CUMULATIVE LACTATION AND METABOLIC DISEASE IN AFRICAN AMERICAN WOMEN

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ABSTRACT

Ellen M. Chetwynd: Cumulative Lactation and Metabolic Disease in African American Women
(Under the direction of Alison Stuebe)

Current literature suggests that breastfeeding duration is inversely associated with maternal metabolic diseases such as diabetes and hypertension. These conditions disproportionately impact black women in the United States, yet breastfeeding prevalence is lower for black women.

We used the Black Women's Health Study (n = 59,001) to quantify the association between breastfeeding and hypertension (Aim 1) and type 2 diabetes (T2D) (Aim 2). We introduce the use of a nested case control design to reduce selection and survival bias. Specifically, we used density sampling, frequency matching controls to cases by questionnaire cycle and risk sets for age. Effect measure modification analyses were performed using likelihood ratio testing for age, parity, and body mass index at age 18.

In Aim 1, 12,513 hypertensive cases were matched 1:2 with controls (n = 37,539) while Aim 2 matched 4505 cases 4:1 to controls (n = 22,525). Breastfeeding was associated with T2D (OR 0.93, 95% CI: 0.85, 1.01) and weakly associated with incident hypertension (OR 0.97, 95% CI: 0.92, 1.02). The association was strongest for women in their forties for both hypertension (OR 0.92, 95% CI: 0.85, 0.99) and T2D (OR 0.86, 95% CI: 0.74, 1.00). As breastfeeding duration increased, rates of both hypertension and T2D decreased in multivariate models (*P* for trend <0.01). For both hypertension and T2D, the strongest associations were present for women who had a normal BMI and fewer children. The outcomes estimates for our study were farther

from the null than previous studies using Cox proportional hazard models, suggesting a reduction in selection bias. They are also closer to the null than previous studies using cross-sectional or retrospective models, suggesting a reduction in survival bias.

Our results extend the existing research by providing outcomes in a high priority group of women previously under-represented, as well as introducing methodology to this area of research that has the potential to reduce bias in future studies on breastfeeding and metabolic health. Our outcomes support the need for inclusive messaging in public health and medical care encouraging breastfeeding for all women, particularly those at risk for T2D or hypertension.

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LIST OF ABBREVIATIONS

BWHS	Black Women's Health Study
CI	Confidence Interval
Hg	Mercury
HR	Hazard Ratio
mPINC	Maternity Practices in Infant Nutrition and Care
NH	Non-Hispanic
NHANES	National Health and Nutrition Examination Study
NHS	Nurse's Health Study
OR	Odds Ratio
PRAMS	Pregnancy Risk Assessment Monitoring System
PROBIT	Promotion of Breastfeeding Intervention Trial
T2D	Type 2 diabetes
US	United States

INTRODUCTION

Lactation: it's foundational to our status as mammals; yet, it is also profoundly affected by cultural trends. During reproductive years, many women weigh the convenience of formula feeding against the health benefits of breastfeeding. Since its nadir in the United States (US) in the 1970s, the rates of breastfeeding have been steadily increasing, as has research on its benefits. While breastfeeding's health benefits are still debated in the popular literature, within the scientific community, there is broad recognition that breastfeeding is the healthiest choice for women and children, and that differential health outcomes for women who have breastfed persist for many years after weaning has occurred (1-3). As such, breastfeeding is an important health behavior that can influence progress towards equalizing health disparities across racial, social and cultural barriers. For breastfeeding to have a positive impact on the health of mothers and children, it needs to be implemented for only a short term period of time, making it a compelling tool in addressing health inequities for public health professionals.

Black women have higher rates of both hypertension and type 2 diabetes (T2D) and lower rates of breastfeeding than the general population in the US. This dissertation assesses the relationship between cumulative lifetime lactation and both hypertension and type 2 diabetes within the Black Women's Health Study, a cohort of 59,001 black women established in 1995. It utilizes a two paper format. Aim 1 estimates the association between breastfeeding and hypertension, and Aim 2 estimates the association between breastfeeding and type 2 diabetes. We use a statistical model designed to reduce selection and survival bias, confirming the results

presented in the existing literature, as well as addressing health inequities by reporting outcomeamong a robust cohort of black women, a high priority population that is underrepresented in the existing literature

CHAPTER 1: LITERATURE REVIEW

1.1 Hypertension

Blood pressure, or the pressure of the blood against the walls of the arteries, is a measure of the force of the heartbeat and the diameter and elasticity of the arterial walls. High blood pressure develops with age as the circulatory system loses elasticity; however, it can also be an indication that the heart is working too hard, increasing the risk of cardiovascular disease. High pressure is diagnosed when systolic blood pressure is ≥ 140 mm Hg (mercury) and diastolic blood pressure is ≥ 90 mm Hg.(4) It is a major treatable contributor to cardiovascular disease, which is the leading cause of death in the United States (5, 6). Hypertension affects nearly one in every three adults (28.6%); (7) however, among adults with hypertension, almost one in five (18.1%) do not know they have the condition, and only 53.3% are able to reach effective levels of control (5).

Black women have a higher age adjusted prevalence of hypertension (45.7%) than black men (43.0%) or white women (28.9%) (8). While treatment levels for hypertension are approximately equivalent across racial groups, effective blood pressure control is less commonly achieved for non-Hispanic (NH) black than for NH white women (8, 9) making the disease potentially more dangerous for black women than for women of other races. Because black women suffer higher morbidity from hypertension and cardiac disease earlier in life, they experience twice the productive life lost from the disease compared to white women (10)

1.2 Type 2 Diabetes

Type 2 diabetes (T2D) accounts for 95% of diabetic disease in the US. It typically begins when the cells of the muscles, liver and fat lose their ability to respond to insulin effectively. As the need for insulin increases, the beta cells in the pancreas become unable to keep up with demand. This eventually leads to high blood glucose levels. Diagnosis of T2D is accomplished by a measurement of glucose or glycohemoglobin in the blood (11). In the US, 29.1 million, or 8.4% of adults have diabetes. Like hypertension, diabetes is more prevalent in black women (9.9%) than in black men (9.2%) or white women (5.3%) (12). Black women with diabetes experience delayed recognition, less effective treatment, and greater morbidity and mortality from the disease than white women (13-15). NH black adults with diabetes are 1.5 times more likely to be hospitalized, and 2.3 times more likely to die from diabetes than their NH white counterparts (16).

1.3 Breastfeeding

Breastfeeding, in its simplest form, is the nourishment of a human infant or child at the breast. Human milk provides all the essential calories, minerals, and nutrients necessary for optimal growth, health, and development (17). The process of milk production begins during pregnancy when levels of prolactin, the primary hormone responsible for milk secretion in the glandular tissue of the breast, begins to increase. Full lactation does not occur until after pregnancy because of the blocking action of progesterone, produced by the placenta. With birth, levels of circulating progesterone drop precipitously, causing the onset of milk secretion (lactogenesis II). Hormonal regulation of this process ensures that lactogenesis II occurs at the end of pregnancy whether or not a woman chooses to breastfeed. After approximately a week, the hormonal regulation of milk production is gradually replaced by autocrine regulation

(lactogenesis III), which is driven by removal of milk from the breast. If a mother chooses not to breastfeed, and thus is not providing any stimulation to the breast, milk production will gradually cease over a period of days to weeks (18).

It is this autocrine regulation that allows women to choose whether they will breastfeed, the proportion of breastfeeding to formula feeding they prefer, and how long they choose to breastfeed their child. All health organizations recommend exclusive breastfeeding for the first six months of life (19), followed by breastfeeding as a part of the complete diet for a year or more (20) or two years or more (21).

The prevalence of breastfeeding initiation in the United States is lowest among NH black women (66.4%) compared with NH white (83.0%), Hispanic (82.4%) and NH Asian (83.2%) women (22). While these percentages indicate that racial differences in breastfeeding initiation and prevalence still exist, there has been some recent improvement in the disparities between black women and other racial groups. In 2008, the rate of initiation of breastfeeding in NH black women was 11.5% higher than initiation rates in 2000, while among NH white women, the increase was only 3.4%. At 6 months, the prevalence of breastfeeding was 13.2% higher in 2008 compared to 2000 in NH black women, while among NH white women, rates were only 8.4% higher. At 12 months of breastfeeding duration, the differences in prevalence between 2000 and 2008 were very similar (6.2% higher in NH black women and 7.2% higher in NH white women) (23). This demonstrates an increasing initiation and duration of breastfeeding among black women, especially up to 6 months.

While continued advocacy and support is needed to eliminate racial disparities in breastfeeding rates, one of the pivotal questions in understanding the disparities in breastfeeding between black women and other racial groups is whether the difference is due to the lower

sociodemographic standing of the African American community overall in the US, or whether the difference is instead driven by social constructs or cultural norms (24). The experience of breastfeeding is assumed to be biologically similar for all women; however, differences in cultural norms and perceived barriers along racial lines have been demonstrated (25-27). Cultural differences, however, don't exist in isolation. Support of breastfeeding from organized health care institutions has been shown to vary depending on race, with black women receiving care that equates or favors formula use over breastfeeding in WIC populations (28-34). For example, a recent study using the 2011 Maternity Practices in Infant Nutrition and Care (mPINC) questionnaire assessed breastfeeding supportive hospital practices. The study authors found that hospitals with catchment areas that had higher percentages of black residents were less likely to have implemented breastfeeding supportive practices (35).

While social constructs are important drivers of breastfeeding duration, sociodemographic differences also play a role (36-39). Several researchers have sought to compensate for the preponderance of studies focused on low income women when assessing racial disparities in breastfeeding. In these studies, sociodemographic factors moderate the relationship between race and breastfeeding duration. For example, in a nationally representative dataset, the Third National Health and Nutrition Examination Questionnaire (NHANES), examined both racial and socioeconomic disparities in breastfeeding rates. While there were disparities in breastfeeding prevalence by sociodemographic group for all races, they were most marked for black women, suggesting that sociodemographic status has a stronger impact on breastfeeding prevalence among black women than among women of other races (36). This is supported by two additional studies that assessed the amount of change that occurred by race with breastfeeding interventions. The study authors found that while change varied by race;

within racial groups, breastfeeding interventions were more effective in urban populations (37) and among women who had higher education (39).

An interesting approach taken by Lundquist et. al. used the Pregnancy Risk Assessment Monitoring System (PRAMS) data to assess the difference in breastfeeding rates between women who were in the military compared with civilian populations. They hypothesized that because the military provides women with stable employment, equal educational opportunities, and universal healthcare including breastfeeding support by pediatric care providers, racial disparities in breastfeeding rates would be minimized. They found that breastfeeding was more prevalent among all women in the military setting than the civilian population, and that the 'black-white gap' in breastfeeding duration was significantly reduced among military affiliates (38).

In summary, both cultural and sociodemographic factors contribute to racial disparities in breastfeeding rates between black women and women of other races in the United States. While the reasons for these disparities are complex, there has been some improvement recently in both understanding the disparities, and in reducing the gap between different racial groups.

1.4 Breastfeeding Research

When assessing breastfeeding for the purposes of research, several dimensions can be measured. Breastfeeding can be either exclusive (the only form of nutrition a child receives), or it can be a portion of a child's nutrition (mixed feeding), supplemented by either formula or other foods (1, 40). When breastfeeding is mixed, it can vary by intensity(1, 41); for instance, a mother of a 4 month old might only breastfeed her child to sleep, which would be a very low intensity of breast milk production, while another mother with the same age child might supplement her

child with a bottle of formula once a week. These two women would have very different measures of breastfeeding intensity, but in a measure of breastfeeding duration at 4 months, they would both be treated equally as having prevalent breastfeeding at that time point.

In breastfeeding research, duration of lactation is typically self-reported. Analysis of duration is accomplished using mean length of any breastfeeding or the presence of breastfeeding at designated time points after birth. The literature suggests that recalled breastfeeding is reasonably reliable, even decades after the index birth. In one study, women as distant as fifty years from birth (69-79 years of age) were asked to recall breastfeeding they had recorded prospectively while they were nursing. In this study, women who had breastfed for a short period of time tended to over-report their breastfeeding duration, while those women with a longer duration of breastfeeding were more likely to report accurately. However, when they misclassified their duration, women with a longer breastfeeding duration tended to under report the length of time they had breastfed. Overall, women were accurate to within +1/-1 three month category of duration 89% of the time (42). Natland et. al. (43) found similar results in a study of women 20 years after birth, with 85% reporting duration accurately within +1/-1 three-month interval. And finally, a study assessing recall reliability after 1 to 3.5 years found modest over-reporting of approximately one month (44).

Recalled breastfeeding duration can be ascertained for each birth, or as an overall lifetime exposure. Cumulative lifetime lactation is typically ascertained by asking women how long they have breastfed all of their children combined. This measure of breastfeeding does not allow for individual measures to be associated with each pregnancy, but instead gives a single value that represents lifetime breastfeeding duration. Cumulative lifetime lactation is typically the variable researchers use when studying the long term associations between breastfeeding and the

development of diseases or conditions later in life. Cumulative lactation data are either used as a whole, or an average breastfeeding duration is calculated by dividing the full cumulative lactation by parity.

Cumulative lactation and parity: When assessing for a dose response relationship between breastfeeding and outcome disease, it is important to take into account how breastfeeding was measured. Cumulative lactation does not account for parity, thus it typically increases as the number of children increase, introducing the possibility that parity may modify the outcome estimate.

Within the studies assessing the relationship between breastfeeding and hypertension or T2D, some authors provided outcome measures using both cumulative lifetime lactation and breastfeeding per birth, allowing for an assessment of how the measures differ. We would expect, for example, that the benefits of breastfeeding for mean duration per child would occur at roughly half the number of months needed for the same level of benefit from cumulative lactation with two children; however, among the studies in this literature, the results were mixed.

Out of the three studies that present both mean breastfeeding duration per birth and cumulative lactation, two studies found the expected relationship between these two measures for breastfeeding exposure and outcome disease. For instance, in a study of Korean women (45), the mean number of children ranged from 1.5 children for women who breastfeed for 1-12 months to 2.1 children for women who breastfed for > 24 months. As expected, the odds of hypertension for a mean duration of 1-9 months of breastfeeding per child (OR 0.90-0.93) were similar to the estimates for cumulative lifetime lactation durations at 1-18 months (OR 0.90-0.93). The same pattern was present in an analysis using the Shanghai Women's Health Study (46). In this dataset, most women had one child (54.4%) and the mean number of months of

breastfeeding per child was 7.2, while the mean number of months of cumulative lactation was 14.6 months. Among women with confirmed cases of T2D, women with >0-0.49 years of breastfeeding per child had similar disease outcome (OR 0.94, 95% CI: 0.73, 1.21) as women who breastfed for >0-0.99 years of cumulative lactation (OR 0.91, 95% CI: 0.73, 1.13). However, in a study using the '45 and Up' dataset from Australia (47), this same pattern was not evident. Instead, the rates of disease outcome were similar in categories of months of breastfeeding duration regardless of whether the breastfeeding exposure measure was mean breastfeeding per child or cumulative lactation.

Another comparison of cumulative lactation and mean duration per child is demonstrated in outcomes from the Nurse's Health Study estimating the association between breastfeeding and hypertension. In this study (48), outcome estimates were analyzed using a dataset restricted to women's first children and then repeated using a variable for mean breastfeeding per child. By assessing cumulative lactation for first children, cumulative lactation was essentially converted into a variable for breastfeeding per child. While we would anticipate that these estimates would be similar given that they both assess the relationship between breastfeeding for one child and outcome disease; in fact, breastfeeding for the first child had a stronger association with hypertension than mean breastfeeding duration, indicating that research using only first births could be confounded by factors independently associated with first births, such as age.

All four of these studies used parity as a covariate to control confounding, yet the results indicate continuing variations in outcomes by parity. These mixed results underscore that including parity as a covariate may not adequately adjust for confounding by parity when cumulative lactation is the variable used to measure exposure to breastfeeding.

Finally, one of the remaining questions about cumulative lifetime lactation as a measure of breastfeeding exposure is whether its inability to measure consistency of breastfeeding, or whether a mother of three children breastfed one child for 18 months or all three children for six months each, is important. We can assess this by using a study by Schwarz et. al. (49) in which a measure of breastfeeding consistency was used in addition to cumulative lactation. Questions on cumulative lactation were asked separately for each child. Women who breastfed all of their children for $\geq$ 1 month were categorized as having consistent breastfeeding practices, while those who did not breastfeed all of their children for a minimum of one month were categorized as having inconsistent breastfeeding. While women who never breastfed were more likely to develop T2D than women who either consistently (OR 1.41, 95% CI: 1.03, 1.92) or inconsistently breastfed (OR 1.42, 95% CI: 0.96, 2.09), consistent breastfeeding was not more protective than inconsistent breastfeeding (OR 0.80, 0.53, 1.21).

In summary, cumulative lactation is a reasonable variable to use in assessing the relationship between breastfeeding and long term outcomes; however, the relationship between parity and cumulative lactation should be carefully considered and multiplicative as well as additive methods should be considered to address the relationship between parity and cumulative lactation in research models.

*Effect Estimates-hypertension***:** There have been eight studies that estimate the association between breastfeeding and hypertension, using hypertension as one of the primary outcomes of interest (45, 47, 48, 50-54) and another six studies that assess the association between breastfeeding and hypertension as a component of metabolic disease (55-60). All but one of the studies (53) found a protective relationship between breastfeeding and rates of hypertension.

This study (53), using the Promotion of Breastfeeding Intervention Trial (PROBIT) data, randomized hospitals and their affiliated outpatient clinics to receive a breastfeeding promotion intervention or usual care. They were able to measure large contrasts in breastfeeding duration and exclusivity between the two groups. The researchers argue that randomization more effectively controls the social patterning inherent in both breastfeeding and weight retention than observational trials. Thus, the contradictory outcomes of the PROBIT study could be demonstrating that breastfeeding is one of a cluster of healthy lifestyle choices that reduce the risk of metabolic poor health, confounding the results of observational studies. However, women needed to have initiated breastfeeding to be included in the PROBIT trial, making it the only study assessing the relationship between breastfeeding and hypertension that did not include women who exclusively formula fed. Additionally, duration was not randomized, but chosen by women in the more supportive setting of the intervention arm, leading to similar rates of breastfeeding in the intervention and control arms, and limiting the ability of randomization to control for social patterning thus reducing the power to detect a difference in outcome rates.

The exclusion of formula feeding women in the PROBIT study also forced the use of short breastfeeding as the referent category when assessing duration, reducing the statistical power to detect differences in outcome measures. Differences in hypertension between breastfeeding and non-breastfeeding women were already present in the chosen referent category used in their trial, which was 1-3 months of cumulative lifetime lactation. For instance, Lupton et. al. (47) found that the odds of hypertension were lower (OR 0.88, 95% CI 0.63, 1.24) at 1-3 months compared to women who had never breastfed. Thus, while the PROBIT study could be shedding light on confounding that is not adequately controlled in observational trials, the

possibility that the contradictory results are due to reduced analytic power to detect differences cannot be eliminated.

Effect Estimates-T2D: There are nine studies that have looked at the relationship between lactation and T2D (46, 49, 50, 54, 61-65). Two of these have considered the effect of lactation in women with a history of gestational diabetes (61, 65). All the studies have shown reduced rates of T2D in women who had a history of any breastfeeding compared to those who did not breastfeed at all; however, there is not consistency regarding differential outcomes by length of cumulative lifetime lactation. It may be that the breastfeeding categories chosen contribute to the differences between studies. The strongest dose responses were demonstrated by Stuebe and Villegas who found differences in categories that varied markedly from the control group. For instance, Stuebe et. al. (62) found the strongest inverse association among women who lactated for more than 23 months (HR 0.88; CI: 0.78, 1.00) and Villegas et. al. (46) among women who lactated for 36 months or more (HR 0.73, 95% CI: 0.58, 0.91). On the other hand, Schwarz et. al. (49) found no difference in women who nursed 1 to 6 months per child (OR 1.04, 95% CI: 0.72, 2.04) compared to the referent of 6 months or more. In an effort to define the dose response relationship between breastfeeding and T2D more clearly, Aune et. al. combined several of the existing cohort studies in a meta-analysis. A linear dose-response relationship was found, indicating that there was a 9% reduction in relative risk for T2D for each 12 month increase in lifetime duration of breastfeeding (66). Thus, while the results vary between trials, the preponderance of evidence indicates that an inverse dose response relationship between breastfeeding and T2D is present.

1.5 Pregnancy, Breastfeeding, and the Reset Hypothesis

Both hypertension and T2D are included in the clustering of health conditions that make up 'metabolic syndrome' (67). While genetics and aging play a role in the development of metabolic conditions, obesity is central to the disease process for both hypertension and diabetes (10, 14, 67). Chronic obesity produces a low grade activation of the immune system triggering inflammatory pathways that dysregulate physiologic maintenance of insulin and leptin sensitivity while increasing immune factors such as cytokines and leukocytes. These pathways together contribute to the development of cardiometabolic diseases by causing end organ damage (68). This end organ damage, in turn, causes physiologic dysfunction in the form of hypertension and type 2 diabetes.

For women, pregnancy introduces increased risk factors for metabolic morbidity and mortality due to the normal changes in metabolic function it incurs. These include increased central adiposity (69), an elevation in blood pressure (70), an atherogenic lipid profile, and insulin resistance (71). Lactation may be an important component of a woman's return to her pre-pregnancy metabolic state. In the postpartum period, lactating women have less inflammation, greater insulin sensitivity (72, 73), beneficial changes in cardiac output (74), and less atherogenic blood lipid profiles compared with formula feeding mothers (55). As a woman breastfeeds, she mobilizes accumulated stores of visceral fat for the caloric needs of milk production (75). If she does not breastfeed, it is possible that her metabolic processes readjust more slowly to baseline levels, and she may have more difficulty in mobilizing fat accumulated during pregnancy (76). Stuebe and Rich-Edwards call this 'The Reset Hypothesis', positing that lactation serves to 'reset' the metabolic processes more efficiently in breastfeeding women, affecting weight retention as well as contributing to improved metabolic functioning later in life.

