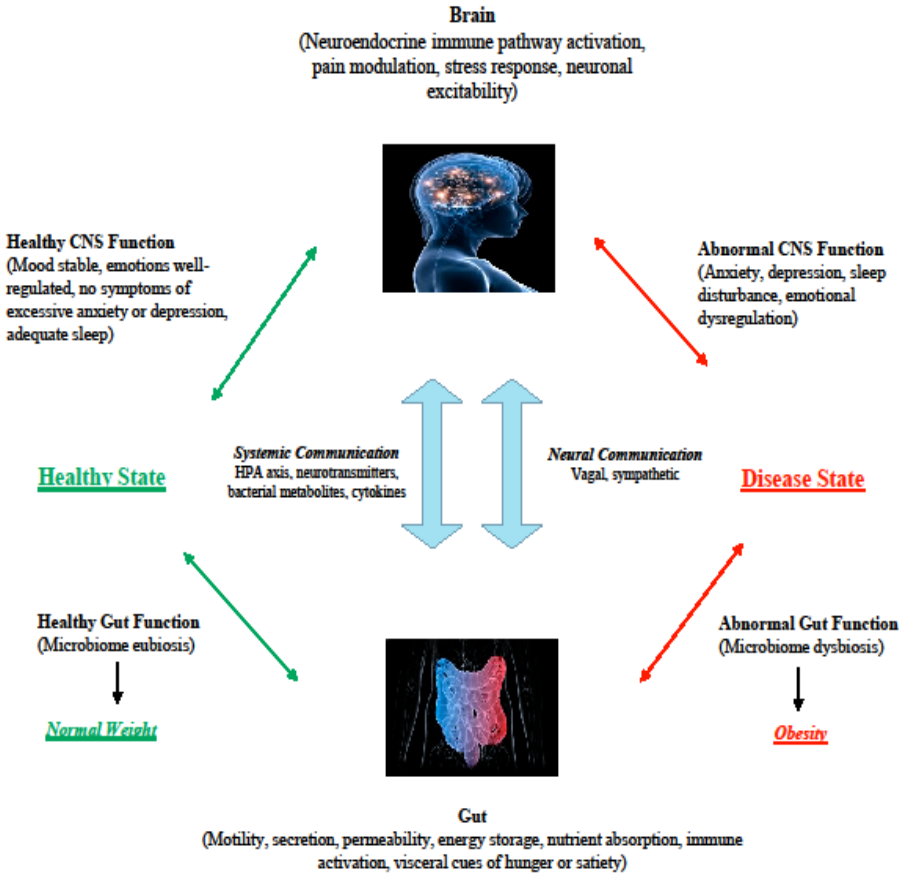

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Table 2

Recent Relevant Studies of Maternal Gut Microbiome and Maternal/Child Outcomes

Author/Year	Study Type	Sample Size	Findings
Bisanz/2014	Prospective, RCT of school-aged children and preg African women in Tanzania	n=26 received probiotic, n=34 no intervention between 12 and 24 weeks pregnant, age 18 to 40	Investigation of yogurt to lower heavy metal levels in at-risk (high contaminated fish intake) group and to examine microbiome in relation to toxin levels. A yogurt containing *L. rhamnosus* GR-1 and supplemented with 4.3 g of Moringa, a micronutrient-rich plant. Yogurt consumption had a protective effect against increases in mercury (3.2nmol/l; P=0.035) and arsenic (2.3nmol/l; P=0.011), but not statistically significant in children.
Brantsaeter/2011	Prospective, nation-wide study in Norway, 1999-2009.	Included n=33,399 primiparas, lifestyle questionnaire at gestational wk 15, then dietary questionnaire between 17-22 wk	Study examining the association between consumption of milk-based probiotic foods in pregnancy and development of preeclampsia and its subtypes in health, Norwegian (presumed Caucasian) primiparous women. Incidence of preeclampsia in study sample (5.3%) versus excluded (7.8%). Dietary questionnaire included how often the women consumed Biola and Cultura probiotic milk products, the only ones widely available during time of study. Biola had *L acidophilus* LA-5*, B. lactis* Bb12, and *L rhamnosus* GG, while Cultura only contained *L acidophilus* LA-5 and *B lactis* Bb12. Intake of these products was associated with reduced risk of preeclampsia, most prominent in severe preeclampsia (adjusted OR=0.79, 95% CI: 0.66, 0.96). Daily intake showed lower risk of all subtypes of preeclampsia (OR=0.80, 95% CI: 0.66, 0.96) and lower risk of severe preeclampsia for weekly (OR=0.75, 95% CI: 0.57, 0.98) and daily (OR=0.61, 95% CI: 0.43, 0.89) intakes.
Dotterud/2010	Prospective randomized, double-blind study of children in Finland	Enrolled n=415: given probiotic milk n=210, or sterile milk n=204, from 36 weeks gestation to age 3 months and breastfed, then followed to age 2	Study (of presumed Caucasians) to evaluate maternal and child gut microbiome effects of 250 cc probiotic milk ingestion (containing *L acidophilus* LA-5, *B. lactis* Bb12, and *L rhamnosus* GG) from 36 wks gestation to 3 months postpartum, while breastfeeding. By three months, both prevalence and relative abundance of each probiotic bacteria (each bacteria P=0.005) were significantly increased among mothers given probiotic milk versus placebo. Only *L rhamnosus* GG colonized the infants at 10 days (P=0.005) and 3 months (P=0.005). These findings suggest varying ability of probiotics to transfer from mother to infant via breastmilk.
Gomez-Arango/2016	Cross-sectional substudy from SPRING (Study of Probiotics in	Enrolled n=205, gut microbiome of overweight n=86 and obese n=119 women; PAI-1	To evaluate the relationships between degrees of overweight, gut microbiome, blood pressure and plasminogen activator inhibitor-1 (PAI-1) levels in overweight and obese pregnant women (race and parity not documented) at 16 weeks gestation. The abundance of butyrate-producing bacteria is significantly negatively associated with

	Gest DM) Study	levels on only 28 overweight, 42 obese.	systolic blood pressure (P=0.055), diastolic (P=0.02) and PAI-1 levels (P=0.0012). Findings suggest increasing butyrate-producing bacteria may contribute to lower BP in overweight and obese pregnant women.
Latinen/2009	Prospective, RCT, 3 parallel-groups of 1st trim preg women, followed to 1 year postpartum in Finland	Enrolled n=256, n=85 dietary counseling/ probiotic, n= 86 counseling/ placebo, and n= 85 control group	To investigate if dietary counseling and probiotic supplementation affects glucose metabolism in healthy, Caucasian, normoglycemic pregnant women. *L rhamnosus* GG and *B lactis* Bb12 containing probiotic capsule and counseling group had lowest blood glucose concentrations during pregnancy (baseline-adjusted means 4.45, 4.60 and 4.56 mmol/l in diet/probiotic, diet/placebo and control/placebo, respectively; P=0.025) and over 12 mon postpartum (baseline-adjusted means 4.87, 5.01 and 5.02 mmol/; P=0/025). Better glucose tolerance in diet/probiotic group confirmed by reduced risk of elevated glucose compared to control/placebo group (OR 0.31; P=0.013), lowest insulin concentration (adjusted means 7.55, 9.32, 9.27 mU/l; P=0.032) along with other similar positive findings in the third trimester and extending over 12-months postpartum.
Luoto/2010	Prospective randomized, double-blind study of children 4 weeks before expected birth and to 6 months of age in Finland	Enrolled n=113: given probiotics n=77, or placebo n=82, then continued up to age 10, probiotics n=54, placebo n=59	Study (of presumed Caucasians) to evaluate effect of probiotic intervention from 3rd trimester to age 6 months. Mothers were given probiotic of *L rhamnosus* GG or placebo in pregnancy and continued as long as breastfeeding. If infant was not breastfeeding, the intervention or placebo was given orally mixed in water. Excessive weight gain was seen in two phases; the initial phase from fetal life to 24-48 months and again at age 4. The perinatal probiotic moderated (P=0.063, analysis of variance for repeated measures) the initial phase of excessive weight, especially if later overweight, but not the second phase. Children exposed to probiotic also appeared to show a tendency of reduction of birthweight-adjusted BMI at age 4 (P=0.080, ANCOVA).
Mokkala/2016	Cross-sectional, healthy, overweight (52%) & obese (48%) women, <17 weeks gestation in Finland	Enrolled n=100, 3-day food diary within 1 week of study of serum zonulin and gut microbiome	Study to investigate whether gut microbiota and diet differ by serum zonulin, a marker of intestinal permeability, in overweight and obese pregnant Finnish women (presumed Caucasian). Divided into low and high zonulin groups by median serum concentration (46.4 ng/mL). The richness and composition of the gut microbiota (P=0.01) and intake of n-3 PUFAs, fiber, and range of vitamins and minerals were higher (P=0.05) in low zonulin group vs. high group. Findings suggest gut microbiota and diet may protect against intestinal permeability, improving metabolic health of both mother and fetus.

(Bisanz et al., 2014; Brantsaeter et al., 2011; Dotterud et al., 2010; Gomez-Arango et al., 2016; Laitinen, Poussa, Isolauri, & Pmid, 2009; Luoto, Laitinen, Nermes, & Isolauri, 2010; Mokkala et al., 2016)

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Chapter 3: *Maternal Gut Composition and Gestational Weight Gain in AA Women*

Weight and Gut Microbiome

A number of genetic and environmental factors are linked to obesity, including hormone release and signaling, diet quality and quantity, cultural and lifestyle behaviors, and socio-economic factors (Angelakis, Merhej, & Raoult, 2013; Bodnar, Siega-Riz, Simhan, Himes, & Abrams, 2010; Chassaing & Gewirtz, 2014; Cox & Blaser, 2013; Louis, Tappu, Damms-Machado, Huson, & Bischoff, 2016; "Share with women. Weight gain during pregnancy," 2010). Also linked to obesity, but perhaps less well recognized, is the composition of the gut microbiome, comprised of bacteria, viruses, archaea, and fungi and their genes (Ursell, Metcalf, Parfrey, & Knight, 2012). In both animal and human studies, the obese state is associated with perturbations of the gut microbiome and its metabolites (Backhed, 2011; Black, Bhattacharya, Philip, Norman, & McLernon, 2016; Bruce-Keller et al., 2017). Microbiota (or bacteria) are most abundant in the gut, affecting health by extracting nutrients and energy from ingested food, producing essential metabolites, serving as a barrier against harmful microbes, and promoting immune function (Wardwell, Huttenhower, & Garrett, 2011). The microbiota colonizing the gut vary among individuals, with some patterns more, and others less, associated with obesity, a phenomenon believed in part due to some microbes over-harvesting and storing energy (Aitken & Gewirtz, 2013; Backhed, 2011; Cox & Blaser, 2013) compared to others. The ratio of two major phyla of bacteria, involving approximately 95% of gut microbes, *Bacteroidetes* (Gram negative) and *Firmicutes* (Gram positive), has been found to be relevant in studies of the gut microbiome that include participants of varying weight categories. Lean individuals have a greater abundance of *Bacteroidetes,* while obese individuals harbor more *Firmicutes,* including *Clostridium* clusters, in their gut (Backhed et al., 2012; Ley, Turnbaugh, Klein, & Gordon, 2006; Santacruz et al., 2010). In one human study of obesity, the proportion of *Bacteriodetes* was found to increase

from 2% to over 20% when participants successfully completed a weight loss intervention over the course of a year (Ley et al., 2006). Similarly, studies of germ-free mice who received colonic microbiota from obese mice gained fat mass without an increase in food consumption (Allen & Kirby, 2012; Turnbaugh & Gordon, 2008; Turnbaugh et al., 2006). In addition, the mice harbored a greater proportion of Firmicutes and less Bacteroidetes compared with lean mice (Turnbaugh & Gordon, 2008; Turnbaugh et al., 2006). This is potential evidence that the gut microbiome plays a causative role in the development of obesity. Other studies have not found such significant changes in the ratio of *Firmicutes* to *Bacteroidetes* as it relates to weight, so this evidence remains controversial. The inconsistency in study findings may be related to a lack of power in many microbiome studies, lack of identification at the species and strain level, and inadequate examination of varied host exposures and comorbidities (Duncan et al., 2008; Weiss et al., 2014).

Gut Microbiome in Pregnancy

The function of the gut microbiome in digestion and metabolism is crucial to the development of obesity, including during pregnancy. Physiologic differences in glucose metabolism and fat storage occur through increased gut transit time and cellular resistance to insulin (Remely et al., 2014) . For instance, in a normal, healthy pregnancy, the immunologic, metabolic and hormonal pathways are altered which promote maternal weight gain, fetal growth and later lactation (Bodnar et al., 2010). Body fat increases starting in early pregnancy, followed later by a reduction in insulin sensitivity (Bodnar et al., 2010). Diminished insulin sensitivity has been correlated with altered immunity in pregnancy, including elevated serum cytokines involved in the metabolic inflammation of obesity (Dello Russo et al., 2013; Ellerbe, Gebregziabher, Korte, Mauldin, & Hunt, 2013). In the context of a healthy pregnancy, these changes in excessive fat deposits and reduction of insulin sensitivity are necessary and beneficial,

yet these same changes outside of pregnancy could be signs of an unhealthy metabolic syndrome. Modulation of gut microbiota composition through certain probiotics has been found to improve glucose metabolism in pregnancy, just as there is evidence that it can reduce the risk of developing obesity, insulin resistance and type 2 diabetes (P. D. Cani, Osto, Geurts, & Everard, 2012; Laitinen, Poussa, Isolauri, & Pmid, 2009). Microbiome studies examining gut composition in predominantly Caucasian pregnant women, however, are inconsistent, with some finding similarly higher ratios of *Firmicutes* to *Bacteroidetes* (FTB) in pregnant women (Savitz, Stein, Siega-Riz, & Herring, 2011) while others finding the opposite (Collado, Isolauri, Laitinen, & Salminen, 2008b). Such differences in gut microbiota between normal versus overweight/obese pregnant women and those consuming high fat, Western-type diets suggest the microbiome may be important in weight management in pregnancy, as in other life stages or circumstances (Aitken & Gewirtz, 2013; P. D. C. O. N. B. J. N. J. Cani & Pmid, 2009; Collado et al., 2008b; Cox & Blaser, 2013). Overweight pregnant women have been found to harbor more *Bacteroidetes* in the gut than women of healthy weight (Collado et al., 2008b). The abundance of these bacteria increases as the pregnancy, and their weights also increase (Collado et al., 2008b). Racial differences in the gut microbiome during pregnancy have not been determined. Few microbiome studies of pregnancy have included AA women, yet even when they do, it is usually a small number of participants. This leaves a broad area of inquiry regarding the role of the microbiome in gestational weight gain that may be involved in the stark obstetric and neonatal health disparities of the AA population. This study will examine the gut composition, represented broadly by FTB ratio, across pregnancy by initial, interval and total gestational weight gain.

