SURVIVAL OF INFANTS WITH SPINA BIFIDA DURING THE FIRST YEAR OF LIFE

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ABSTRACT

Nelson D. Pace: Survival of Infants with Spina Bifida during the First Year of Life.
(Under the direction of Anna Maria Siega-Riz)

Birth defects are a leading cause of infant mortality in the U.S. accounting for 20% of infant deaths. Spina bifida, a neural tube defect, is characterized by the protrusion of the spinal cord through a boney defect in the vertebral column. First-year mortality occurs among 8% of infants with spina bifida, thirteen times higher than the national average risk of infant mortality for all U.S. births.

Pre-pregnancy obesity (body mass index $\geq$30) is common occurring in more than 1 in 5 pregnant women in the U.S. Furthermore, the average childbearing woman has a diet that is considered poor quality. We conducted a retrospective cohort study using the National Birth Defects Prevention Study linked to state death records to examine the role of pre-pregnancy body mass index and maternal dietary patterns on survival among infants born with spina bifida. Overall first-year mortality risk among infants with spina bifida was 4.4% (95% CI: 3.52, 5.60%). Infants who had multiple co-occurring defects, were born very preterm, were one of multiples, had high-level spina bifida lesions, or had non-Hispanic Black mothers were at highest risk of infant mortality. Maternal pre-pregnancy underweight and obesity were associated with higher infant mortality risk (15.7% (95% CI: 7.20, 32.30%) and 5.8% (95% CI: 3.60, 9.35%), respectively) compared to normal weight mothers (2.2% (95% CI: 1.19, 4.18%)).

Cox models adjusted for maternal age, education, race/ethnicity, and folic acid supplementation showed that underweight and obese mothers had greater hazard of infant mortality compared to normal weight mothers (HR: 4.5 (95% CI: 1.08, 16.72) and 2.6 (1.36,

8.02), respectively). Mothers that scored low (poorer diet quality) in both the Healthy Eating Index and the Diet Quality Index for Pregnancy had higher hazard of infant mortality compared to mothers with high (better diet quality) scores (HR: 1.4 (0.54, 4.33) and 2.4 (0.93, 5.78), respectively) though the estimates were imprecise.

Our results support maternal pre-pregnancy body mass index as a modifiable factor that may help in efforts to improve infant survival. This study provides suggestive evidence that maternal pre-pregnancy diet may be associated with infant survival among babies born with spina bifida.

To my wife, Ginnie Kim. No one has made a greater sacrifice than her to make this possible. Hours, days, weeks, and years have passed since I began this pursuit, and with it many adventures, opportunities, and sacrifices. For her support and faith in me, I am truly blessed and grateful.

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CHAPTER 1: INTRODUCTION AND SPECIFIC AIMS

Birth defects are the leading cause of infant death in the United States accounting for 1 in every 5 infant deaths. Neural tube defects (NTDs), defined as the failure of the neural tube to close normally during development, are the second most prevalent major birth defect worldwide. Spina bifida (SB) is the most common NTD occurring in 1 out of every 3,000 live births. Infant mortality for children born with SB in the United States is 8%, thirteen times higher than the national average of 0.6%.

Considerable research has been conducted to identify risk factors and causes of NTDs. Notably folic acid supplementation and food fortification has caused a decline in the prevalence of SB and NTDs overall. Risk factors such as maternal pre-pregnancy obesity is associated with increased risk of spina bifida. Maternal diet plays a key role in embryonic and fetal development: NTDs occur with less frequency among mothers with prudent dietary patterns compared to other dietary patterns (Western, low-calorie Western, and Mexican). Additional research has investigated risk factors of infant mortality among babies born with SB. Identified risk factors include maternal age, race, ethnicity, low birth weight for gestational age, co-occurrence of multiple birth defects, nativity, and parity. Moreover, one study showed that infants with SB who were born after mandatory food fortification in the United States had modestly improved first-year survival compared to infants with SB born before the era of fortification. This finding highlights the possibility that folic acid not only prevents NTDs but might also ameliorate the severity of SB.

We hypothesize that among infants born with spina bifida (1) pre-pregnancy obesity may increase the risk of infant mortality and (2) higher maternal diet quality or

certain dietary patterns may reduce infant mortality. This study will use data from the National Birth Defects Prevention Study (NBDPS), a ten state case-control study which investigates more than 30 different birth defects. Estimated delivery dates of study participants are from October 1997 through December 2011. NBDPS data linked with infant mortality data collected from each state's vital records will be used to investigate survival among infants with SB. All birth defect classifications were reviewed by clinical geneticists to prevent misclassification of SB. The primary exposure of interest, maternal diet quality, will be evaluated using data from a shortened food frequency questionnaire. Our primary outcome of interest is time to infant mortality among infants with SB. The overall objective of this study is to describe first-year survival of infants born with SB and investigate whether pre-pregnancy body mass index (BMI), maternal diet quality, and other risk factors are associated with improved survival. We propose the following study aims.

Aim 1. Investigate first-year mortality among infants born with spina bifida overall and by pre-pregnancy BMI. Survival estimates vary by time interval and by mother-infant characteristics.

Subaim 1. Present cumulative incidence curves for all infants with SB as well by pre-pregnancy BMI categories. Report estimated survival for the following intervals: one day, early neonatal ($<$ 7 days), neonatal ($<$28 days), and infant ($<$ 1 year).

Subaim 2. Calculate survival estimates stratified by key risk factors. We will stratify results by race/ethnicity, gestational age, isolated/multiple defects, and plurality.

Aim 2. Examine the relationship between maternal diet quality and infant survival among infants with spina bifida. Using three methods, we will investigate whether maternal diet is associated with improved survival. We will measure diet quality using the Diet Quality Index for Pregnancy and the 2010 Healthy Eating Index and examine dietary patterns through latent class analysis.

Aim 3. Investigate the potential for bias to affect observed survival estimates.

Non-participation and competing events can produce non-generalizable results and biased survival estimates.

Subaim 1. Describe differences in mortality between interviewed and non-interviewed mothers and their infant. Using classification files for both interviewed and non-interviewed will allow us to compare survival by participation and a number of key characteristics.

Subaim 2. Adjust for competing events caused by the exclusion of induced abortions and fetal death in survival analyses. Starting follow-up at 20 weeks gestation will allow us to address competing events.

Note: This subaim was not completed as part of the dissertation though the background research and planning was done and is included in this document because of its crucial nature to fully understanding the subject matter.

This study marks the first assessment of the relation between pre-pregnancy BMI, maternal diet and survival of infants with SB. Conducting this study will extend scientific understanding of these factors as not only protective against SB but also a potential factor to improve survival. This research will help inform health education and recommendations in clinical and population settings to thereby reduce mortality of infants born with spina bifida.

CHAPTER 2: BACKGROUND

In this chapter, I present an overview of a category of birth defects, known as neural tube defects. Frequency of occurrence for each type of neural tube defect as well as corresponding infant mortality is then discussed. I then describe how pre-pregnancy body mass index (BMI), considered in this study as another fetal exposure, is measured. Measures of maternal diet, another fetal exposure, are then outlined to convey their differences, strengths, and weaknesses. After covering these topics, I describe the different epidemiologic and statistical methods needed to appropriately perform the survival analysis for the specific aims.

2.1 Neural Tube Defects

Neural tube defects (NTDs) are the second most prevalent major birth defect worldwide with congenital heart defects being the most prevalent (1). Defined as the failure of the neural tube to close normally during development, NTDs can affect the spinal cord or brain and are thought to primarily originate during embryonic development in the first trimester of pregnancy. Between approximately weeks 5 and 6 of gestation (3rd and 4th week of embryonic life) the neural tube closes beginning at what will later develop into the forebrain and closes at the end of the spinal cord within a few days (2). Figure 2.1 illustrates the various types of NTDs, of which the major ones include anencephaly, encephalocele, and spina bifida. Anencephaly occurs approximately in 1 out of every 5,000 live births (3) with a survival period that rarely goes beyond the first year of life. Encephalocele occurs in approximately 1 out of every 12,000 live births (3) with an infant mortality of 28% (4).

The most prevalent NTD, spina bifida (excluding infants with co-occurring anencephaly) occurs in approximately 1 in every 3,000 births though this varies by race/ethnicity and has an infant mortality of 8% (4). In the US, spina bifida prevalence is higher for Hispanic and non-Hispanic whites than for African Americans and Asians (5–7).