1.6 Obesity

Pregnancy and the postpartum period are times of high risk for increasing baseline BMI. Increasing gestational weight gain is a good predictor of increasing postpartum weight retention; however, it is the most predictive of postpartum weight retention in women who are not obese prior to pregnancy (77-79). Conversely, BMI prior to pregnancy is predictive of gestational weight gain (78, 80-85), at least in the first pregnancy (86-88). Women who are obese prior to pregnancy have higher rates of gestational weight gain. Rates of obesity are higher in black women (56%) than in the general US population (35%) (89) and black women retain more weight after pregnancy than white women (90-93). Thus higher pre-pregnancy obesity, as well as higher gestational weight gain among African American women, both have the potential to contribute to increased weight retention after pregnancy.

Qualitative research on obesity and pregnancy weight gain in the African American population has described reinforcement of 'eating for two' during pregnancy, as well as a cultural concern that vigorous exercise could harm the growing child (94, 95). However, cultural norms cannot be considered in isolation. In order to understand trends in obesity among black women, inequalities in income, access to quality education and health care, as well as stable and affordable housing with access to healthy foods and safe places to exercise must all be considered (96). This is demonstrated when sociodemographic covariates in predictive models attenuate racial differences in health outcomes. For instance, in a recent study by Endres et. al. (93), African American race was initially a strong predictor of postpartum weight retention (aOR 2.21, 95% CI: 1.31, 3.71), but was attenuated with the addition of further sociodemographic variables (aOR 1.75, 95% CI: 0.95, 3.23) and behavioral variables (aOR 1.51, 95% CI: 0.81,

2.81) (93). BMI is thus an important confounder to consider in the association between breastfeeding and both T2D and hypertension, particularly among African American women.

1.7 Age

Age is an important modifier of the relationship between breastfeeding and metabolic disease. Blood pressure naturally increases with age in industrialized countries, from childhood through adulthood (97). As a result, prevalence of unhealthy blood pressure, or hypertension, also increases. By age 75, most US women (78.5%) have hypertension. According to the Framingham Heart Study, if a woman is free of hypertension at age 65, she has a 91% risk of developing it during the rest of her lifespan (98). The risk of T2D also increases with age, particularly after age 45. While there is a natural progression toward higher blood pressure, T2D rates increase with age primarily due to secondary factors, such as weight gain and decreased physical activity. The majority of T2D (63%) is diagnosed between ages 40 and 64 (99). Given the rising risk of both outcomes with advancing age, we therefore included an assessment of age as a modifier in both aims.

1.8 Knowledge Gaps

Few of the previous studies on breastfeeding and hypertension (54) or breastfeeding and T2D women (54, 100) include robust numbers of black women. While evidence exists on the relationship between breastfeeding and metabolic syndrome in majority white populations, there are significant differences documented between rates of metabolic syndrome in black and white women (101). We also know that obesity rates are rising faster in the African American population than they are among whites (102) and central adiposity is one of the primary risk factors for both diabetes and hypertension. In previously studied cohorts where black women make up a minority of the study population, all of these differences, as well as the social,

cultural, and economic racial disparities in the US, can confound the relationship between breastfeeding and metabolic health (103). By using the racially defined dataset available to us through the BWHS, we will be able to disentangle racial differences from the primary relationship of interest. An analysis of all African American women necessarily provides more commensurate measures of cultural factors than a dataset of mixed race. And finally, precision around our measures of association for black women will be higher in this analysis than other analyses simply because of the number of black women available in the dataset. The proposed analysis of exclusively African American women provides a rich opportunity to extend the existing research into this high priority population.

Women who are obese have a higher risk of developing both hypertension (6) and type 2 diabetes (104). Obesity has also been shown to be associated with lower rates of breastfeeding (105-110). Interestingly, several authors have found racial differences in the relationship between obesity and breastfeeding (110-112). For example, one study found a 3% lower chance of breastfeeding for each unit increase in prepregnancy BMI for white women (HR 1.03, 95% CI: 1.01, 1.04), but not among black women (HR 1.00, 95% CI: 0.98, 1.02) (110). The same pattern was demonstrated when comparing Hispanic women to black women. Obese Hispanic women were more likely to discontinue breastfeeding in the first six months after birth (RR 1.5, 95% CI: 1.1, 2.1), but obese black women were not, (RR 1.04, 95% CI: 0.78, 1.39) (111). In contrast with these studies, in our earlier work with this dataset, we found lower breastfeeding rates among obese black women than black women who were of normal weight.

The proposed moderation analysis of BMI will address several specific gaps in the literature: 1) there is not a study of the relationship between breastfeeding and T2D that assesses BMI as a moderator, 2) among the studies that assess breastfeeding and hypertension, results

assessing BMI as a moderator were mixed and in need of further exploration, and 3) the studies that have assessed BMI as a moderator have not had sufficient numbers of black women to determine whether the existing hypothesized racial differences are relevant.

1.9 Significance

Rates of metabolic diseases such as hypertension and type 2 diabetes and associated obesity are rising in the US population. There is a disproportionate burden of these cardiometabolic disease states among black women. It is therefore a critical public health priority to evaluate health behaviors that have the potential to reduce rates of these important metabolic conditions. Addressing breastfeeding as a preventative health behavior is particularly compelling because it is a modifiable health behavior requiring a time-limited commitment and is associated with differences in metabolic health outcomes that last well beyond weaning. Quantifying the association in black women, who have a lower rate of breastfeeding but higher rates of hypertension, T2D, and obesity, provides an important opportunity for informing public health messages specific to this high priority population.

1.10 Specific Aims

Aim 1: To estimate the association between cumulative lifetime lactation and reduced onset of hypertension in African American women. A nested case cohort model will be used to quantify the association between cumulative lifetime lactation, collected retrospectively from approximately 40,000 parous women who were < 40 with their last pregnancy, and hypertension from ages 40 to 65. The resulting odds ratios will be assessed for effect measure modification by obesity at age 18.

Hypothesis 1a: Longer cumulative lifetime lactation is associated with lower rates of hypertension.

Hypothesis 1b: The association between cumulative lifetime lactation and onset of hypertension will be stronger in women who are not obese at age 18.

Aim 2: To estimate the association between cumulative lifetime lactation and reduced onset of type 2 diabetes in African American women. A nested case cohort model will be used to quantify the association between cumulative lifetime lactation, collected retrospectively from approximately 40,000 parous women who were < 40 with their last pregnancy, and type 2 diabetes from ages 40 to 65. The resulting odds ratios will be assessed for effect measure modification by obesity at age 18.

Hypothesis 2a: Longer cumulative lifetime lactation is associated with lower rates of type 2 diabetes.

Hypothesis 2b: The association between cumulative lifetime lactation and onset of type 2 diabetes will be stronger in women who are not obese at age 18.

CHAPTER 2: APPROACH

2.1 Population

The *Black Women's Health Study* is a prospective cohort study initiated in 1995 by researchers from Boston University and Georgetown University's Lombardi Comprehensive Cancer Center in response to the scarcity of black women in cohorts used to study women's health. The research was funded by the National Institutes of Health. Participants for the Black Women's Health Study (BWHS) were recruited through the National Education Association, the personnel office of the national government, Essence magazine, the Black Nurses' Association, and friends and relatives of respondents. Follow-up is ongoing, with 59,001 participants receiving questionnaires every two years. As of 2013, 87% of person-time was complete. BWHS participants represent all quadrants of the United States. The most common medical conditions reported at baseline were uterine fibroids, hypertension, high cholesterol, and diabetes (113). Respondents' deaths are reported through friends, relatives, the postal service, and the National Death Index. For women who give consent, illnesses are classified through the request for, and review of, medical records.

2.2 Analytic Sample

To be eligible for inclusion in Aim 1 or Aim 2, participants must have completed the cumulative lactation questions on either the 1995 or 2011 questionnaire. The analytic sample for this dissertation consists of all parous women for whom there are complete data on cumulative lifetime lactation and outcome disease. A detailed description of the covariates is included in

'Covariates', but are briefly described here to illustrate their relation to analytic sampling strategies.

Lactation exposure was reported cumulatively in 1995 and 2011 (Figure 2.1). Since the data were collected cumulatively, we could not verify whether breastfeeding was consistent (i.e. had a similar duration for each pregnancy), nor can we determine whether it occurred with each pregnancy. Consequently, lifetime lactation was maintained as a cumulative value inclusive of all the months of breastfeeding a woman had been exposed to in her life, rather than estimating a mean lactation per birth. In order to minimize recall bias, the 1995 baseline question on cumulative lactation was used for women who had their last pregnancy before 1995, and the 2011 questionnaire for women who had a pregnancy in or after 1995.

Disease onset was reported differently depending on whether it occurred prior to the 1995 baseline questionnaire or in one of the subsequent questionnaires. If it occurred prior to cohort entry (1995), women reported decade of onset (for example, between ages 30-39) whereas those who developed disease after 1995 reported whether disease had occurred since the previous biennial questionnaire.

Study Design: One of the complexities inherent in assessing the relationship between breastfeeding and outcome diseases (Figure 2.2) is designing a model in which the exposure dependably precedes the onset of disease without inducing selection bias. A common approach to this in longitudinal cohort studies has been to measure exposure retrospectively, at the time of a baseline questionnaire, and incident disease from baseline questionnaire onward using a Cox proportional hazards model (45, 46, 48, 54, 62, 114). While this method compares disease outcome to like risk sets based on age or time, it may induce selection bias by excluding study participants with prevalent disease at cohort entry.

In a typical model utilizing this statistical method, excluded participants with prevalent disease were not cohort participants at the time of exposure; however, exposure, in essence, marks the entry of all participants into the study timeline. This inference is based on the analytic use or exclusion of the exposure variable based on the outcome disease. If a participant does not develop disease, she would be included in the analytic population; however, if disease occurs before baseline, she is excluded. In this way, exposure data are differentially utilized based on the outcome. The time between exposure and outcome thus becomes immortal time, or time during which the participant cannot develop disease and remain in the analytic sample (115).

One alternative to this conundrum is to utilize a cross sectional design (47, 50, 56). This type of analysis treats all cases of disease similarly, whether they occurred prior to, or after, cohort entry, thus utilizing all cases of disease. To operationalize this study design, women who develop disease at any age or point in time are compared to women who are disease free at the end of the study. While correcting for the bias created by differential treatment of exposure based on outcome, this design compares women who reach the end of the study period without disease to those who developed disease at every age and time equally. Women who reach the end of the study without disease, particularly a very common condition such as hypertension, are thus a disproportionately healthy population compared to the cases. While correcting for selection bias, this model does not allow the comparison of cases to like risk sets by age or at set points in time.

In order to both use all the cases present in the cohort, and compare women with disease to women in like risk sets, we used a nested case control design with cases frequency matched to controls within age categories according to the 2-year period in which the case was diagnosed. Controls were sampled with replacement and were eligible to become a case in a later 2-year period allowing them to represent the exposure profile of the baseline population (116).

Case Control Sampling Design: The primary challenge in carrying out this study design was the difference in the data on disease onset between women who developed disease from 1995 onward and those who developed disease before the study baseline. For women with disease onset after the baseline questionnaire, the age of each participant at disease onset could be derived by assigning the median age between the disease onset and immediate previous questionnaires. For example, if a woman with a new onset of hypertension was 52 when she completed the 1997 questionnaire, we knew her hypertension developed between ages 50 and 52. She would be assigned the median age, 51, as the age when her hypertension developed. Age categories for control selection were as follows: 40-44, 45-49, 50-54, 55-59, and 60-65. Her risk set would then include all women who were without hypertension in 1997 and were between the ages of 50 and 54.

If, instead, hypertension occurred prior to the baseline questionnaire, we had less information. For instance, if a woman was 52 at the time of the baseline questionnaire in 1995 and had an outcome disease, she would be asked 'if a doctor ever told [her] that [she] had [hypertension] (not during pregnancy)' and the 'age at which it was first diagnosed' with response options for: under 30, 30-39, 40-49, or $\geq$ 50. If she responded that her hypertension developed between ages 40-49, we still would not have enough data to select her control from a 5 year risk set. In order to assign an age of onset of hypertension within each category of age using the available data, the following formula was used: let A = age, C = the age category for disease onset, Y = the youngest age in each age category of disease onset, and B = the participant's age at baseline. The assigned age was defined as:

$$A = Y + (\tfrac{1}{2} (B - Y))$$

For the participant who was 52 at baseline with hypertension onset between 40-49, age would be assigned as follows: B = 52; Y = 40 and age (A) = 40 + (½ (52-40)) = 46. For instances in which ½ (B - Y) > 10, the age at the midpoint of the decade of onset was assigned (i.e. if a participant was age 65 at baseline with prevalent diabetes diagnosed at age 40-49, the assigned age at onset would be 45.

To correctly assign risk sets to participants, one further modification was enacted. We wanted each risk set to include women who were disease free and were her age at the questionnaire cycle in which she reported disease, but if we used the questionnaire from 1995 to obtain risk sets for all women with disease onset prior to 1995, her risk set would be artificially restricted. If the risk set for our example participant was selected based on the age at which she *reported* hypertension (age 52 in 1995), her risk set would include women who had not developed hypertension prior to cohort entry; however, women who were in her risk set at the time she developed disease, but themselves developed disease prior to cohort entry (for example, age 50), would no longer be eligible for sampling. We addressed this issue by deriving risk set samples for each 2-year interval from 1975, when the oldest women in our sample became eligible for selection, through 1995. We used these 2-year risk sets to select controls for each case.

Temporality: The BWHS recruited women who were between the ages of 20 and 70. The median age at cohort entry was 38; therefore, some women were past their reproductive years at cohort entry, and some gave birth after becoming members of the cohort. Cumulative lactation was assessed in 1995 and again in 2011; therefore, it was not linked to a particular age or year. In order to control for temporality, (i.e. to ensure that exposure occurred before disease onset), we

limited our analysis to women who were < 40 at the time of their last birth and reported disease onset at or after age 40.

Exclusion by age: The risk of newly acquiring hypertension or type 2 diabetes peaks between ages 45 and 65. (Figure 2.3).(12, 117). We limited the dataset to women who were ≤ 65 at the age of disease onset to focus on middle aged onset of hypertension and T2D (Figure 2.4).

2.3 Exposure Assessment

Women in the Black Women's Health study have been giving birth since 1941. Between 1941 and 2011, breastfeeding practices have changed dramatically. The nadir in breastfeeding initiation occurred in the early 1970s when breastfeeding initiation rates dropped as low as 22 % in the US population (118). Currently 80% of mothers in the United States initiate breastfeeding. Overall, the initiation rate in the BWHS is 61% (Figure 2.5). However, since this figure represents women initiating breastfeeding from 1941 through 2011, it is not an accurate representation of how women in the BWHS compare to women exposed to different cultural trends over time. To more accurately assess breastfeeding rates in this population, an average breastfeeding rate per pregnancy was calculated (cumulative lactation/parity) bifurcated at 1995, based on the year of each woman's last birth. When the data for women giving birth to all their children before 1995 were compared to women who gave birth to their last child in 1995 or later, a different pattern emerged.

The median year for giving birth in this cohort prior to 1995 was 1978. In 1978, the US breastfeeding rate of any breastfeeding was 44.3% (national data by race not available) (118) and the initiation rate for women in the BWHS prior to 1995 was 57%. The closest national statistic for black women specifically was in 1973, when only 15% of NH black women were initiating

breastfeeding (119) . After 1995, the breastfeeding initiation rate for the BWHS was 80%, which is comparable to current overall trends in the United States, and higher than rates of black women specifically, suggesting that women in the BWHS continued to breastfeed at higher rates than black women overall, and similarly to the general population.

Women in the Black Women's Health Study were asked about cumulative lifetime lactation or 'How many months, in total, have you breastfed your children' in 1995 and again in 2011. Response options in 1995 included 'no children', 'never tried', 'tried but couldn't', and the following categories for months: < 1, 1-3, 4-6, 7-11, 12-17, 18-23, 24-35, 36-47, and $\geq$ 48. In 2011, in response to the same question, participants reported the number of months of cumulative lactation as an integer. To align categories across these two variables, we tested internal validity among women who responded in both 1995 and 2011 (Figure 2.6). Based on these results, we collapsed 1995 responses for 'never tried' or 'tried but couldn't' to match 'none' from 2011, and coded women reporting 'less than 1 month' in 1995 as '1-3 months' from 2011. We additionally collapsed breastfeeding categories over 24 months due to sparse data. For our analysis, we used the following categories, in months: none, 1-3, 4-6, 7-11, 12-17, 18-23, and $\geq$ 24.

Because the categorical boundaries for the cumulative lactation variable were set in the 1995 questionnaire, it was not possible to adjust them for more direct comparison to previous studies on breastfeeding and metabolic disease. It is noteworthy; however, and indicative of the need for consensus measures within the field of breastfeeding research, that the categorical boundaries for lactation vary considerably among cohorts as to how categories of breastfeeding duration were assigned.

While it was necessary to collapse the 1995 questionnaire with the 2011 questionnaire to create a consistent variable for both years, the information contained in the category for 'Tried but couldn't' was considered important information because it could contain information. Women who tried to breastfeed but could not might have experienced lack of social support, lack of medical support for breastfeeding, or postpartum complications for mother or child. We therefore used this variable to conduct sensitivity analyses in both papers.

2.4 Outcome Assessment

Hypertension in the BWHS is a report of antihypertensive drug use, or report of physician diagnosed hypertension together with use of diuretics in the same questionnaire cycle. Validation of this measure in the Black Women's Health Study has been reported previously in a subset of 139 women in which hypertension was confirmed in 99% of cases (120). The overall prevalence of hypertension in the BWHS was 38% by 2011, similar to the 42.1% of adult women with hypertension in the US (8).

A diagnosis of diabetes was determined by self-report alone (see section on 'Case Control Selection' for exact wording of questionnaire question), without report of medication use. While this question does not differentiate between type 1 and type 2 diabetes, elimination of diabetic cases that developed at ages < 40 effectively limited the diabetic population to only cases of type 2 diabetes (121-124). By 2011, 13% of women in BWHS were diagnosed with T2D, which is similar to the 13% (99) prevalence of T2D among black women in the general population. Self-reported diabetes was validated in the Black Women's Health Study population in 2011 by sending questionnaires to each participant's treating physician. From a sample of 656 randomly selected and consenting participants, 229 physicians returned questionnaires, and of those, 219 (96%) confirmed a diagnosis of diabetes (123).

2.5 Covariate Assessment

Covariates were selected from the BWHS questionnaires based on a review of the existing literature and directed acyclic graphs. Each variable was individually qualitatively assessed and missingness was considered. A subset of variables was selected for consideration in each model. These variables are listed below (exclusive of variables created for sensitivity analyses).

Covariates fall into four general categories: 1) structural variables, 2) metabolic risk factors and health behaviors, 3) demographic variables, and 4) concurrent metabolic diseases. Definitions, coding decisions, variable structure, biases and background literature are discussed below.

STRUCTURAL VARIABLES

Age *[categorical 40-44, 45-49, 50-54, 55-59, 60-65]:*

Age was used structurally to control temporality between exposure and outcome, as well as being used as a matching criterion for control selection.

Parity [1, 2, 3, 4+]:

Parity was defined as any pregnancy, including stillbirths. Parity was important to control when using cumulative lifetime lactation because parity, particularly high parity, is correlated with cumulative lactation, and thus parity may moderate the association between exposure and outcome. Categories above four were collapsed for ease of interpretation. Parity data were also used to derive year of first and last birth.

METABOLIC RISK FACTORS

BMI [<25 | 25 to 29.9 | 30 to 34.9 | 35 or more]:

Weight was measured at multiple time points and in multiple ways in the BWHS. At baseline, participants gave their height, weight, weight at age 18, and self-reported hip and waist measurements. With respect to hip and waist measurements, although abdominal fat is more predictive of metabolic disease, particularly among black women, there are significant concerns about the validity of self-measurement and self-report of these values. Weight gain during pregnancy was also considered, but due to its limited availability (asked in 1997, 1999, 2001, and 2003 only), it was not included as a covariate. Based on previous research using this dataset we know that most of the changes in BMI from age 18 to prepregnancy BMI were movement from a normal BMI into higher BMI categories, while obese and very obese teens at age 18 typically remained either obese or very obese women later in life. In a previous analysis of these data, BMI at age 18 was a stronger confounder of the relationship between pre-pregnancy diabetes and breastfeeding duration than BMI prior to pregnancy; thus, BMI at age 18 was chosen as the primary BMI variable for use in both models. Categories were created as defined by the World Health Organization (125). Categories were limited to normal weight (<25) and overweight/obese ($\geq$ 25) based on earlier analyses for two reasons: 1) at age 18, the data for overweight and obese were sparse, and 2) overweight adolescents were typically obese by the baseline questionnaire, suggesting that combining overweight and obese at age 18 would reflect their similar weight categories in adulthood.

Family History of Metabolic Disease [no | yes >50 | yes < 50]

At baseline, study participants were asked to report parental history of heart attack, stroke or diabetes. Response options were "No / Don't know", "Yes" and "Yes, before age 50."

This variable is at risk for misclassification because the positive responses were not mutually exclusive and the negative response included an option for uncertainty. Thus, a positive response may underrepresent parental history of metabolic disease. Furthermore, parental onset of these conditions after 1995 was not assessed. Regardless of these limitations, it was considered as a variable in each model because family history is a strong predictor of disease, and could provide control for lifestyle exposures during childhood.

Gestational Diabetes [no | yes]

Pre-eclampsia [no | yes]

History of pre-eclampsia or gestational diabetes: In 2009, participants were asked if they had ever had a pregnancy with pre-eclampsia or gestational diabetes. Women who were older at cohort entry may under-report true occurrence for two reasons. First, recall bias might disproportionally affect the births that occur prior to 1995; however, reliability studies demonstrate that women have good recall of pregnancy complications ($r = 0.74$), even when the event occurred 30 years in the past (126). Second, bias may occur due to differences in reporting and treating these diseases historically. In particular, practices for screening and diagnosis of gestational diabetes has evolved during the period that study participants gave birth.