Methods

The study includes a subset of women participating in a larger 5-year parent study (*Biobehavioral Influences on the Microbiome and Preterm Birth*, 1RO1 NR

014800, “Parent Study”) with on-going recruitment of a socioeconomically (SES) diverse cohort of up to 540 pregnant AA women. In the Parent Study, women are recruited during the 1st trimester (8-14 weeks) and followed through delivery with data collected at 3 times; twice via in-person contact during prenatal appointments (8-14 & 24-30 weeks), and once via medical records review post-delivery. In the ongoing Parent Study, swab samples are collected to determine the composition of the oral, vaginal, and gut microbiome, and blood samples for micronutrient status and biological indicators of stress and inflammation. Questionnaires are administered to assess demographics, multiple measures of stress and mood, experiences of racism and discrimination, and dietary intake. Medical record abstraction is ascertained for weight and height at the first and subsequent prenatal visits, as well as complications and outcomes of the pregnancy.

For this sub-study an additional (3rd) prenatal patient encounter visit with data collection was added for a subset of 27 women who consented to participate during the period of February 2015 and November 2015. This visit occurred between 35-40 weeks’ gestation and involved a 3rd rectal swab to determine late pregnancy gut microbiome and to repeat completion of some of the questionnaires on stress, mood, and diet and add data on activity. Data from the Parent Study relevant to the specific aims of the proposed study, as well as the data collected at this added visit have been used in analyses.

Study Sample and Sites

In the Parent Study, pregnant AA women were recruited among 18 to 40 year-old women presenting to prenatal care clinics of Emory Midtown and Grady Hospitals, which provide prenatal care to ~3,000 women annually. Approximately one-third of AA women present for prenatal care prior to 14 weeks gestation (Dunlop et al., 2015). Emory Midtown, a private hospital, and Grady Hospital, a public one, have prenatal clinics

staffed by the Emory obstetrical faculty. For Emory and Grady Hospitals, respectively, the percentage of AA deliveries to married women are 29% and 7%, to women with Medicaid are 68% and 91%, and with less than high school education are 19% and 45% (Dunlop et al., 2015). Given that SES is a determinant of the stress, nutrition, and health behaviors under study, the diversity across these hospitals has provided us with sufficient variation in the biobehavioral factors of interest to probe their impact on the brain-gut axis and gestational weight gain using method of insurance (Medicaid versus Private, employer) as a proxy for SES.

Recruitment

For the Parent Study, AA women who presented to Emory and Grady prenatal clinics between 8-14 weeks' (determined by standard criteria based upon last menstrual period and/or first trimester ultrasound), and who met baseline eligibility criteria (see below) were offered a "Dear New-Mom-to-Be" pamphlet describing the study and asked by the clinic nurse if they were interested in speaking with a research coordinator present at the site to learn more; the coordinator then met with those who expressed interest to verify eligibility, fully described the study, and completed the consent process. Those who consented were offered up to $30 for completing study procedures at the first encounter and an additional $30 at the second encounter, up to $60 total.

Study Eligibility

Inclusion criteria for the Parent Study were: 1) AA race (via self-report). By limiting participation to AAs, this study identified intra-race risk variation in the gut microbiome and 2) Singleton pregnancy between 8-14 weeks' gestation (verified by clinical record). 3) Ability to comprehend written and spoken English. 4) Age 18 to 40 years (inclusive). 5) Parity zero to four (verified by prenatal record), to limit factors known to impact weight (*e.g.,* number of prior births). 6) No chronic medical conditions or chronic medications (verified by prenatal record), as these could impact gut microbiome

and stress. There were no additional criteria for inclusion in this sub-study with the exception that the women must not have yet delivered.

Selection of Participants for the Sub-Study

During the 24-30-week visit, Parent Study participants were asked by the research coordinator or the primary investigator of this sub-study (Edwards) if they would be willing to return for an additional data collection visit during their 35th-41st week. If so, arrangements were made to meet during an upcoming prenatal clinic visit at one time between the 35th to 41st weeks. At that visit, the primary investigator of this sub-study (Edwards) reviewed with the subject a new Informed Consent and, once signed, the woman completed the demographic, stress, activity and mood questionnaires, and collected a rectal swab for determination of late-pregnancy gut microbiome composition. Subjects were given an additional $20 for this extra sub-study visit.

Data Collection

Collection of data for the Parent Study and the sub-study combined occurred at four time points (prenatal visits between 8-14, 24-30, and 35-41 weeks and post-delivery medical chart review); these data were combined with data gleaned from the medical charts (See **Table 1**). Data collection occurred prospectively for the entire cohort and was conducted by an experienced research team, trained in all aspects of the protocol and in the ethical conduction of human subjects' research. All biologic samples were collected and processed using universal precautions and methods consistent with the Human Microbiome Project.

Rectal Swabs: Participants were given verbal and pictorial instruction to obtain (in a private room) the rectal swab specimen using Sterile Catch-All™ Sample Collection Swabs (Epicentre Biotechnologies, Madison WI). The gloved research attendant or PI (Edwards) took the swab and immediately swirled in 750 uL of MoBio buffer contained in sterile MoBio bead tubes (MoBio Laboratories, Inc., Carlsbad, CA). The MoBio tubes

were then placed on ice, put securely into two plastic bags marked “BIOHAZARD” until transported to the School of Nursing Biobehavioral Core Lab for storage until DNA extraction was performed. Later, when the DNA was extracted, the V3 and V4 regions of the 16S rRNA gene were sequenced. Processing and mapping were completed with QIIME 1 and OTUs were mapped to Greengenes version 13_8. Raw sequencing data were processed using Qiime1. Samples with less than 10,000 reads were dropped. Data was rarified at 14,900 reads. The ratio of two major phyla of the gut, *Firmicutes* and *Bacteroidetes*, found to be relevant in studies including lean and obese participants, was determined at each time point. Finally, a log 10 transformation of the *Firmicutes* to *Bacteroidetes* ratio was used for the analyses.

Questionnaire Data Collection: Questionnaires validated in similar populations were given to the women by the research coordinators or primary investigator.

1. *Socio-demographic Survey* using self-report and prenatal administrative record review, family size and household income for determination of age, years of education, marital status, and insurance status.

2. *Health Survey* ascertained, within the prior month, all diagnoses (including infections), and medications (including antibiotics and supplements.)

Clinical Data Collection: Maternal Medical Chart Abstraction was completed under the supervision of one of the primary investigators of the parent study, Dr. Anne Dunlop, using a standardized abstraction tool to ascertain the following conditions and birth outcomes:

1. *Total weight gain during pregnancy* was calculated by using the 2009 IOM guidelines for rates of weight gain in each trimester by BMI and following methods described by Chung et al (Chung et al., 2013). A woman’s GWG per week in the second and third trimesters, adjusted for gestation age at delivery, was determined using the formula: GWG (kg/week) = Total weight gain (kg/week at final measurement – Week at first visit

measurement) (Chung et al., 2013). *Interval weight gain during pregnancy* was calculated by subtracting the weights at the midpoint (defined as a weight taken sometime between 20 weeks and 24 weeks, 6 days) the other measurement points at each prenatal visit from the 'calculated early pregnancy weight' and the 'self-report pre-pregnancy weight'.

2. *Early and Pre-pregnancy BMI* was calculated from measured height and weight at the first prenatal visit (between 8-14 weeks', referred to as the 'measured early pregnancy BMI') and subsequent weight measured at prenatal visits; in addition, women were asked to self-report their pre-pregnancy weight to estimate a 'self-report pre-pregnancy BMI'. BMI was categorized according to accepted definitions (obesity ≥ 30 kg/m^2, overweight 25-29.99 kg/m^2, healthy weight 18.5-24.99 kg/m^2, and underweight <18.5 kg/m^2). Interval and total weight gain was determined by considering the initial pre-, early, and any subsequent pregnancy measured weights in relation to the gestational age at those measurement points.

Data Management

Questionnaire and clinical data were entered into computer tablets via REDCap management software. For ready access and analyses, data were stored in five databases, which contained a unique ID for each subject within each record so that data could be easily linked across the parent and substudy-merged databases. The first database contained the sociodemographic and questionnaire data; the second contained clinical medical record and clinical microbiology data; the third contained biomeasures of stress data; the fourth contained nutrient measures; and the fifth contained 16S rRNA sequencing pertaining to the gut microbiome.

Data Analyses

For the analysis of the data in this proposed study, only the first trimester, the midpoint gestational weight gain and third trimester data were included, along with the

medical chart abstraction. The addition of the third trimester data point was important given total and interval GWG being assessed and that most women deliver at or near term. Initial data exploration included: 1) determining the distributions of variables and assessing the need for data transformations, 2) insuring assumptions of statistical analyses were satisfied, 3) identifying co-linearity and outliers that require further investigation. For categorical variables, we checked for sparse cells and regrouped categories as needed. In building predictive models, we checked linearity assumptions for continuous predictors and considered higher-order terms if needed. For missing data, we performed sensitivity analysis of the impact of missing data. We proceeded to analyze the data to examine the relationship between maternal gut microbiome composition during pregnancy and interval and total GWG.

Results

A total of 27 women were included in this study. **Table 2** outlines the baseline characteristics for the sample. The mean age of the women in this study was 25.2; most were insured by Medicaid (77.8%); had at least attended some college (55.5%); and were single (85.2%). Of the women who were single, 77.7% were in a relationship, yet varied in whether they lived with their partner (44.4%) or they lived separately (33.3%); 22.2% of the women reported not being in a relationship.

Table 3 outlines the weight-related characteristics of the participants. While the percent of overweight (11.1%) and obese (44.4%) participants at baseline combined to be the majority of the group, this was lower than the national rate of overweight and obese childbearing-age AA women combined, which is nearly 80% (Savitz et al., 2011). Almost half of the women (48.1%) gained less than recommended for their BMI (calculated at the first prenatal visit) by the midpoint of pregnancy (20-25 estimated weeks' gestation). Only 29.6% gained in the recommended range by the midpoint. Total weight gain over the entire pregnancy was more often either less than

recommended (33.3%) or more than recommended (40.7%), this is a pattern consistent with other studies of gestational weight gain in AA women (Savitz et al., 2011). **Table 4** shows the category of total weight gain by prenatal BMI category established at the first prenatal study visit. Consistent with the literature, the largest percentage of subjects combined in all BMI categories gained excessively (40.1%) and the next largest group gained inadequately (33.3%). When divided by BMI category, almost half of the combined overweight and obese gained excessively (n=7, 46.6%) yet an additional percentage gained inadequately (n=5, 33%).

The significant associations among the key variables of weight and FTB ratio are presented in **Table 5**. The lower the weight at time 1, the more they gained over the pregnancy (r = -.38, p = .05), as is recommended (*Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009). Given the small sample size, initial weight was examined as a continuous variable. Those at a higher weight at time 1 had a higher FTB ratio at time 1 and the greater the ratio dropped as the pregnancy advanced (r = .42, p = .03). Thus, the FTB change was negatively associated with FTB at time 1. Finally, the weight by mid-gestation was significantly associated with the total gain in the pregnancy (r = .75, p < .001).

Figure 1 shows the log 10 transformation of the Firmicutes to Bacteroidetes ratio at each of the three time points in the study for each category of total gestational weight gain: those who gained inadequately as determined by their prepregnant BMI, those who gained within the recommended range for their initial BMI, and those who gained more than recommended for their initial BMI. The change in ratio over the pregnancy is revealed by these unique categories of weight gain patterns.

One-way between-groups analysis of variance was conducted to explore the ratio of *Firmicutes* to *Bacteroidetes* (FTB) at time one, time three and the change from time 1 to 3, by initial BMI categories revealing no significant variance among the groups.

See **Table 6**. Also, a one-way between-groups analysis of variance was conducted to examine the relationship of categories of weight gain at the midpoint of pregnancy and the change in FTB ratio during the pregnancy. The participants were categorized as those who by the midpoint (20-25 weeks gestation) had inadequate, adequate, or excessive weight gain according to the recommendations by initial BMI. The category of weight gain at the midpoint was found to be significantly associated at the p = .05 level in the change in FTB ratio from the first to the third time points in pregnancy (*f* = 3.48, *p* = .05). The large effect size was calculated using eta squared and equaled .22. Post-hoc comparisons using Tukey's Honestly Significant Difference test indicated the mean score for inadequate gainers (*Mean* = 1.28, *SD* = 3.27) was significantly different from the excessive gainers (*Mean* =-17.60, *SD* = 32.68). The adequate gainers did not differ significantly from either other group. See **Table 7**. This test was also repeated to explore the initial BMI category on the change in FTB ratio during the pregnancy and revealed no association (*f* =.77, *p* = .43). Finally, the category of total weight gain (inadequate, adequate, or excessive) was also not associated with the change in FTB ratio during the pregnancy (*f* =1.54, *p* =.24). See **Table 8**.

Standard multiple linear regression was then used to determine the association of total gestational weight gain and the amount of weight gain at the midpoint with the FTB ratio change from 1st to 3rd time point, controlling for the initial FTB. The initial models controlled for select covariates, age, insurance (as a proxy for socioeconomic status) and height, based on support from the literature and none of the covariates were significant. Preliminary analyses of the final models were conducted to determine any violation of the assumptions of normality, linearity, multicollinearity, and homoscedasticity. There was multicollinearity between FTB at time 1 and change in FTB from time 1 to time 3 in Table 8, thus making this a poor regression model. The total variance explained by entering the variables into the model was 96%, with the adjusted

R square = 95.6%, *F* (3, 26) = 195.28, *p*=.000. Due to multicollinearity, only the initial FTB ratio was significant (*beta* =-20.79, *p* = .000) and neither midpoint weight (*beta*= -.17, *p*=.87) nor total weight gain (*beta* = -1.69, *p*= .11) had a statistically significant unique contribution to explaining the change in FTB ratio from the 1st to 3rd time point. When FTB at time 1 was then removed from the model and the change in FTB ratio was reexamined with only the weight measures, no significance existed in the model as a whole or in either variable. The total variance was poorly explained by only entering the weight variables into the model with the adjusted R square = 5%, F (2, 26) = .37, p=.70. See **Tables 9** and **10**.