Several spina bifida subtypes exist, four of which are depicted in the bottom row of Figure 2.1. The most well-studied and most common, myelomeningocele, is characterized by a cystic protrusion of the spinal cord, the meninges (layers of connective tissue that act as a membrane covering the spinal cord), and cerebrospinal fluid through a vertebral defect (8). Additional spina bifida subtypes include meningocele (Figure 2.1, bottom row, second from the right), myelocele, lipomeningocele, and lipomyelomeningocele. These subtypes are both less common and less thoroughly studied. Meningocele involves protrusion of meninges and cerebrospinal fluid only but not of the spinal cord through a spinal defect. Myelocele, similarly to myelomeningocele, is characterized by a protruding spinal cord though in this case it is not covered by the meninges. Lipomeningocele and lipomyelomeningocele are distinguished from myelomeningocele, meningocele, and myelocele by the presence of excessive lipomatous (fatty) tissue which is connected to the either the spinal cord or filum terminale (a fibrous strand of tissue that provides support to the spinal cord) (8). Figure 2.1 (bottom row, second from the left) depicts a form of lipomeningocele. Etiologies of myelomeningocele, meningocele, and myelocele are hypothesized to be related to one another and to differ from those of lipomyelomeningocele and lipomeningocele (9). Epidemiologic studies of spina bifida often consider all subtypes together collectively rather than examining them separately (10–13).

In addition to subtypes, spina bifida can also be characterized by the anatomical location along the vertebrae of the protrusion, from top to bottom, cervical, thoracic, lumbar, sacral (see Figure 2.2). The vast majority of spina bifida occurs in the lumbar region of the spine (8).

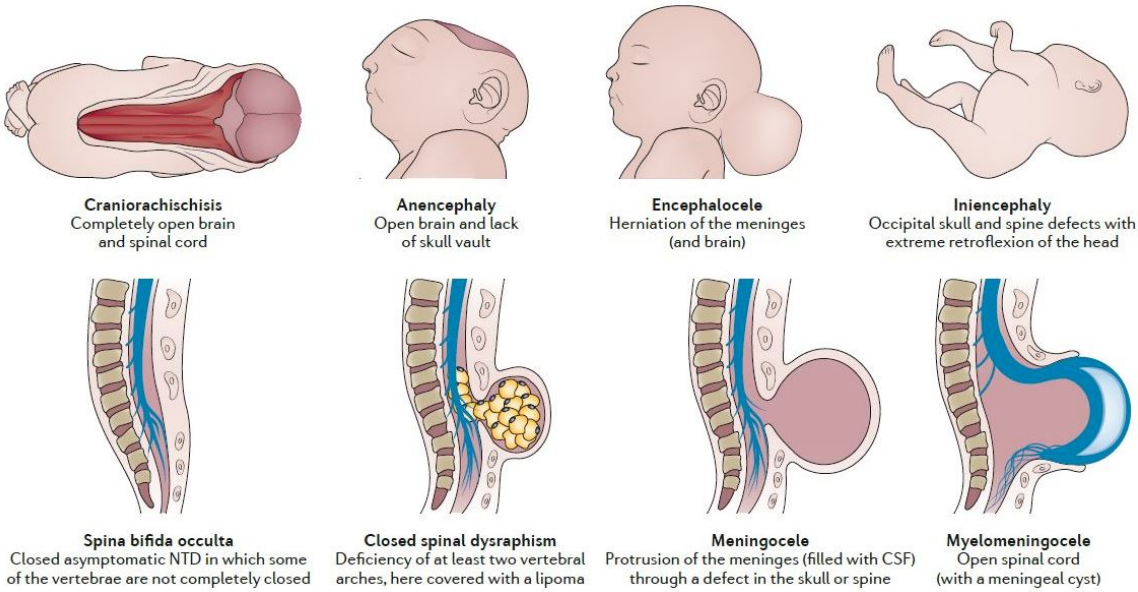

Figure 2.1: Overview of neural tube defects. Schematic representation of several neural tube defects (NTDs). CSF, cerebrospinal fluid. [Borrowed from Copp et al. 2015 (14).]

As stated previously, some NTDs exhibit high infant mortality and many result in significant disability throughout life, therefore prevention is of critical importance. A considerable amount of research has been done to investigate ways to prevent NTDs. Most notably, the discovery that increased consumption of folic acid can prevent spina bifida has had a large impact on the occurrence of NTD (15, 16). Clinical recommendations for prenatal supplementation and policies for food fortification have led to a decline in the prevalence of spina bifida, anencephaly, and neural tube defects overall (15, 17–21). Additional epidemiologic research has shown that a healthy maternal diet is associated with a decreased risk of NTD and orofacial clefts (22). Also NTDs occur with less frequency among mothers with prudent dietary patterns compared to other dietary patterns (Western, low-calorie Western, and Mexican; details of the foods in these diets can be found in the section: Data-Driven Dietary Patterns Analysis) (23). These studies and accompanying policy changes have demonstrated the key role of maternal nutrition in embryonic and fetal development.

Overtime, infant mortality of among infants with spina bifida has greatly improved.

Prior to the 1960s, only 10 to 12% of infants with spina bifida survived (24). In England, Lorber et al. observed, among infants treated for spina bifida, a 50% two-year survival from 1959-1963 and a 64% two-year survival from 1967-1968 (24). A twenty-year study of all live births in New York State had a first-year mortality among infants with spina bifida of 11.5% on average from 1983 to 2006 (25). While national level data from 1999-2007 had an infant mortality risk of 8.1% among infants with spina bifida (4). Improvements in clinical care and medical technology have paved the way for this reduction in mortality (26). The introduction of antibiotics in the 1940s substantially reduced the risk of meningitis which then affected most infants with spina bifida (26). Shunts, developed in the late 1950s, helped prevent hydrocephalus a major contributor to mortality (26). Continuing technological advancements from the 1960s to the 1990s provided marked improvements in shunts and management, catheterization protocols to reduce renal complications, more frequent implementation of early surgery, and the development of spina bifida clinics. All of which have helped reduce spina bifida related mortality (24, 27). Notably, the fetal surgery to treat myelomeningocele was shown in the MOMS trial to reduce the need for postnatal shunts, prevent hindbrain herniation, and preserve neurologic function (28). Unfortunately, the MOMS trial suggested no improvement in perinatal mortality comparing infants with fetal surgery to infants with postnatal surgery. Fetal surgery also increased the risk of preterm delivery and uterine dehiscence (28). While fetal surgery may not improve mortality, improvements in prenatal and postnatal care have led to reductions in mortality for infants born with spina bifida.

Additional research has been conducted investigating risk factors of infant mortality among infants born with spina bifida. Identified risk factors include maternal age, race, ethnicity, low birth weight for gestational age, existence of multiple birth defects (non-isolated defects), nativity, and parity (17, 25, 29–31). Among infants with spina bifida, lesions high on the spine showed significantly lower survival probability compared to lower

lesions (29). Moreover, one observational study showed that infants with spina bifida who were born after mandatory food fortification in the United States had modestly improved first-year survival compared to infants with spina bifida born before the era of fortification (30). This finding highlights the possibility that folic acid not only prevents NTDs but might also ameliorate the severity of spina bifida. Just as folic acid supplementation may ameliorate the severity of spina bifida, we hypothesize that higher maternal diet quality may improve survival of infants born with spina bifida.

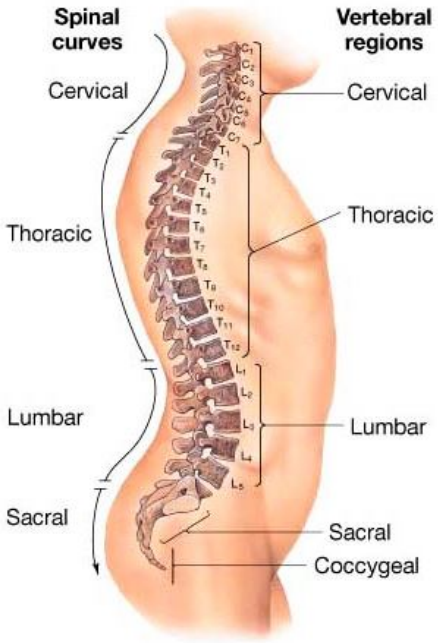

Figure 2.2: The vertebral column. The major regions of the vertebral column. [Borrowed from Martini 2005 (32).]

2.2 Pre-pregnancy Body Mass Index

Body mass index (BMI) is a proxy measure for body fatness. First suggested by Adolophe Quetelet in 1832 (33), BMI is a ratio of height to weight as mass (in kilograms) divided by height (in meters) squared (kg/m^2). While it more accurately represents excess weight given ones height (34), the measure has long been used as an indicator of risk

of mortality and morbidity. The measure is inexpensive and easily obtainable (35). BMI has also been shown to predict body fat percentage well (35, 36). Among women ages 20-39 years old, body mass index explained 83.9% of the variability in percentage of body fat measured via dual-energy X-ray absorptiometry (DXA) a gold standard for body fat measurement (Pearson's r=0.839) (36).