Gestational diabetes was particularly important in Aim 2 as there was a subset of the studies assessing breastfeeding and later T2D in women with a history of gestational diabetes (65, 127-130), and this variable is used for a sub-analysis of the population in Aim 2.

HEALTH BEHAVIORS

Diet [>28 | 26-28 | 23-25 | 19-22 | <19]:

A DASH (Dietary Approaches to Stop Hypertension) variable was derived from the food frequency questionnaire administered at baseline. Unhealthy components of the diet (sodium, red meat, SSDs) were reverse coded and the overall DASH score was made up of the sum of rankings for each of the components. The energy from each of the food groups was summed, creating a variable with full caloric intakes (energy). These were ranked using quintiles. The dairy quintiles were re-calibrated in order to take into account the low consumption of these food groups (131).

Dietary questionnaires were administered in 1995 and 2001. The quintiles created in 1995 were used for all participants due to missingness in 2001.

Vigorous exercise [>3 | >0 to 3 | none]:

Vigorous exercise (On average, hours per week, over the last year) was recorded in every questionnaire cycle except 2003 and 2005. Baseline scores were used for all participants.

Vigorous activity was assessed using Actigraph testing in 2006. Results indicated that vigorous activity had a higher correlation ($r=.40$, $P<.001$) with reported activity than moderate ($r=.26$, $P<.05$) activity in a study using a 7 day activity diary to validate the physical activity instruments used in the Black Women's Health Study (132).

We evaluated correlation coefficients between proximal questionnaire cycles to determine whether vigorous activity from one questionnaire could be a proxy for vigorous activity in another questionnaire. Correlation coefficients were between $r = 0.43$- 0.51 for consecutive

questionnaires and r = 0.32 – 0.48 when comparing questionnaire cycles four years apart, suggesting that vigorous exercise remains relatively stable.

The energy required for milk production in humans is preferentially obtained from decreased activity and increased caloric intake rather than from mobilization of fat. Therefore, dietary patterns and exercise are particularly important variables to include in any analysis of the effect of lactation on health outcome. Although this study's measures may be remote from lactation, they represent general trends in health behaviors.

Both diet and exercise were measured at baseline; therefore, for women who developed the outcome disease prior to 1995, these variables were measured after, rather than before, the outcome. We hypothesized that diet and exercise after disease onset were a reasonable estimate of the diet and exercise patterns of an individual prior to the onset of the outcome disease. To test this hypothesis, we performed a sensitivity analysis in which the dataset was divided into women who developed disease before and after 1995, with adjustment for diet and exercise ascertained in 1995. For both Aim 1 and Aim 2 the outcome estimate was compared between models and to the overall outcome estimate using an *a priori* change in estimate of 5%. Outcome estimates were similar across all models suggesting that timing of diet and exercise assessment with respect to onset of disease did not materially affect results. We therefore used baseline diet and exercise for all participants.

Smoking [pack-years: never | <20 | >20:

The overall rate of any smoking in this population was low (14%) and smoking initiation after 1995 was almost absent. Pack-years was calculated based on responses in 1995 from the number of cigarettes smoked per day (# cigs smoked/20 = packs/day). This was added up over the

number of years a person smoked, also reported in 1995. Categories were based on those constructed in the Framingham studies (133).

Alcohol per week [none | 1-7 | >7]:

Alcohol exposure was measured using data from 1995. The prevalence of abstinence in this dataset (48%) is similar to national rate among black women (47%) (134).

DEMOGRAPHIC FACTORS

Education [> college | some college | HS or less]:

Education was measured in 1995 and 2003. The cultural span of this longitudinal sample was quite wide, including changes, such as desegregation, that had a profound impact on the meaning of educational attainment, and the availability of education over the years. Thus, the meaning of education was different for women who were older when they began participating in the questionnaire; however, the confounding across time is likely much less marked than it would be across races (24) and controlled by using age and questionnaire cycle as matching criteria for cases and controls.

Marital status [never | ever]:

Marital status was assessed in 1995, 1997, 1999, and 2005. Marital status was derived using data from 2005 and the most proximal earlier responses were used for missing data.

Childhood neighborhood urbanity [urban | suburban | rural | combination]:

Childhood urbanity was ascertained in 1997. Neighborhood characteristics can affect general health and wellbeing that set patterns of behavior into adulthood.

Childhood neighborhood segregation [predominantly black | mixed | predominantly white]

As with urbanity, childhood neighborhood segregation can set patterns of behavior that last well into adulthood.

METABOLIC DISEASE

Prevalent Hypertension and type 2 diabetes [no | yes]:

For Aim, 1 a variable for T2D prevalent at case selection was created, and for Aim 2, a similar variable was created for hypertension.

Final adjustment sets: To determine the final variables for inclusion in models for Aim 1 and Aim 2, all variables were assessed for changes in prevalence across levels of cumulative lactation among the controls. Additionally, a model with all potential covariates was examined to assess for changes in estimate (*a priori* change in estimate $\geq$ 5%), removing each covariate individually from largest to smallest Wald *P*-value (135). Change in estimate values were balanced against an assessment of change in precision (*a priori* <0.01) calculated using the difference in confidence limit ratios (confidence limit ratio = upper confidence limit/lower confidence limit) (136). We also used *DAGitty* (137) to construct a directional acyclic graph and calculate a minimally sufficient set. Each of these methods of assessing variables was compared. Using these various results, the minimally sufficient set determined through backward elimination was used as a model to which all variables with any significant findings were added individually or in like groups cumulatively. Effect estimates were again assessed for a > 5% change. The final adjustment set included variables that caused a >5% change in beta. Additional variables with less than 5% change in beta were included if they were judged to be important based on previous literature and did not affect precision. Categorical levels of adolescent BMI

(ascertained at age 18) was included in both models because of *a priori* plans for moderation analyses.

2.6 Data Analysis

We used unconditional logistic regression to estimate the association between cumulative lifetime lactation and outcome disease. Conditional analyses are always appropriate to use, but can be computationally taxing, particularly in large datasets. Unconditional maximum likelihood methods are appropriate in large datasets in which all strata will have more than 10 individuals (116). Both Aim 1 and Aim 2 have large final populations and outcome measures that are either common (hypertension) or relatively common (T2D); therefore unconditional likelihood methods are appropriate.

With frequency matching, each case is matched to a stratum of like controls rather than a single individual control. This method ensures that there are equal distributions of the matching factors within cases and controls (138).

We used density or risk set sampling, which draws controls from the risk set of each case at the time that case occurs. Controls are present in multiple risk sets based on the amount of time they spend in the study. It is for this reason that odds ratios approximate the incidence rate ratios that would have been estimated in the full cohort. It was important to our study design that the controls were selected at the age and 2-year epoch of the cases so that we were controlling for age and time in our control selection. Risk set sampling facilitated this design element.

It is not typically recommended to match more than 4 controls to a case. In Aim 1, hypertension was very common (42.1%) leaving a relatively small sets of controls in each

stratum. For this reason, we decreased the number of controls from 4 to 2:1; but for Aim 2, diabetes was less common (13%), allowing for the higher ratio of 4:1.

The datasets for these analyses were large (Aim 1 n = 37,539; Aim 2 n = 25,325). Additionally, almost all proportions for missing values were under 10%, with the exception of gestational diabetes (missing 27.6%). These two study characteristics allowed for use of complete case analysis. For Aim 1, 7455 (20.0%) observations were eliminated, and for Aim 2 6845 (30.4%) were eliminated.

In each aim, age, parity, and adolescent BMI were assessed as moderators. If moderation existed, outcome estimates were stratified by the moderator and presented in the paper as either a figure or table.

The same two sensitivity analyses were performed in each aim. First, we were interested in the difference between adolescent BMI, which is a moderator of the relationship between breastfeeding and outcome disease, and adult BMI, which could be a moderator or a mediator, dependent on when it was measured in relation to a participant's reproductive years. A variable for adult BMI at age 40 was created to standardize the age of measurement, and to ensure that it occurred after the last birth, and before disease onset. We further limited this analysis to women who were of normal weight during adolescence because we were interested in isolating obesity that developed after adolescence exclusive of women who had been obese all their lives. In order to achieve these selection criteria, we had to limit the dataset to women whose disease occurred after 1995. The variable for adolescent obesity was then replaced with adult onset of obesity, and a stratified model was assessed (139).

The second sensitivity analysis utilized the 1995 variable for cumulative lactation, which included 'tried but couldn't' and 'less than 1 month'. There is evidence that metabolic risk factors prior to or during pregnancy such as obesity (106, 140-142), insulin resistance (143), and glucose homeostasis in pregnancy (144) adversely impact lactation. It is possible that the category of failed lactation (Tried but couldn't) could represent either pre-existing metabolic risk factors or perinatal complications thus providing insight on the possibility of reverse causality. For Aim 2, we also restricted this dataset to women who reported gestational diabetes, therefore additionally assessing failed lactation among women with known diabetic risk. It is also possible that 'tried but couldn't' reflects lack of social support for breastfeeding or barriers to access to sufficient medical support for lactation.

2.7 Tables and Figures

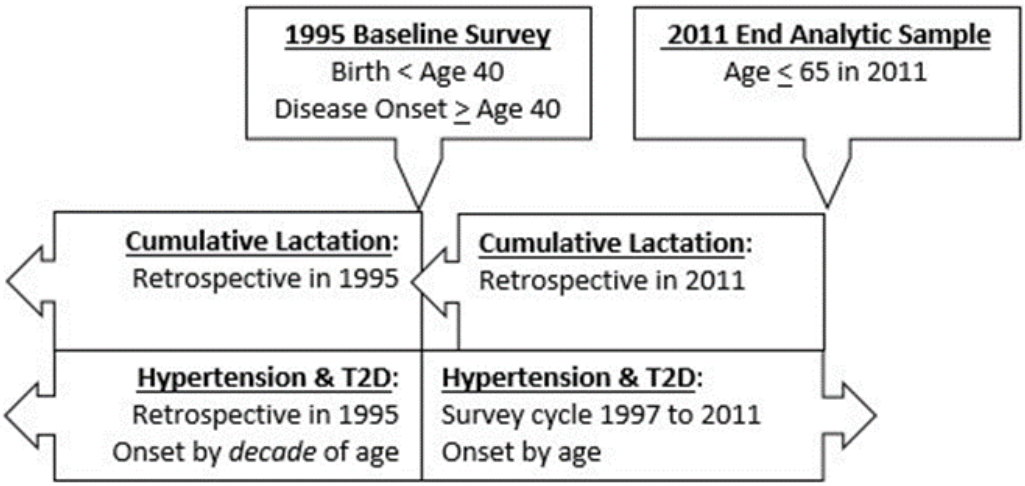

Figure 2.1. Analytic sample parameters illustrating prospective and retrospective ascertainment of cumulative lactation and disease onset.

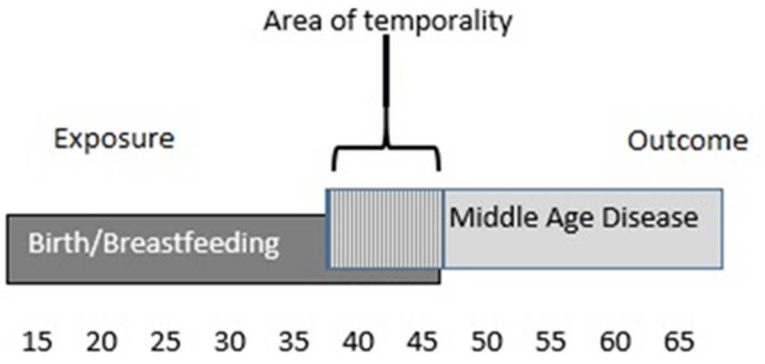

Figure 2.2 Potential area of temporality in which sequencing of exposure and middle aged disease might cause error.

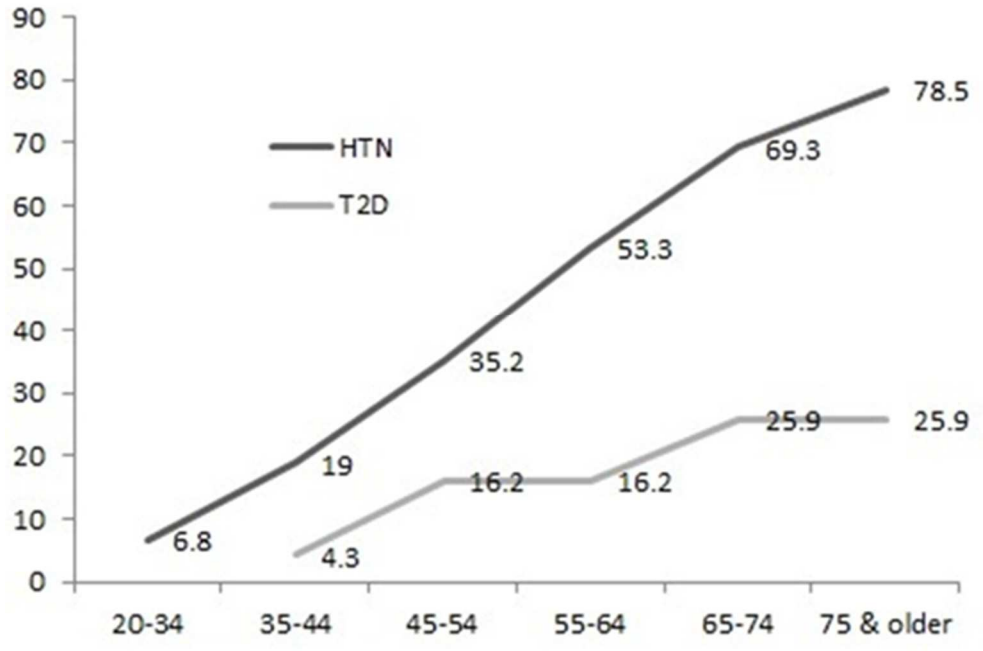

Figure 2.3 Prevalence of hypertension (HTN) and type 2 diabetes (T2D) for women in the United States. Statistics for hypertension are from the American Heart Association, 2015 update(8) while those for T2D are from 2009-2012 NHANES(104)

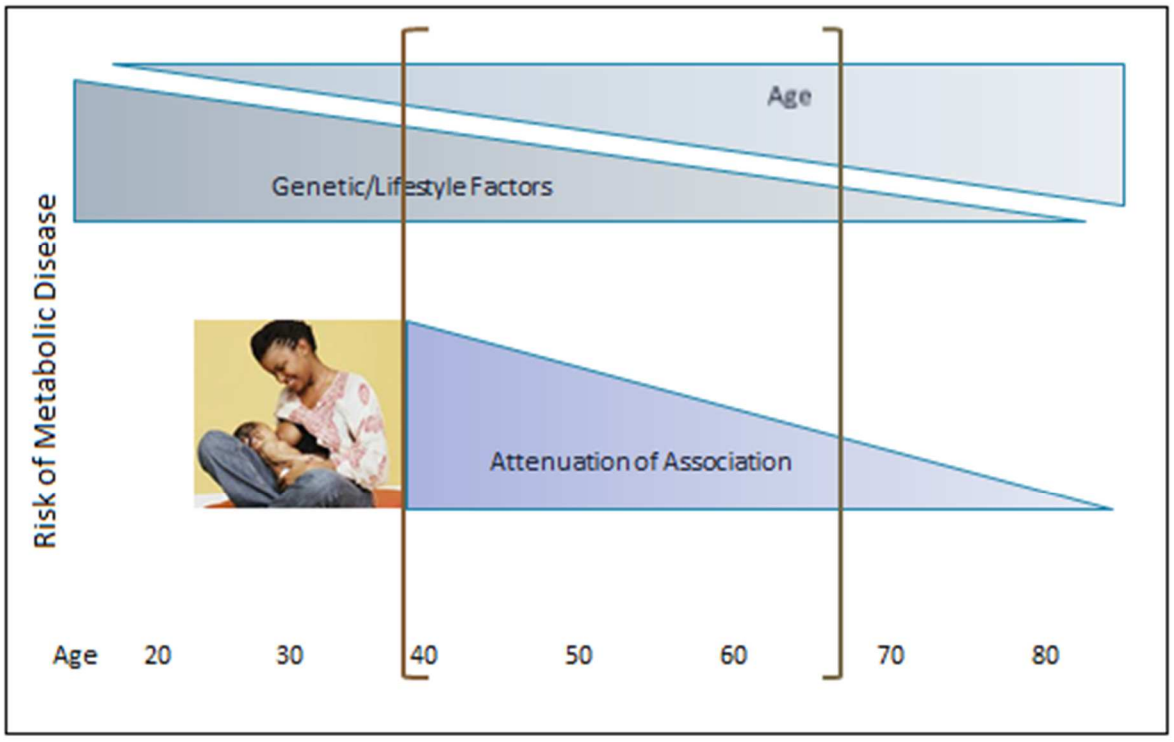

Figure 2.4 As women age, their risk of metabolic disease increases. Women more vulnerable to disease acquire it at younger ages, and the protective association with breastfeeding attenuates with time. Brackets encompass the age range targeted for this analysis.

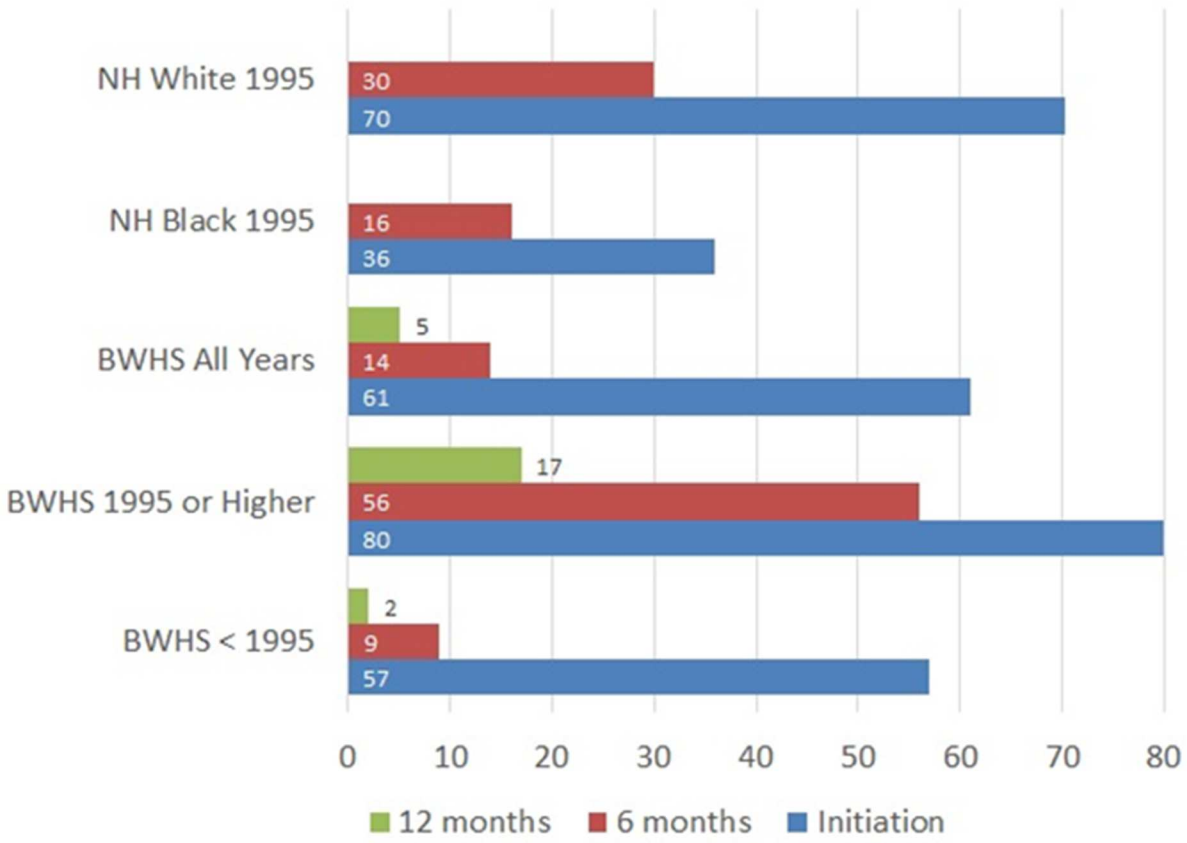

Figure 2.5 Prevalence of any breastfeeding for the women in the Black Women's Health Study at initiation, 6 months, and 12 months compared to initiation and 6 month breastfeeding rated in the general US population for NH black and NH white women in 1995 (191).