Discussion

The purpose of this longitudinal study was to describe the relationship between the maternal gut microbiome composition, in this case represented by the *Firmicutes* to *Bacteroidetes* ratio, a frequently reported indicator of general gut health and weight, and interval and total gestational weight gain in AA women. The findings do not indicate that any significant relationships exist among the initial BMI or weight or the category of total weight gain of the mother and the change in the FTB ratio during the pregnancy. The associations that were discovered were in the patterns of the ratio of FTB over time. The category of weight gain *at the midpoint* was found to be significantly (at the p = .05 level) associated with the change in FTB ratio across the pregnancy (*f* = 3.48, *p* = .05), a large effect size with the mean differences between the inadequate and excessive gainers being statistically significant. The difference in the change in ratio of FTB from time 1 to time 3 also showed a negative correlation with the ratio at the first time point (*r* = -.98, *p* < .001), suggesting that the greater the FTB ratio at the start of pregnancy, the greater the decline in the FTB ratio over the pregnancy. Thus, there is a relative drop in *Firmicutes* levels or rise in *Bacteroidetes* levels as the pregnancy advances. This finding

supports previous research that *Bacteriodetes* levels rise during pregnancy and that the ratio of FTB declines (Collado, Isolauri, Laitinen, & Salminen, 2008a; Collado et al., 2008b; Gronlund, Grzeskowiak, Isolauri, & Salminen, 2011; Mokkala et al., 2016). Gut microbiome studies in pregnant women have had inconsistent findings related to the change in FTB ratio over time; this finding supports that there is a decline (Collado et al., 2008a; Savitz et al., 2011).

Pregnancy is, at times, an inflammatory condition, aggravated by maternal obesity and inappropriate gestational weight gain (P. D. Cani et al., 2012; Chassaing & Gewirtz, 2014). On the other hand, mid-pregnancy is typically a time of low inflammation in order to not reject the growing fetus (Jabbour, Sales, Catalano, & Norman, 2009; Kim, Romero, Chaemsaithong, & Kim, 2015). The gut microbiome is affected by all these factors, as well (Chassaing & Gewirtz, 2014). As in most other studies of pregnant women, it was revealed that the gut microbiome of the overweight and obese AA women in early pregnancy had a much greater relative abundance of *Firmicutes* compared to *Bacteroidetes*, similar to other obese populations, and the ratio dropped steadily during the pregnancy (Collado et al., 2008a; Savitz et al., 2011). The women who were at a healthy BMI at the start of pregnancy also experienced a drop in the FTB ratio, though minor and not statistically significant.

While gut composition is affected by many factors, including weight, diet quality, physical activity and genetics, the protective changes to the maternal physiology in pregnancy may include changes in gut composition to allow weight gain and adiposity to support fetal demands and later lactation (Koren et al., 2012). The composition of the gut microbiome and the permeability that is derived from a dysbiotic gut have become novel considerations in the study of the complex etiology of such obesity-associated obstetric complications as preeclampsia and gestational diabetes (Duca, Sakar, & Covasa, 2013; Hildebrandt et al., 2009). Mouse models suggest factors in the early

weeks of pregnancy when the placenta vascularization into the uterine wall occurs may be altered by hematogenous spread of endotoxins, notably increasing risk for later preeclampsia. Hypertensive disorders, including preeclampsia, are complications of pregnancy more commonly experienced by AA women as compared to women of other races/ethnicities (Sakawi et al., 2000). Defective spiral artery formation, oxidative stress, chronic low-grade infections like asymptomatic bacteriuria or periodontitis have all been associated with an increased risk of preeclampsia, as well (DiGiulio et al., 2010; Sakawi et al., 2000). Interestingly, AA women have a relatively low prevalence of gestational diabetes as compared to women of other races, even at high BMI categories, and the reasons remain unanswered (Hedderson et al., 2012). The prevalence and severity of preeclampsia among AA women, however, is higher than in other races (Breathett, Muhlestein, Foraker, & Gulati, 2014). Cardiovascular disease risks are higher later in life among women with a history of these gestational conditions (Main, McCain, Morton, Holtby, & Lawton, 2015; Marshall, Guild, Cheng, Caughey, & Halloran, 2014; McClure, Catov, Ness, & Bodnar, 2013; Ng, Cameron, Hills, McClure, & Scuffham, 2014). Additionally, childbearing-age AA women have been found to have uniquely lower trunk and total body fat deposits compared to women of other races (Rahman, Temple, Breitkopf, & Berenson, 2009). The findings of this study may suggest the distinct gestational weight gain patterns (highest inadequate and high rates of excessive when compared to other races) and specific gestational risks more prevalent in AA women could be related to the intra-racial differences in the gut microbiome that promote vascular and metabolic inflammation more than energy harvest in this population. The risks may be modifiable if weight gain patterns are normalized to ensure recommended rates by mid-pregnancy and at term, especially for women entering pregnancy overweight or obese.

Limitations

As this study is exploratory, hypothesis testing was not the primary focus of the statistical analysis. Rather the data was analyzed with the specific intent of establishing population parameters (e.g., effects sizes) for the variables being investigated. Further, given the high cost and extensive time required of microbiome analysis, the budget limits and time constraints of the number of subjects and time points to be used in the analysis, the yield of subjects with all data was 27, less than the original target of 80. Also, the focus at the Phylum level of the FTB ratio limits the conclusions as it fails to reflect the range of their function, which contain many species that are both commensal and pathogenic. At the Class, Order, Family, Genus and Species and Strain levels, much greater insight can be gained given consideration of function and effect of specific bacteria. The benefit of the within-race analysis can be also seen as a limitation in terms of generalizability to other races. Finally, while this study does not establish causation of FTB change over the pregnancy by category of weight gain by mid-gestation, further examination of this phenomenon is warranted.

Table 1.
Overview of Data Collection for this Study

Items Collected for Entire Cohort	**Type of Assay/Analysis**	**Purpose**
Rectal swab	Gut microbiome 16S rRNA gene sequencing	Characterize the microbiome
Sociodemographic questionnaire (1st time point only)	Family size and household income (for determination of poverty/income ratio), marital status, insurance status	For adjustment in statistical models
Chronic Stressor exposure questionnaires	Adverse Childhood Experiences-Household Dysfunction Questionnaire (ACE-HD), Childhood Trauma Questionnaire (CTQ-SF)	Measures of chronic stressors for impact on microbiome
Health surveys	Illnesses, diagnoses, medications, Pregnancy Physical Activity Questionnaire (PPAQ)	Measure health & behavior for impact on microbiome and energy consumption
Modified Block-Bodnar Food Frequency Questionnaire	Average Intake of kilocalories per day	Measure daily caloric intake for impact on microbiome
Medical record abstraction	Measured weight, height, and gestational age at first and subsequent prenatal care visit; health history, prenatal Infections, complications, and treatments	Calculation of total and interval gestational weight gain and BMI; Measure health & treatments for impact on microbiome
4th Time Point: After delivery		
Medical record abstraction	Measured weight and gestational age at last prenatal visit; Birth outcome (gestational age, birth weight, size-for-age), Delivery type & method, Complications	Calculation of total and interval gestational weight gain and BMI; Determination of birth and pregnancy outcomes.

Table 2.
Sample Characteristics

Sociodemographic Characteristic	Distribution
Age, Mean (Range, Min-Max)	25.2 years (17, 18-35)
Insurance Type, n (%) Medicaid Private	 21 (77.8) 6 (22.2)
Race, n (%) Black, African American	 27 (100)
Educational Level, n (%) HS or less College or greater	 12 (44.4) 15 (55.5)
Marital Status, n (%) Married Single	 4 (14.8) 23 (85.2)
Relationship Status, n (%) Not in relationship In relationship, no cohabitation In a relationship, cohabitation	 6 (22.2) 9 (33.3) 12 (44.4)
Parity, n (%) 0 1 2 or 3	 11 (40.7) 9 (33.3) 7 (25.9)

Table 3.
Weight-Related Characteristics

Weight-Related Clinical Characteristics (N=27)	Frequency (Percent)
BMI at First prenatal care visit Underweight (<18.5) Healthy Weight (18.5-24.9) Overweight (25-29.9) Obese (30 or more)	 1 (3.7) 11 (40.7) 3 (11.1) 12 (44.4)
Gain by recommendation* at midpoint of pregnancy** Inadequate Adequate Excessive	 13 (48.1) 8 (29.6) 6 (22.2)
Total Gain by recommendation for pregnancy Inadequate Adequate Excessive	 9 (33.3) 7 (25.9) 11 (40.7)

*IOM Guidelines for Weight Gain During Pregnancy(*Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009)
**Midpoint of pregnancy: 20-25 weeks gestation

Table 4.

Category of Total Weight Gain by Prenatal BMI Category

		Prenatal BMI Category								
		Underweight and Healthy Weight			Overweight			Obese		
		Count	Row N %	Column N %	Count	Row N %	Column N %	Count	Row N %	Column N %
Total Weight Gained by BMI Category	Inadequate	4	44.4%	33.3%	2	22.2%	66.7%	3	33.3%	25.0%
	Adequate	4	57.1%	33.3%	0	0.0%	0.0%	3	42.9%	25.0%
	Excessive	4	36.4%	33.3%	1	9.1%	33.3%	6	54.5%	50.0%

Note: Prenatal BMI established at 1st prenatal visit or Time 1.

Table 5.

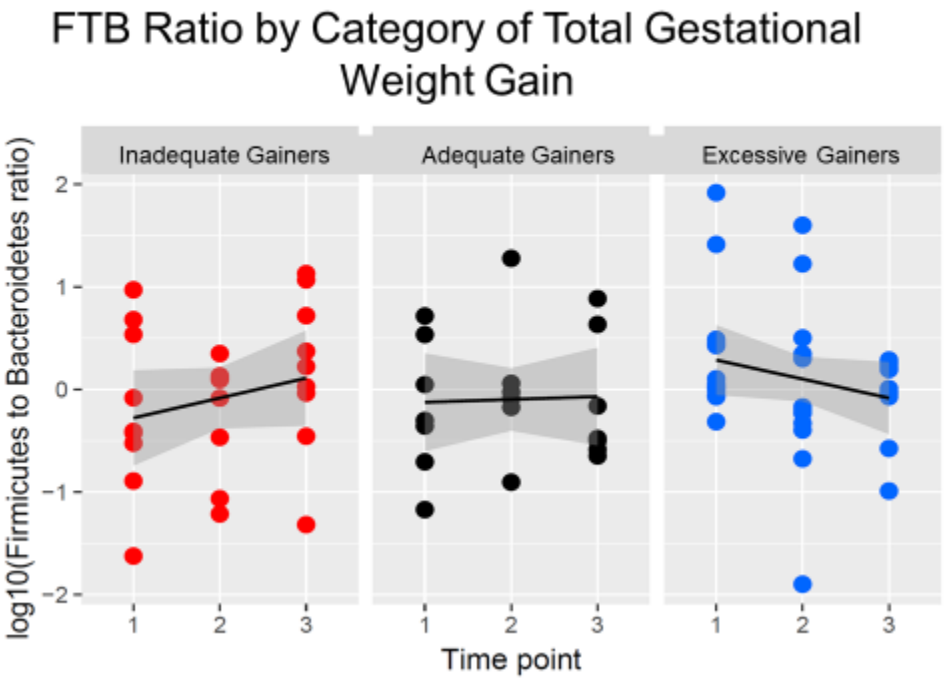
Correlation Matrix of Significant Associations among Key Variables

	1. FTB 1st visit	2. FTB Change 1-3 visit	3. Weight 1st visit	4. Weight Gained 1st to Midgestation	5. Total GWG 1-3 visit
1. *Firmicutes* to *Bacteroidetes* (FTB) Ratio 1st visit	1 27	***r* = -.98**** ***p* < .001**	***r* = .42*** ***p*=.03**	.10 .63	.08 .69
2. *Firmicutes* to *Bacteroidetes* (FTB) Ratio Change 1st to 3rd visit	***r* = -.98**** ***p* <.001**	1 27	***r* =-.39*** ***p* =.05**	-.16 .40	-.15 .46
3. Weight 1st Visit	***r* =.42*** ***p* = .03**	***r* = -.39*** ***p*= .05**	1 27	-.23 .25	***r*= -.38*** ***p* =.05**
4. Weight Gained 1st to Midgestation	.10 .63	-.17 .40	-.23 .25	1 27	***r*=.75**** ***p* < .001**
5. Total Gestational Weight Gain (GWG), 1st to 3rd Visit	.08 .69	-.15 .46	***r* = -.38*** ***p* = .05**	***r*= .75**** ***p* < .001**	1 27

*Correlation significant at 0.05 level (2-tailed)

**Correlation significant at the 0.01 level (2-tailed

Figure 1.



One-Way Analysis of Variance Results

Table 6.

Firmicutes to Bacteroidetes (FTB) ratio by Initial BMI

FTB Ratio	Underweight & Healthy Weight (< 25, n=12, 44.4%)	Overweight (25-29.9, n=3, 11.1%)	Obese (30 or more, n=12, 44.4%)	One-way between-groups ANOVA
FTB ratio at 1st visit Mean (SD)	1.49 (2.60)	2.45 (1.42)	10.57 (23.92)	$F(2, 24)=1.00$, $p=.38$
FTB ratio at 3rd visit Mean (SD)	2.14 (3.33)	1.23 (1.06)	2.90 (3.95)	$F(2, 24)=0.32$, $p=.73$
FTB change from 1st to 3rd visit Mean (SD)	0.66 (0.46)	-1.22 (2.39)	-7.66 (24.55)	$F(2, 24)=0.77$, $p=.43$

All ANOVA results with Levene statistic >.05, thus no violation of assumption of homogeneity of variance.

Table 7.

Firmicutes to Bacteroidetes (FTB) ratio by Weight Gain at mid-gestation (20-25 weeks gestation)

Weight Gain at midpoint gestation	Inadequate Gain (n=13, 48.1%)	Adequate Gain (n=8, 29.6%)	Excessive Gain (n=6, 22.2%)	One-way between-groups ANOVA
FTB ratio at 1st visit Mean (SD)	2.34 (2.79)	1.18 (1.17)	18.69 (33.08)	$F(2, 24)=2.85$, $p=.08$
FTB ratio at 3rd visit Mean (SD)	3.62 (4.49)	1.33 (1.71)	1.09 (0.70)	$F(2, 24)=1.76$, $p=.19$
FTB change from 1st to 3rd visit Mean (SD)	1.28 (3.27)	0.15 (2.23)	-17.59 (32.68)	$F(2, 24)=3.48$, $p=.05$*

*Correlation significant at 0.05 level (2-tailed), Eta squared= .22, Tukey HSD revealed Mean difference between inadequate gainers was significantly different from the excessive gainers. The adequate gainers did not differ significantly from either other group.
All ANOVA results revealed Levene statistic >.05, thus no violation of assumption of homogeneity of variance.