Pre-pregnancy BMI refers to maternal BMI just prior to the start of pregnancy. Recent estimates indicate that among U.S. women ages 20-39, 24% are overweight and 32% are obese (37). Pre-pregnancy obesity, defined as a BMI greater than or equal to 30 at the start of pregnancy, is common, occurring in more than 1 in 5 pregnant women in the U.S. (21% in 2009) with increases in prevalence during the last decade (38). Maternal obesity is the number one obstetrical risk factor for a multitude of negative maternal and infant conditions (39). Overweight and obese pre-pregnancy BMI has been associated with infant mortality, preterm birth, stillbirth, and longer duration in neonatal intensive care (40). A recent meta-analysis showed a 42% increased odds of infant mortality among infants born to mother who were obese relative to normal weight women (95% CI: 1.24-1.63) with an even greater elevated odds among the most obese category (>35 BMI) (odds ratio (OR): 2.02, 95% CI: 1.61-2.56). Underweight pre-pregnancy BMI (<18.5) has also been associated with negative infant outcomes (e.g. indicated preterm delivery, neonatal intensive care, infant mortality) (40, 41). One large study with data from women in 38 U.S. states found the association of maternal pre-pregnancy BMI demonstrated a "J" shaped pattern, with infants of underweight mothers having births with higher infant mortality (5.4/1,000 live births) compared to births of mothers with normal pre-pregnancy weight (4.2/1,000 live births) and infant mortality rapidly increasing with severity of maternal pre-pregnancy obesity: (5.9/1,000 for $30 \leq BMI < 35$; 6.8/1,000 for $35 \leq BMI < 40$; 8.2/1,000 for $BMI \geq 40$ among live births) (41).

Prior research suggests that maternal pre-pregnancy obesity is associated with increased

risk of spina bifida (42–46). However, no prior studies, to our knowledge, have examined the influence of maternal pre-pregnancy BMI in relation to infant mortality among infants with spina bifida. The purpose of this study is to investigate the first-year survival of infants born with spina bifida and examine the association of pre-pregnancy BMI with infant mortality.

2.3 Maternal Diet

Pregnancy is a critical period of rapid development and heightened metabolic activity for a growing fetus. Imbalances in specific nutrient and overall energy intakes during pregnancy have been associated with adverse pregnancy outcomes (47–55). Increased emphasis has been placed on promoting nutrient-rich diets during pregnancy to help offset the widespread energy-dense yet nutrient-poor food environment and consumption patterns in the U.S. (56–58). Nutrient rich diets for pregnancy should emphasize consuming fruits and vegetables, foods rich in iron and calcium, and adequate folic acid/folate consumption (59). Dietary patterns are utilized to capture the quality and habits of the entire diet consumed by a given population which may be more informative than isolating specific nutrients or foods that tend to be highly correlated.

Considering maternal diet as a whole rather than with the examination of individual foods or nutrients has several distinct advantages. First, biomarker assessments for specific nutrients are limited and can only assess a single nutrient at a time. Second, people do not eat specific nutrients but rather foods and combinations of certain foods overtime. Subsequently, nutrients and foods are highly correlated. Third, the combination of foods and nutrients can have synergistic effects that are greater than the sum of the effect of each nutrient. Therefore dietary patterns are an ideal way to assess the overall impact of diet on health and provide for meaningful interpretation.

Overall measures of diet fall generally into two categories: (1) dietary pattern indexes

by which maternal diet is assessed against some scale of diet quality and (2) data-driven approaches by which foods are grouped to represent different seemingly naturally occurring dietary patterns. The former approach includes the Diet Quality Index for Pregnancy (60) and Healthy Eating Index (61), Alternative Health Eating Index (62), Recommended Food Score (56, 63), and Mediterranean Diet Score(22). The scoring systems effectively use the wealth of data captured by dietary assessment tools (such as food records, food frequency questionnaires (FFQ), and multiple 24-hour dietary recalls) and produce a composite score representing overall diet quality (60). The data-driven approach is often broadly termed dietary pattern analysis. This analytic form takes multidimensional dietary data and forms groups with similar foods. We next describe in detail the key dietary pattern indexes mentioned above and the most pertinent form of data-driven dietary pattern analysis.

2.4 Measures of Diet Quality during Pregnancy

2.4.1 Recommended Food Score

Developed by Kant et al., the Recommended Food Score (RFS) focuses on the consumption of healthy foods and awards points to an individual's overall score for each recommended (i.e. healthy) food consumed at a given frequency (e.g. weekly) (56). Foods considered recommended or healthy on the scoring system are fruit, vegetables, whole grains, lean meats or meat alternates, and low-fat dairy products. For instance, as originally published (56), the RFS included the following 23 foods items:

1. apples or pears
2. oranges
3. cantaloupe
4. orange or grapefruit juice
5. grapefruit
6. other fruit juices
7. dried beans
8. tomatoes
9. broccoli
10. spinach
11. mustard, turnip, or collard greens
12. carrots or mixed vegetables with carrots
13. green salad

14. sweet potatoes, yams
15. other potatoes
16. baked or stewed chicken or turkey
17. baked or broiled fish
18. dark breads like whole wheat, rye, or pumpernickel
19. cornbread, tortillas, and grits
20. high-fiber cereals, such as bran, granola, or shredded wheat
21. cooked cereals
22. 2% milk and beverages with 2% milk
23. 1% or skim milk

The RFS is calculated by awarding 1 point for each recommended food that is consumed at least weekly and then summing the points for a maximum score of 23. Consumption of non-recommended foods do not contribute to or take away from the score. This list of foods was intended to reflect the, then current, dietary guidelines. To implement the RFS, now, more than 15 since years since its development, investigators should adapt the list to reflect current dietary guidelines as their tool for capturing diet as has been done previously (64). The RFS, differently from the other diet scoring systems, only considers healthy elements of a subject's diet ignoring the consumption of unhealthy foods. One can imagine, that a subject who consumes well beyond the guidelines for caloric intake of both recommended and non-recommended foods can have a higher RFS than a subject with more prudent eating habits that only consumes recommended foods but with less variety. Such nuances of this scoring system need to be carefully considered when implemented.

2.4.2 Mediterranean Diet Score

Dietary indices for the Mediterranean diet show adherence to the traditional Mediterranean diet first described by Trichopoulou et al. (65) and further specified to include fish (66, 67). Briefly, as described by Trichopoulou et al., the traditional Mediterranean diet is characterized by a high intake of vegetables, legumes, fruits and nuts, and cereals (that in the past were largely unrefined), and a high intake of olive oil but a low intake of saturated lipids, a moderately high intake of fish (depending on the proximity of the sea), a low-to-moderate intake of dairy products (and then mostly in the form of cheese or yogurt), a low

intake of meat and poultry, and a regular but moderate intake of ethanol, primarily in the form of wine and generally during meals. (66) Differently from other dietary indices, this diet placed greater emphasis on the consumption of fish over other forms of meat, healthy fats, nuts, and wine.

The Mediterranean diet has been scored for dietary adherence in multiple ways (22, 65, 66, 68). Here we describe the scoring specification given in Feldkamp et al. 2014, called the Mediterranean Diet Score (MDS), which was adapted for use in a population of pregnant women. The MDS consist of 9 component scores each equally weighted with 0-9 range based on decile of consumption with total scores ranging from 0 to 81; 81 indicating the highest possible adherence to this dietary pattern. For beneficial components (fruits, vegetables, legumes, fish, grains, and fat ratio (high monounsaturated fatty acids to saturated fatty acids) higher scores are given for greater consumption. For less favorable components (meat, dairy, and sweets), higher scores are given for lesser consumption. Alcohol consumption is notably excluded from the MDS as it is not recommended for consumption during pregnancy.

Use of the MDS in the NBDPS has shown a trend of decreasing risk of gastroschisis with increasing MDS among Hispanics and all ethnicities collectively (68). A weak protective association was found between higher MDS and anencephaly(22). No association was found between MDS and spina bifida(22), cleft lip(22), cleft plate(22), microtia(69) and hypospadias (70).

2.4.3 Diet Quality Index for Pregnancy

The Diet Quality Index for Pregnancy (DQI-P), developed by Bodnar and Siega-Riz, is a diet score uniquely adapted for pregnancy based on recommendations from the 2000 Dietary Guidelines for American (DGA) and the Food Guide Pyramid from the U.S. Department of Agriculture (USDA) (71), the Dietary Recommended Intakes (RDA, EAR and

AI) for pregnancy by the Food and Nutrition Board (72), and the Institute of Medicine 1992 report, Nutrition during Pregnancy and Lactation: An Implementation Guide (60, 73).

The DQI-P is an ordinal score based on consumption of 8 components (60). Implementation of the DQI-P was first done using data collected from a food frequency questionnaire (FFQ) but can be implemented using other dietary assessments such as from 24-hour recalls. The DQI-P consists of 8 components, each component having equal weight with a maximum of 10 points for a maximum total score of 80 points. DQI-P scores as calculated in the NBDPS are typically grouped into quantiles (tertiles or quartiles) to compare the highest to the lowest quantile of DQI scores for a given outcome (22, 68). The components are: % recommended servings of grains, vegetables, and fruits, % of recommended dietary allowances (RDA) for folate and iron, % of adequate intake for calcium, % of energy from fat, and a meal/snack patterning score (see Table 2.1 from Bodnar et al. 2002)(60).

Table 2.1: Dietary components included in the Diet Quality Index for Pregnancy. [Borrowed from Bodnar et al. 2002 (60).]