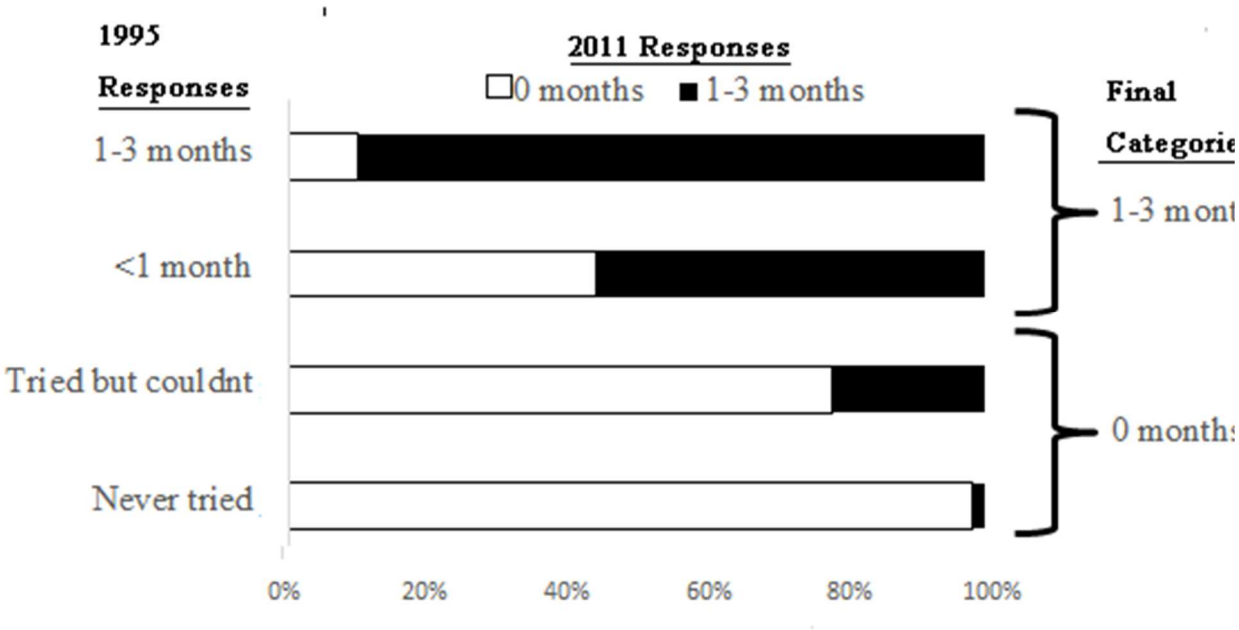

Figure 2.6 Among women whose last birth occurred prior to 1995, responses to the categorical 1995 cumulative lactation question were compared to their integer responses in 2011. Based on response patterns to both questionnaires, women who 'Tried but couldn't' breastfeed and 'Never tried' were categorized as '0 months' in the final lactation variable and women who breastfed for < 1 month were categorized as '1 month'.

Table 2.1 Final adjustment set for Aim 1 and Aim 2

Final Adjustment Sets	
Aim 1: Breastfeeding → Hypertension	**Aim 2: Breastfeeding → T2D**
• Age • Questionnaire cycle • Parity • Exercise • Adolescent BMI • Age of first birth • Diet • Smoking • Family history of myocardial infarction	• Age • Questionnaire cycle • Parity • Exercise • Age at first birth • Adolescent BMI • Education • Gestational Diabetes

CHAPTER 3. CUMULATIVE LACTATION AND MIDDLE AGED ONSET OF HYPERTENSION IN AFRICAN AMERICAN WOMEN

3.1 Introduction

Hypertension affects nearly one in every three women (8) and is a contributor to cardiovascular disease, which is the leading cause of death in the United States (5, 6). It is hypothesized that the metabolic changes that occur during pregnancy may influence risk of cardiovascular diseases later in life (145-147). These metabolic changes include increased central adiposity (69), an elevation in blood pressure (70), an atherogenic lipid profile, and insulin resistance (71). After pregnancy, lactation may be an important component of a woman's return to her pre-pregnancy metabolic state. Lactating women have less inflammation, greater insulin sensitivity (73), beneficial changes in cardiac output (74) and less atherogenic blood lipid profiles (55) than formula feeding women. These favorable changes during lactation have been termed the 'Reset Hypothesis' and have been posited as a possible mechanism underlying differential risks of metabolic diseases later in life for women who breastfed compared to women who formula fed (76).

The association between lactation and metabolic conditions has important implications for health disparities. Black women in the United States have a disproportionately high prevalence of hypertension (42.1%) compared with other populations (white 28.0%, Hispanic 26.0%) (8). Higher morbidity from hypertension and cardiac disease earlier in the life cycle among black women results in nearly twice the productive life lost compared with white

women (10). Additionally, black women have lower breastfeeding initiation (66.4%) compared with white (83.0%), and Hispanic (82.4%) women (22). These differences in breast feeding may contribute to a greater burden of disease among black women. However, none of the studies of breastfeeding and hypertension include sufficient numbers of black women for informative results (45, 47, 48, 50-52), and only a few of the studies of breastfeeding and general metabolic or cardiac health include this population (54, 56, 57, 148).

To address these gaps in the literature, we conducted a nested case control analysis within a follow-up study of black women, the Black Women's Health Study, to estimate the association between lactation history and incident hypertension, with control for individual metabolic risk factors, health behaviors, and demographic characteristics.

3.2 Methods

DATA

The Black Women's Health Study: We used data from the Black Women's Health Study, a longitudinal cohort of 59,001 black women enrolled in 1995. Health and demographic data were collected at baseline, and participants complete follow-up questionnaires every two years. Ages at enrollment ranged from 21 to 69, with a median age of 38. Participants represent all regions of the United States, and almost half (44%) had completed college at the time of recruitment. The most common medical conditions reported at baseline were uterine fibroids, hypertension, high cholesterol, and diabetes (149). Follow-up through the 2013 questionnaire cycle is complete for 87% of person-time.

MEASURES

Cumulative lifetime lactation: Women completed questions on their lactation history in 1995 and 2011. Participants were asked "How many months, in total, have you breastfed your children" with categories of response options in 1995 that included 'no children', 'never tried', 'tried but couldn't', and the following categories of months: <1, 1-3, 4-6, 7-11, 12-17, 18-23, 24-35, 36-47, and ≥ 48. In 2011, the same question provided a space for participants to fill in the number of months of cumulative lifetime lactation as an integer. Responses for women who answered the questionnaire twice were compared. Based on their responses, the categories for 1995 were collapsed such that 'never tried' and 'tried but couldn't' were coded to equal 'none' from 2011, and the category for 'less than 1 month' in 1995 was categorized as '1-3 months'. The final cumulative lactation variables for 1995 and 2011 contained the following categories in months: none, 1-3, 4-6, 7-11, 12-17, 18-23, ≥24. Cumulative lifetime lactation was ascertained from the most proximal response to the participant's last birth.

Hypertension during middle age: Hypertension in the BWHS is a report of antihypertensive drug use, or report of physician diagnosed hypertension together with use of diuretics in the same questionnaire cycle. For women who already had hypertension at the time of enrollment, hypertension onset was reported by decade of age (< 30, 30-39, 40-49, or ≥ 50). For these women, a specific age of onset was allocated using the available categorical responses to disease onset with the following formula. Let A = age, C = the category for decade of age of onset, B = the participant's age at baseline, and Y = the youngest age in each category of hypertensive onset. The assigned age would thus be:

$$A = Y + (½ (B - Y))$$

For example, if a participant aged 55 at baseline reported prevalent hypertension (B = 55), with a diagnosis of hypertension at age 40-49 (Y = 40), then age (A) = 40 + (½ (55-40)) = 47.5. For instances in which ½ (B - Y) > 10, the age at the midpoint of the decade of onset was assigned (i.e. if a participant was age 65 at baseline with prevalent hypertension diagnosed at age 40-49, the assigned age would be 45).

In follow-up questionnaires, women were asked if they had been diagnosed with hypertension in the past two years. Their age at diagnosis was assumed to be the median age between completion of that questionnaire and the previously completed questionnaire. Age was then categorized as follows: 40-44, 45-49, 50-54, 55-59, and 60-65.

Covariates: Parity and age at last birth were obtained in 1995 and updated with each subsequent questionnaire. Diet (Dietary Approaches to Stop Hypertension [DASH] scores) (131), vigorous exercise, and smoking (pack-years) were ascertained from the baseline questionnaire. Body Mass Index (BMI) (kilograms/m^2) in adolescence was derived from recalled weights at age 18 and height at baseline and categorized as normal (<25 kg/m^2) or overweight/obese ($\leq$ 25 kg/m^2). Education was reported in 1995 and updated in 2003. Histories of pre-eclampsia and gestational diabetes were obtained in 2009. Diabetes was ascertained on each questionnaire, and a family history of metabolic disease before or after age 50 (parent with diabetes, stroke, or myocardial infarction) were each ascertained separately at baseline.

Analytic population: We restricted this analysis to parous women (n = 44,350) who had responded to questions about cumulative lifetime lactation (n = 42,068). We excluded 7,109 women whose onset of hypertension occurred before age 40 and 1,012 women who were aged 40 or more at the birth of their last child. We limited the analytic sample to women who had developed hypertension during ages 40 through 65; thus we excluded an additional 690 women

who were over 65 at the time of diagnosis. This gave us a sampling population of 33,257 women in which we identified 12,513 cases of incident hypertension. We used density sampling to frequency match two controls to each case for a final analytic population of 37,539. Controls were randomly selected from risk sets matched on age and questionnaire cycle. Risk sets included all women who were in the same age category, and were disease free at the time the risk set was sampled. Hypertension cases in the analyses could have occurred as early as 1975; therefore the risk sets included each two-year period from 1975 through 1993, as well as the years from 1995 on. A single participant could be selected as a control more than once, and a control could become a case in a later risk set (116). Because we used density sampling for the controls, the odds ratios (OR) provide an estimate of the incident rate ratios that would have been obtained from the full cohort (138, 150).

Statistical analysis: We used unconditional logistic regression to estimate odds ratios and 95% confidence intervals (CI) for the association between cumulative lifetime lactation and incident hypertension. Missing values were handled using complete data analysis (7,455 women excluded).

Potential confounders and effect measure modifiers were identified through a review of the literature and examination of causal diagrams. Inclusion was based on associations with cumulative lactation among the controls and a greater than 5% change in estimate criterion (135) balanced against an assessment of change in precision (*a priori* <0.01) calculated using the difference in confidence limit ratios (confidence limit ratio = upper confidence limit/lower confidence limit) (136).

Age, adolescent BMI, and parity were assessed for effect measure modification using likelihood ratio tests (LRTs). An *a priori P*-value for significance was set at <0.05. Within three

age restricted models, we performed tests for trend by including the summary variable for cumulative lactation in the model as an ordinal variable among women who breastfed.

Sensitivity analyses: Weight retention after pregnancy has been hypothesized as a risk factor for later hypertensive disease. We were therefore interested in whether overweight/obesity that developed during the reproductive years had a stronger effect on the relationship between breastfeeding and hypertension than adolescent BMI. This measure was not available for women whose disease occurred prior to the baseline questionnaire, so a restricted dataset was used including only women whose disease onset occurred after the baseline questionnaire (n=9,205). In this subset, we were able to derive a variable that measured BMI that developed between ages 18 and 40 (adult onset of BMI) by ascertaining BMI at age 40 (inclusive of age 41) in women who reported normal adolescent BMI (BMI at age 18).We repeated the assessment of effect measure modification for BMI, replacing adolescent BMI with adult onset BMI.

It is possible that very short periods of breastfeeding might be associated with perinatal morbidities rather than a choice not to breastfeed. The responses available in the 1995 questionnaire allowed us to compare women who 'never tried' to breastfeed with those who failed to meet their expectations ('tried but couldn't' and '<1 month'). For this analysis, we restricted the dataset to women who had given birth to their last child prior to baseline in 1995 (n=21,991), whose complete breastfeeding experience would be captured on the 1995 questionnaire.

All analyses were conducted using SAS (version 9.3; SAS Institute, Cary, NC). The study protocol was approved by the Boston University Institutional Review Board.

3.3 Results

Characteristics by duration of lactation among the population of controls are shown in Table 3.1. Women with longer breastfeeding durations were more likely to have had three or more children, consumed a healthier diet, exercised more, smoked less, and were more likely to have completed college.

Table 3.2 shows the relationship between cumulative lactation and hypertension in a model adjusted for the selection factors (age and survey cycle) as well as the full multivariate model which was adjusted for age, survey cycle, parity, age of first birth, diet, exercise, adolescent BMI, smoking, and family history of myocardial infarction. The OR for any versus no breastfeeding in the multivariate model was 0.97, 95% CI: 0.92, 1.02. When cumulative duration of breastfeeding was further broken down into months, there was an inverse relationship between breastfeeding and hypertension (*P* for trend <0.001) In age-specific models (Figure 3.1) (LRT chi^2 = 4.006, *P* = 0.045), any breastfeeding had the strongest inverse association with hypertensive onset between ages 40-49 (OR 0.92, 95% CI: 0.85, 0.99). As breastfeeding duration increased for these women, rates of hypertension decreased (OR for $\geq$ 24 months, 0.82, 95% CI: 0.69, 0.98; *P* for trend <0.001). Breastfeeding was not associated with risk of hypertension in women age 50 and older.

Parity (Figure 3.2) modified the association between hypertension and breastfeeding (LRT chi^2 = 14.182, *P* <0.001). Breastfeeding for $\geq$ 24 months was associated with reduced risk regardless of parity, but for shorter durations of breastfeeding, the association was observed only in primiparous women.

There was no effect measure modification of the relationship between breastfeeding and hypertension by adolescent BMI (LRT chi^2 = 3.696, *P* <0.545). However, in the subset of

women for whom we had data on BMI at age 40 (among women who were normal weight at age 18 and after childbearing was complete), there was significant effect modification with BMI category (normal, overweight, obese) (LRT $chi^2 = 10.735$, $P = 0.001$). Any breastfeeding (Table 3.3) had the strongest inverse association with hypertensive onset in women who were normal weight at age 18 and at age 40 (OR 0.77, 95% CI: 0.65, 0.92) but as breastfeeding duration increased for these women, risk of hypertension was not consistently reduced. As BMI increased, within each stratum of breastfeeding duration greater than 3 months, risks of hypertension within that stratum increased as well.

When the difference in the association between breastfeeding and hypertension was assessed using reported planned formula feeding versus failed lactation, ORs were 1.05, 95% CI: 0.97, 1.14 for women who failed to successfully breastfeed and 1.01, 95% CI: 0.90, 1.13 for women who breastfed for less than one month when compared to a referent of women who planned formula feeding.

3.4 Discussion

In this large nested case control study, we found that breastfeeding was associated with reduced risk of hypertension in women aged 40-49. No association was observed for women whose hypertension was diagnosed at ages 50 and 65. The inverse association increased with longer cumulative lactation, and was strongest among women who breastfed for 24 months or more. As parity increased, the inverse association for women with shorter cumulative lactation was attenuated, but persisted for women who breastfed for $\geq$24 months. Our results are consistent with the existing evidence for a dose-dependent association between cumulative lifetime lactation and hypertension.

Our finding that the association between lactation and hypertension attenuated with age are consistent with previous cross-sectional studies (47, 52). The attenuation of associations over time may reflect the cumulative impact of the aging pr9ocess on vascular function. During the aging process, blood pressure naturally rises due to calcification and stiffening of elastine in the blood vessels (151). By age 75, most American women (78.5%) have hypertension (8).

One of the risk factors for hypertension is obesity (6). The risk of obesity, especially central adiposity, increases with increased parity (82, 85, 152-154). The association between lactation and weight retention after pregnancy has been studied extensively, and the results are mixed (79, 155-159). We assessed obesity in two ways by ascertaining adolescent BMI and adult onset of overweight/obese status at age 40. We did not directly assess the association between pregnancy and postpartum weight retention in this study, but previous research using this population demonstrated greater weight gain for women who had children than among those who did not (160) and an inverse association between lactation and postpartum weight retention limited to women who were normal weight prior to pregnancy (161). Interestingly, we found that the inverse association between cumulative lactation and hypertension was also strongest in normal weight women. By contrast, in a study of young Korean women also stratified by BMI measured in adulthood, the inverse association between lactation and hypertension was stronger in obese women (45). However, these two studies differed in the age of obesity measured, and also differed in the covariates used and population demographics, impeding direct comparison.

To our knowledge, this study is the first to assess the association of cumulative lactation and hypertension comparing failed lactation to no breastfeeding. Because of the hormonal complexity of the first few days of breastfeeding, it is possible that simply initiating breastfeeding could change the hypothalamic-pituitary axis (162) thus ‘turning on’ the

mechanism that resets metabolic processes. In contrast, failed or very short lactation could also be associated with high levels of perinatal morbidity which would argue for the possibility of reverse causality in this subgroup of women. Our results do not support an association between very short periods of lactation and hypertension. While women who report failed lactation have higher rates of hypertension than women who chose not to breastfeed, the difference was very small. A trend toward reverse causality could exist, but our results are relatively uninformative. Additionally a lack of social or medical support cannot be ruled out, both of which can reduce breastfeeding rates (33), as well as increase the risk of hypertension (163). Although this population of women had high uptake of prenatal care, hospital postpartum care has been shown to be inconsistently supportive of breastfeeding in communities with higher populations of black women (35), which might also affect the association.

One of the strengths of our study was that we utilized all cases of hypertension reported by women in the Black Women's Health Study, rather than limiting the analysis to women whose hypertension was diagnosed after they joined the cohort. This approach reduced the potential bias that could have been introduced by ascertaining lactation history retrospectively but excluding all cases of prevalent disease at baseline. This bias could move the point estimate toward the null by eliminating disease that occurred at earlier ages. However, inclusion of these cases introduced imprecision as to the age of diagnosis.

Our study must be interpreted within the limitations of the study design. We used cumulative lifetime lactation as the measure of breastfeeding exposure. This measure is based on recall, sometimes over long periods of time; however, the literature suggests that recalled breastfeeding is reasonably reliable, even decades after the index birth (42-44). Furthermore, we were not able to assess lactation intensity, as cumulative lactation only measures the duration of

breastfeeding. Finally, the Black women's Health Study population differs from the general population of African American women in the United States: they are better educated (164) and breastfeeding rates were higher (22). Thus, our results may be less generalizable to black women of lower socioeconomic status or who had lower rates of breastfeeding uptake for other reasons. Lastly, cumulative lactation was assessed in 1995, after the onset of hypertension for some study participants. In theory, this could lead to biased recall of the exposure; however, this is unlikely, given that a protective association between breastfeeding and hypertension had not been described.

There is a disproportionate burden of hypertension and cardiac disease among black women. This study contributes to the existing literature on the relationship between breastfeeding and hypertension, extending the results to this understudied and high priority population. Quantifying the association between lactation and hypertension in black women, who have a lower rate of breastfeeding but higher rates of hypertension, provides an important opportunity for informing targeted public health messages. Addressing breastfeeding as a potential preventative health behavior is particularly compelling because it is a modifiable health behavior requiring a time-limited effort. Future research assessing this relationship would benefit from more specific measures of breastfeeding that account for intensity as well as breastfeeding duration, and from collecting more detailed information on perinatal events.

3.5 Tables and Figures

Table 3.1 Demographic characteristics by months of lifetime lactation in the control population. Black Women's Health Study 1995-2011

	Cumulative months of lifetime lactation				
	None (n = 20568)	**1-3 (n =6174)**	**4-6 (n =3893)**	**7-11 (n =2688)**	**≥12 (n = 4216)**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Age at first birth[1] [Med, IQR]	20 (18, 24)	22 (19, 27)	23 (19, 27)	23 (19, 28)	23 (19, 28)
BMI at age 18[1,2] [Med, IQR]	20 (19, 23)	20 (19, 22)	20 (19, 22)	20 (19, 22)	20 (19, 22)
Parity					
1	34.1	32.8	30.4	20.4	11.8
2	35.8	34.0	40.7	40.3	32.9
3	30.1	33.2	28.9	39.3	55.3
DASH Diet (higher = healthier)[2]					
<19	16.9	14.6	11.6	10.1	10.2
19-22	25.9	23.1	19.2	21.2	18.0
23-25	21.1	21.8	21.5	21.6	20.9
26-28	17.9	20.4	22.6	20.1	23.5
>28	18.2	20.2	25.1	26.9	27.3
Vigorous Exercise[1]					
None	44.7	38.7	32.9	32.2	29.9
< 3 hours	34.4	39.2	40.5	41.3	40.9
≥ 3 hours	20.9	22.1	26.7	26.5	29.2
Smoking (pack-years)[1]					
Never	53.4	58.6	61.9	59.7	65.6
< 20	32.9	30.4	29.7	31.7	29.2
≥ 20	13.6	11.0	8.5	8.5	5.2

	None (n = 20568) n (%)	1-3 (n =6174) n (%)	4-6 (n=3896) n (%)	7-11 (n =2688) n (%)	≥12 (n = 4216) n (%)
Education					
High school or less	25.7	17.5	13.1	12.7	13.5
Some college	34.4	31.4	27.4	27.3	26.0
College or more	39.9	51.1	59.5	59.9	60.4
Family History of MI[1]					
Age < 50	6.8	6.2	7.1	6.4	5.7
Age ≥ 50	19.9	20.0	17.6	17.7	15.1
Pre-eclampsia[2]	7.3	6.3	6.6	8.1	8.3
Prevalent Diabetes	1.4	1.0	1.2	1.3	0.8

Abbreviation: BMI = Body Mass Index; Kg = kilograms; m = meters; Med = median; IQR = inter-quartile range; HS = high school; MI = myocardial infarction; DASH = Dietary Approaches to Stop Hypertension

[1] Missing: Age at first birth (1.5%); BMI at age 18(1.8%); DASH Diet (8.4%); Exercise (5.1%); Smoking (3.1%); Education (0.1%); Family History of MI (5%); Pre-eclampsia (30.7%)

[2] BMI = kg/m^2

Table 3.2 Odds Ratios for hypertension by duration of cumulative lactation. Black Women's Health Study 1995-2011

	Cumulative Lactation N = 30,084				
		Model 1[1]		Model 2[2]	
	Cases / Controls	OR	(95% CI)	OR	(95% CI)
None		1.00		1.00	
Any	10,108 / 19,976	0.95	(0.90, 0.99)	0.97	(0.92, 1.02)
1-3	1649 / 3328	0.95	(0.89, 1.02)	0.96	(0.90, 1.03)
4-6	1073 / 2112	0.98	(0.90, 1.06)	1.01	(0.93, 1.09)
7-11	726 / 1513	0.92	(0.84, 1.02)	0.94	(0.86, 1.04)
12-17	516 / 1018	0.97	(0.87, 1.09)	0.99	(0.88, 1.11)
18-23	241 / 523	0.88	(0.75, 1.03)	0.90	(0.77, 1.06)
≥24	380 / 841	0.87	(0.76, 0.98)	0.89	(0.78, 1.01)
***P* for trend[3]**			0.772		<0.001

OR: Odds ratio, CI: Confidence interval

[1] Model 1 adjusted for age and survey cycle

[2] Model 2 adjusted for age, survey cycle, parity, age of first birth, diet, exercise, BMI at age 18, smoking, and family history of myocardial infarction