Table 8.

Firmicutes to Bacteroidetes (FTB) Ratio by Total Weight Gain in Pregnancy

	Inadequate Gain (n=9, 33%)	Adequate Gain (n=7, 25.9%)	Excessive Gain (n=11, 40.7%)	One-way between-groups ANOVA
FTB ratio at 1st visit (Mean, SD)	2.19, 3.18	1.57, 1.98	11.04, 25.00	$F(2, 24)=1.03$, $p=.37$
FTB ratio at 3rd visit (Mean, SD)	4.10, 5.08	1.98, 2.92	1.23, 0.64	$F(2, 24)=1.93$, $p=.17$
FTB change from 1st to 3rd visit (Mean, SD)	1.91, 3.24	1.23, 0.64	-9.80, 24.80	$F(2, 24)=1.54$, $p=.24$

All ANOVA results with Levene statistic >.05, thus no violation of assumption of homogeneity of variance.

Final Standard Multiple Linear Regression Results

Table 9.

Firmicutes to Bacteroidetes (FTB) Ratio Change from Time 1 to Time 3 by Initial FTB and Weight Gain Measures

Variable	Exp (B)	95% CI		SE	t	p-value
		Lower	Upper			
Constant		-2.31	12.22		1.41	.17
FTB at 1st visit	-.97	-1.08	-.89	.05	-20.79	.000**
Weight Gained at Midpoint~	-.01	-.08	.07	.04	-.17	.87
Total Weight Gain~~	-.07	-.43	.04	.11	-1.69	.11

~In Kilograms, weight gained from time 1 to prenatal visit at 20-25 weeks gestation
~~Total Weight Gain from time 1 to time 3
**Correlation significant at the 0.01 level (2-tailed)
Adjusted R Square = .96, p < .001**

Table 10.

Firmicutes to Bacteroidetes (FTB) Ratio Change from Time 1 to Time 3 by Weight Gain Measures

Variable	Exp (B)	95% CI		SE	t	p-value
		Lower	Upper			
Constant		-13.90	14.87		.070	.95
Weight Gained at Midpoint~	-.13	-3.56	2.32	.04	-.17	.87
Total Weight Gain~~	-.05	-1.78	1.53	.11	-1.69	.11

~In Kilograms, weight gained from time 1 to prenatal visit at 20-25 weeks gestation
~~Total Weight Gain from time 1 to time 3
**Correlation significant at the 0.01 level (2-tailed)
Adjusted R Square = -.05, p = .70

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Chapter 4: *The Brain-Gut Axis and Its Association with Gestational Weight Gain in African-American Women*

The Brain-Gut Axis in Pregnancy

In both animal and human studies, there is growing evidence of a significant bidirectional association between brain and gut involving various systems, including the autonomic nervous system (ANS), the enteric nervous system (ENS), the hypothalamic-pituitary-adrenal (HPA) axis, and the immune system (Cepeda, Katz, & Blacketer, 2017; Foster & McVey Neufeld, 2013). The relationship between the brain and gut is often referred to as the "brain-gut axis" or the "microbiota-gut-brain axis" (Foster & McVey Neufeld, 2013). Central nervous system function is affected by the status of the gut microbiome, either "eubiosis", optimal microbiome composition, or gut dysfunction and a pathogenic state, called "dysbiosis"; at the same time, the brain influences gut function, permeability, and composition of the microbiome (Aitken & Gewirtz, 2013). The gut microbiome composition acts directly and indirectly, through systemic and neural pathways, to influence the stress response, immune status, and weight of an individual (Mayer, Knight, Mazmanian, Cryan, & Tillisch, 2014). Acute and chronic stress and unhealthy lifestyle choices, such as inactivity or poor quality dietary intake, magnify risks, creating higher systemic inflammation from hematogenous transfer of pathogenic bacteria from the gut into the circulation (Wadhwa, Culhane, Rauh, & Barve, 2001). See **Figure 1**. With a more penetrable gut wall, junctions of proteins that link adjacent intestinal epithelial cells are fragile and the intestinal wall barrier is breached by bacteria and bacterial components like lipopolysaccharides (Power, O'Toole, Stanton, Ross, & Fitzgerald, 2014). Greater intestinal permeability is hypothesized to be the mechanism by which systemic inflammatory conditions such as metabolic endotoxemia, insulin resistance and type 2 diabetes mellitus are derived (Cani, Osto, Geurts, & Everard, 2012; Mokkala et al., 2016). Alternatively, a eubiotic gut microbiome protects the system

from bacterial translocation through a “leaky gut” via epithelial cell renewal and management of intestinal integrity.

While the gut microbiome is a critical regulator of metabolism, immunity and neurologic function, it is often overlooked in the care of pregnant women. An unhealthy or “dysbiotic” gut microbiome profile is seen in obese as compared to populations of non-pregnant lean individuals, and in those with metabolic conditions (Angelakis, Merhej, & Raoult, 2013; de la Cuesta-Zuluaga et al., 2017; Xu & Knight, 2015). There is also mounting research that the gut microbiome plays a role in some obstetric outcomes, especially the incidence and severity of such complications as gestational diabetes and gestational hypertension (Bauer, Hamr, & Duca, 2016; Chen, Eslamfam, Fang, Qiao, & Ma, 2016). When a woman enters pregnancy overweight or obese, she is at higher risk of preexisting or gestational metabolic and cardiovascular conditions that affect the placenta and embryo from the earliest stages of development (Creanga et al., 2015; Zhao, 2013). Further, the risk of obesity and excessive gestational weight gain extends beyond the time of the pregnancy and confers additional health risks later in life for both mother and child (Davies et al., 2010; E. M. Davis, Stange, & Horwitz, 2012; Flegal, Carroll, Kit, Ogden, & Pmid, 2012; Gaillard et al., 2013; *Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009). On a population level, the prevalence of obesity has grave implications. A majority of all childbearing-age women in the United States, and nearly eighty percent of those who are African American, are overweight or obese, and this rate is continuing to rise (Branum, 2016; de Jongh, Paul, Hoffman, & Locke, 2013; Janjua, Mahmood, Islam, & Goldenberg, 2012; Poston et al., 2016).

Weight and the Brain-Gut Axis in African American Women

The origins of obesity, while complex, are conferred through microbiome-driven energy harvest, metabolism, hypothalamic regulation (including psychosocial stressors)

and immune status (Bauer et al., 2016; David et al., 2014). Composition of the gut microbiome and weight fluctuations are also bidirectionally linked; quality and quantity of diet and activity determine energy homeostasis, controlled in part by gut microbes and their metabolites, just as weight changes influence the gut's composition (E. F. Murphy et al., 2010; E. F. Murphy et al., 2013). High fat, sugar and processed foods that are hallmarks of the "Western Diet" promote a dysbiotic gut and enhance intestinal permeability (Backhed, 2011; Morrison & Regnault, 2016).The degree of physical activity also has a direct effect on multiple systems and disease states (Choi, Fukuoka, & Lee, 2013; Cohen & Koski, 2013). Metabolic syndromes, including Type 2 and gestational diabetes, and many mood disorders can be prevented or improved through regular, moderately intense physical activity (Bennett et al., 2013; Braun, Park, & Conboy, 2012; Egshatyan et al., 2016; Forsythe, Kunze, & Bienenstock, 2016; Guszkowska, Langwald, Dudziak, & Zaremba, 2013). Some studies have shown that the ratio of the predominant phyla in the gut, *Firmicutes* to *Bacteroidetes* (FTB), are higher in those with obesity and lowers with weight loss (David et al., 2014; Ley et al., 2005; Million, Lagier, Yahav, & Paul, 2013). Body mass index is significantly associated with early pregnancy gut microbiome composition at the phylum level but there have been inconsistent findings related to FTB ratio as pregnancy advances (Gomez-Arango et al., 2016). These findings are perhaps inconsistent due to limited power in many microbiome studies, as well as differences in host exposure or comorbidities (Duncan et al., 2008).

Chronic stress can play a significant role in the composition of the gut microbiome, as well as in weight status and obesity-related conditions throughout life (Luna & Foster, 2015). Murine studies of maternal separation have shown that in critical periods during infancy and adolescence, stress leads to life-long changes in the diversity and composition of the gut microbiome (Garcia-Rodenas et al., 2006; O'Mahony et al., 2009). A large cross-sectional study of pregnant women found after adjusting for

sociodemographic factors, behavioral risk and perceived stress, the report of chronic stressors was the most significant factor in higher rates of bacterial vaginosis among Black women (Culhane, Rauh, McCollum, Elo, & Hogan, 2002). There are many similar findings of microbiome changes in the gut from exposures to chronic stress (Golubeva et al., 2015; Inui, Chen, & Meguid, 2015; Kerr et al., 2015; Rogers et al., 2016). Chronic stress from a history of childhood trauma, especially emotional, physical and sexual abuse, has been found to lead to disordered eating, restrictive weight control behaviors and excessive exercising, each independently associated with gut dysbiosis, HPA axis dysfunction and increased circulation of inflammatory cytokines (Borre, Moloney, Clarke, Dinan, & Cryan, 2014; Cryan & Dinan, 2012; Grenham, Clarke, Cryan, & Dinan, 2011; Isohookana, Marttunen, Hakko, Riipinen, & Riala, 2016; Kerr et al., 2015). Acute stress has a less powerful and more temporary impact on the status of the gut microbiome and systemic inflammation (Culhane, Rauh, & Goldenberg, 2006; Dinan & Cryan, 2012; Emge et al., 2016).

Knowledge, motivation and the ability to access healthier lifestyles and dietary choices can be severely limited by stress and poverty. Thus, the health disparities of many serious chronic disorders are borne by the poor and disenfranchised. In pregnancy, if these chronic conditions are preexisting, maternal/fetal health outcomes are compromised (Bodnar, Siega-Riz, Simhan, Himes, & Abrams, 2010; Zilberlicht et al., 2016). Morbidity and mortality of both mother and infant are higher among racial minorities, with the highest rates among African Americans (R. R. Davis, Hofferth, & Shenassa, 2014; Gage, Fang, O'Neill, & DiRienzo, 2010; Gonzalez et al., 2009). Pregnancy-related deaths of African American women compared to Whites is 2-5 times higher, widening with increasing age (Creanga et al., 2015). The mortality ratio has increased from 10 per 100,000 live births in the 1990s to 16 deaths per 100,000 for the years 2006-2010, with persistent racial disparities due to increases in the incidence of

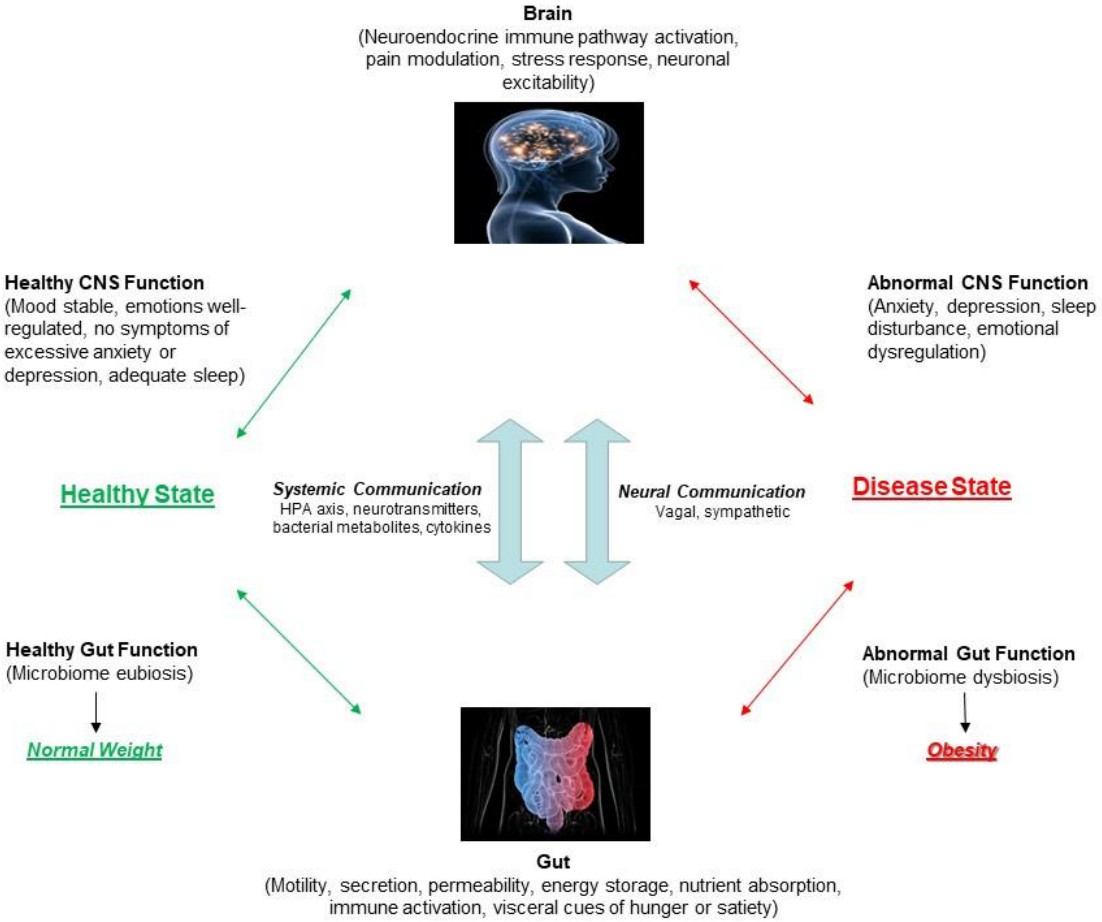
chronic conditions, especially cardiovascular disease, among African American women (Creanga et al., 2015). Preterm birth is also approximately twice as high among African Americans as compared to Whites, including both spontaneous deliveries and medically-induced deliveries for complications such as preeclampsia (de Jongh et al., 2013; Whitehead & Helms, 2010; Wise, Palmer, Heffner, & Rosenberg, 2010). Contributors to these worse outcomes are multifactorial and poorly understood (Dominguez, 2011; Gonzalez et al., 2009; Main, McCain, Morton, Holtby, & Lawton, 2015). Suspected contributors include that African American women are more likely than Whites to live in neighborhoods of socioeconomic disadvantage; a more significant social stressor among African-American women than Whites, and one that is associated with inadequate gestational weight gain or even weight loss during pregnancy (Dailey, 2009; Janevic et al., 2010; Mendez et al., 2013). Poor neighborhoods, in addition, have multiple barriers to resources essential for health: healthy food options, safe sidewalks and green space for recreation, and proper medical facilities (Vinikoor-Imler, Messer, Evenson, & Laraia, 2011). Interestingly, this inadequate physical environment is associated with adverse maternal health behaviors but not worse obstetric outcomes in African American women, yet there is an association with both adverse behaviors and obstetric outcomes in White women (Vinikoor-Imler et al., 2011).