Component	Score	Score categories	% Population in subgroup
6–11 servings of grains per day, % recommended servings[1,2]	0–10	≥100%	1.2
		99%–50%	19.5
		<50%	79.3
3–5 servings of vegetables per day, % recommended servings[1,2]	0–10	≥100%	37.4
		99%–50%	36.7
		<50%	25.9
2–4 servings of fruits per day, % recommended servings[1,2]	0–10	≥100%	51.3
		99%–50%	25.8
		<50%	23.0
Folate intake as % RDA[2,3]	0–10	≥100%	43.8
		99%–50%	43.8
		<50%	12.4
Iron intake as % RDA[2]	0–10	≥100%	19.4
		99%–50%	52.2
		<50%	28.4
Calcium intake as % AI for age[2]	0–10	≥100%	56.5
		99%–50%	33.5
		<50%	10.0
Total fat ≤30% energy intake[4]	0–10	≤30%	28.8
		>30%, ≤35%	30.9
		>35%, ≤40%	25.3
		>40%	15.1
Meal pattern[5]	0–10	3 meals/2 snacks	71.6
		3 meals/0–1 snack(s) or 2 meals/2 snacks	12.4
		2 meals/0–1 snack(s) or 1 meal/snacks	16.1

RDA – Recommended Dietary Allowance; AI – Adequate Intake.
[1] Based on Food Guide Pyramid[6] recommendations for diets containing ≤1600, 1601–<1900, 1900–<2500, 2500–<2800 and ≥2800 kcal.
[2] Used as a continuous percentage (0% to 100%) corresponding to a continuous DQI-P score of 0 to 10 points.
[3] As dietary folate equivalents.
[4] Scoring based on the following categories: ≤ 30% = 10 points; >30%, ≤ 35% = 7 points, >35%, ≤ 40% = 4 points, > 40% = 0 points.
[5] Scoring based on the following categories: 3 meals/2 snacks = 10 points; 3 meals/0–1 snack(s) or 2 meals/2 snacks = 5 points; 2 meals/0–1 snack(s) or 1 meal/snacks = 0 points.

Of the 8 components; three measure adequate consumption of important food groups (grains, vegetables, and fruits), 3 measure adequate intake of key vitamins and minerals (folate, iron, and calcium), 1 reflects moderation in consumption of a macronutrient (fat), and the final component captures the frequency and type (meal/snack) of food consumed. The first three components' requirements are based on recommended daily servings (71). Components 4, 5, and 6 relate to satisfying the RDA and AI guidelines. Component 7, like components 1 through 3, follows the recommendation given by Dietary Guidelines for Americans 2000 to consume $\leq$30% of total calories from fat (71). Like many recommendations provided for pregnant women, the recommendation applicable to all adults. Meal/snack pattern score is incorporated in the DQI-P to reflect the Institute of Medicine (73) recommendations for the number of eating occasions during pregnancy.

Notably missing from the DQI-P is a component related to dietary variety and dietary moderation. These aspects of diet while important are not part of the DQI-P as the original authors of the index felt that such scores would create burdensome complexity for those wishing to implement the measure (a strength of which is its simplicity) in public health settings. (60)

The DQI-P is consistent with other indices in that it does not include nutrient intake from dietary supplements. The goal of creating the DQI-P was to assess diet in its most traditional sense (60). If one's objective is to measure total nutrient intake, nutrient intake from supplements should then also be included in the assessment.

Use of the DQI-P in the National Birth Defects Prevention Study (NBDPS) has shown higher diet quality to be associated with a reduced risk of some conotruncal and septal heart defects (74), anencephaly (22), cleft lip (22), and cleft palate (22). No association was found between DQI-P and hypospadias (70). A trend of decreasing risk with increasing DQI-P score was found for both spina bifida and microtia but precision was lacking and resulting confidence intervals included the null value. A statistically significant trend of

decreasing risk of gastroschisis with increasing DQI-P among Hispanics and all ethnicities together was also shown(68). Among Hispanics, every quartile of DQI-P scores had a statistically significant reduced risk of gastroschisis when compared to the referent lowest quartile of scores (68).

While the DQI-P was published in 2002 and does not implement the most recent Dietary Guidelines for Americans (75), many of the underlying components recommendations remain unchanged. RDAs for folate and iron are the same as well as adequate intake (AI) for calcium. The meal pattern recommendations from the Institute of Medicine are also the same today. Noteworthy updates to the DQI-P to reflect the most recent DGA would be the emphasis on whole grain consumption rather than all grains in general. Furthermore, recommendations to limit saturated fat consumption rather than simply overall total fat is a major change to dietary recommendations that has occurred since 2000. To allow for comparison of results to numerous other studies using the original DQI-P published as recently as August 2015, we will use the DQI-P as it was originally proposed by Bodnar and Siega-Riz (60). To complement this approach and address how updated dietary guidelines may improving our understanding of how dietary patterns may play a role in the development of NTDs, we employ the Health Eating Index 2010 score as our alternative dietary exposure.

2.4.4 Healthy Eating Index

The Healthy Eating Index (HEI) is a measure of diet quality that assesses adherence to the Dietary Guidelines for Americans (DGA). The DGA is national dietary guidance applicable uniformly to all individuals ages 2 and over including pregnant women. The DGA serves as the foundation for all nutrition guidance and policies provided by the United States government. The HEI is a scoring metric based on the DGA that can be applied to any defined set of foods, such as dietary data collect from 24-recalls or food frequency questionnaires, a specific menu, or a selection of grocery items. The DGA are issued every

5 years by the USDA and U.S. Department of Health and Human Services. A corresponding HEI is updated approximately every five years by a federal working group, led by Center for Nutrition Policy and Promotion (CNPP) with members from the National Cancer Institute and the USDA Food and Nutrition Service. The most recently publish HEI is that the HEI-2010 based off the 2010 DGA.

To take the DGA from a set of recommendations to a scoring systems based on quantified standards of consumption requires the USDA Food Patterns. The USDA Food Patterns translates the recommendations of the DGA into specific, quantified recommended standards for types and amounts of foods. The HEI scoring system directly reflects the standards set forth in the USDA Food Patterns.

The HEI-2010 has 12 components: 9 adequacy and 3 moderation components (see Table 2.2). The score for each adequacy component increases as more is consumed. The score for each moderation component decreases as less is consumed. Standards for scores are density-based (i.e. servings divided by calories). For DGA recommendations that vary by energy level, sex, and/or age, the corresponding component is the least-restrictive among the varying recommendations. Therefore the consumption level needed to obtain the maximum score for that component is the least-restrictive. Possible composite scores range from 0 to 100 with individual components with maximum scores of 5, 10, or 20. For all components, a higher scores indicates closer adherence to dietary guidance and ideally also indicating a healthier diet.

The HEI is designed for the principal purpose of monitoring diet quality in the overall U.S. population as well as low-income subpopulations. The Center for Nutrition Policy and Promotion (CNPP) routinely gathers national survey data via 24-hour recalls of dietary intake and uses the HEI for this very purpose. Importantly, the HEI is also used evaluate the US food supply, examine relationships between diet and health-related outcomes (77–81) including mortality (82–84) and between diet cost and diet quality (85, 86), to determine

Table 2.2: Healthy Eating Index components and scoring system. [Borrowed from Guenther et al. 2013 (76).]

HEI-2010 components and scoring system

HEI–2010[1] component	Maximum	Standard for maximum score	Standard for minimum score of zero
▲ Adequacy *(higher score indicates higher consumption)*			
Total Fruit[2]	5	≥ 0.8 cup equiv. / 1,000 kcal[10]	No fruit
Whole Fruit[3]	5	≥ 0.4 cup equiv. / 1,000 kcal	No whole fruit
Total Vegetables[4]	5	≥ 1.1 cup equiv. / 1,000 kcal	No vegetables
Greens and Beans[4]	5	≥ 0.2 cup equiv. / 1,000 kcal	No dark-green vegetables, beans, or peas
Whole Grains	10	≥ 1.5 ounce equiv. / 1,000 kcal	No whole grains
Dairy[5]	10	≥ 1.3 cup equiv. / 1,000 kcal	No dairy
Total Protein Foods[6]	5	≥ 2.5 ounce equiv. / 1,000 kcal	No protein foods
Seafood and Plant Proteins[6,7]	5	≥ 0.8 ounce equiv. / 1,000 kcal	No seafood or plant proteins
Fatty Acids[8]	10	(PUFAs + MUFAs) / SFAs ≥ 2.5	(PUFAs + MUFAs) / SFAs ≤ 1.2
▼ Moderation *(higher score indicates lower consumption)*			
Refined Grains	10	≤ 1.8 ounce equiv. / 1,000 kcal	≥ 4.3 ounce equiv. / 1,000 kcal
Sodium	10	≤ 1.1 gram / 1,000 kcal	≥ 2.0 grams / 1,000 kcal
Empty Calories[9]	20	≤ 19% of energy	≥ 50% of energy

[1] Intakes between the minimum and maximum standards are scored proportionately.
[2] Includes 100% fruit juice.
[3] Includes all forms except juice.
[4] Includes any beans and peas not counted as Total Protein Foods.
[5] Includes all milk products, such as fluid milk, yogurt, and cheese, and fortified soy beverages.
[6] Beans and peas are included here (and not with vegetables) when the Total Protein Foods standard is otherwise not met.
[7] Includes seafood, nuts, seeds, soy products (other than beverages) as well as beans and peas counted as Total Protein Foods.
[8] Ratio of poly- and monounsaturated fatty acids (PUFAs and MUFAs) to saturated fatty acids (SFAs).
[9] Calories from solid fats, alcohol, and added sugars; threshold for counting alcohol is > 13 grams/1,000 kcal.
[10] Equiv. = equivalent, kcal = kilocalories.

the quality of nutrition intervention programs (87) and food assistance packages (88), and to assess how diet quality changes over time (89).