[3] *P* for trend calculated among women who breastfed (N = 13,920)

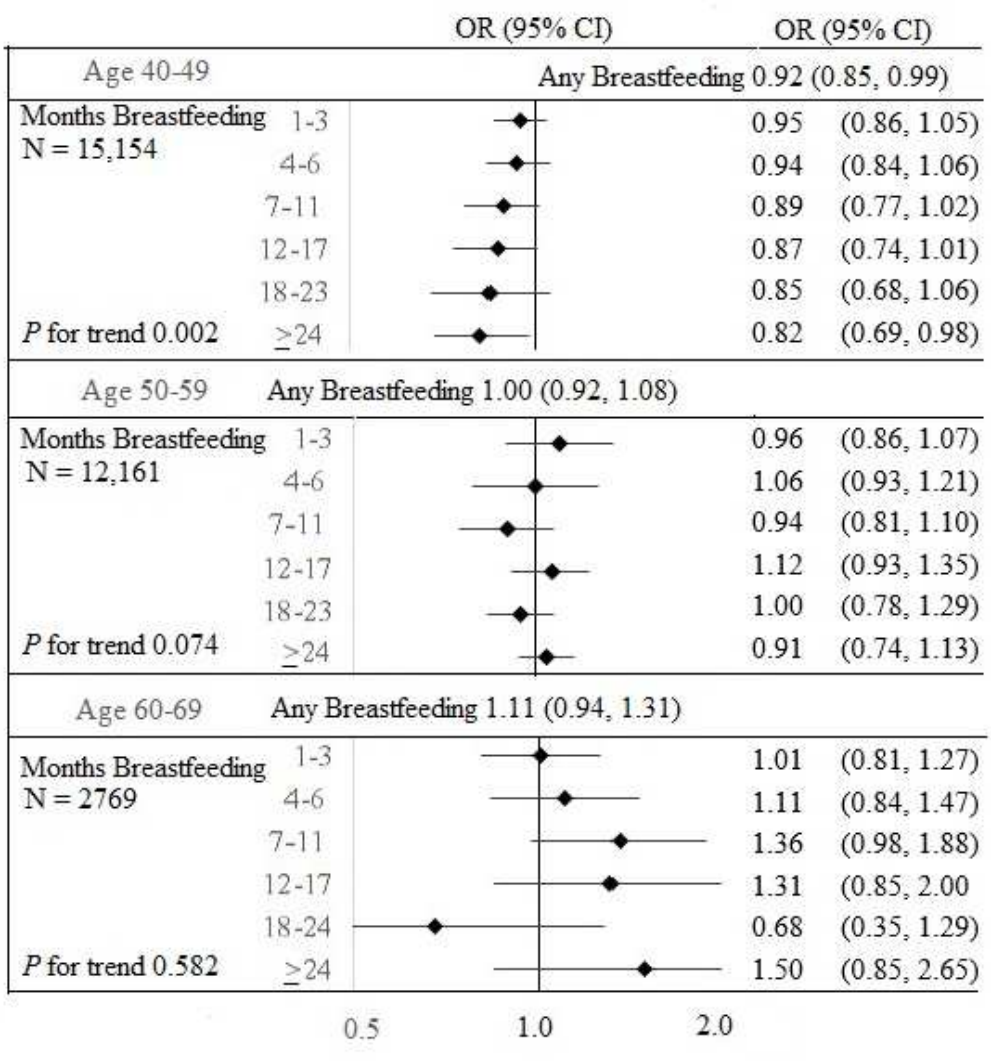

Figure 3. 1 Odds ratios (OR) and 95% Confidence Intervals (CI) for the association between hypertension and cumulative lifetime lactation, for any breastfeeding and months of duration, compared to no breastfeeding among datasets restricted to age epochs (40-49, 50-59, and 60-65). The results were adjusted for age, survey cycle, parity, age at first birth, diet, exercise, BMI at age 18, smoking, and family history of myocardial infarction. P for trend is among women who breastfed. Black Women's Health Study 1995-2011.

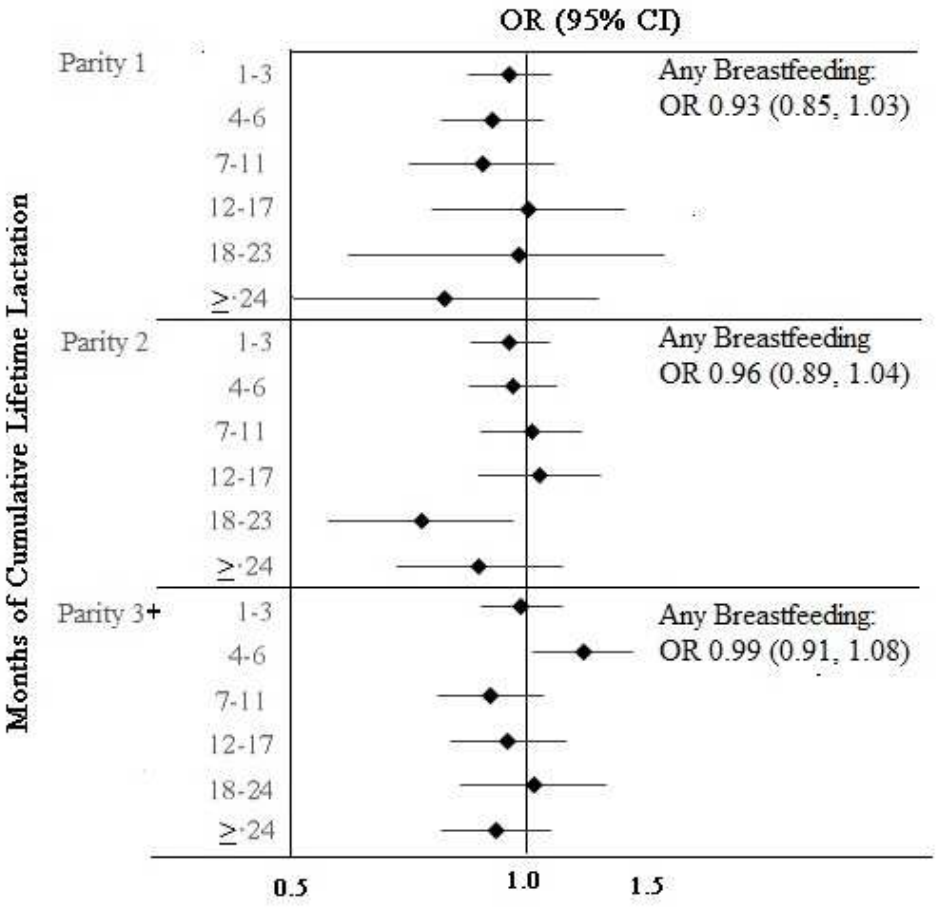

Figure 3.2 Odds ratios (OR) and 95% Confidence Intervals (CI) for the association between hypertension at ages 40-65 and cumulative lifetime lactation, in months of duration, compared to no breastfeeding, stratified by parity. N = 30,084. The results were adjusted for age, survey cycle, parity, age at first birth, diet, exercise, BMI at age 18, smoking, and family history of myocardial infarction. Black Women's Health Study 1995-2011.

Table 3.3 Odds ratios for hypertension by duration of cumulative lactation stratified by adult onset of obesity attained at age 40 among women who developed hypertension after baseline (1995). N = 9205. Black Women's Health Study 1995-2011

	Months of Cumulative Lactation by BMI Status (Kg/m^2) N = 9205						
		Normal Weight (< 25)		**Overweight (25-29.9)**		**Obese (≥30)**	
	Cases / Controls	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
None	1365 / 2625	1.00		1.00		1.00	
Any	1668 / 3556	0.77	(0.65, 0.92)	0.99	(0.85, 1.15)	1.06	(0.90, 1.24)
1-3	510 / 1066	0.86	(0.68, 1.09)	0.99	(0.81, 1.22)	0.97	(0.78, 1.21)
4-6	368 / 788	0.73	(0.56, 0.96)	0.95	(0.75, 1.19)	1.21	(0.94, 1.55)
7-11	275 / 618	0.67	(0.49, 0.90)	1.01	(0.78, 1.31)	1.04	(0.78, 1.38)
12-23	341 / 700	0.83	(0.62, 1.20)	1.04	(0.81, 1.33)	1.05	(0.81, 1.36)
≥24	174 / 384	0.74	(0.52, 1.07)	0.96	(0.69, 1.33)	1.07	(0.76, 1.50)

OR: Odds Ratio, CI: Confidence interval

Adjusted for age, survey cycle, parity, age of first birth, diet, exercise, BMI at age 18, smoking, and family history of myocardial infarction

CHAPTER 4: CUMULATIVE LACTATION AND MIDDLE AGED ONSET OF TYPE 2 DIABETES IN AFRICAN AMERICAN WOMEN

4.1 Introduction

Type 2 diabetes (T2D) is of growing concern in the United States, affecting 11% of adult women (104). The risk of type 2 diabetes (T2D) is higher among parous women than among women without children (165). Pregnancy is a state of metabolic burden as a mother's body adjusts to foster fetal growth. It nourishes the fetus by increasing visceral fat storage (166) increasing insulin resistance, and maintaining higher levels of circulating lipids (167). In the postpartum period, lactation is associated with improved glucose and lipid metabolism (61) as well as reduced inflammatory markers (168) among women who breastfeed, compared with postpartum women who don't breastfeed. After cessation of lactation, a woman's breastfeeding history continues to be associated with decreased metabolic syndrome (56), less hypertension (47), reduced risk of cardiac diseases(52, 58), including myocardial infarction (114), and a variety of other conditions which can broadly be considered as reflective of metabolic health (169). Several authors have also found a decreased risk of T2D in association with longer breastfeeding duration (46, 54, 62, 63).

The prevalence of diabetes is higher in the non-Hispanic black population (16%) than among non-Hispanic white (8%), Asian American (9%), or Hispanic (13%) populations (104). Additionally, black women have lower rates of breastfeeding initiation (66% vs. 80%) than

women in the general population, suggesting the possibility that reducing breastfeeding disparities may lessen the diabetes disease burden in black women. However, only one of the study populations used to quantify the association between breastfeeding and T2D has included a substantial number of black women (49).

The objective of this study was to quantify the relationship between cumulative lifetime lactation and diabetes among black women in the United States. We therefore conducted a case-control analysis nested within the Black Women's Health Study. We hypothesized that longer cumulative lactation would be associated with reduced onset of type 2 diabetes in middle aged women.

4.2 Methods

DATA

The Black Women's Health Study: The Black Women's Health Study is a longitudinal cohort of 59,001 black women from across the United States. Baseline health and demographic data were collected in 1995, and subsequent health questionnaires are completed every two years. At enrollment, participants had a median age of 38, and type 2 diabetes was among the most common health conditions reported (149). Follow-up is ongoing and complete for 87% of person-time through 2013.

Cumulative lifetime lactation: Women were asked about cumulative lifetime lactation or 'How many months, in total, have you breastfed your children' in 1995 and again in 2011. Response options in 1995 included 'no children', 'never tried', 'tried but couldn't', and the following categories for months: < 1, 1-3, 4-6, 7-11, 12-17, 18-23, 24-35, 36-47, and $\geq$ 48. In 2011, in response to the same question, participants reported the number of months of

cumulative lactation as an integer. To align categories across these two variables, we tested internal validity among women whose last child was born before 1995, and who responded in both 1995 and 2011. Based on these results, we collapsed 1995 responses for 'never tried' or 'tried but couldn't' to match 'none' from 2011, and coded women reporting 'less than 1 month' in 1995 in the same way as the category for '1-3 months' from 2011. For our analysis, we used the following categories, in months: none, 1-3, 4-6, 7-11, 12-17, 18-23, and $\geq$ 24. For women who completed childbearing before 1995, we ascertained cumulative lifetime lactation from the 1995 questionnaire, and for women who completed childbearing after 1995, we used the 2011 questionnaire.

Type 2 diabetes during middle age: At baseline, participants' were asked 'if a doctor ever told you that you have [diabetes] (not during pregnancy)' and the 'age at which it was first diagnosed' with response options for: under 30, 30-39, 40-49, or $\geq$ 50. In each subsequent questionnaire year (every 2 years), they reported whether they had been newly diagnosed since the previous questionnaire. While this question does not differentiate between type 1 and type 2 diabetes, elimination of diabetic cases that developed at ages < 40 effectively eliminated type 1 diabetics from the population (121-124). Self-reported diabetes was validated in the Black Women's Health Study population in 2011 by sending questionnaires to participant's treating physician. From a sample of 656 randomly selected and consenting participants, 229 physicians returned questionnaires, and of those, 219 (96%) confirmed a diabetic diagnosis (123).

Half of the respondents were older than 38 at cohort entry; therefore, much of the breastfeeding exposure and some of the diabetes onset was ascertained in response to retrospective questions at the time of the baseline questionnaire. In our analysis, we included all cases of T2D - cases reported retrospectively as well as those reported in each 2-year

questionnaire cycle. This reduced the selection bias that would have been induced by exclusion of disease that was present at the time of the baseline survey (115, 170), a technique commonly used when assessing the relationship between breastfeeding and disease onset using a Cox proportional hazard model.

Participants with prevalent diabetes at the time of the 1995 baseline questionnaire reported the age at which they received their diagnosis by decades (age less than 30, 30-39, 40-49, and 50 or more years old). In order to estimate 5-year epochs of disease onset, we used the following approach. Age of onset of T2D that occurred prior to baseline was allocated using the available data (age by decade of onset) with the following formula: let A = age, Y = the youngest age in each age category of disease onset, and B = the participant's age at baseline. The assigned age would thus be:

$$A = Y + (½ (B - Y))$$

For example, if a 55-year old participant reported prevalent T2D at baseline (B = 55), with a diagnosis of T2D at age 40-49 (Y = 40), then age (A) = 40 + (½ (55-40)) = 47.5. For instances in which ½ (B - Y) > 10, the age at the midpoint of the decade of onset was assigned (i.e. if a participant was age 65 at baseline with prevalent T2D diagnosed at age 40-49, the assigned age would be 45). If diagnosis of T2D was reported after the baseline questionnaire, age of disease onset was assigned the median age between the 2-year questionnaire cycle in which it was reported and the prior questionnaire. These assigned ages were used to derive the following categories for age of incident disease: 40-44, 45-49, 50-54, 55-59, and 60-65.

Cases were frequency matched to four controls (1:4) within age categories according to the 2-year period in which the case was identified. In order to appropriately assign controls to

risk sets prior to baseline, we derived 2-year risk sets every 2 years from 1975 to 1993, and used the existing questionnaire cycles to create risk sets from 1995 - 2011. Controls were sampled with replacement. Each control could be in multiple risk sets and was eligible to become a case in a later risk set (116).

Eligible participants were parous Black Women's Health Study respondents (n = 44,350) who answered questions on cumulative lifetime lactation (n = 42,068). In total, these eligibility criteria excluded 14,651 women who were non-parous, and 2282 who did not respond to questions on cumulative lactation. For diabetic women, disease onset was limited to ages 40 to 65, excluding 1355 diabetic women who were < 40, and 525 who were > 65 at diagnosis. To ensure that lactation exposure preceded the onset of diabetes, we further limited our sample to women who were < 40 at the time of their last birth (1128 excluded), yielding a sampling population of 39,060 women. We identified 4505 eligible cases of T2D and randomly sampled 18,020 controls from like risk sets for a final analytic population of 22,525. Controls were sampled more than once 3.8% of the time.

Covariates: Confounders and effect measure modifiers were identified through a review of the literature. Covariates considered for the final model included demographic information, health behaviors, metabolic risk factors, and reproductive history. Education (high school or less, some college, and college or more) was established at baseline and updated in 2003. Urbanity and the racial make-up of each participant's childhood neighborhood were ascertained in 1997. The following covariates were ascertained at baseline: Diet (Dietary Approaches to Stop Hypertension [DASH] scores), vigorous exercise, smoking (pack-years), body mass index or kilograms/m^2 (BMI) at age 18 (< 25, 25-29.9, and $\geq$ 30 kg/m^2) and family history of diabetes or myocardial infarction before or after age 50. Participants were asked about pregnancy

complications (gestational diabetes and pre-eclampsia) in 2009. Parity and age were reported in 1995 and updated with each subsequent questionnaire. This information was used to derive age for each participant's first and last birth. Hypertension was ascertained at baseline and updated with subsequent questionnaire cycles. These data were used to derive a variable that indicated prevalent hypertension at the time of diabetic case or control selection.

Statistical analysis: We used unconditional logistic regression to estimate the association between cumulative lifetime lactation and incident type 2 diabetes in middle age. Missing values were handled by using a complete data analysis. Most variables were missing values for less than 10% of the population, with the exception of gestational diabetes (GDM), for which 27.6% was missing. In total, 6845 observations (30.4%) were excluded with missing data. Since GDM was the primary variable with missing data, we compared the complete case model to two models without adjustment for GDM: one without missing values for GDM, and a second which included all missing GDM values. An *a priori* change in estimate of 10% was not met in either alternate model (Appendix 1). We report odds ratios (OR) and 95% confidence intervals (CI). Given our use of density sampling to select controls, the ORs provide an estimate of the incident rate ratios that would have been obtained from a cohort design (138, 150).

To determine the final adjustment set, we examined the association between all potential covariates and cumulative lifetime lactation among the controls using frequencies for categorical variables and median (25^{th} and 75^{th} percentile interquartile range) for continuous or ordinal variables. Additionally, a model with all potential covariates was examined to assess for changes in estimate (*a priori* change in estimate $\geq$ 5%), removing each covariate individually from largest to smallest Wald *P*-value (135). A minimally sufficient set of confounders (age and year of case or control selection, education, parity (as a categorical variable), age of first birth, gestational

diabetes, and exercise) was identified, to which we added an ordinal variable for parity and BMI at age 18.

Interactions by age, parity, and BMI status were hypothesized. Age was of interest because there is an attenuation of the association between breastfeeding and T2D over time (62) and the risk of T2D increases with age (62). We stratify by parity because rates of T2D increase as parity increases (165, 171) and because cumulative lactation is correlated with parity, introducing the possibility of a multiplicative association between breastfeeding and parity. BMI has a complicated relationship with breastfeeding. Women who are obese prior to pregnancy have lower rates of breastfeeding, (106-110, 172, 173) and, while the literature is mixed, there is some evidence that without breastfeeding, postpartum weight retention may be increased (79, 155-157). This weight retention has been hypothesized to be on the causal pathway between pregnancy and T2D (76). Thus, stratification by obesity prior to pregnancy is of interest as a modifier and weight gain during the reproductive years, while not a direct measure of weight retention after pregnancy per se, provides the opportunity to explore the potential mediating effect of weight on the relationship between breastfeeding and T2D.

To assess for attenuation of the relationship between cumulative lifetime lactation and T2D by age, estimates were compared across three age restricted models (40-49, 50-59, and 60-65). Within each model, *P* for trend was calculated by including the summary variable for cumulative lactation in the model as an ordinal variable among only women who had initiated any breastfeeding. We similarly performed stratified analyses by BMI at age 18 and by parity. Effect measure modification using likelihood ratio tests (LRT *a priori P*-value <0.05) was used to assess all three potential modifiers.

Sensitivity Analyses: Our measure of BMI utilized recalled weight for age 18 and height at baseline. While most of the women in the analytic sample were of normal BMI at age 18 (89%), the proportions were reversed at the time of disease onset (19% normal BMI). To begin to disentangle the temporality of the effect of BMI on the relationship between breastfeeding and T2D, we conducted an analysis stratified by post-childbearing BMI, rather than by BMI at age 18. We ascertained BMI during the reproductive years at 40 (inclusive of women who were either 40 or 41) and ensured adult onset of obesity by limiting the dataset to women who were of normal weight at age 18. Because BMI data at age 40 were only collected on study questionnaires beginning in 1995, this analysis was also limited to women who were $\leq$ 40 years of age at study baseline in 1995.

The primary direction of effect in this analysis was from lactation to T2D, but there is also evidence that metabolic risk factors prior to, or during pregnancy, such as obesity (140-142, 173), insulin resistance (143), and glucose homeostasis in pregnancy (144) decrease lactation, introducing the possibility of reverse causality. By assessing the baseline questionnaire response options for cumulative lactation which included two categories we categorized as 'failed lactation' ('tried but couldn't' and 'less than 1 month'), we were able to assess the association between T2D and failed lactation in comparison to no breastfeeding, exploring the possibility that short breastfeeding duration may be a proxy for metabolic risk. Since these response options were only offered in 1995, we limited our analysis to women who gave birth to their last child before 1995. We additionally assessed this same relationship between breastfeeding and T2D in a dataset restricted to women who reported gestational diabetes, therefore additionally assessing failed lactation among women with known diabetic risk.

All analyses were conducted using SAS (version 9.3; SAS Institute, Cary, NC). The study protocol was approved by the Boston University Institutional Review Board.

4.3 Results

In our study sample, 43% of women initiated breastfeeding and 10% had a lifetime breastfeeding duration > 1 year. Consistent with secular trends in breastfeeding rates (119, 174), women whose last child was born before 1995 were less likely to have ever breastfed than women whose last child was born after 1995 (42% vs. 78%).

Among controls (Table 4.1), longer lifetime breastfeeding duration was associated with higher parity, decreasing obesity, increased DASH diet scores, increased exercise, decreased alcohol consumption, and smoking, lower family history of diabetes, lower prevalent hypertension, and greater education. However, gestational diabetes tended to be more common with increasing durations of breastfeeding.