Studies have been conducted on the relationship between the gut microbiome and weight in other populations but are lacking related to weight gain patterns in African American women during pregnancy and the role it plays in their alarming maternal-child health disparities. The guiding conceptual framework of this study posits that stress, nutrition, and health behaviors (operative within the context of African American women's lives) impact the maternal gut microbiome directly and via neuro-endocrine immune pathway activation. The gut microbiome, in turn, influences nutrient absorption, energy storage, mood and behavior which ultimately influence the pattern and degree of

gestational weight gain (**Figure 1**). It is unclear, however, whether the gut microbiome has a measurable association with prepregnancy weight or gestational weight gain patterns in this population. This study will explore the possible influence of caloric intake, physical activity, and measures of chronic stress on the association between maternal gut microbiome composition and interval and total gestational weight gain in AA women.

Figure 1.

The Brain-Gut Axis and Weight



Methods

The study population consisted of African American women who were receiving prenatal care at a publicly-funded inner-city hospital and a university hospital system in metropolitan Atlanta, both of which are affiliated with Emory University. Participants were a subsample from a larger 5-year "Parent Study" (*Biobehavioral Influences on the Microbiome and Preterm Birth*, 1RO1 NR 014800) with on-going recruitment of a socioeconomically (SES) diverse cohort of 540 pregnant AA women. Women were recruited during the 1st trimester (8-14 weeks) and followed through delivery with data collected at 3 times; twice via in-person contact during prenatal appointments (8-14 & 24-30 weeks), and once via medical records review post-delivery. Swab samples were collected to determine the composition of the oral, vaginal, and gut microbiome, and blood samples for micronutrient status and biological indicators of stress and inflammation. Questionnaires were administered to assess demographics, multiple measures of stress and mood, experiences of racism and discrimination, and dietary intake. Medical record abstraction was ascertained for weight and height at the first and at each subsequent prenatal visit, as well as complications and outcomes of the pregnancy. For this sub-study, an additional (3rd) prenatal patient encounter visit with data collection was added for a subset of 27 women who consented to participate during the period of February 2015 and November 2015. This visit occurred between 35-41 weeks' gestation and involved a 3rd rectal swab to determine late pregnancy gut microbiome and as well as completion of questionnaires on stress, mood, and caloric intake and additional data on physical activity. Both the parent and sub-studies were approved by the Emory Institutional Review Board. Further information regarding the recruitment, enrollment, study eligibility and data collection and analysis procedures have been described in a previously published article (Corwin et al., 2017).

Data Collection

For women enrolled in both the Parent Study and the sub-study, collection of data occurred at four time points: prenatal visits between 8-14, 24-30, and 35-41 weeks estimated gestational age and post-delivery medical chart review (**Table 1**). Data collection occurred prospectively for the entire cohort and was conducted by an experienced research team, trained in all aspects of the protocol and in the ethical conduction of human subjects' research.

Table 1.
Overview of Data Collection

Items Collected for Entire Cohort	**Type of Assay/Analysis**	**Purpose**
Rectal swab	Gut microbiome 16S rRNA gene sequencing	Characterize the microbiome
Sociodemographic questionnaire (1st time point only)	Family size and household income (for determination of poverty/income ratio), age, insurance status	For adjustment in statistical models
Chronic Stressor exposure questionnaires	Adverse Childhood Experiences-Household Dysfunction Questionnaire (ACE-HD), Childhood Trauma Questionnaire (CTQ-SF)	Measures of chronic stressors for impact on microbiome
Health surveys	Illnesses, diagnoses, medications, Pregnancy Physical Activity Questionnaire (PPAQ)	Measure health & behavior for impact on microbiome and energy consumption
Modified Block-Bodnar Food Frequency Questionnaire	Average intake of kilocalories per day over the previous 4 months	Measure daily caloric intake for impact on microbiome
Medical record abstraction	Measured weight, height, and gestational age at first and subsequent prenatal care visit; health history, prenatal Infections, complications, and treatments	Calculation of total and interval gestational weight gain and BMI; Measure health & treatments for impact on microbiome
4th Time Point: After delivery		
Medical record abstraction	Measured weight and gestational age at last prenatal visit; Birth outcome (gestational age, birth weight, size-for-age), Delivery type & method, Complications	Calculation of total and interval gestational weight gain and BMI; Determination of birth and pregnancy outcomes.

Table 2

Measures of Psychosocial Chronic Stress, and Physical Activity

MEASUREMENT TOOLS	DESCRIPTION AND RELEVANCE	SCORING IN LITERATURE
Childhood Trauma Questionnaire (CTQ-SF)	This short form version of the original 70-item CTQ tool elicits information about abuse and neglect in childhood by asking about 25 clinical items and three validity items and rating their occurrence on a 5-point Likert scale, in categories of emotional, physical and sexual abuse and emotional and physical neglect. The CTQ-SF has been extensively used in high-risk populations of AA and in a prospective study of pregnant women (Glaesmer et al., 2013; Spinhoven et al., 2014). The correlation coefficients for the sub-category items in the CTQ-SF range from .61 (physical neglect) to .95 (sexual abuse) (Bernstein et al., 2003).	Total Score Median 36
Adverse Child Experiences-Household Dysfunction Questionnaire (ACE-HD)	This tool ascertains experiences related to dysfunction in the childhood home by asking about 10 experiences with each being scored 0-1 and identifying any family members who exhibited the behavior. ACE is used extensively among women of reproductive age. Elevated scores correlate with increased risk for adverse outcomes and lifelong gut dysbiosis (A. Murphy et al., 2014).	High Score cut-off = 2
Pregnancy Physical Activity Questionnaire (PPAQ)	32-item semi-quantitative questionnaire of the previous week that measures type, duration, and frequency of physical activities performed by pregnant women known to be relevant in microbiome, mood and weight gain including household/caregiving, occupational, sports/exercise, transportation, and inactivity (Cohen & Koski, 2013; Currie, Woolcott, Fell, Armson, & Dodds, 2013).	Total MET hours/week Median (IQR) 180 (137)

All biological samples were collected and processed using universal precautions and methods consistent with the Human Microbiome Project. Participants were given verbal and pictorial instructions to obtain (in a private room) all swab samples using Sterile Catch-All™ Sample Collection Swabs (Epicentre Biotechnologies, Madison WI). The gloved research attendant or primary investigator for the sub-study (Edwards) took the swab and immediately swirled it in 750 uL of MoBio buffer contained in sterile MoBio bead tubes (MoBio Laboratories, Inc., Carlsbad, CA). The MoBio tubes were then placed on ice, put securely into two plastic bags marked “BIOHAZARD” until transported to the School of Nursing Biobehavioral Core Lab for storage until DNA extraction was performed. When the DNA was later extracted and V3 and V4 regions of the 16S rRNA gene were sequenced. Processing and mapping were completed with QIIME 1 and

OTUs were mapped to Greengenes version 13_8. Raw sequencing data was processed using Qiime1. Samples with less than 10,000 reads were dropped. Data was rarified at 14,900 reads. The ratio of two major phyla of the gut, *Firmicutes* and *Bacteroidetes*, found to be relevant in studies including lean and obese participants, was determined at each time point. Finally, a log 10 transformation of the *Firmicutes* to *Bacteroidetes* ratio was used for the analyses.

Questionnaire data were collected by items being either self-read or read to the women by the research coordinators or PI.

1. Socio-demographic surveys by self-report and prenatal administrative record review, family size and household income for determination of age, years of education, marital status, and insurance status were ascertained.

2. Health surveys established all diagnoses (including infections), and medications (including antibiotics and supplements) within the last month.

3. The Food Frequency Questionnaire (FFQ) ascertained dietary and supplement intake over the previous four months of: quality and quantity of nutrient and caloric intake, known to affect gut microbiome. The original full-length dietary questionnaire was developed by Gladys Block at the National Cancer Institute (Block et al., 1986). Dietary information in this study was collected using a modified Block-Bodnar semi-quantitative FFQ developed to address intake of calories and nutrients by pregnant women and validated in hundreds of studies with women of various races and low literacy since its launch in 1986 (Block, Hartman, & Naughton, 1990; Block, Woods, Potosky, & Clifford, 1990). Correlations between the questionnaire and dietary intake ranged from 0.5-0.7 (Block, Woods, et al., 1990; Boucher et al., 2006; Johnson, Herring, Ibrahim, & Siega-Riz, 2007). Data was collected and analyzed through the NutritionQuest online Food

Frequency Questionnaire. Usual frequency of consumption and portion size from a broad list of foods was used in determining average daily kilocalorie intake, the key component of intake relevant to weight gain considered in this study.

4. Measures of Psychosocial Stress, Mood, and Physical Activity: We employed instruments to measure these matters, using only those validated in minority pregnant women. (**Table 2**.)

Clinical Data Collection: Maternal Medical Chart Abstraction was completed under the supervision of one of the primary investigators of the parent study, Dr. Anne Dunlop, using a standardized abstraction tool to ascertain for the following conditions and birth outcomes:

1. Total weight gain during pregnancy was calculated by using the 2009 IOM guidelines for rates of weight gain in each trimester by BMI and following methods described by Chung et al. Weight gain ranges for the pregnancy are based on prepregnant BMI calculated at the first visit: BMI <18.5: 12.5-18 kg; BMI=18.5-24.9: 11.5-16 kg; BMI= 25-29.9: 7-11.5 kg; BMI = 30 or more: 5-9 kg (*Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009). A woman's GWG per week in the second and third trimesters, adjusted for gestation age at delivery, was determined using the formula: GWG (kg/week) = Total weight gain (kg/week at final measurement – Week at first visit measurement) [110]. Interval weight gain during pregnancy was calculated by subtracting the weights at the midpoint (defined as a weight taken sometime between 20 weeks and 24 weeks, 6 days) the other measurement points at each prenatal visit from the 'calculated early pregnancy weight' and the 'self-report pre-pregnancy weight' (Chung et al., 2013; *Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009).

2. Pregnancy Physical Activity Questionnaire (PPAQ) is a self-administered questionnaire that measures the approximate time spent in 33 activities that, together with caloric intake, have the greatest impact on gestational weight gain. The activities range from household/caregiving, occupational, sports/exercise, transportation, and sedentary behavior during the past 12 weeks (Chasan-Taber et al., 2004). The duration of time in each activity is multiplied by its intensity to determine the average weekly energy expenditure in MET-hours/week. One MET, which is equal to 3.5 ml/kg per minute, is considered the average resting energy expenditure of a typical adult. The intensity of exercise is usually expressed as multiples of resting energy expenditure (Chasan-Taber et al., 2004; Evenson, Chasan-Taber, Symons Downs, & Pearce, 2012). Current recommendations in pregnancy are that women "may safely engage in 30 min or more of moderate physical activity (an intensity of 3-6 METS) on most, if not all, days of the week" (Melzer, Schutz, Boulvain, & Kayser, 2010). Correlational coefficients of the PPAQ are 0.78 for total activity, 0.82 for moderate activity, 0.81 for vigorous activity, and ranged from 0.83 for sports/exercise to 0.93 for occupational activity [55].

3. Childhood Trauma Questionnaire short form (CTQ-SF), Adverse Childhood Experiences- Household Dysfunction Questionnaire (ACE-HD), Perceived Stress Survey (PSS), Edinburgh Depression Scale (EDS) are all self-administered questionnaires that reflects brain function by measuring either acute (PSS and EDS) or chronic (CTQ-SF and ACE-HD) stress that negatively impacts the hypothalamic-pituitary-adrenal axis, reduces some health-promoting behaviors, increases some health-risking behaviors and promotes gut dysbiosis (Beydoun & Saftlas, 2008; Blakeley, Capron, Jensen, O'Donnell, & Glover, 2013; Bock & Braun, 2011; Foster & McVey Neufeld, 2013).

4. Gestational age assessments for all participants occurred via early pregnancy dating by LMP and/or early ultrasound, given enrollment criteria. Gestational age at birth (late

preterm 35 weeks to term 41 weeks) and birth weight was determined from the delivery record.

Data Management and Analysis

Questionnaire and clinical data were entered by the research coordinator or sub-study primary investigator into computer tablets via REDCap management software. For ready access and analyses, data were stored in five databases, which contained a unique ID for each subject within each record so that data could be easily linked across the Parent and sub-study-merged databases. The first database contained the sociodemographic and questionnaire data; the second contained clinical medical record and clinical microbiology data; the third contained biomeasures of stress data; the fourth contained nutrient measures; and the fifth contained 16S rRNA sequencing pertaining to the gut microbiome.

For the analysis of the data, the first trimester data, the midpoint gestational weight gain and average daily caloric intake data, and third trimester data were included, along with the medical chart abstraction. The addition of the third trimester data point at for the sub-study at 35 to 41 weeks gestation was important in order to assess total and interval GWG being assessed and because most women deliver at or near term.

Data were analyzed by descriptive and inferential test statistics. Analysis included measures of central tendency and dispersion to examine frequencies, means and variability measures for each variable selected. Nonparametric tests were used for variables not normally distributed, using a Chi-square test. Relationships among normally distributed variables were evaluated using Pearson correlations and Student t-tests. Controlling for select covariates, additional linear regression analyses were conducted to assess the impact of significant variables on the change in either weight at

time 1 or time 3 of pregnancy or the change in FTB ratio during the pregnancy. The data were analyzed using SPSS 24.0. Statistical tests were two-sided with a p-value <0.05 used to determine statistical significance.

Results

A total of 27 participants were included in this sub-study and the baseline characteristics are presented in **Table 3**. The women were between 18 and 35 years of age, with the mean 25.2 years. Most of the women had Medicaid insurance (77.8%), had attended some college (55.5%), were single (85.2%), were less likely to be overweight or obese than the national average for childbearing-age AA women (55.5% versus nearly 80%) and were either null- or primiparous (74%).