Table 2.3: The Alternative Healthy Eating Index 2010 scoring method[1]. [Borrowed from Chiuve et. al 2012 (62).]

Component	Criteria for minimum score (0)	Criteria for maximum score (10)
Vegetables,[2] *servings/d*	0	≥5
Fruit,[3] *servings/d*	0	≥4
Whole grains,[4] *g/d*	0	
Women		75
Men		90
Sugar-sweetened beverages and fruit juice,[5] *servings/d*	≥1	≤1
Nuts and legumes,[6] *servings/d*	0	≥1
Red/processed meat,[7] *servings/d*	≥1.5	≤1
trans Fat,[8] *% of energy*	≥4	≥0.5
Long-chain (n-3) fats (EPA + DHA),[9] *mg/d*	0	250
PUFA,[10],*% of energy*	≤2	≥1
Sodium,[11] *mg/d*	Highest decile	Lowest decile
Alcohol,[12] d*rinks/d*		
Women	≥2.5	0.5–1.5
Men	≥3.5	0.5–2.0
Total	0	110

2.4.5 Alternate Healthy Eating Index 2010

First created in 2002 by McCullough et al., the Alternative Healthy Eating Index was designed as diet quality score to include foods and nutrients associated with chronic disease risk with particular emphasis on cardiovascular disease. The index serves as an alternative to the HEI and stresses the role of nuts and types of fats in a diet. Prior research has shown higher AHEI scores associated with lower risk of broad categories of major chronic diseases (90), diabetes (91), colorectal cancer (92), cardiovascular disease (90, 93, 94), and overall mortality (94). Neither the HEI nor AHEI has been used to determine the risk of birth defects by diet score.

[1]For the extensive clarifications and details referenced by the superscripts in the table, please see *Alternative Dietary Indices Both Strongly Predict Risk of Chronic Disease* by Chiuve et al. 2012.

The Alternate Healthy Eating Index 2010 (AHEI-2010) (62) is an update to the original AHEI that incorporates scientific evidence that has emerged to support the role of particular dietary factors in the prevention of chronic disease. Unlike the HEI, the AHEI is not based on the Dietary Guidelines for Americans but rather other research (62). Similarly to the HEI, it is composed of several component scores and is used to calculate an overall diet score with a higher score indicating a healthier diet. The AHEI is made up of 11 components: six adequacy components with greater consumption resulting in a higher score, one component (alcohol) for which moderate intake results in the highest score ideal, and four components for which the lower the consumption, the higher the score. Scores from all components are summed to obtain a total AHEI score ranging from worst (0) to best (110). Scoring criteria are further outlined in Table 2.3.

The discussion of these five diet quality scoring systems highlights the strengths and weaknesses of each method for capturing dietary patterns. Among these scoring systems, we have selected the DQI-P and HEI 2010 as measures to employ in this study due to their unique applicability to the pregnancy period. The DQI-P was specifically designed for to assess diet quality during pregnancy by including measures of adherence to pregnancy-specific recommendations. Furthermore, several NBDPS studies have confirmed the relevance of the DQI-P diet score as a measure being associated with reduced risk of a number of birth defects. The HEI is designed in such away to be applicable to both pregnant and non-pregnant adults. Additionally, the HEI is a national standard that has wide applicability and use. For these reasons, we will use these two systems to study how maternal diet quality can have an impact of infant mortality.

We now describe the most relevant form of data driven analysis for studying the effect of dietary patterns.

2.4.6 Data-Driven Dietary Patterns Analysis

The full complexity of the relationship between diet and health outcomes cannot be appreciated by examining the impact of individual nutrients and foods on health outcomes (67). Further, collinearity of many nutrients and foods make it difficult to attribute effects to a specific component of a diet. The creation of dietary patterns via latent class analysis (LCA) inherently accounts for interactions between single nutrients and foods that are unobserved and thereby not anticipated in dietary patterns based on diet quality scoring systems. Such a method can provide a more robust means of determining proper exposure-outcome associations.

An alternative yet complementary approach, to capturing overall diet via some specified measure of diet quality, is to examine data-driven dietary patterns. Compared to diet quality indexes, which are based on prevailing hypotheses and guidance from current dietary recommendations for scoring (95), LCA, factor, and cluster analysis are data driven approaches used to derive dietary patterns. These methods allow dietary data to determine variables or groups that represent a variety of diets existing in a study population. Data driven statistical method include: factor analysis, principal component analysis, mixture modeling, and latent class analysis. Latent class analysis (LCA) can be used to identify a set of discrete and exclusive latent (unmeasured, unknown) classes among participants based on their responses to a set of observed (measured and known) categorical variables. LCA is particularly useful methodologic approach to understanding diet as it can identify patterns within the collective dietary data of participants. Each pattern can be considered a given exposure type and examined in relation to the health outcome of interest.

Consider Figure 2.3, what we observe from initially collected data are statistical associations that are present between several measured variables, here labeled components. Basic statistical analyses show that the components are related to one another, for instance, more

frequent consumption of component 1 (tortillas) is associated with more frequent consumption of component 2 (beans). While this statistical association exists, eating tortillas does not cause one to eat beans, but rather there is often an underlying latent variable of a dietary pattern (faded cicles of classes 1, 2, and 3)that can capture the true relationship of these components as well as many other food items. Figure 2.4 reveals the true relationship between the components that is due to latent classes, which in our context, are different dietary patterns. The relationship between tortilla and bean consumption could be explained by a Mexican dietary pattern such as was identified in Sotres-Alvarez et al. (23).

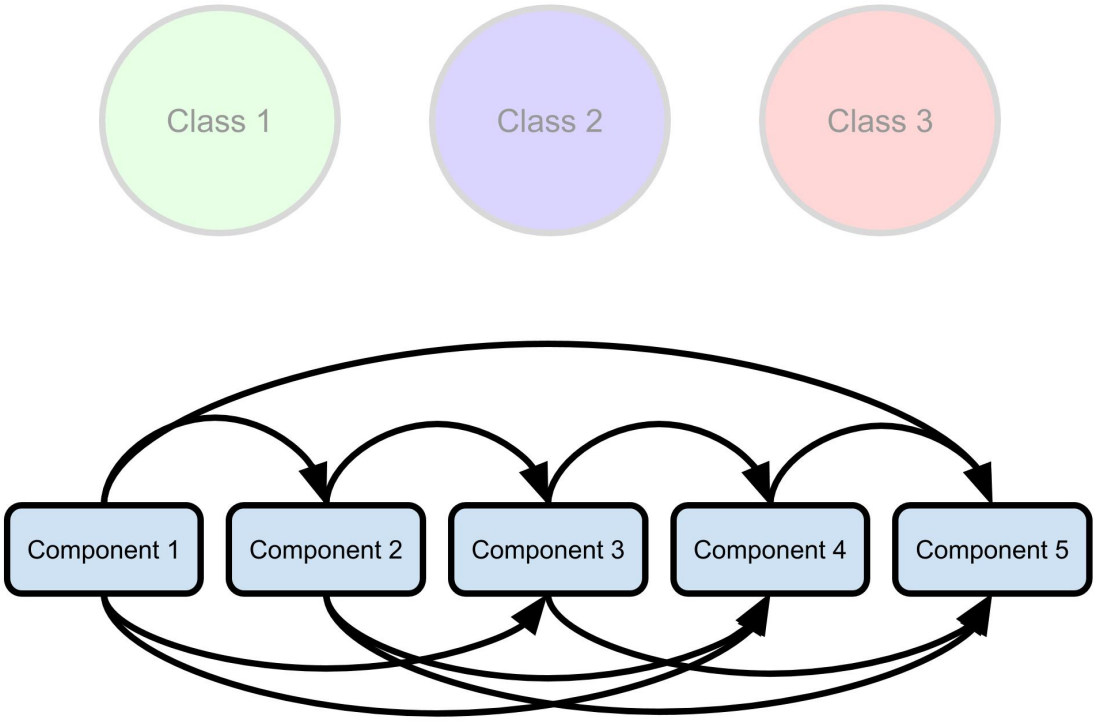

Figure 2.3: Statistical associations among components with unidentified (hidden) latent classes.