The association between cumulative lifetime lactation in crude and adjusted models is illustrated in Table 4.2. When we assessed this association using any breastfeeding (ever vs. never), ever breastfed was inversely associated with T2D (OR 0.87, 95% CI: 0.80, 0.94), but the association was attenuated when the model was adjusted for age, survey cycle, parity[categorical], parity[ordinal], age at first birth, GDM, exercise, education, and BMI at age 18 (OR 0.93, 95% CI: 0.85, 1.01). Cumulative lifetime lactation was further categorized by months of lactation. Within categories of duration, a dose-response relationship was evident (p for trend 0<0.01); however, at $\geq$ 24 months of cumulative lactation, the dose response association between breastfeeding and T2D was attenuated (OR for 18-23 months, 0.74 95% CI: 0.56, 0.97; OR for $\geq$ 24 months 0.87, 95% CI: 0.70, 1.08).

We further used age restricted datasets to assess for effect measure modification of the relationship between breastfeeding and T2D by age (Figure 4.1). The inverse association was strongest for women with onset of T2D at ages 40-49 (OR 0.86, 95% CI: 0.74, 1.00) and attenuated with increasing age at disease onset (ages 50-59 OR 0.96, 95% CI: 0.85, 1.08; ages 60-65, OR 0.99, 95% CI: 0.82, 1.20). For women in their 50s and early 60s cumulative lifetime lactation < 11 months attenuated as age increased, but for women who breastfed for 12-23 months, the association trended toward a protective relationship through all ages examined.

In Figure 4.2, we examined the association between breastfeeding and T2D stratified by parity (LRT 16.90, $p < 0.01$). Women with $\geq$ 24 months of cumulative lactation were considerably more likely to have $\geq$ 4 children compared to women who breastfed for 18-23 months. For example, 11.2% of women with a lifetime lactation $\geq$ 24 months had 5 children, compared with 2.3% of women with a lifetime lactation of 18-23 months [data not shown]; however, there was no pattern of change to the association between breastfeeding and T2D with increasing parity.

Figure 4.3 illustrates stratification by overweight/obese status. We found that women who were overweight/obese at age 18 had higher rates of T2D than women who were of normal weight at age 18. When we further assessed moderation by BMI at age 40 among women who were normal weight at age 18, the most protective associations were present among women who had normal BMIs at both ages.

When we assessed failed lactation in comparison to women who reported planned formula feeding, overall, the rate of T2D was highest among women who tried but couldn't breastfeed (OR 1.10, 95% CI: 0.96, 1.26) (Table 4.3). Among women with gestational diabetes, while the confidence limits were still inclusive of the null, we found higher rates of T2D among

women who 'tried but couldn't (OR 1.14, 95% CI: 0.69, 1.87) and who 'breastfed < 1 month' (OR 1.22, 95% CI: 0.60, 2.49) compared to women who planned to formula feed their babies.

4.4 Discussion

In this nested case-control analysis conducted in the Black Women's Health Study, we found that any breastfeeding was associated with reduced maternal onset of type 2 diabetes in middle age. Our findings confirm and extend earlier work on the association between breastfeeding and T2D (49, 54, 62, 66, 175). Previous studies have demonstrated an attenuation of the association between lactation and T2D with 15 years (62) and 10 years (46) since last birth. We assessed maternal age rather than time since birth, and found a similar attenuation over time as maternal age increased. This attenuation could reflect the increasing risk of T2D with age, or a diminution of the positive physical effects of breastfeeding over time and distance from the exposure.

We also found that the risk of T2D decreased as breastfeeding duration increased; however, this relationship was attenuated among women with a lifetime breastfeeding duration of ≥ 24 months. Previous studies also demonstrate this attenuation of the association between lactation and maternal metabolic disease risk (50, 54). For example, in the Women's Health Initiative, the same pattern was present in a study on metabolic syndrome (54). In this study assessing multiple components of metabolic syndrome, attenuation was present among women with lifetime breastfeeding duration ≥ 24 months for diabetes and obesity, but not hypertension or hyperlipidemia in adjusted models. In our study population, we speculated that grand multiparity among women with ≥ 24 months of lifetime lactation may underlie the dose response relationship between breastfeeding and T2D. We found higher parity among women with longer lifetime lactation duration. Among women in the ≥ 24 month group, 11.2% had 5 children,

compared with 2.3% of women in the 18-23 month group; however, a corresponding weakening of the association between breastfeeding and T2D at ≥24 months was present for women with 3 children, but the pattern did not persist for women with 4 or more children. Previous studies have assessed modification by parity for the relationship between breastfeeding and T2D (46, 62, 63); however, the results have been mixed. It is worth noting that among the studies that showed no modification, one utilized a dataset of urban Chinese women whose parity was low overall (median, 1 child; and 10.5% of the population had 4 or 5 children) (46), and the second used breastfeeding per birth (175), which, unlike the cumulative lactation variable used in all the studies finding modification, accounts for parity by definition. The absence of a simple relationship between cumulative lactation and T2D by strata of parity in our study underscores the difficulty in characterizing women with four or more children. This category could equally include women who lack access to good health care, as well as women who have many children by choice, a group who might breastfeed for longer durations of time in association with positive health behaviors.

Obesity rates, as with diabetes, are higher in black women than in the general US population (56% vs. 33%) (90-93). Pregnancy is a time when many women gain weight; and, although adult onset of overweight/obesity is not a direct measure of postpartum weight retention, previous work with the Black Women's Health Study found that parous women gained more weight during the reproductive years than nulliparous women (160). Increased weight gain associated with parity suggests that childbirth plays a role in the weight gain that occurs with age. Additionally, there is some evidence that women who breastfeed are less likely to retain weight postpartum (161, 176). The differential role of lactation on postpartum weight loss has been studied extensively, and the results are mixed (79, 155-157). Our study, as with most of the

existing studies on the topic, assessed BMI as an important confounder of the relationship between breastfeeding and T2D. Consistent with the existing literature (46, 49, 54, 63, 66, 175), modification of the relationship between breastfeeding and T2D by BMI was minimal; however, our results demonstrate greater attenuation of the relationship with adult onset of overweight/obesity status than adolescent overweight/obesity. This lends support to the possibility that weight gained during the reproductive years may be on the causal pathway between breastfeeding and T2D; however, future research that includes specific data on postpartum weight retention is needed to fully address this possibility.

Events in the immediate postpartum period reflect endocrine and metabolic changes that may be affected by a mother's overall health as well as social and physical support systems. To our knowledge, this is the first study to measure associations between failed lactation in the early postpartum period and its association with T2D. We found women who reported failed lactation had higher rates of T2D than women who reported planned formula feeding. This association was stronger among women who had gestational diabetes and also reported failed lactation, lending support to the hypothesis that failed lactation may be a marker for metabolic risk. However, there is also evidence that black women receive disparate lactation support from the medical community (32-35). Thus, the possibility that these relationships are confounded by inadequate medical or social support for breastfeeding cannot be eliminated.

Our findings must be interpreted within the limitations of the study design. Our assessment of the exposure was limited in that we used recalled cumulative lifetime lactation to measure breastfeeding duration. Cumulative lifetime lactation relies on recall of breastfeeding duration and thus may be susceptible to bias; however, the literature on breastfeeding recall indicates that it is a reasonably dependable measure, even if it occurs decades after the index

birth (42, 43, 177). Recalled total duration is also limited in that this measure does not account for breastfeeding intensity or duration for individual births; thus, we were unable to determine whether breastfeeding occurred with all pregnancies, or differentially with some and not others. These limitations are important because intensity and consistency of breastfeeding may be associated with different levels of protection (49). Furthermore, we had incomplete data on pregnancy complications beyond gestational diabetes and preeclampsia, as well as no information about access to lactation support in the perinatal period. Finally, the generalizability of our findings may be limited in that the participants in this cohort are highly educated and a higher proportion chose to breastfeed than black women in the general population of the United States.

One of the strengths of our study was the use of a case control design with density sampling allowing estimation of the incident rate ratio. Use of a nested case control design was also innovative and beneficial to this line of research because it reduced selection bias without inducing survival bias. Selection bias was reduced by including cases of T2D that occurred both prior to and after cohort entry. In this way, we reduced the 'immortal' time between retrospective exposure and incident disease (115). Retrospective or cross-sectional designs also use all cases of disease; however, they correct selection bias by introducing survival bias by treating all disease as prevalent and thus losing the specificity of comparing disease onset to like risk sets across time. Our study design reduced both selection and survival bias while preserving the benefits of retrospective studies (use of all cases), and Cox proportional hazard models (comparison to risk sets at specific times). However, the steps taken to assign a specific age of onset for disease diagnosis among women who developed disease prior to 1995 introduced imprecision in our measure of age disease onset.

In conclusion, we found that longer cumulative lifetime lactation is associated with reduced rates of T2D in a population of black women. These findings are important for public health, because black women experience substantial disparities in breastfeeding as well as an increased burden of diabetic disease, including delayed recognition, less effective treatment, and greater morbidity and mortality (13-16). Breastfeeding is compelling as a preventative health behavior because it requires a time-limited commitment. This study supports targeted interventions by health care providers and public health advocates to encourage and support breastfeeding among black women as well as highlighting the need to prioritize support of women who are working to initiate breastfeeding, particularly if other risk factors for T2D, such as gestational diabetes, exist.

4.5 Tables and Figures

Table 4.1 Demographic characteristics among the control population by duration of cumulative lifetime lactation (months). n = 12,510 controls. Black Women's Health Study 1995-2011

	Cumulative lactation (months)				
	None n= 6670 %	**1-3 months n = 2101 %**	**4-6 months n = 1353 %**	**7-23 months n = 1870 %**	**≥ 24 months n = 516 %**
Age 1st birth[Med (IQR)]	20 (18, 24)	23 (19, 27)	23 (19, 28)	23 (20, 28)	23 (19, 28)
Parity					
1	34.3	34.6	32.5	15.3	7.4
2	36.6	35.5	30.6	43.3	26.4
3	18.1	17.4	16.2	24.3	29.3
4+	11.1	12.6	10.7	17.1	37.0
Overweight/Obese at age 18 (≥ 25 kg/m^2)					
	10.1	9.4	7.8	8.9	6.8
DASH Diet (higher = healthier)[1]					
< 19	16.7	14.0	13.5	12.2	7.3
19-28	64.4	65.9	62.7	63.7	63.6
> 28	18.9	20.0	23.8	24.2	29.1
Vigorous Exercise[1]					
none	43.0	33.3	31.6	30.3	26.7
< 3 hours	36.2	44.3	43.3	42.4	43.4
≥ 3 hours	20.8	22.4	25.1	27.3	29.8
Alcohol (drinks/week)					
none	68.6	68.6	68.0	72.8	78.5
1-7	25.0	25.2	26.1	23.1	17.0
> 7	6.3	6.2	5.8	4.0	4.5
Smoking (pack-years)					
never	55.9	57.3	61.4	63.9	71.6
< 20	32.2	33.8	29.6	30.5	24.9
≥ 20	11.9	8.9	8.9	5.6	3.6
Family history of Diabetes [1]					
None	70.0	71.6	72.5	72.4	73.7
Age > 50	20.7	19.9	19.0	19.1	18.1
Age < 50	9.3	8.5	8.5	8.5	8.2
Prevalent hypertension	30.7	26.7	27.1	25.9	25,4
Gestational Diabetes[1]	3.7	4.0	3.8	6.7	8.3
Education					
≤ High School	21.6	12.7	11.3	10.3	7.4
Some College	33.7	29.0	27.9	25.1	26.2
≥ College	44.7	58.4	60.8	64.6	66.5

[1]Missing: Smoking (2.9%); Family history of diabetes (6.4%); Urbanity (11.6%)

Table 4.1 Odds ratios for type 2 diabetes by duration of cumulative lactation for crude and multivariate model. Black Women's Health Study 1995-2011.

	Cumulative Lactation N = 15,680				
		Model 1[a]		Model 2[b]	
	Cases / Controls	OR	(95% CI)	OR	(95% CI)
None	1885 / 6557	1.00		1.00	
Any	1452 / 5786	0.87	(0.80, 0.94)	0.93	(0.85, 1.01)
1-3	547/ 2080	0.91	(0.82, 1.01)	0.98	(0.87, 1.09)
4-6	324 / 1338	0.84	(0.74, 0.96)	0.93	(0.81, 1.06)
7-11	239 / 873	0.95	(0.81, 1.10)	1.00	(0.86, 1.18)
12-17	144/ 649	0.77	(0.63, 0.93)	0.79	(0.65, 0.96)
18-23	68/ 336	0.69	(0.53, 0.90)	0.74	(0.56, 0.97)
≥24	130 / 510	0.88	(0.72, 1.08)	0.87	(0.70, 1.08)
***P* for trend[c]**			0.64		<0.01

Odds Ratio, CI: Confidence interval

[a] Model 1 adjusted for age and survey cycle

[b] Model 2 adjusted for age, survey cycle, parity[categorical], parity[ordinal] age at first birth, gestational diabetes, education, vigorous exercise, and BMI at age 18

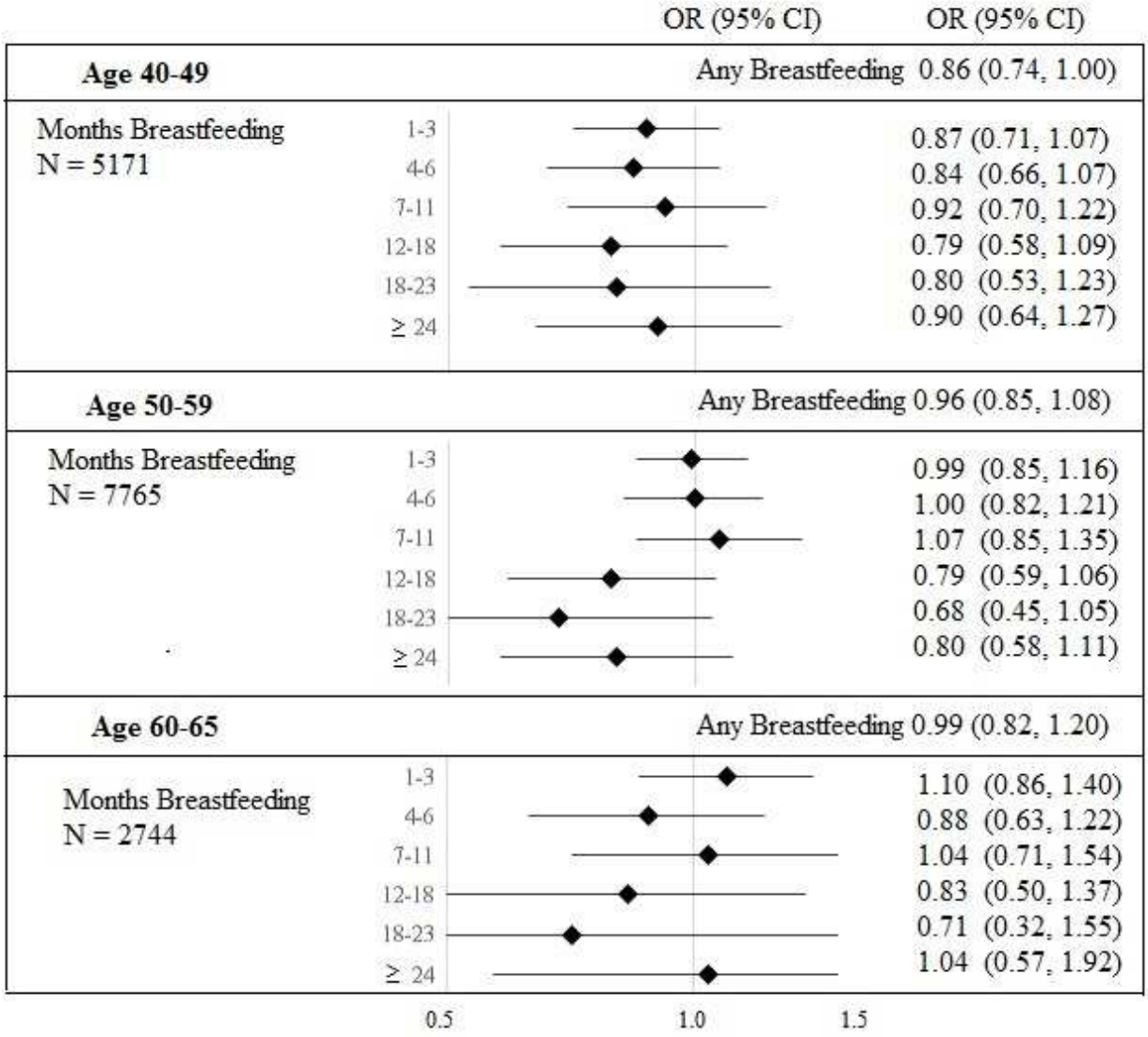

Figure 4.1 Odds ratios (OR) and 95% Confidence Intervals (CI) for the association between type 2 diabetes and cumulative lifetime lactation, for any breastfeeding and months of duration, compared to no breastfeeding among datasets restricted to age epochs (40-49, 50-59, and 60-65). N = 15,680. The results were adjusted for age, survey cycle, parity[categorical], parity[ordinal], age at first birth, exercise, gestational diabetes, education, and BMI at age 18. Black Women's Health Study 1995-2011.

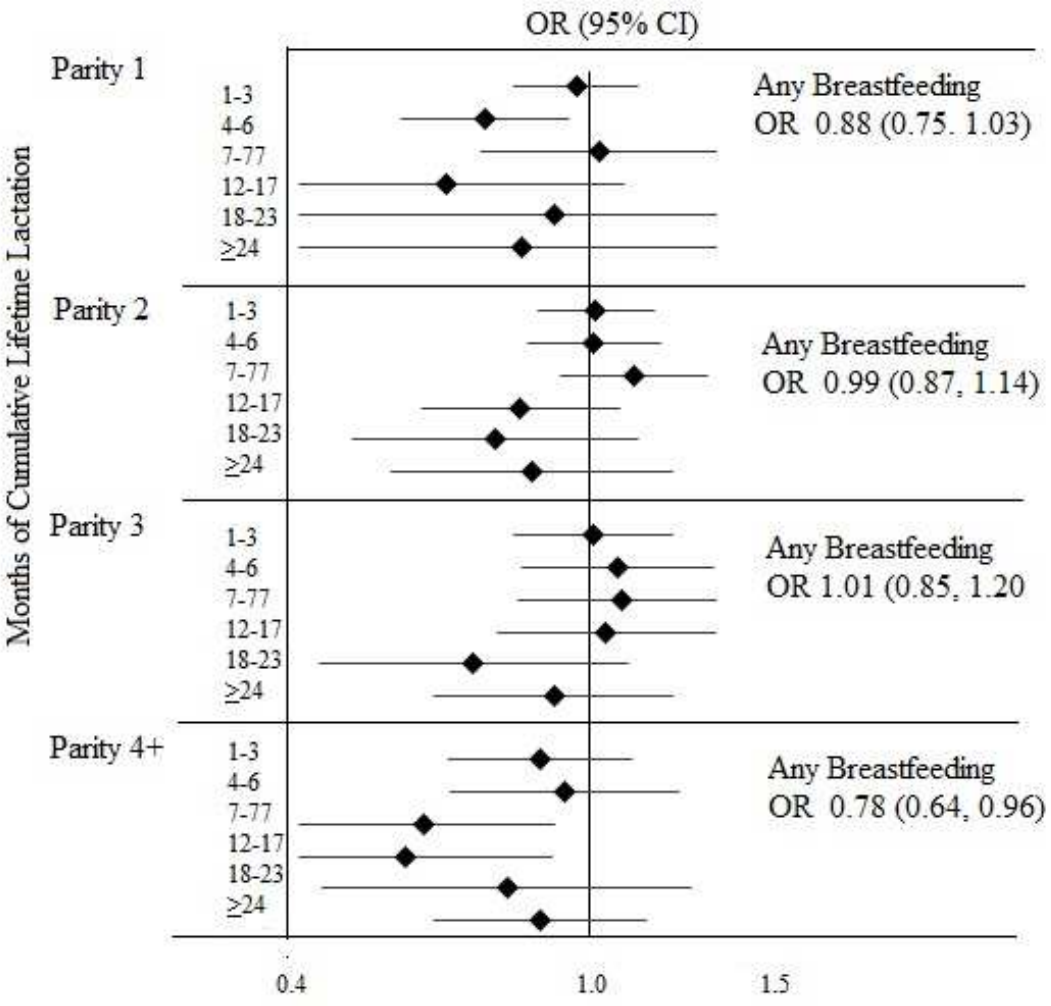

Figure 4.2 Odds ratios (OR) and 95% confidence intervals (CI) for type 2 diabetes comparing cumulative lifetime lactation (in months) to no breastfeeding by levels of parity. ORs were controlled for age, year, parity[cat], parity[ordinal], age at first birth, education, gestational diabetes, exercise, BMI at age 18, and an interaction term between parity and cumulative lifetime lactation. N = 15,680. Black Women's Health Study 1995 – 2011.