Weight-related characteristics are found in **Table 4**. The overweight and obese categories combine to be the largest group (55.5%) yet was lower than the national average for AA women of childbearing age, which is reported to be nearly 80% of AA women are overweight or obese (Alavi, Haley, Chow, & McDonald, 2013; Bodnar et al., 2010). The average total daily kilocalorie intake calculated using proprietary software by NutritionQuest which measured reported frequency and quantity of food and drink via the Modified Block-Bodnar food frequency questionnaire and weekly energy expenditure, which was calculated by the Pregnancy Physical Activity Questionnaire (PPAQ), comprise **Table 5**. Twenty subjects completed the food frequency questionnaire. Of the 25 completed activity-focused PPAQ tools, two were discarded as they were considered implausible in healthy women without handicaps (29.93 and 40.74), thus the total number of scores used for analysis was 23. The daily kilocalorie intake was positively associated with calculated energy expenditure (r = 54, p =.03), as expected. The amount of weight gain at mid-gestation was highly correlated with the total weight gained for the pregnancy (r= .75, p <.001). To best measure physical

activity, only light activity or higher was included in the analysis, eliminating the few sedentary behavior scores that would negatively skew the results. Degree of physical activity was estimated for various categories of activities, including light, moderate, and vigorous intensity sports and exercise; occupation; transportation activity (such as walking to places); household/caregiving activities, sedentary activity and total physical activity. As aforementioned, the duration of time in each activity is multiplied by its intensity to determine the average weekly energy expenditure in MET-hours/week. One MET, which is equal to 3.5 ml/kg per minute, is considered the average resting energy expenditure of a typical adult. The intensity of exercise is usually expressed as multiples of resting energy expenditure (Chasan-Taber et al., 2004; Evenson et al., 2012). The self-report of weekly MET-hours per week of combined physical activity ranged widely from a minimum of 89.95 to a maximum of 675.18, with a median (IQR) of 243.99 (244.77).

The chronic stress measures of childhood trauma and household dysfunction are found in **Tables 6** and **7**. These scores were moderately high, a finding similar to previous reports among this minority population (Culhane et al., 2002; Dominguez, 2011). In order to more fully examine chronic stress, the household dysfunction portion of the Adverse Childhood Experiences Questionnaire was targeted. This chronic stress measure had a significant association with weight, in this case an inverse relationship with the weight at first visit: (r = -.45, p =.02). There were no associations found among the chronic stress measures and FTB ratios at any time in pregnancy. Initial weight (defined as the measured weight at the first prenatal visit) was also positively associated with initial FTB ratio (r = .42, p = .03), negatively associated with FTB ratio change during pregnancy (r = -.39, p = .05) and negatively associated with total weight gain (r = -.38, p = .05.) Because acute stress has a less profound and more temporary effect on

gut microbiome, no acute stress measures were included in this study. See **Tables 8 and 9**, where the descriptive statistics and significant associations among all the variables are presented.

Controlling for select covariates based on significance from correlation analyses or supported by the literature (age, BMI, number of previous term and preterm births, and public or private insurance type as proxy for socioeconomic status), linear regression analyses were conducted to assess the impact of these variables on the change in either weight at time 1 or time 3 of pregnancy or the change in FTB ratio during the pregnancy. Preliminary analyses revealed no violation of the assumptions of normality, linearity, or homoscedasticity in any models. Violations of multicollinearity did occur in some of the initial models but not the final models. The first of these final standard multiple regression models evaluated the degree to which FTB at time 1 and ACE-HD were predictive of weight at time 1. A modest degree, 25%, of the total variance was explained by entering these two variables into the model with the adjusted R square = .25, F (2, 26) = 5.33, p = .01. See **Table 10**. A second standard multiple regression model evaluated how predictive weight at time 1, and ACE-HD were on weight at time 3. Preliminary analyses of this model also revealed no violation of the assumptions of normality, linearity, multicollinearity or homoscedasticity. A moderate to large percentage of the total variance, 31.4%, was explained by the two variables entered into the model with the adjusted R square = .31, F (2, 26) = 6.95, p < .01. See **Table 11**.

Discussion

The conceptual model of the brain-gut axis influencing weight gain and gut microbiome composition during pregnancy was examined in this study of AA women and some novel findings were identified. While the majority (55.5%) of the subjects of

this study were overweight or obese at the start of pregnancy, this percentage was considerably lower than the national average for AA childbearing-age women, nearly 80%. Interestingly, while the recommended dietary intake for all women during pregnancy is 1900-2500 kilocalories per day, the mean and standard deviation of these mostly overweight and obese subjects was 2858.70 (1813.26). African American women by race have been found to consume the highest numbers of calories in pregnancy compared to other races of women, M (SD) = 3136 (1356) yet are also the only race of women that exhibit a pattern of both high rates of inadequate and excessive gestational weight gain regardless of prepregnant BMI (Brooten, Youngblut, Golembeski, Magnus, & Hannan, 2012). The same pattern was also exhibited in this study with 33.3% of participants having inadequate total weight gain and 40.7% having excessive total weight gain. Given this study group was leaner at the start of pregnancy than the national average for AA women, this group's lower than expected mean caloric intake was consistent. The daily caloric intake (Mean = 2858.70) was also significantly associated with physical activity (r =.54, p = .03). A large study of 826 ethnically-diverse pregnant women in Denver, Colorado found the median total energy expenditure was 195.8 MET-hours per week (95% CI 189.50, 202.00) (Harrod et al., 2014). Given the participants also reported a greater energy expenditure than in this comparison group, these factors may have together resulted in this AA group being leaner than expected.

The study subjects also had a significant risk of self-reported childhood-related household dysfunction and the finding that the higher the total ACE-HD score, the lower their initial weight in pregnancy. Higher childhood trauma scores are generally associated with higher risk of obesity yet also of disordered weight control behaviors (Fuemmeler, Dedert, McClernon, & Beckham, 2009; Isohookana et al., 2016; McDonnell & Garbers, 2017; A. Murphy et al., 2014). The childhood trauma scores for this study

group and a similar pregnant cohort of healthy Turkish women, the only cohort for whom childhood trauma data have been reported by category of abuse or neglect, are presented for comparison in **Table 6** (Cirak, Yilmaz, Demir, Dalkilinc, & Yaman, 2015). Other than the higher rate of self-reported emotional neglect among this study population, however, the mean rates of other categories of neglect and abuse were similar. The significance of the specific measure of household dysfunction in childhood may be related to the subjects reporting conditions of more food insecurity, or now having more restrictive weight control instead of overeating behaviors, or may simply be due in part to having lower parity (74% were null- or primiparous), all variables with the lower mean caloric intake and lower initial weights in these participants. There were no associations between the ACE-HD score and the FTB ratio at any time point. The other measure of chronic stress, the Childhood Trauma Questionnaire, did not show any association with caloric intake, physical activity, weight, or FTB ratio measurements at any time point.

The most critical element for these women was the initial weight in pregnancy, which was found to be negatively associated with: 1) childhood household dysfunction; 2) total weight gain; and 3) change in FTB during the pregnancy. See **Figure 2**. The initial weight was also positively associated with the initial FTB ratio with the greater the ratio of *Firmicutes* to *Bacteroidetes* at baseline, the greater the drop in the ratio over the pregnancy. The FTB ratio becomes more dominated by *Bacteroidetes* and less dominated by *Firmicutes* with time in pregnancy. This supports current clinical knowledge that the state of the mother's health at the start of pregnancy is predictive of the course of the pregnancy. Those who start pregnancy at a healthy weight have a more optimal gut microbiome composition and gain more gestational weight than those at a higher starting weight. Also, the initial gut composition and degree of chronic stress

from childhood household dysfunction together are moderately predictive of a third of the variance in the total weight gained during the pregnancy. Further, the trajectory of weight gain at the midpoint is highly predictive of total gestational weight gain ($r = .75$ $p < .001$.) This makes preconception counseling and close monitoring of weight gain during the first half of pregnancy key to optimizing initial weight, total weight gain and even gut composition at the end of pregnancy. Moreover, while chronic stress has been found to lead to long-term gut dysbiosis in other populations, this association (as represented by FTB ratio) was not found in this study. AA women with a history of significant childhood household dysfunction appear to enter pregnancy at a lower weight than their counterparts with less exposure to childhood household dysfunction. The effects of this specific type of chronic stress may be missed if not targeted at the preconception or, the more likely attended, first obstetric visit. Results from the ACE-HD questionnaire also revealed that specific family history of substance use/abuse and criminal history may impart greater insight into the type and degree of dysfunction particularly common and apparently impactful on initial weight in pregnancy. Answers to the ACE-HD did allow us to discern which member of the woman's childhood family participated in the substance use/abuse but not when the substance abuse occurred. It may be that the women who in our study presented at the start of pregnancy at lower relative weight had mothers who used substances during their own pregnancies, and as such may have been exposed in utero. Exposures to many different chronic stressors at a critical young age can alter physical development and weight (de Jongh et al., 2013; Golubeva et al., 2015; van Reedt Dortland et al., 2012). In addition, intergenerational effects on the gut-brain axis can be passed from mother to child and later to grandchild via decreased gut microbial diversity and increased permeability in pregnancy that influences the developing gut with potential lifelong health effects on future pregnancies and offspring (Fujimura, Slusher, Cabana, & Lynch, 2010; Levin et al., 2016). The child's gut

microbiome is established through the health of the mother in pregnancy and by early feeding and home care practices (Levin et al., 2016; Marques et al., 2010). Whether and how these are factors in the stark differences in maternal weight and obstetric disparities between AA and other races of women should be further examined in future studies.

Limitations

Objective measures of physical activity, dietary intake and sleep would have enhanced the rigor of this study but were seen as unduly burdensome for the participants and cost-prohibitive. In addition, given there were only 20 dietary evaluations available for this study (many of the subjects did not complete the 40-minute assessment online at home, if not done at the time of enrollment) and only 23 physical activity questionnaires, the regression models with these variables included may have lacked power to detect potential significance. The gut microbiome was also evaluated only through assessment at the phylum level by FTB ratio. Classification at a lower taxonomic rank, such as genus or species level, would provide more clarity regarding degrees of commensal or pathogenic bacteria present and their action on the individual's health during pregnancy.

Table 3

Sample Characteristics

Sociodemographic Characteristic	Distribution
Age, Mean (SD), Min-Max	25.2 (4.80) years, 18-35
Insurance Type, n (%) Medicaid Private	 21 (77.8) 6 (22.2)
Race, n (%) Black, African American	 27 (100)
Educational Level, n (%) HS or less College or greater	 12 (44.4) 15 (55.5)
Marital Status, n (%) Married Single	 4 (14.8) 23 (85.2)
Relationship Status, n (%) Not in relationship In relationship, no cohabitation In a relationship, cohabitation	 6 (22.2) 9 (33.3) 12 (44.4)
Parity, n (%) 0 1 2 or 3	 11 (40.7) 9 (33.3) 7 (25.9)

Table 4

Weight-Related Characteristics

Weight-Related Clinical Characteristics (N=27)	Frequency (Percent)
BMI at First prenatal care visit Underweight (<18.5) Healthy Weight (18.5-24.9) Overweight (25-29.9) Obese (30 or more)	 1 (3.7) 11 (40.7) 3 (11.1) 12 (44.4)
Gain by recommendation at midpoint of pregnancy Inadequate Adequate Excessive	 13 (48.1) 8 (29.6) 6 (22.2)
Total Gain by recommendation for pregnancy Inadequate Adequate Excessive	 9 (33.3) 7 (25.9) 11 (40.7)

Table 5

Physical Activity and Daily Kilocalorie Intake***

	Physical Activity n=23	**Daily Kilocalories n=20**	***r***	***p* value**
Mean (SD)	297.93 (189.01)	2858.70 (1813.26)	.54	.03
Median (IQR)	258.25 (260.77)	2221.84 (1851.75)		

*Physical Activity (light and above) measured in MET-h.week via Pregnancy Physical Activity Questionnaire
**Total Daily Kilocalorie by Food Frequency Questionnaire measure of average intake in the previous four months.

Table 6

Childhood Trauma Questionnaire (n=26 or 27)

	Mean (SD)	**Mean (SD)*	Median	Minimum	Maximum
Emotional Abuse n=26	7.12 (3.97)	*6.40 (1.82)*	5.00	5	19
Physical Abuse n=27	6.37 (1.50)	*5.74 (1.92)*	6.00	5	11
Sexual Abuse n=26	6.50 (4.50)	*6.23 (2.11)*	5.00	5	25
Emotional Neglect n=27	9.81 (5.08)	*8.81 (4.09)*	9.00	5	24
Physical Neglect n=27	6.78 (2.48)	*7.09 (2.49)*	5.00	5	13
Total CTQ Score n=26	36.69 (13.34)		33.50	25	79

*Comparative group of similarly aged, healthy, non-depressed pregnant Turkish women (Yildiz Inanici, Inanici, & Yoldemir, 2017)

Table 7

Adverse Childhood Experiences: Household Dysfunction Score (n=27)

	Frequency	Percent	Parent	Sibling/Other
Ever live with alcoholic	4	15	4	2
Ever live with street drug user	8	30	7	2
Parents divorced/separated	17	63		
Live with stepfather or stepmother	12	44		
Ever live in foster home	2	7		
Ever run away for >1 day	4	15		
Anyone in household depressed/mentally ill	3	11	2	1
Did anyone attempt/commit suicide	0	0		
Did anyone go to prison	8	30	6	3
Did anyone commit a serious crime	3	11	2	1
Total Score	Mean (SD) 2.26 (1.77)	Median 2.0	Minimum 0	Maximum 7

Table 8

Descriptive Statistics of Dependent and Independent Variables

Variable	**Mean (SD) or Median (IQR)***	**N**
Pregnancy Physical Activity Questionnaire	258.25 (260.77)*	23
Daily Kilocalories	2221.84 (1851.75)*	20
Firmicutes to *Bacteroidetes* Ratio 1st visit	5.63 (16.28)	27
Firmicutes to *Bacteroidetes* Ratio Change 1st to 3rd visit	-3.25 (16.52)	27
Weight at 1st visit (kg)	79.18 (23.26)	27
Weight Change from 1st to Mid-gestation (20-25 weeks)	3.70 (3.53)	27
Total Weight Gain, 1st to 3rd visit	11.27 (6.27)	27
Childhood Trauma Questionnaire	36.69 (13.34)	26
Adverse Childhood Experiences-Household Dysfunction	2.26 (1.77)	27