LCA takes into account how variables are related to one another. Each is not given equal weight (e.g. lettuce maybe given less weight than avocados in determining a dietary

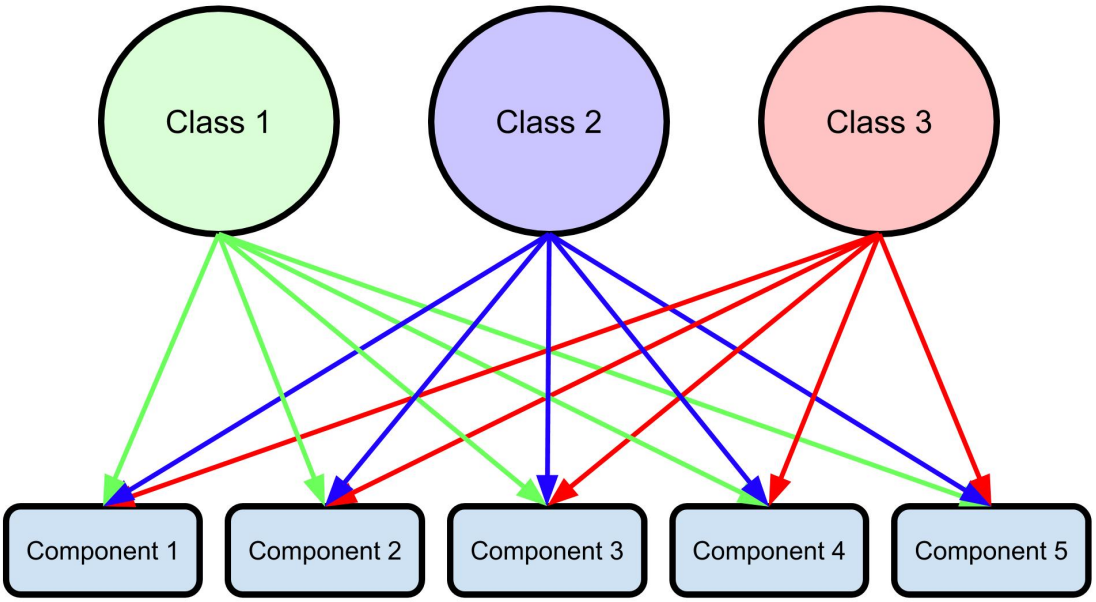

Figure 2.4: Incorporation of latent classes to determine true relationships between components.

pattern). In LCA, the underlying latent variables are classes which are discrete and categorical. LCA is especially useful to classify subjects into groups when there is no gold standard for these classifications as is the case with dietary patterns.

To date, there have been 2 studies that have examined the association between dietary patterns and neural tube defects (23, 96). Specifically, Sotres-Alvarez et al. investigated NTDs collectively and Vujkovic et al. investigated spina bifida alone. In the US-based study by Sotres-Alvarez et al. using data from the NBDPS, a prudent dietary pattern (characterized by consuming fruits, yogurt, reduced-fat milk, whole-wheat bread, fortified cereal, and fish) was found to be protective and used as a referent group. Specifically, women who did not take a folic acid containing supplement in the Mexican[2] (characterized by high intake of chili peppers, avocados, salsa, tortillas, refried beans, and chicken or beef by-products), Western, and low-calorie Western classes (both Western diets were characterized by low intakes of fruits and vegetables and high intake of French fries, white bread, soda, potato chips, and bacon), which were also identified via LCA, were significantly more likely (AOR (95%CI): 1.6 (1.15-2.19), 1.5 (1.10-1.90), and 1.4 (1.05-1.83) respectively) to have offspring born with NTDs than were those in the prudent class. These associations were not significant among supplement users.

In the Netherlands-based study, by Vujkovic et al., data-driven principal component analysis identified a dietary pattern similar to a Mediterranean diet. This Mediterranean-like diet was high in vegetables, vegetable oil, legumes, fruits, fish, cereal products, butter and alcohol, and low in sugars, potatoes and sweets. Mothers were grouped into quartiles of observance to this dietary pattern. Mothers who followed this dietary pattern the most (i.e. highest quartile) showed a reduced risk of 63% (95% CI: 16.7-83.6%) for having a child with spina bifida compared to the lowest quartile of mothers over the study period

[2]Current research is moving away from giving different derived patterns names but rather identifying the food components in these patterns to emphasize that what is important about the diet are the foods that are in it.

(96). In both of these studies, the healthier dietary patterns characterized by higher intake of fruits, vegetables, and fish, were associated with reductions in the risk of NTDs.

While there is great of importance in identifying health benefits of specific nutrients, humans eat a variety of foods that contain a variety of nutrients as well as non-nutrients that cannot be captured individual nutrients. Further, data driven dietary patterns can describe patterns that are born of the data and represent unique features of a population which are not likely to be fully captured by a pre-defined score (i.e DQI-P, HEI, AHEI, etc.), thus identifying data-driven dietary patterns has potential to expand upon our knowledge of spina bifida, complementing diet quality scores, and improve survival. We seek to examine the influence of dietary patterns on spina bifida mortality. We hypothesize that mothers with data-driven dietary patterns characterized by energy-dense and nutrient-poor or processed foods will be associated with poorer infant survival compared to mothers with healthy dietary patterns during pregnancy.

The following section provides a background of the survival analysis techniques and methods needed to address the aims of this study. A description of external validity, as it relates to this study, is also covered.

2.5 Survival Analysis

2.5.1 Kaplan-Meier Estimate

Epidemiology, as a science, is concerned with the occurrence and distribution of disease or other health related events in a population. Survival analysis focuses on the "when" of the outcome's occurrence if it occurs at all (i.e. time to an event). Survival analysis is therefore inherently longitudinal. Subjects are followed over time. Time to event data could be: time to death, time to onset of disease or reoccurrence (e.g. cancer), length of hospital stay, time to full recovery. With such data, drop-out or loss-to-follow-up often occurs for a variety of reasons. This is commonly termed censoring. Due to this censoring,

we can only observe the survival experience up to a certain point in time. When and if the outcome occurs is unknown. We only know that they survived to a certain point, but we do not know the exact time of failure (i.e. time at which they experienced the event). To still be able to calculate survival probabilities for the event at given time points despite censoring, Kaplan and Meier proposed a way to non-parametrically estimate survival, even in the presence of censoring, using the method of maximum likelihood (97). This survival probability is called the Kaplan-Meier or product-limit estimate. A survival function (i.e. survival curve) can be constructed from the Kaplan-Meier estimates across the study period (Figure 2.5).

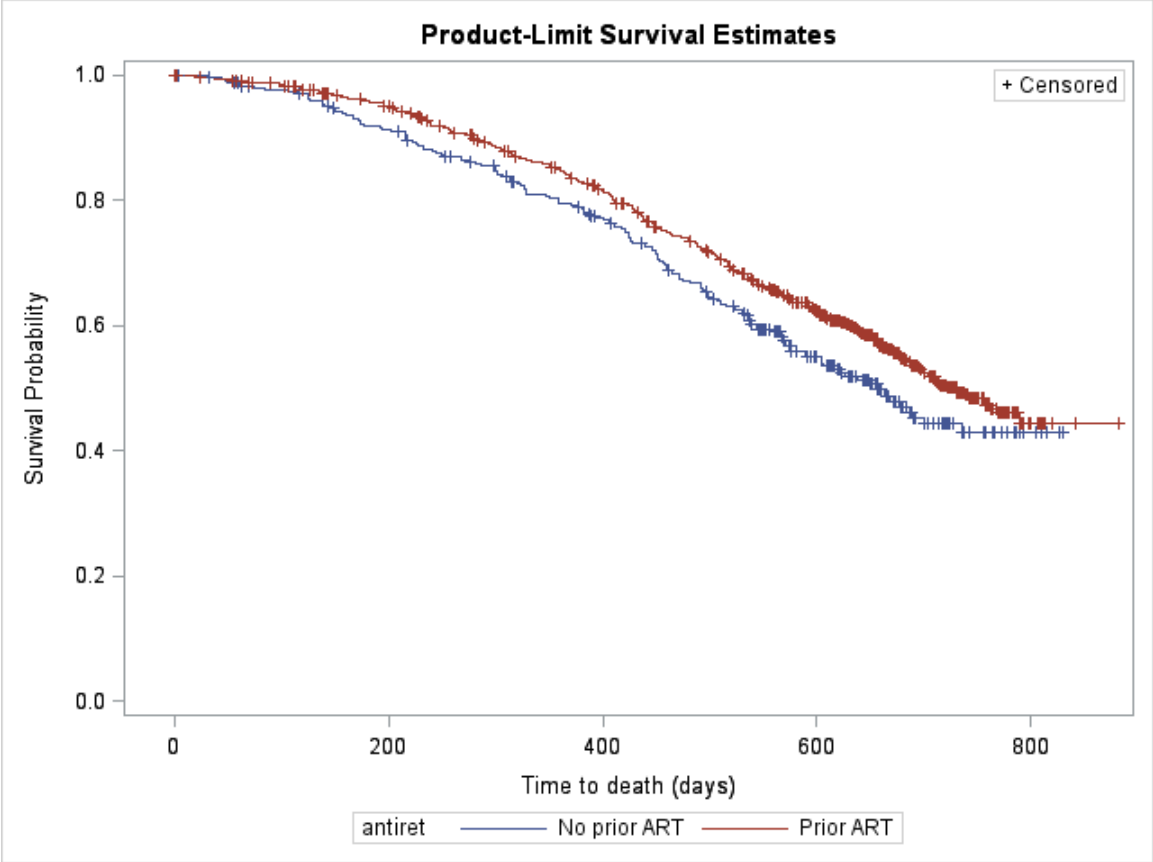

Figure 2.5: Example of survival curves calculated using the Kaplan-Meier estimate.