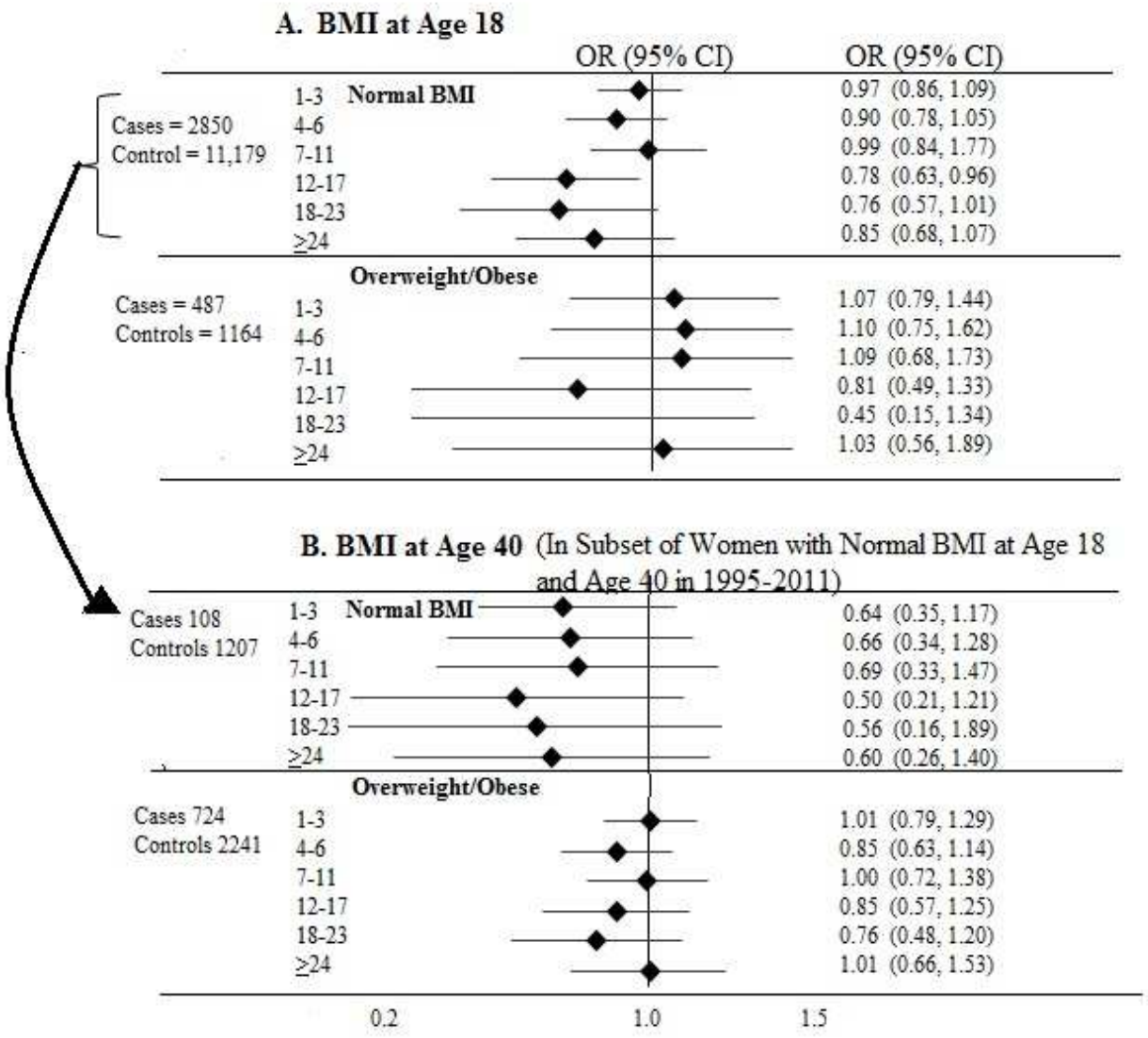

Figure 4.3 Odds ratios (OR) and 95% Confidence Intervals (CI) for the association between type 2 diabetes and cumulative lifetime lactation, compared to no breastfeeding by categories of BMI: normal (< 25 kilograms/meter2); overweight/obese (> 25 kilograms/meter2). A. N = 15,680. Adjusted for age, year, parity[cat], parity[ordinal], age at first birth, education, gestational diabetes, exercise, BMI at age 18, and an interaction term between BMI at age 18 and cumulative lifetime lactation. B. N = 4280. Adjusted for age, year, parity[cat], parity[ordinal], age at first birth, education, gestational diabetes, exercise, BMI at age 40, and an interaction term between BMI at age 40 and cumulative lifetime lactation. Subset limited to women who were of normal weight at age 18, and who were <40 when they joined the cohort. Black Women's Health Study 1995 – 2011.

Table 4.2 The association between type 2 diabetes and cumulative lifetime lactation among women who gave birth prior to 1995. Comparison of 'Failed Lactation' (tried but couldn't breastfeed, or breastfed for < 1 month) to women who reported planned formula feeding

Cumulative Lactation	Total Population N=14,058		GDM N=833		No GDM N = 13,225	
	OR	95% CI	OR	95% CI	OR	95% CI
None	1.00	Referent	1.00	Referent	1.00	Referent
Tried but couldn't	1.10	(0.96, 1.26)	1.14	(0.69, 1.87)	1.10	(0.96, 1.27)
<1 month	0.96	(0.80, 1.15)	1.22	(0.60, 2.49)	0.94	(0.78, 1.14)

OR: odds ratio, CI: confidence interval; GDM, gestational diabetes
Adjusted for: age, year, parity[cat], parity[ordinal], age at first birth, education, gestational diabetes, exercise, BMI at age 18

CHAPTER 5: CONCLUSIONS

5.1 Introduction

The purpose of this dissertation was three-fold. Primarily, we wanted to assess the association between breastfeeding and maternal metabolic health, specifically hypertension and type 2 diabetes. Our second goal was to quantify this relationship among black women, a population under-represented in the current literature. And our third goal was to introduce a methodology to this literature that might reduce selection and survival bias, while maintaining some of the benefits of both time to event and retrospective models.

This dissertation was designed within the theoretical constructs of the Reset Hypothesis, which posits that the normal metabolic processes of pregnancy facilitate accumulation of fat stores and changes in glucose and lipid metabolism, providing nutrition in utero and accumulating stores for breastfeeding. Within these constructs, lactation plays a role in resetting maternal metabolism to its pre-pregnancy state. The result of formula feeding, then, is a disruption in the return to prepregnancy metabolic processes with a resulting increase in disease risk.

In the existing literature, two methods have typically been used for assessing the association between breastfeeding and disease outcome: Cox proportional hazards models, and cross-sectional or retrospective designs. Studies using hazards models to assess the associations between breastfeeding and incident disease typically use retrospectively ascertained

breastfeeding history at baseline. Women with prevalent disease are excluded, and incident disease is assessed from baseline until the end of the study, thereby eliminating cases of disease that occur after exposure to breastfeeding, but prior to study enrollment. On the other hand, retrospective designs do not eliminate any cases of disease; however, prevalent disease is ascertained at study end. Using logistic regression, women who developed disease at any time and at any age are compared with women who survived the full study period without developing disease. Both of these approaches introduce bias. The association between breastfeeding and metabolic disease varies with age, and the strongest associations have been found in younger women, or those closest to the exposure. Thus, hazards models eliminate women who develop disease closest to the exposure, treating exposed women differently based on the age at which their disease occurred. While retrospective studies include all cases of disease, comparing women with disease occurring across all ages and times to women who have survived the full study period without developing disease reduces selection bias at the cost of introducing survival bias. To address these limitations of the extant literature, we used a case control model which matched on age and time of disease onset, while allowing for all cases to be included. Our design further maintained risk sets that were similar by age and time, thus reducing the selection bias present in hazard models without inducing the survival bias present in retrospective designs. We anticipated that our outcome estimates would be further from the null than what has reported in Cox proportional hazards analyses, because women with a younger onset of disease were excluded from these models. We further anticipated that our results would be closer to the null than retrospective models, because we compare women of similar ages and times.

We were also interested in exploring the use of cumulative lactation as a measure of breastfeeding exposure. Cumulative lactation is constructed such that as parity increases,

cumulative lifetime lactation typically also increases. Thus the effects of higher parity may confound the relationship between cumulative breastfeeding and the onset of diseases later in life. We anticipated that the associations between cumulative lactation and disease onset would be modified by parity.

We hypothesized that women who were obese prior to pregnancy would experience less protection from breastfeeding than women of normal weight. Specifically, if women were overweight or obese prior to pregnancy, indexed in our analysis by BMI at age 18 (adolescence), we expected a higher rate of disease regardless of breastfeeding status because a woman's weight prior to pregnancy would more strongly influence risk than the differential changes in metabolism due to breastfeeding after pregnancy. Thus, women who breastfed, but were overweight or obese as adolescents, would experience a smaller decrease in rates of hypertension and diabetes, compared with women who breastfed but were of normal weight as adolescents.

Selection of referent groups in studies of breastfeeding & maternal health: An important methodological consideration in studies of breastfeeding and maternal health is how to model the breastfeeding exposure. Extant studies typically use two approaches: authors compare parous women who have ever breastfed with parous women who never breastfed, or they compare outcomes among various durations of breastfeeding. These analyses of duration may define categories of breastfeeding duration, or may model a linear association between breastfeeding duration and health outcomes.

When assessing dose response relationships, the choice of referent category impacts the interpretation of study results. Some studies have used medical recommendations for breastfeeding duration as the referent, framing the discussion of results around the risk of reduced breastfeeding (48). However, the majority of studies in the existing literature use 'no

breastfeeding' as the referent category, with the one exception of the PROBIT study (53). In this study, breastfeeding initiation was an inclusion criteria; therefore, the study authors were not able to assess whether ever breastfeeding was associated with hypertension. For the dose response analysis, they used 1-3 months as their referent category, and found no association. While the mathematical differences between strata are not changed by the choice of referent category, using 'no breastfeeding' as a referent could over-represent the dose response relationship in situations in which ever breastfeeding is inversely associated with the outcome disease. This is of particular concern when modeling trends in associations, in that including '0' for never-exposed individuals may produce a significant p for trend when there is actually not a dose-response association. However, for nominal variables, selection of a different referent group can also affect the precision of estimates, in that a smaller referent group will widen confidence intervals.

We explore the effect of different modeling strategies for nominal breastfeeding duration variables in Table 5.1. Here, we present odds ratios for hypertension using either never breastfed or breastfed 1-3 months as the referent group. In order to determine whether using 1-3 months as a referent category would significantly alter our outcomes, we here present an alternate table for the primary outcomes in T2D (Table 5.1). As anticipated, the confidence limit ratio increased when we used a smaller referent group, reducing the precision of our estimates.

For our analyses, we chose to use 'no breastfeeding' as the referent category for three reasons. First, as noted above, these models were more statistically stable. For example, in the study on T2D, there were 14,355 women who had not initiated breastfeeding, compared with 4110 women who breastfed for 1-3 months. Second, because we are asking whether breastfeeding 'resets' metabolism after pregnancy within the Reset Hypothesis paradigm, the

population of interest for comparison was all parous women. After pregnancy, lactation occurs whether or not women choose to breastfeed, and thus, the option not to breastfeed is an active choice, rather than a passive one without exposure. For example, consider recreational drug use as a comparator. In this instance, the category of 'no drug use' is likely to be inclusive of women who actively choose not to use drugs, as well as women who did not use drugs as a passive decision because they were never exposed or sought out the choice. The category of 'no breastfeeding' in our analysis includes only parous women. This is different from studies inclusive of both parous and non-parous women, which would be similar to women who both actively and passively did not use recreational drugs. Finally, to facilitate comparison of our results to others, we chose to use the same referent group as other published studies.

5.2 Summary of Findings

Differences between hypertension and T2D: As anticipated, our results are consistent with existing evidence of a protective association between breastfeeding and both hypertension and T2D. However, we found a more marked association between breastfeeding and T2D than breastfeeding and hypertension. For example, compared with never having breastfed, the association between breastfeeding and hypertension with any breastfeeding was only marginally protective (OR 0.97, 95% CI: 0.92, 1.02) while the association between any breastfeeding and T2D demonstrated evidence of a stronger inverse association (OR 0.87, 95% CI: 0.81, 0.94).

Most studies report dose response measures, or the effect estimates for the relationship between varying lengths of breastfeeding and hypertension or T2D. Our results confirm the results of previous research suggesting that there is an inverse dose response relationship between breastfeeding and each outcome disease. As in other studies, the relationship is stronger for T2D than for hypertension (50, 54). Among women ages 40-49 in our analysis of lactation

and hypertension, the minimum "dose" of lifetime lactation associated with a protective association exclusive of the null was ≥24 months (OR 0.82, 95% CI: 0.69, 0.98, vs. never lactated), whereas for T2D 4-6 months of breastfeeding was associated with a reduced risk (OR 0.72, 95% CI: 0.57, 0.91 vs never lactated). Additionally, the dose response relationship in our results was present for women of all ages with T2D (p for trend across all age categories < 0.01) but was only present for hypertension in women ages 40-49 (p for trend < 0.01) compared to women aged 50-59 (p for trend = 0.07) and women aged 60-65 (p for trend = 0.58).

Interestingly, for T2D but not for hypertension, increasing exposure to breastfeeding decreased the rate of disease up to 23 months, but this inverse dose-response association was attenuated at ≥ 24 months. For instance, the rate of T2D in 40-49 year old women who breastfed from 18-23 months compared with women who did not breastfeed was 0.58 (95% CI 0.37, 0.90), but for women who breastfed for ≥ 24 months, the protective association was attenuated (OR 0.83, 95% CI: 0.60, 1.16), creating a 'J-shape' to the dose response relationship (i.e. a dose response relationship that was progressively stronger from 1-3 up to 18-23 months, and then weaker at ≥ 24 months) that persisted through all age categories examined.

One possible explanation for this J-shaped relationship between breastfeeding and T2D in our study could be the use of the variable for cumulative lactation as a measure of exposure. If the outcome disease is associated with parity, increasing parity may confound the relationship between breastfeeding and outcome disease. As parity increases, women have more opportunities to lactate, and therefore, parity and cumulative lactation are correlated. Thus, the use of cumulative lactation to measure breastfeeding exposure could increase the possibility for residual confounding by parity in the relationship between increasing lactation and the outcome

disease. Parity is associated with both T2D (165) and hypertension (178) yet we only found the J-shaped dose-response relationship in the study on T2D.

We initially stratified parity into 3 categories for T2D and found the anticipated pattern for parity most clearly in the highest level of parity (3+), i.e., an attenuation of the association between breastfeeding and T2D among women with ≥ 24 months of breastfeeding (Figure 5.1). To further define this attenuation, we added an additional category to parity so that we could stratify by 1, 2, 3, and 4+ children. Counter to our expectations, rather than further defining this attenuation at ≥ 24 months, the associations between breastfeeding and T2D were stronger among women who had 4 or more children, and although there was attenuation of the association, it occurred at 18-23 months as well as ≥ 24 months (Figure 4.2).

These results highlight the multiple factors that determine parity. For instance, women with many children may have less opportunity for self-care, or may have higher rates of obesity due to childbirth occurring at older ages as parity increases, each time, increasing the risk of postpartum weight retention. A high parity could correlate with an inability to control fertility, which might also be an indication of irregular health care or health maintenance. Any of these factors complicate the ability to interpret strata of parity, and may alter associations between breastfeeding and T2D due to varying maternal risk of T2D within each strata. Thus, while there is effect measure modification of the association between breastfeeding and both hypertension and T2D by parity, the pattern is not predictable or consistent. These results underscore the importance of considering determinants of parity when studying the associations between reproductive history and maternal health, as well as the interpretation of results within the limitations of the measurement of breastfeeding exposure.

One possibility is that there is something about the disease process itself that leads to this relationship. This hypothesis is supported by the absence of a J-shaped pattern in the hypertensive study. There are two additional studies that examine both hypertension and diabetes within the same populations that are consistent with our results. They both find a similar J-shaped dose response relationship for T2D but not hypertension (50, 100).

When these additional studies are further examined, we find the relationship persists despite different methods of controlling for confounding by parity. In the study using data from the Women's Health Initiative (100), Schwarz et. al. note that women who breastfed for 13-23 months had lower rates of T2D (OR 0.75, 95% CI: 0.66, 0.85), than women who breastfed for 24+ months (OR 0.88, 95% CI: 0.76, 1.01) in multivariate models. While cumulative lifetime lactation was used for the breastfeeding exposure variable, thus introducing the possibility of confounding by parity, only the additive effect of parity was controlled and not the multiplicative interaction, allowing for the possibility that the use of cumulative lactation to quantify breastfeeding exposure might have led to residual confounding by parity. On the other hand, in a study of Chinese women, the analytic population was limited to women who only had one child, thus controlling the multiplicative effect of breastfeeding and parity; yet still, the rate of T2D for >12 months was closer to the null (OR 0.57, 95% CI: 0.31, 1.02) than the outcome estimate for 6-12 months (OR 0.47, 95% CI: 0.26, 0.86). In summary, because the J-shaped dose response relationship is present whether the multiplicative association between breastfeeding and parity was controlled (50) or not (100), we must assume that while measuring the exposure using cumulative lactation may contribute to the confounding of the relationship by parity, there are additional complexities to the relationship between breastfeeding and T2D that go beyond residual confounding by parity.

In this dissertation, we modeled associations with both hypertension and T2D. These data afforded the opportunity to compare the association between breastfeeding and these two diseases together, and we therefore assessed whether there was effect measure modification of T2D by pre-existing hypertension, present at the time of T2D diagnosis (Table 5.2). The protective association between breastfeeding duration and T2D was attenuated for women with hypertension within each category of breastfeeding, except for women who had breastfed for $\geq$ 24 months (LRT chisq = 9.252 p = 0.002), except for women who had breastfed for $\geq$ 24 months, for whom the point estimates were very similar, although the confidence limit ratios were wide (OR for non-hypertensive women who breastfed for $\geq$24 months, compared with no breastfeeding 0.82, 95% CI: 0.61, 1.09, CLR 1.79, and for hypertensive women 0.83, 95% CI 0.58, 1.21, CLR 2.09).

Differences in associations between breastfeeding and T2D vs. hypertension may reflect the life course of these diseases. Due to the loss of elasticity in blood vessels with aging, most women will develop hypertension regardless of their health behaviors, and prevalence thus increases steadily with age (Figure 2.3). On the other hand, although age also affects the prevalence of T2D, the causal mechanisms underlying T2D are not an integral part of the aging process. T2D begins when the cells of the muscles, liver and fat lose their ability to respond to insulin effectively, increasing the need for insulin and the load on the beta cells in the pancreas, until the pancreas cannot produce enough insulin to modulate glucose levels, causing hyperglycemia (11). Thus, the break-down of normal pancreatic and cellular function can be exacerbated or caused by health behaviors such as a sedentary lifestyle and poor eating habits.

Alternatively, the differences we see in the associations between breastfeeding and hypertension vs. diabetes could also be related to the different mechanisms of action through

which breastfeeding might affect the disease risk. While the risks of both diseases increase with increasing adiposity, thus suggesting that lactation could be differentially affecting postpartum weight retention, there are different hormonal pathways at work with both diseases as well. Oxytocin, the primary neuropeptide responsible for the milk ejection reflex, is essential to lactation (179). Each time the baby goes to breast, the mother's oxytocin levels rise in response to suckling. Oxytocin also has been associated with lower blood pressure, vasodilation, and metabolic effects, as well as having anti-inflammatory properties (180, 181). These mechanisms have been suggested pathways through which breastfeeding might affect maternal cardiac function. T2D, on the other hand, is regulated by insulin. When insulin resistance and insulin secretory defects are measured in women with a history of gestational diabetes, lactation has a positive effect on insulin and glucose responses (182, 183). But the relationship between insulin and lactation is even more intimate. Not only might maternal insulin homeostasis influence breastfeeding success, but recent research also demonstrates that insulin is essential to mammary differentiation and stimulation of the genes that are directly responsible for milk protein synthesis (184-187). Thus, pre-existing insulin dysregulation can affect milk production, reducing the chances for successful breastfeeding in the postpartum period. These potential mechanisms of are particular interest given our findings linking failed lactation with subsequent diabetes risk. In summary, while breastfeeding has an inverse dose-response relationship with both hypertension and T2D, the mechanisms underlying these associations might be quite different for each disease process.

Attenuation: In our study, there was attenuation of the relationship between breastfeeding and hypertension as well as breastfeeding and T2D as women aged; however, it is difficult to determine whether attenuation of these positive associations was because of age, or time from

exposure. Several studies address attenuation of the relationship between breastfeeding and later hypertension with age or time since exposure. For instance, Lupton et. al. (47) found that the association between 24+ months of cumulative breastfeeding and hypertension was strongest among women aged 45 to <54 years (OR 0.58, 95% CI: 0.44, 0.77) compared with women who were 54 to <64 (OR 0.60, 95% CI: 0.50, 0.73) or age 64 or more (OR 1.03, 95% CI: 0.88, 1.22). Natland et. al. (52) found similar results when stratified by age 50. Parous women 50 years of age or younger who reported no lactation had higher blood pressure than the reference group of women who had lactated for 24 months or more (OR 1.88, 95% CI: 1.41, 2.51). For women over age 50, however, the association between ≥24 months' lactation and hypertension included the null (OR 1.26, 95% CI: 0.96, 1.65). The same attenuation with age is seen in the relationship between breastfeeding and death by cardiovascular disease (188). Among parous women younger than 65 years, those who reported never lactating had an adjusted HR of 2.86 (95% CI: 1.51, 5.39) for death from cardiovascular disease compared with women who had ever lactated.

Among women 65 or older, the association was much weaker (HR 1.11, 95% CI: 0.77, 1.69). While none of the study authors assessing the relationship between breastfeeding and T2D stratify results based on age, Stuebe et. al.(62) assessed attenuation over time by performing an analysis restricted to women whose last birth occurred <15 years from the onset of disease. For these women, nursing for > 23 months had a stronger association with T2D (0.59 (95% CI: 0.44, 0.79) than the analysis of the full population (0.67, 95% CI: 0.54, 0.84) (62). Similar results were found in the Shanghai Women's Study (46), where the outcome estimates for the relationship between breastfeeding and T2D were stratified at ≤ 10 years and >10 years since birth.

Attenuation with age and attenuation with years from last birth both appear to be consistent across studies; however, it is not clear whether this is due to time since exposure, or a

reduction in the statistical power to detect small differences in outcome due to the increasing disease prevalence, particularly in the case of hypertension. None of the studies to date have investigated whether there is a difference in attenuation using time from exposure compared with the overall age of the population or the menopausal state of the participants. Our study confirms the literature on the attenuation of the relationship between breastfeeding and hypertension with age, and extends the existing literature by assessing attenuation by age in the relationship between breastfeeding and T2D.

Body mass index: We hypothesized that breastfeeding could be associated with reduced rates of metabolic disease, but in the presence of overweight/obesity in adolescence, the association would not be as strong. For hypertension, our results did not support this hypothesis. There was no moderating effect of adolescent overweight/obesity on the relationship between breastfeeding and hypertension (LRT $chi^2 = 3.696$, $P = 0.545$). However, this was not the case for the study on T2D (LRT $chi^2 = 17.884$, $P < 0.001$) for which adolescent BMI was a modifier. As anticipated, women who were of normal weight as adolescents experienced the greatest inverse dose-dependent association between breastfeeding and T2D.