Table 9

Associations among Independent and Dependent Variables

Independent Variables/Covariates	Weight at Time 1	Total Weight Gain
Independent Variables		
Pregnancy Physical Activity	-	r = -.13 p =.53
Daily Kcal	r = -.08 p = .75	r = -.13 p =.60
CTQ	r = -.09 .65	r = .12 p =.55
ACE-HD	r= -.45* p=.02	r = .02 p =.91
FTB at time 1	r = .42* p =.03	r = .08 p =.69
FTB Change 1-3	r= -.39* p=.05	r = -.15 p =.46
Covariates		
Age (years)	r = .20 p =.31	r = -.23 p =.91
BMI (kg/m2)	r = .95** p =.000	r = -.42* p =.03
Number of Previous Births (term/preterm)	t= -1.26 p =.22	t = .87 p =.40
Insurance Type (public or private)	t = .08 p =.94	t = -.85 p =.40
Height (meters)	r = .36 p = .07	r = .03 p = .87

Final Standard Multiple Linear Regression Results

Table 10

*Weight at time **1** by FTB ratio at time 1 and ACE-HD score~*

Variable	*B*	95% CI		*t*	*p*-value
		Lower	Upper		
FTB at time 1	.33	-.05	.99	1.88	.07
ACE-HD score	-.37	-9.66	-.13	-2.12	.04*

~Dependent Variable: Weight at time 1
Predictor Variables: FTB at time 1, Adverse Childhood Experiences-Household Dysfunction (ACE-HD)
*Significant at 0.05 level (2-tailed)
**Significant at the 0.01 level (2-tailed)
Adjusted R Square = .25, p = .01**

Table 11

***Total** weight gained in pregnancy by FTB ratio at time 1 and ACE-HD score~*

Variable	*B*	95% CI		*t*	*p*-value
		Lower	Upper		
FTB at time 1	.09	-.13	.20	.43	.67
ACE-HD score	.05	-1.38	1.70	.21	.83

~Dependent Variable: Weight gained from time 1 to time 3
Predictor Variables: FTB at time 1, Adverse Childhood Experiences-Household Dysfunction (ACE-HD)
*Significant at 0.05 level (2-tailed)
**Significant at the 0.01 level (2-tailed)
Adjusted R Square = .07, p = .91

Figure 2

Associations Among Key Variables

	Negative Association	Positive Association
Weight at Time 1	Total Weight Gain ACE-HD FTB Change Time 1 to 3	FTB at Time 1
FTB Change Time 1 to 3	FTB at Time 1	
Total Weight Gain		Weight at Mid-gestation

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Chapter 5: Discussion and Conclusion to the Study: *Brain-Gut Axis and Its Influence on Gestational Weight Gain*

The purpose of this dissertation was to first describe the relationship between the maternal gut composition, as represented by *Firmicutes* and *Bacteroidetes* ratio, and interval and total gestational weight gain in AA women. The second aim was to explore the possible influence of dietary intake, physical activity, and measures of chronic stress on the association between maternal gut microbiome composition and interval and total gestational weight gain in African American (AA) women. The aims were analyzed and presented in Chapters 2, 3 and 4 of this dissertation. Chapter 2, "The Maternal Gut Microbiome in Pregnancy", was published in the American Journal of Maternal Child Nursing (Edwards, Cunningham, Dunlop, & Corwin, 2017). Chapters 3 and 4 will be submitted for publication in order to further disseminate the findings of this study. This final chapter summarizes the findings and presents implications for future research and translation to practice.

Nearly two-thirds of US women of childbearing age are overweight or obese and nearly half, once pregnant, gain excess gestational weight (*Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009). Among AA childbearing-age women, the rate of self-reported overweight and obesity is nearly 80% (Flegal, Carroll, Kit, Ogden, & Pmid, 2012; Ogden, Carroll, Kit, & Flegal, 2014). In 2009, the Institute of Medicine (IOM) published revised recommendations for trimester-specific weight gain and total gestational weight gain (GWG) by pre-pregnancy body mass index (BMI) compared to their original recommendations published in 1990 (*Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009). The more restrictive recommendations presented a particular challenge for African-American (AA) women, given they are more likely than women of other races to enter pregnancy overweight or obese (Bowers et al., 2013; Brooten, Youngblut, Golembeski, Magnus, & Hannan, 2012; Gould Rothberg,

Magriples, Kershaw, Rising, & Ickovics, 2011; Headen, Davis, Mujahid, & Abrams, 2012; Krukowski, Bursac, McGehee, & West, 2013; Marshall, Guild, Cheng, Caughey, & Halloran, 2014; Misra, Hobel, & Sing, 2010; Paul, Graham, & Olson, 2013; Wise, Palmer, Heffner, & Rosenberg, 2010). Most studies of pregnant women have considered pre-pregnant weight and total gestational weight gain, ignoring interval gain. While risks for poor obstetric outcomes were greatest among women in the highest BMI categories, this was especially so if excessive weight was gained (Althuizen, van Poppel, Seidell, & van Mechelen, 2009; Blomberg, 2011; Bodnar, Siega-Riz, Simhan, Himes, & Abrams, 2010; Davies, Maxwell, McLeod, Gagnon, Basso, Bos, Delisle, Farine, Hudon, Menticoglou, Mundle, Murphy-Kaulbeck, Ouellet, Pressey, Roggensack, Leduc, Ballerman, Biringer, Duperron, Jones, Lee, Shepherd, Wilson, et al., 2010; R. R. Davis, Hofferth, & Shenassa, 2014; Facco, 2011; Fontaine, Hellerstedt, Dayman, Wall, & Sherwood, 2012; McClure, Catov, Ness, & Bodnar, 2013). Timing of weight gain is significant to the risk of adverse obstetrical outcomes and onset or worsening of obesity in the mother and child (Davenport, Ruchat, Giroux, Sopper, & Mottola, 2013; Durie, Thornburg, & Glantz, 2011; Fontaine et al., 2012; "Share with women. Weight gain during pregnancy," 2010). Inadequate weight gain in the first 20 weeks', in all BMI categories, increases the risk of preterm birth and fetal growth restriction (Beydoun, Tamim, Lincoln, Dooley, & Beydoun, 2011; R. R. Davis, Hofferth, & author reply, 2012; Headen et al., 2012; Hinkle, Sharma, & Dietz, 2010), whereas excessive GWG during the first (but not the second 20 weeks gestation) increases the risk of excessive neonatal body fat, a precursor to childhood obesity (Davenport et al., 2013). Although the strongest predictor of excessive GWG is prepregnant BMI, most AA women exhibit a unique gestational weight gain pattern of either inadequate or excessive weight gain in pregnancy, regardless of initial BMI (Hunt, Alanis, Johnson, Mayorga, & Korte, 2013). Research, including this study, is beginning to examine the complex socio- and bio-

behavioral contributors to racial differences in pre-pregnant weight and inappropriate GWG (Flegal et al., 2012; Krukowski et al., 2013).

The Brain-Gut Axis

In this study the role of an understudied, but potentially important, biological contributor to maternal obesity and GWG among AA women, the maternal gut microbiome, was examined. Increasing evidence supports that the gut microbiome influences the bidirectional signaling between the gastrointestinal tract and the brain by integrating central nervous system function with endocrine and inflammatory pathways (Karlsson, Tremaroli, Nielsen, & Backhed, 2013; Khanna & Tosh, 2014; Zhao, 2013). The “brain-gut microbiota axis”, or more simply called the “brain-gut axis”, it is regulated at the neural, hormonal, and immunologic levels, and alterations of these systems are linked with dysfunction in the stress response, behaviors, and obesity (Karlsson et al., 2013; Zhao, 2013). Another key mechanism of the brain-gut axis is through the stress response which, largely via elevated cortisol levels, increases gut permeability, allowing spread of microbiota across the intestinal mucosa into the systemic circulation, affecting immune and neuronal cells of the enteric nervous system (Huurre et al., 2008; Karlsson et al., 2013; Manco, Putignani, & Bottazzo, 2010; Yang, Keshavarzian, & Rose, 2013). This migration occurs through a mechanism of intestinal permeability sometimes referred to as “leaky gut” (Power, O'Toole, Stanton, Ross, & Fitzgerald, 2014). In a leaky gut, the junctions, composed of various proteins that bind adjacent intestinal epithelial cells, are weak and fail to act as an integral barrier to migrating pathogens (Power et al., 2014). Increased intestinal permeability is thought to be the mechanism by which a dysbiotic gut leads to such low-grade inflammatory disorders as obesity and its associated conditions, such as insulin-resistance and Type 2 diabetes mellitus (Mokkala et al., 2016). High permeability also allows the leakage of bacteria

themselves, or lipopolysaccharides (LPS) contained in the cell walls of gram-negative bacteria, thereby inducing a systemic inflammatory condition called metabolic endotoxemia (P. D. Cani, Osto, Geurts, & Everard, 2012; P. D. C. O. N. B. J. N. J. Cani & Pmid, 2009). During pregnancy, this inflammatory state can lead to vascular dysfunction of the placenta with deleterious effects such as placental abruption and fetal growth restriction (Kashtanova et al., 2016; Kim, Romero, Chaemsaithong, & Kim, 2015).

Weight and the Gut Microbiome in Pregnancy

The function of the gut microbiome in digestion and metabolism is crucial to the weight gain and development of obesity. Digestion and metabolism are just some of the functions that change in pregnancy. In a healthy pregnancy, the immunologic, metabolic and hormonal pathways change to support maternal weight gain, fetal growth and later lactation (Bodnar et al., 2010). Body fat increases starting in early pregnancy, followed later by a reduction in insulin sensitivity (Bodnar et al., 2010). Diminished insulin sensitivity has been correlated with altered immunity in pregnancy, including elevated serum cytokines involved in the metabolic inflammation of obesity (Dello Russo et al., 2013; Ellerbe, Gebregziabher, Korte, Mauldin, & Hunt, 2013). In the context of a healthy pregnancy, these alterations in fat deposits and reduction of insulin sensitivity naturally occur, whereas these same changes outside of pregnancy would signal an unhealthy metabolic syndrome.

The ratio of the two major phyla of intestinal bacteria, involving approximately 95% of gut microbes, *Bacteroidetes* (Gram negative) and *Firmicutes* (Gram positive), has been found to be relevant in studies of the gut microbiome that include participants of varying weight categories. Lean individuals have a greater abundance of *Bacteroidetes,* while obese individuals harbor more *Firmicutes,* including *Clostridium*

clusters, in their gut (Backhed et al., 2012; Ley, Turnbaugh, Klein, & Gordon, 2006; Santacruz et al., 2010). Pregnant women entering pregnancy overweight have been found to harbor more *Bacteroidetes* in the gut than women who enter pregnancy at a healthy weight (Collado, Isolauri, Laitinen, & Salminen, 2008a). The abundance of these bacteria has often been found to increase as the pregnancy progresses, as weights also increase (Collado et al., 2008a). Gut microbiome studies in pregnant women, however, are inconsistent, with some finding higher ratios of *Firmicutes* to *Bacteroidetes* (FTB) in pregnant versus non-pregnant women (Savitz, Stein, Siega-Riz, & Herring, 2011) while others finding the opposite (Collado et al., 2008a). Few microbiome studies of pregnancy have included AA women, yet even when they do, it is usually only a small number of participants. This has left a large gap in the literature regarding the role of the microbiome in gestational weight gain that may be involved in the stark obstetric and neonatal health disparities of the AA population. This study examined the gut composition, represented broadly by *Firmicutes* to *Bacteroidetes* (FTB) ratio, through the pregnancy by initial, interval and total gestational weight gain in AA women.

Summary of Research Findings

Chapter 2

The first published manuscript, Chapter 2: The Maternal Gut Microbiome during Pregnancy, summarized the state of the science regarding the gut microbiome, the Brain-Gut Axis, and the hormonal and metabolic alterations to the gut during pregnancy (Edwards et al., 2017). In this article the conceptual framework of the study was presented: bidirectional communication through systemic and neural pathways that together comprise the brain-gut axis. The study posited that through this axis, a healthy state exists, characterized by healthy central nervous system function, gut eubiosis and

normal weight. Alternatively, a disease state exists, characterized by abnormal central nervous system function, gut dysbiosis and a heightened risk of obesity.

Clinical implications of the Gut Microbiome in Pregnancy were then discussed. Given the critical prenatal period when the maternal and fetal microbiome are particularly sensitive, negative interference with the brain-gut axis during these times might be expected to be associated with a higher maternal and child risk for chronic intestinal disorders, conditions which affect weight and growth, and even neuropsychiatric disorders (Mayer, Savidge, & Shulman, 2014; Mayer, Tillisch, & Gupta, 2015). Chapter summaries included that dietary and lifestyle patterns that affect the brain-gut axis must be considered in the care of childbearing-age and pregnant women. While there are no current clinical guidelines to facilitate discussion and care related to the microbiome in pregnancy, healthy lifestyles can prove beneficial to the pregnancy and gut microbiome. Pre- and probiotic use and increasing dietary fiber and fermented foods (such as yogurt, kefir, kombucha, and miso) have been suggested to improve constipation and other gastrointestinal conditions commonly seen in pregnant women, while also promoting eubiosis of the gut (Griffin, 2015). Recommendations that primary care and preconception evaluations should also include dietary assessment and counseling to facilitate reaching a healthy prepregnant body mass index and optimize overall health concluded the chapter (Egan et al., 2014).

Chapter 3

The next chapter, “Maternal Gut Composition and Gestational Weight Gain in AA Women”, presented the analysis of the first aim, to examine the gut composition, represented broadly by FTB ratio, across the pregnancy by initial, interval and total gestational weight gain. The study hypothesis for aim 1 was: Maternal gut microbiome composition at the 1st and/or 3rd trimester of pregnancy, or the change in composition

from the 1st to 3rd trimester, would be associated with interval and/or total GWG. Analyses were done to explore the weight-related characteristics of the participants. A total of 27 women were included in this study, the mean age was 25.2; most were insured by Medicaid (77.8%); had at least attended some college (55.5%); and were single (85.2%). While the 55.5% of the participants at baseline were either overweight or obese, this was lower than the national rate of overweight and obese childbearing-age AA women, which is nearly 80% (Savitz et al., 2011). Almost half of the women (48.1%) gained less than recommended for their BMI (calculated at the first prenatal visit) by the midpoint of pregnancy (20-25 estimated weeks' gestation). Only 29.6% gained in the recommended range by the midpoint. Total weight gain over the entire pregnancy was more often either less than recommended (33.3%) or more than recommended (40.7%), a pattern consistent with other studies of gestational weight gain in AA women (Savitz et al., 2011).