Independence of events is a key assumption for Kaplan-Meier estimate and for survival analysis in general. Specifically we assume that the event of interest (e.g. death) occurs

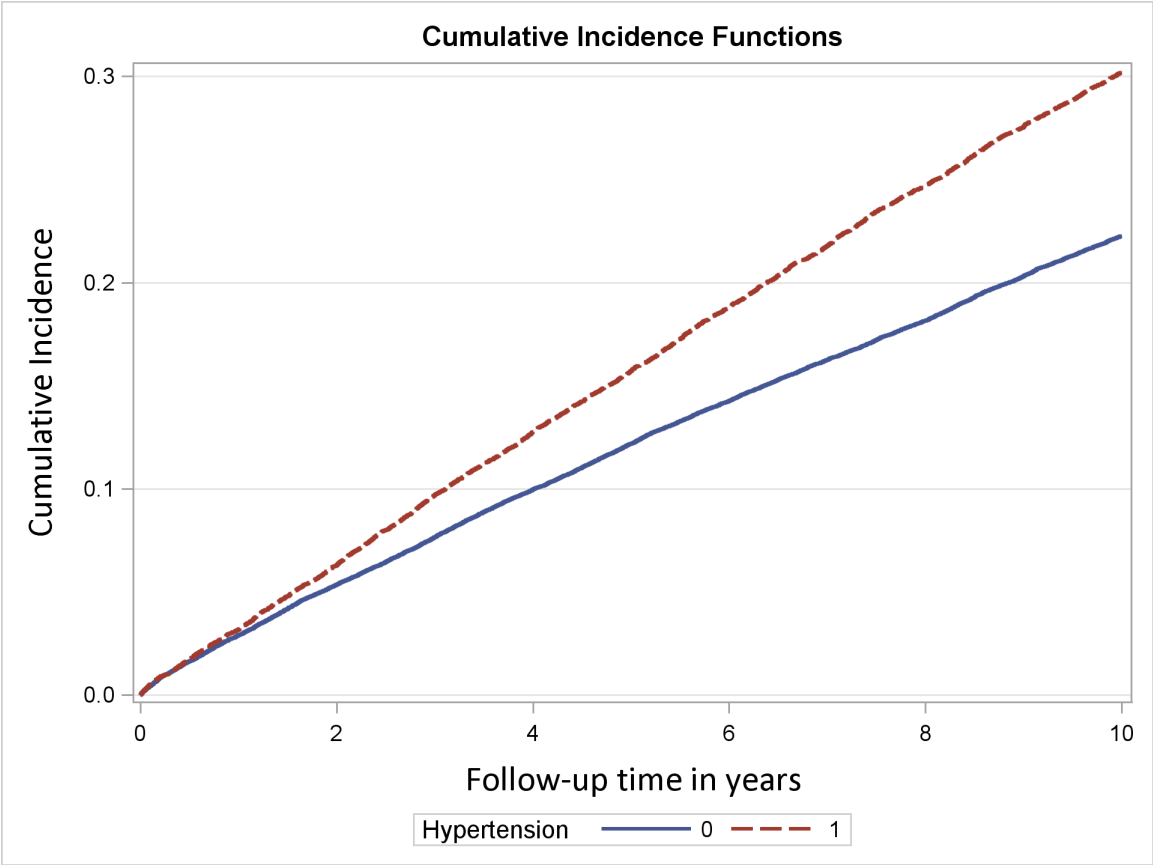

Figure 2.6: Example of cumulative incidence curves.

independently of other events (i.e. deaths). Further, we assume that censoring is independent of survival time, i.e. we assume that at any time patients who are censored have the same survival prospect of survival as those who continue to be followed. For example, the assumption implies that if a subject becomes lost to follow up, this lost subject is not at any greater risk of the outcome occurring than those that remain in the study under observation. Independent censoring is key to having the Kaplan-Meier estimate reflect truth as censored data is assumed to behave as uncensored data. If censoring is related to having event of interest (e.g. censored observations are near end-of-life) this can leader to bias survival estimates (e.g. overestimated infant mortality rate). Further consideration to this assumption being violated will be given later on.

2.5.1.1 Comparison of Kaplan-Meier Survival Curves

Survival data can be stratified by variables to examine survival curves within levels of that variable. Two or more survival curves can be compared both formally through statistical tests and visually. The two options are complementary and should be used in tandem. Visual inspection can reveal distinct differences in survival though without the accompanying statistical testing, in some cases, it can be hard to tell whether differences should be attributed to chance variation or not. Statistical tests for comparing survival curves are built around the null hypothesis that the two curves are equivalent ($S_1(t) = S_2(t)$) and the alternative hypothesis that the curves are not equivalent ($S_1(t) \neq S_2(t)$). For formal comparison of survival curves, the standard test is the log-rank test (98, 99).

The log-rank test compares estimates of each group's hazard function (instantaneous risk of the event as it depends on survival time) (100) at each unique failure time. In addition to the assumptions inherent to the use of Kaplan-Meier estimates, the log-rank test also assumes proportional hazards across the two survival functions. This test has great statistical power if the assumption is upheld though the test is still valid when the assumption is violated. The assumption can be assessed by examining the survival curves.

If the survival curves cross the proportional hazards assumption is violated and the power of the test is reduced (101).

Alternative tests, include Gehan's Wilcoxon, Tarone-Ware, Fleming-Harrington, and Peto-Prentice among others. The Wilcoxon test is of particular importance in studies of highly lethal birth defects, as it weighs failures by the observed number currently at risk for the event. In other words, as survival decreases, weights decrease to reflect the reduced amount of information available. Such weighting can assess exposures that might matter more earlier on and that might be less influential later on.

2.5.2 Cox Proportional Hazards Model

Kaplan Meier methods allow for non-parametric examination of survival but does not have the flexibility to consider multiple variables at once. The Cox Proportional hazards model for survival data, developed by David Cox (102), is analogous to linear regression for cross-sectional data with a continuous outcome. Cox proportional hazard model can investigate how survival varies across treatment or exposure levels while controlling for potential confounding. From the model hazard ratios are produced comparing the hazard among the exposed to the hazard among the unexposed. The hazard is instantaneous rate of experiencing the event of interest conditional on surviving up to the given time. The hazard ratio (HR) is the ratio of the hazard in the exposed compared to the unexposed. In practice, one can roughly consider hazards as incidence rates, hazard ratios as incidence rate ratio, and both can be interpreted accordingly (103). The average hazard over the study period is equivalent to the incidence rate over the study period.

Key assumptions of the Cox model include: (1) independence of events, (2) non-informative censoring, conditional on explanatory variables, and (3) proportional hazards. New to this model is the proportional hazards assumption. Hazards are assumed to be proportional over time (i.e. constant hazard ratio). For instance, if the hazard of infant death

is in one group is 3 times that of another group in the first week of life (HR = 3.0), this modeling assumption implies that the hazard ratio is the same at 8 months old. The hazard ratio, therefore, should be independent of time (104). This assumption can be tested graphically and by use of interaction terms for time. No assumption is made directly about the hazard itself only that the hazard ratio is constant. For this reason the model is considered semi-parametric and estimates are determined using partial maximum likelihood.

2.5.3 Competing Events Analysis

The perinatal window involves the time surrounding birth. Here we will defined perinatal mortality as an encompassing term to refer to death in utero after 20 weeks gestation (fetal death) or within the first year of life (infant mortality). Prior literature has mistakenly assumed the absence of competing events among studies of perinatal mortality (105). In the study of naturally occurring perinatal mortality, induced abortions preclude the observation of the natural perinatal death. While there are 3.5 infants born with spina bifida per 10,000 live births in the U.S., the actual prevalence of spina bifida is difficult to determine due to prenatal diagnosis and often subsequent elective termination of pregnancy (3). Among infants prenatally diagnosed with spina bifida, the approximate percent of electively terminated pregnancies in the North America is 50% (95% CI: 35-64%) (106).

This is a situation of competing events. Commonly in longitudinal studies, one event's observation may preclude the observation of another event. We call these events, competing events.