In the existing literature, multivariate models almost always include BMI when assessing the association between breastfeeding and metabolic disease; however, the age at ascertainment of BMI differs across the studies. Some, as with ours, utilize BMI ascertained at age 18 (46, 48, 54, 56, 62), while others use BMI measured before (65, 189), or after (50) pregnancy, or at study baseline (47, 50, 60, 63, 100). Due to these differences in ascertainment of BMI, some studies were controlling for BMI as a confounder, while others were assessing BMI as a mediator, and finally, in some studies BMI could occur either before or after breastfeeding, depending on the age of the participant at the baseline questionnaire. The Reset Hypothesis posits that postpartum

weight retention could be a mediator of the relationship between breastfeeding and outcome metabolic disease, but none of the studies specifically control for postpartum weight retention.

Techniques used to examine the effect of BMI on the primary association also differ between studies. Some studies compare results between models with and without BMI looking for a change in the effect estimate (46, 48). While this helps to determine whether BMI is a significant contributor to the model overall, it does not untangle the temporality of BMI in the relationship between breastfeeding and metabolic diseases. There are three studies that, within the limits of the data available for analysis, assess mediation by BMI. In a study of women ages 40-81, Zhang et. al. (50) controls for BMI at baseline as well as controlling for recalled BMI in the postpartum period. In a study of women aged 40-70 at baseline Villegas et. al. (46) controlled for BMI at age 20, age 40, and the difference between the two. And finally, using a Cox model, Stuebe et. al. (62) used BMI at age 18, and updated it with baseline BMI, as well as subsequent BMI throughout the observational period. When updated BMI was added to the model in addition to BMI at age 18, the association between breastfeeding and T2D was marginally attenuated but remained significant, suggesting that weight changes during lactation play only a minor role in the association between breastfeeding and T2D.

Only two studies take the further step of stratifying results based on BMI. Lee et. al. (45) measured BMI at baseline in a cohort of young Korean women, many of whom were still in their reproductive years. Interestingly, they found a stronger inverse association between breastfeeding and hypertension among obese women than among women of normal weight. For women who were obese, defined as BMI $\geq$ 23.05, ever breastfeeding decreased the risk of hypertension by 20%, while among women who had a normal BMI, ever breastfeeding only decreased the relative risk of hypertension by 6%. In a study of T2D, Liu et. al. (110), used a

dataset of women who were over 45 at baseline to assess the association between ever breastfeeding and T2D. BMI, measured at baseline (BMI <25 and BMI $\geq$ 25), did not change the effect estimates when the referent value was nulliparous women; however, this measure of BMI was ascertained after breastfeeding and diagnosis of T2D, so does not provide results that test either mediation or modification of the relationship between breastfeeding and T2D

We attempted to address BMI as a mediator in a sensitivity analysis. Our primary variable for BMI was created using weight recalled for age 18. We did not have consistent information about weight gain during or after pregnancy in this population. Since adolescent obesity does not account for obesity that developed between pregnancy and disease onset, we isolated women who were of normal weight during adolescence. In a sub-analysis among these women, we assessed the effect of overweight/obesity at age 40 on the relationship between breastfeeding and both hypertension and diabetes. In our analysis, we excluded women with births after age 40; thus, we isolated weight gain to the reproductive years. However, we still could not determine whether obesity that developed between ages 18 and 40 occurred before or after the woman's first birth. It is likely that the preponderance of weight gain that occurred during these years was likely to have been due to postpartum weight retention, based on previous research demonstrating that weight gain occurred more often with pregnancy than in nulliparous women in this dataset (160). For both hypertension and T2D, adult onset of overweight/obese status measured at age 40 was a stronger effect measure modifier of the association between breastfeeding and metabolic disease than overweight/obesity reported for age 18. From this, we can determine that whether women were overweight/obese in adolescence did not influence the association between breastfeeding and incident disease as much as the accumulation of weight over their reproductive years, lending support to the hypothesis that postpartum weight retention

could be on the causal pathway. Further studies that include a more accurate measure of postpartum weight retention would be necessary to address the possibility of mediation directly.

Failed Lactation: One of the difficulties in using retrospective assessment cumulative of cumulative lifetime lactation is the absence of information regarding breastfeeding intention. We were able to indirectly measure breastfeeding intention using the 1995 questionnaire which specifically asks women if they 'never tried' to breastfeed, or 'tried but couldn't', as well as breastfeeding for 'less than one month'. These categories could hypothetically differentiate between planned formula feeding ('never tried') and failed intention to breastfeed ('tried but couldn't' and '< 1 month'). A sub-analysis limited to women whose last child was born prior to the baseline questionnaire allowed us to compare women who failed to initiate breastfeeding to those who never attempted to breastfeed. We hypothesized that breastfeeding failure might be a marker for underlying metabolic risk. This underlying metabolic risk could confound associations between breastfeeding duration and health in later life. In both studies, women with failed lactation had greater rates of outcome disease than women who chose to formula feed, although the magnitude of the difference was negligible in the study on hypertension. This difference suggests that some of the observed association between lactation and T2D may be confounded by women with metabolic dysregulation being both more likely to develop T2D later in life as well as being less likely to successfully initiate breastfeeding. To further explore this possibility, we limited our analytic sample to women with a history of gestational diabetes. Such women have known insulin dysregulation which can delay lactogenesis and disrupt milk production (190). Among these women, the difference between planned formula feeding and failed lactation was even more pronounced, lending support to our hypothesis that failed lactation may reflect underlying metabolic dysregulation or reverse causality. These results

should be treated cautiously, however, because it is also possible that failed lactation was caused by lack of medical support, lack of social support, or other medical conditions not related to metabolic function, all of which might also increase the risk of disease onset later in life.

Selection Bias: We hypothesized that studies using a Cox proportional hazard model excluding prevalent disease at study baseline, would selectively eliminate women who developed hypertension and T2D while they were young or closer to their exposure to pregnancy and breastfeeding. We further hypothesized that this selection bias would result in effect estimates that were closer to the null than our results would be. Here we compare our results for T2D to a study using this method (62) (Table 5.1), although it is important to note that women in the Nurse's Health Study were older as well as having lower breastfeeding initiation rates (64%) than women in the Black Women's Health Study. While caution in this direct comparison is necessary, the results of these two studies are particularly intriguing because Stuebe et. al. stratify by time since exposure, allowing for the comparison of our results to those within the full population, and an isolated group of women who were younger at exposure. The women who are <15 years from their exposure to breastfeeding are the same women who we hypothesize would be affected by the selection bias we describe.

As anticipated, the Cox proportional hazard ratios for the full population in the previous literature were closer to the null than the effect estimates in our study; however, when the dataset was restricted to women who gave birth in the last 15 years, the hazard ratios drop below those in our study (Table 5.3).

Survival Bias: We hypothesized that using a retrospective analysis would induce survival bias by comparing women of different ages at study end. Hypertension is a disease whose prevalence increases with age. By comparing women of different ages at study end, older women

will have had more time to develop disease than younger women. The result would be an over-estimation of the association between breastfeeding and outcome disease in these studies. This bias is further influenced by secular trends in breastfeeding, because younger women are more likely to breastfeed for longer periods. The use of a case control design comparing women in like risk sets by age and survey cycle controls for both age and secular trends, which can be seen in the lower point estimates in our study compared with that of Lupton et al.(47) (Table 5.4).

While effect estimates from different studies cannot be directly compared, these patterns are suggestive of a shift toward the null using a time to event model that selectively eliminates the youngest disease onset in the population, and a shift away from the null using a retrospective model confounded by both age and secular trends over time. While these comparisons lend support to the possibility that our model corrects for both selection and survival, a full methodological study would be needed to effectively address this hypothesis

5.3 Strengths and Limitations

These studies must be interpreted within the limits of their designs. All measures were recalled, and some of the variables might be particularly subject to recall bias, for instance, the variables for gestational diabetes and pre-eclampsia were ascertained in 2009. Reports of these conditions are likely to be weighted toward births that occurred more recently since the first births included in this dataset could have occurred as early as approximately 1945, when many women, particularly in rural areas, were still giving birth at home, and screening for gestational diabetes and pre-eclampsia were less precise. In both table 3.1 and table 4.1 pre-eclampsia and gestational diabetes were more common among women with longer cumulative lactation durations. Thus, the rates for these two maternity conditions are likely to have increased over time, along with the durations of breastfeeding for the population.

We assessed the effect measure modification of BMI on the relationship between breastfeeding and metabolic disease; however, the primary hypothesized causal pathway was through weight retention after pregnancy. While we were able to ascertain adolescent weight as well as weight gained between age 18 and age 40, we were unable to utilize a direct measure of weight retention; therefore, the measures of BMI are an approximation the actual causal pathway. The absence of perinatal data also might cause unmeasured confounding due to reverse causality. Pregnancy is considered a window to future metabolic health; therefore, more sensitive measures of metabolic health during pregnancy would have improved our ability to control confounding. We analyzed a sub-population in which we had a variable for failed lactation. We hypothesized that this variable was a proxy for high metabolic risk, such as gestational diabetes. It is possible that, rather than being a proxy for physiologic mechanisms in the breastfeeding process, failed lactation could instead indicate a lack of support for breastfeeding, or of emotional and physical support in the hospital or the home setting.

Among the strengths of this study was the use of all cases of disease from both before and after cohort entry, limiting the possibility of selection bias. Our use of a case control design while matching on age and questionnaire cycle, allowed for comparisons across similar risk sets; however, our assignment of age of disease onset for women who developed disease prior to the baseline questionnaire introduced imprecision to this variable.

We stratified results based on parity. Any time cumulative lifetime lactation is used as a measure of breastfeeding exposure with an outcome that might be affected by parity, there should be some test of the multiplicative interaction between parity and cumulative lactation. Additionally, the determinants of parity need to be carefully considered in order to interpret the associations between reproductive history and maternal health.

In the United States, breastfeeding rates differ along racial lines. The prevalence of breastfeeding initiation is lowest in black women compared with NH white, and Hispanic women (22). Despite these disparities, it is more common to find research that under-represents black women, or focuses only on black women from low income populations. Given the current inequalities in the existing research, this dissertation, utilizing a cohort of black women, adds a necessary breadth to our current understanding of the relationship between breastfeeding and both hypertension and T2D. This added breadth allows for a more inclusive presentation of the benefits of breastfeeding in targeted public health messaging as well as the provision of individualized health care advice.

5.4 Future Research

Continued examination of these associations is warranted as we further define the health benefits of breastfeeding. Specifically, the confounding effect of our hospital systems on the early days of breastfeeding and its association with metabolic disease have yet to be addressed. We were able to address failed lactation, but the addition of breastfeeding intention would allow for a much more direct measure of breastfeeding success. The concept of failed lactation is an important one worthy of further assessment, particularly given the sensitivity of breastfeeding to failure in the earliest days postpartum, both for physical and emotional reasons. Our understanding of the mediating effects of postpartum weight retention on the relationship between breastfeeding and both hypertension and T2D would be improved with more specific measures of weight during the reproductive years. We additionally need to pursue the methodologic biases that might be present in the use of hazard models that do not utilize all of the cases of disease in observational datasets as this is a common methodologic approach to determining the relationship between reproductive exposures and later disease onset. Further

consideration of the impact of choice of referent group on the interpretation of associations between breastfeeding and maternal health is also warranted. And finally, it is important to explore whether public health messaging about the benefits of breastfeeding actually changes practice in the medical field, or breastfeeding behaviors.

5.5 Tables and Figures

Table 5. 1 Odds ratios for type 2 diabetes by duration of cumulative lactation for crude and multivariate models. Black Women's Health Study 1995-2011

	Cumulative Lactation N = 15,897						
		Model 1[a]			Model 2[b]		
	Cases / Controls	OR	(95% CI)	CLR	OR	(95% CI)	CLR
None	1918 / 6670	1.00	Referent		1.00	Referent	
Any	1469 / 5840	0.87	(0.81, 0.94)		0.91	(0.84, 0.99)	
1-3	555/ 2101	0.91	(0.82, 1.02)	1.24	0.96	(0.86, 1.08)	1.26
4-6	325 / 1353	0.83	(0.73, 0.95)	1.30	0.90	(0.79, 1.03)	1.30
7-11	242 / 884	0.95	(0.81, 1.10)	1.36	0.97	(0.83, 1.14)	1.37
12-17	147/ 649	0.78	(0.65, 0.94)	1.45	0.78	(0.64, 0.95)	1.48
18-23	69 / 337	0.70	(0.54, 0.91)	1.69	0.72	(0.55, 0.95)	1.73
≥24	131 / 516	0.88	(0.72, 1.07)	1.49	0.84	(0.68, 1.03)	1.51
Calculating OR Using 1-3 Months as Referent							
1-3	555/ 2101	1.00	Referent		1.00	Referent	
4-6	325 / 1353	0.91	(0.78, 1.06)	1.36	0.93	(0.80, 1.09)	1.36
7-11	242 / 884	1.03	(0.87, 1.23)	1.41	1.01	(0.85, 1.20)	1.41
12-17	147/ 649	0.85	(0.70, 1.05)	1.50	0.81	(0.66, 1.00)	1.52
18-23	69 / 337	0.77	(0.58, 1.01)	1.74	0.75	(0.56, 0.99)	1.77
≥24	131 / 516	0.96	(0.77, 1.09)	1.42	0.87	(0.69, 1.09)	1.58

OR, Odds Ratio; CI, Confidence interval; CLR, confidence limit ratio

[a] Model 1 adjusted for age and survey cycle

[b] Model 2 adjusted for age, survey cycle, parity[categorical], parity[ordinal], age at first birth, gestational diabetes, education, vigorous exercise, and BMI at age 18

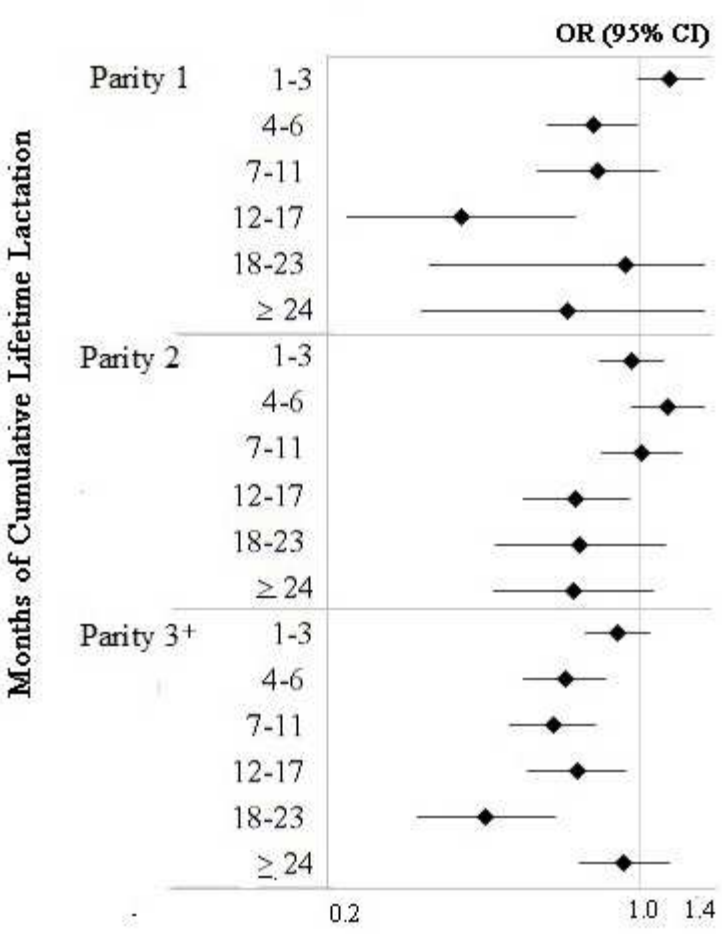

Figure 5. 1 Odds ratios (OR) and 95% confidence intervals (CI) for type 2 diabetes comparing cumulative lifetime lactation (in months) to no breastfeeding by levels of parity. ORs were controlled for age, year, parity, education, gestational diabetes, exercise, BMI at age 18, and an interaction term between parity and cumulative lifetime lactation. N = 15,680. Black Women's Health Study 1995 – 2011.

Table 5.2 Effect measure modification of the association between breastfeeding and type 2 diabetes by hypertension, present at the time of onset of T2D or control selection

	Cumulative Lactation N = 15,680					
	Non-Hypertensive			Hypertensive		
	OR	(95% CI)	CLR	OR	(95% CI)	CLR
None	1.00	Referent		1.00	Referent	
1-3	0.89	(0.76, 1.04)	1.37	1.07	(0.89, 1.28)	1.44
4-6	0.76	(0.62, 0.92)	1.48	0.92	(0.73, 1.15)	1.58
7-11	0.73	(0.58, 0.92)	1.59	1.03	(0.80, 1.32)	1.65
12-17	0.70	(0.53, 0.93)	1.75	0.93	(0.67, 1.29)	1.93
18-23	0.53	(0.34, 0.80)	2.35	0.91	(0.57, 1.45)	2.54
≥24	0.82	(0.61, 1.09)	1.79	0.83	(0.58, 1.21)	2.09

CI, Confidence interval; CLR, Confidence limit ratio; OR, Odds ratio

Table 5.3 A comparison of the hazard ratios (HR) and 95% confidence interval (CI) from the Nurse's Health Study (NHS) to the odds ratios (OR) and 95% confidence intervals from the Black Women's Health Study (BWHS) for the relationship between months of breastfeeding and type 2 diabetes (T2D). Referent category for both studies is formula feeding.

BWHS				**NHS**		**NHS < 15 years**	
	OR	(95% CI)		HR	(95% CI)	HR	(95% CI)
None	1.00	Referent	None	1.00	Referent	1.00	Referent
1-3	0.98	(0.87, 1.09)	>0-3	0.98	(0.91, 1.05)	0.72	(0.44, 1.18)
4-6	0.93	(0.81, 1.06)	>3-6	1.03	(0.94, 1.13)	0.74	(0.42, 1.32)
7-11	1.00	(0.86, 1.18)	>6-11	0.96	(0.87, 1.06)	0.64	(0.35, 1.17)
12-17	0.79	(0.65, 0.96)	>11-23	0.92	(0.84, 1.02)	0.70	(0.42, 1.15)
18-23	0.74	(0.56, 0.97)					
≥24	0.87	(0.70, 1.08)	>23	0.88	(0.78, 1.00)	0.47	(0.27, 0.81)

BWHS, Black Women's Health Study; CI, Confidence interval; CLR, Confidence limit ratio; NHS, Nurse's Health Study; OR, Odds ratio

Table 5.4 A comparison of the odds ratios (OR) and 95% confidence interval (CI) from the 45 and Up Study with the OR and 95% CI from the Black Women's Health Study (BWHS) for the relationship between months of breastfeeding and hypertension. Referent category for both studies is no breastfeeding.

Breastfeeding	**BWHS**			**45 and Up**	
(months)	OR	(95% CI)		HR	(95% CI)
None	1.00	Referent	None	1.00	Referent
1-3	0.95	(0.86, 1.05)	1-<3	0.88	(0.63, 1.24)
4-6	0.94	(0.84, 1.06)	3-<6	0.87	(0.62, 1.20)
7-11	0.89	(0.77, 1.02)	6-<12	0.74	(0.55, 0.98)
12-17	0.85	(0.57, 0.81)	12-<18	0.71	(0.53, 0.95)
18-23	0.85	(0.68, 1.06)	18-<24	0.57	(0.41, 0.79)
≥24	0.82	(0.69, 0.98)	≥24	0.58	(0.44, 0.77)

BWHS, Black Women's Health Study; Nurse's Health Study; OR, Odds ratio

APPENDIX

Exploration of missing gestational diabetes values in Aim 2 comparing complete case analysis with known gestational diabetes status to models unadjusted for gestational diabetes both inclusive and exclusive of missing values. Black Women's Health Study, 1995-2011.

	Cumulative Lactation (months)	**Cases**	**Control**	**OR**	**(95% CI)**	***Model***
All Ages (n = 15897)	None	1918	6670	1.00		Known GDM—no adjustment
	Ever	1469	5840	0.92	(0.85, 1.00)	
All Ages (n = 15897)	None	1918	6670	1.00		Known GDM—adjustment*
	Ever	1469	5840	0.91	(0.84, 0.99)	
All Ages (n = 21101)	None	2453	9339	1.00		Known and unknown—no adjustment
	Ever	1750	7559	0.91	(0.85, 0.98)	
All Ages (n = 15897)	None	1918	6670	1.00		Known GDM—no adjustment
	1-3	555	2101	0.96	(0.86, 1.07)	
	4-6	325	1353	0.90	(0.78, 1.03)	
	7-11	242	884	1.01	(0.86, 1.17)	
	12-17	147	649	0.81	(0.67, 0.98)	
	18-23	69	337	0.76	(0.58, 1.00)	
	≥ 24	131	516	0.89	(0.72, 1.09)	
All Ages (n = 15897)	None	1918	6670	1.00		Known GDM— adjustment*
	1-3	555	2101	0.96	(0.86, 1.08)	
	4-6	325	1353	0.90	(0.79, 1.03)	
	7-11	242	884	0.97	(0.83, 1.14)	
	12-17	147	649	0.78	(0.64, 0.95)	
	18-23	69	337	0.72	(0.55, 0.95)	
	≥ 24	131	516	0.84	(0.68, 1.03)	
All Ages (n = 21101)	None	2453	9339	1.00		Known and unknown—no adjustment
	1-3	668	2787	0.94	(0.85, 1.03)	
	4-6	396	1760	0.90	(0.80, 1.02)	
	7-11	279	1124	0.98	(0.85, 1.13)	
	12-17	176	815	0.83	(0.70, 0.99)	
	18-23	80	428	0.72	(0.57, 0.93)	
	≥ 24	151	645	0.87	(0.72, 1.05)	

*model used in paper

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