One-way between-groups analysis of variance (ANOVA) tests were conducted to explore the ratio of *Firmicutes* to *Bacteroidetes* (FTB) at time one, time three and change from time 1 to 3 and no significant variance was found among the women by initial BMI categories and among women categorized by inadequate, adequate and excessive total gestational weight gain. No significant variance was found by FTB at time one, time three and change from time 1 to 3 in any of these ANOVA tests. The only significant ANOVA test examined the relationship of categories of weight gain at the midpoint of pregnancy and the change in FTB ratio during the pregnancy. The participants were categorized as those who by the midpoint (20-25 weeks gestation) had inadequate, adequate, or excessive weight gain according to the recommendations by initial BMI. The category of weight gain at the midpoint was found to be significantly associated at the $p = .05$ level in the change in FTB ratio from the first to the third time points in

pregnancy (*f* = 3.48, *p* = .05). The large effect size was calculated using eta squared and equaled .22. Post-hoc comparisons using Tukey's Honestly Significant Difference test indicated the mean score for inadequate gainers (Mean = 1.28, SD = 3.27) was significantly different from the excessive gainers (Mean = -17.60, SD = 32.68). The adequate gainers did not differ significantly from either other group.

The findings did not, however, indicate any significant relationships existed among the initial BMI or weight or the category of total weight gain of the mother and the change in the FTB ratio during the pregnancy. The associations that were discovered were in the patterns of the ratio of FTB over time. The category of weight gain at the midpoint was found to be significantly associated with the change in FTB ratio during the pregnancy (f = 3.48, p = .05), a large effect size with the mean differences between the inadequate and excessive gainers being statistically significant. The difference in the change in ratio of FTB also showed a correlation with the ratio at the first time point (r = -2.71, p = .03), revealing that the greater the FTB ratio at the start of pregnancy, the greater the decline in the relative abundance of *Firmicutes* to *Bacteroidetes* over the pregnancy. Thus, there is a drop in *Firmicutes* levels and/or rise in *Bacteroidetes* levels as the pregnancy advances. Gut microbiome studies in pregnant women have had inconsistent findings related to the change in FTB ratio over time; this finding suggests there is a decline (Collado, Isolauri, Laitinen, & Salminen, 2008b; Savitz et al., 2011).

Finally, standard multiple linear regression analyses were applied to examine if total gestational weight gain and the amount of weight gain at the midpoint were correlated with the FTB ratio change from 1st to 3rd time point, controlling for the initial FTB. The total variance explained by entering the variables into the model was 96%, with the adjusted R square = 95.6%, *F* (3, 26) = 195.28, *p*=.000. No relationships were identified due to significant multicollinearity between FTB at time 1 and change in FTB

from time 1 to time 3, thus making a poor regression model. When the variable of FTB at time 1 was then removed from the model and the change in FTB ratio was reexamined with only the weight measures, no significance existed in either variable or the model as a whole, adjusted R square = 5%, F (2, 26) = .37, p=.70.

In summary, the significant factor in FTB ratio during the pregnancy was category of weight gain at the midpoint interval of pregnancy. There exists a significant difference in the change of FTB over the pregnancy when comparing the women who inadequately and those who excessively gained by the midpoint. Those who inadequately gained had a slight elevation in FTB ratio during the pregnancy while those who gained excessively experienced a dramatic drop over the three time points in pregnancy. While this study did not establish causation or consider the clinical implications of FTB change over the pregnancy by category of weight gain at mid-gestation, further examination of this phenomenon is warranted.

Chapter 4

The final chapter, The Brain-Gut Axis and Its Association with Gestational Weight Gain in African-American Women, addressed the second aim of this dissertation: to explore the possible influence of caloric intake, physical activity, and/or measures of chronic stress on the association between maternal gut microbiome composition and interval and total gestational weight gain in AA women. The study hypothesis for aim 2 was: caloric intake, physical activity, and/or measures of chronic stress will influence the association between maternal gut microbiome composition and interval and total gestational weight gain in AA women. While the gut microbiome is a critical regulator of metabolism, immunity and neurologic function, it is often overlooked in the care of pregnant women. An unhealthy or “dysbiotic” gut microbiome profile is seen in obese as compared to lean populations and in those with metabolic conditions (Angelakis, Merhej,

& Raoult, 2013; de la Cuesta-Zuluaga et al., 2017; Xu & Knight, 2015). There is mounting evidence that the gut microbiome plays a role in some obstetric outcomes, especially the incidence and severity of such complications as gestational diabetes, preeclampsia, and gestational hypertension (Bauer, Hamr, & Duca, 2016; Chen, Eslamfam, Fang, Qiao, & Ma, 2016). When a woman enters pregnancy overweight or obese, she is at higher risk of preexisting or gestational metabolic and cardiovascular conditions that affect the placenta and embryo from the earliest stages of development (Creanga et al., 2015; Zhao, 2013). Further, the risk of obesity and excessive gestational weight gain extends beyond the time of the pregnancy and confers additional health risks later in life for both mother and child (Davies, Maxwell, McLeod, Gagnon, Basso, Bos, Delisle, Farine, Hudon, Menticoglou, Mundle, Murphy-Kaulbeck, Ouellet, Pressey, Roggensack, Leduc, Ballerman, Biringer, Duperron, Jones, Lee, Shepherd, & Wilson, 2010; E. M. Davis, Stange, & Horwitz, 2012; Flegal et al., 2012; Gaillard et al., 2013; *Weight Gain During Pregnancy: Reexamining the Guidelines*, 2009).

Chronic stress can play a significant role in the composition of the gut microbiome, as well as weight status and obesity-related conditions throughout life (Luna & Foster, 2015). Chronic stress from a history of childhood trauma, especially emotional, physical and sexual abuse, has been found to lead to disordered eating, weight control behaviors and excessive exercising, all associated with gut dysbiosis, HPA axis dysfunction and increased circulation of inflammatory cytokines (Borre, Moloney, Clarke, Dinan, & Cryan, 2014; Cryan & Dinan, 2012; Grenham, Clarke, Cryan, & Dinan, 2011; Isohookana, Marttunen, Hakko, Riipinen, & Riala, 2016; Kerr et al., 2015). Studies have been conducted on the relationship between the gut microbiome and weight in other populations but are lacking in regard to weight gain patterns in African American women during pregnancy and the potential role weight gain patterns play in their alarming

maternal-child health disparities. Morbidity and mortality of both mother and infant are higher among racial minorities, with the highest rates among African Americans (R. R. Davis et al., 2014; Gage, Fang, O'Neill, & DiRienzo, 2010; Gonzalez et al., 2009). Pregnancy-related deaths of African American women compared to Whites is 2-5 times higher, widening with increasing age (Creanga et al., 2015). Specifically, the pregnancy-related mortality ratio has increased from 10 per 100,000 live births in the 1990s to 16 deaths per 100,000 for the years 2006-2010, with persistent racial disparities due to increases in the incidence of chronic conditions, especially cardiovascular disease, among African American women (Creanga et al., 2015). Preterm birth is also approximately twice as high among African Americans as compared to Whites (de Jongh, Paul, Hoffman, & Locke, 2013; Whitehead & Helms, 2010; Wise et al., 2010). Contributors to these worse outcomes are multifactorial and poorly understood (Dominguez, 2011; Gonzalez et al., 2009; Main, McCain, Morton, Holtby, & Lawton, 2015). The guiding conceptual framework of this study posits that stress, nutrition, and health behaviors (operative within the context of African American women's lives) impact the maternal gut microbiome directly and via neuro-endocrine immune pathway activation. The gut microbiome, in turn, influences nutrient absorption, energy storage, mood and behavior which ultimately influence the pattern and degree of gestational weight gain.

The results of the analyses revealed daily kilocalorie intake was positively associated with energy expenditure (r = 54, p =.03). The amount of weight gain by mid-gestation was highly correlated with the total weight gained for the pregnancy (r= .75, p <.001), the trajectory of gain remained consistent for the duration of the pregnancy. The self-report of weekly MET-hours per week of combined physical activity was widely ranging with a median (IQR) of 243.99 (244.77). The chronic stress measures of

Childhood Trauma Questionnaire and Adverse Childhood Experiences Questionnaire-Household Dysfunction were used in this study. The household dysfunction portion of the Adverse Childhood Experiences Questionnaire was focused upon to better target specific exposures to chronic stress. There was a significant association with the ACE-HD and weight, in this case an inverse relationship with initial weight: (r = -.45, p =.02). There were no associations found among the chronic stress measures and FTB ratios at any time in pregnancy. Initial weight was also positively associated with initial FTB ratio (r = .42, p = .03), negatively associated with FTB ratio change during pregnancy (r = -.39, p = .05) and negatively associated with total weight gain (r = -.38, p = .05).

Various regression models were then assessed. The first standard multiple regression model evaluated the degree to which FTB at time 1 and ACE-HD predicted weight at time 1. A modest degree, 25%, of the total variance was explained by entering these two variables into the model with the adjusted R square = .25, F (2, 26) = 5.33, p = .01. A second standard multiple regression model evaluated how predictive weight at time 1, and ACE-HD were on weight at time 3. A moderate percentage of the total variance, 31.4%, was explained by the two variables entered into the model with the adjusted R square = .31, F (2, 26) = 6.95, p < .01.

The most critical element for these women was their initial weight in pregnancy, which in turn was negatively associated with childhood household dysfunction, total weight gain and change in FTB during the pregnancy. The initial weight was also positively associated with the initial FTB ratio. Further, the greater the initial ratio of *Firmicutes* to *Bacteroidetes*, the greater the drop in the ratio over the pregnancy. The FTB ratio becomes more dominated by *Bacteroidetes* and less dominated by *Firmicutes* with time in pregnancy. Those who start pregnancy at a healthy weight have a FTB ratio typically associated with other lean populations and tend to gain more gestational weight

than those at a higher starting weight, as is recommended (Bowers et al., 2013; Egshatyan et al., 2016; Li et al., 2013; Million, Lagier, Yahav, & Paul, 2013). The initial gut composition and degree of chronic stress from childhood household dysfunction together also are moderately predictive of a third of the variance in the total weight gained during the pregnancy. Finally, in this study, the trajectory of weight gain at the midpoint was highly predictive of total gestational weight gain ($r = .75$, $p < .001$.) This makes preconception counseling and close monitoring of weight gain during the first half of pregnancy key to optimizing initial weight, total weight gain and gut composition at the end of pregnancy. While such chronic stress has been found to lead to long-term gut dysbiosis in other populations, this association was not found in this study. AA women with a history of significant childhood household dysfunction appear to enter pregnancy at a lower weight than their counterparts with less exposure to childhood household dysfunction. The effects of this specific type of chronic stress may be missed if not targeted at the preconception or, the more likely attended, first obstetric visit.

Summary and Implications for Research and Practice

Clinically, it is known that a woman's health prior to and in early pregnancy are important considerations in determining her risk for adverse obstetrical outcomes (Hambidge et al., 2014; Teede & Moran, 2016). These study findings suggest that the initial gut composition and the interval weight gain by mid-pregnancy are significant in the change in gut composition throughout the pregnancy, as well. Given the racial disparities in preterm birth and fetal growth restriction, complications more likely among women at lower initial weights and with inadequate weight gain by mid-pregnancy, these study findings imply that close monitoring and creative counseling regarding preconception health and optimizing early gut eubiosis and pregnancy weight gain may help reduce the risk of these complications among AA women (Davenport et al., 2013).

Prepregnancy obesity prevalence has increased in the United States by an average of 0.5 percentage points per year, from 17.6% in 2003 to 20.5% in 2009 (Fisher, Kim, Sharma, Rochat, & Morrow, 2013; Lynch et al., 2014). Since prepregnancy overweight and obesity prevalence continues to rise, with up to nearly 80% of childbearing-age AA women, these findings highlight the need to address obesity as a key component of preconception care, particularly among this high-risk groups (Brooten et al., 2012; Cahill et al., 2018; Clifton et al., 2016; de Jongh et al., 2013).

This study is innovative in that it addresses at least four gaps in the literature with the potential to influence practice and reduce health disparities:

1. Although interval and total GWG clearly impact maternal, fetal, and infant outcomes, and although in non-pregnant populations the gut microbiome and weight gain are closely linked, little research to date has explored the association between the gut microbiome during pregnancy and interval or total GWG. Given the potential to modify the gut microbiome, a better understanding of its contribution to a healthy pregnancy is essential. There are few intervention studies with AA women examining the effect of probiotic foods or supplements on gestational weight gain patterns and obstetric outcomes and more are needed (Ilmonen, Isolauri, Poussa, & Laitinen, 2011; Luoto, Kalliomaki, Laitinen, & Isolauri, 2010).

2. Consistent with recommended frameworks for studying racial disparities, a requisite first step is to examine factors within the disparate group before examining differences among races, thus this study focused on the understudied population of AA women (Blackmore et al., 1993; Gareau, 2014; Jin & Flavell, 2013; Manco, 2012; Rowley, 2001). Expanding this study to include other racial groups is necessary to determine if these findings are unique to AA women or shared among other races.

3. As AA women have been under-represented in human microbiome studies, the findings of this study elucidate intra-racial differences in the gut microbiome in the population most likely to experience poor birth outcomes and unhealthy GWG (Gareau, 2014; Jin & Flavell, 2013; Manco, 2012). AA women who plan a pregnancy or those in early pregnancy would benefit from counseling to assess history of exposure to childhood trauma, promote healthy lifestyle choices to promote gut eubiosis and meet recommended weight gain parameters based on prepregnant BMI. If preconception care is missed, clinicians should ensure instruction and close surveillance of recommended weight gain, especially by the midpoint of pregnancy, to possibly mitigate the higher likelihood of inappropriate total weight gain and such adverse obstetric risks as preterm birth, fetal growth restriction, gestational hypertension and preeclampsia in this population.

Ultimately, severe maternal morbidity and mortality affects over 60,000 women and babies in the United States each year, and this burden is rising and is borne at a disproportionate rate among AA women (Creanga et al., 2015). This rise and racial disparity in morbidity and mortality are likely due to a combination of factors including advancing maternal age, prepregnancy obesity, preexisting chronic medical conditions and operative deliveries (Creanga et al., 2015; Dominguez, 2011), and, based on this study, maternal weight gain during pregnancy. The societal and economic consequences of these adverse outcomes are extensive and include greater utilization of healthcare services, higher direct and indirect medical costs, prolonged hospitalization for mother and baby, and need for long-term rehabilitation (Creanga et al., 2015). It is my sincere hope that the findings of this study may one day serve to improve the care and obstetric outcomes of AA women and children.

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