We will distinguish competing event from competing risks. A competing event refers to the occurrence of the outcome of interest or any other event that preclude another event from occurring or being observed. A competing risk refers only to an event (that is not the outcome of interest) that can preclude the outcome of interest from being observed. In natural perinatal mortality (i.e. death via fetal death or infant mortality), induced abortions are

a competing risk. Induced abortions and fetal death are competing events for one another.

Integral to the calculation of Kaplan-Meier estimates and their corresponding survival curves is the assumption of non-informative censoring. When a competing risk occurs, that observation would be censored as it can no longer experience the event of interest. This may lead to biased results as the censoring is informative (107). There are methods to address this in a non-parametric (Kaplan-Meier) paradigm (108, 109). For semi-parametric models, such as the Cox proportional hazard model, additional methods exist to address competing events in this context (110). Survival analysis studies are complicated by competing events which are by nature exclusive and dependent. We address how this problem is addressed in non-parametric and semi-parametric settings in the following sections.

2.5.3.1 Nonparametric Handling of Competing Events

The Kaplan-Meier method does not yield valid estimates and survival curves for a given outcome when failures due to competing risks are consider censored observations like subjects who are lost to follow-up or choose to remove themselves from the study. A common mistake in the analysis of competing risks is to compute Kaplan-Meier based results for event of interest while treating failures from other causes as censored events. This typically results in the overestimation of cumulative incidence estimates (111). The cumulative incidence function for the event of interest, accounting for competing risk events, is estimated in a two-step process (112, 113). First, one calculates the KaplanMeier estimate of the overall survival from any competing event (i.e. failure due to the event of interest or any competing risk). Second, the conditional probabilities of experiencing only the event of interest are calculated. These conditional probabilities correspond directly to the cause-specific hazard function. The cause-specific cumulative incidence curve is then estimated using these two quantities (109).(108)

Automated methods for calculating the cause-specific cumulative incidence curve have been developed. The SAS %CIF macro use the method described above and can be used

to compare cause-specific cumulative incidence across exposures or other variables (109).

In the context of competing events, the log-rank test is no longer valid. The log-rank test compares the effect of the levels of one variable on the cumulative incidence function (the null hypothesis being that the cumulative incidence functions are the same across levels of the tested variable). In order to test differences across exposures or other variables, a test equivalent to the log-rank test, but for scenarios with competing risks, was developed by Gray in 1988 (114). This test can likewise be performed use the SAS %CIF macro (109).

2.5.3.2 Cox Proportional Hazard Model and Competing Events

Cox proportional hazard models, in the presence of competing risks, can be used to calculate two key and distinct survival analysis quantities: (1) the cause-specific hazard ratio (csHR) and (2) the subdistribution hazard ratio (sdHR). Which type of hazard ratio that should be reported depends upon the scientific question at hand. The Cause-Specific Hazard model lends itself more to etiologic research, while the subdistribution hazard model more closely reflects individual risk and can be helpful for allocating resources (110). We describe each method and its uses in greater detail below.

The Cause-Specific Hazard Model.

Consider the following illustration from Lau et al. 2009 to describe the cause-specific hazard in discrete time (Figure 2.7). The black circles represent the at-risk individuals in the population. The gray squares represent individuals that experience event 1 (the event of interest) at a given time. The triangles represent individuals that experience event 2 (the competing risk) at a given time. The risk-set for the cause-specific hazard only includes individuals that have not experienced previously either event (i.e. the event of interest or the competing risk).

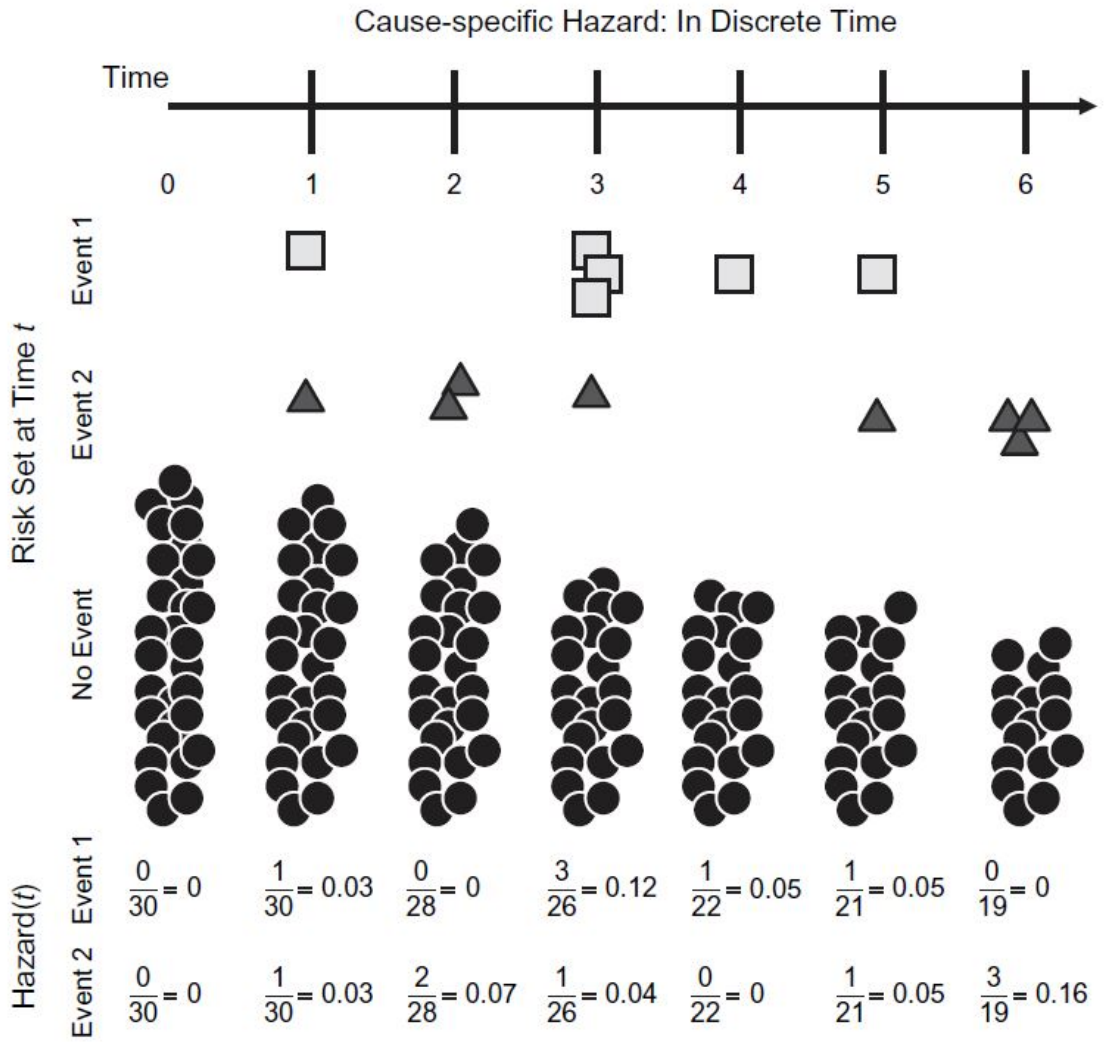

Figure 2.7: Cause-specific Hazard: In Discrete Time. [Borrowed from Lau et al. 2009 (110).]

A proportional hazard model can be constructed for the event of interest in the presence of competing risks. Individuals that experience a competing risk are treated as censored making this model mathematical the same as a model constructed by an investigator who ignored competing risks altogether. With this treatment of competing risks and censored observations, comes the assumption of non-informative censoring which now includes cases of a competing risk, drop-out, or loss to follow-up. Further independence of competing events is necessary to be able to use cumulative incidence to quantify the risk of the event of interest. Moreover, the interpretation of the cause-specific hazard ratio can be stated as: the relative change in the cause-specific hazard of a given event corresponding to a 1-unit increase in the exposure. This interpretation, given the prior assumptions hold, applies to the hazard ratio we would observe in hypothetical world in which the competing risk is eliminated, all else being equal (110).

The cause-specific hazard ratio is automatically report by default through common statistical survival analysis procedures like PROC PHREG (SAS) or coxph() (R console). As a result, the cause-specific hazard ratio is more frequently reported than its counterpart, the subdistribution hazard ratio.

The Subdistribution Hazard Model.

To model survival data in the presence of competing risks, Fine and Gray developed the subdistribution proportional hazard model (also known as the Fine and Gray regression model or competing risks regression) (115, 116). Consider the following illustration from Lau et al. (2009) to describe the subdistribution hazard in discrete time (Figure 2.8). The black circles represent the at-risk individuals in the population. The gray squares represent individuals that experience event 1 (the event of interest) at a given time. The triangles represent individuals that experience event 2 (the competing risk) at a given time. The subdistribution hazard includes in its denominator individuals that have not experienced previously event 1 (i.e. the event of interest) as well as individuals that have experienced

event 2 previously (i.e the competing risk) even though they are not eligible to experience event 1.

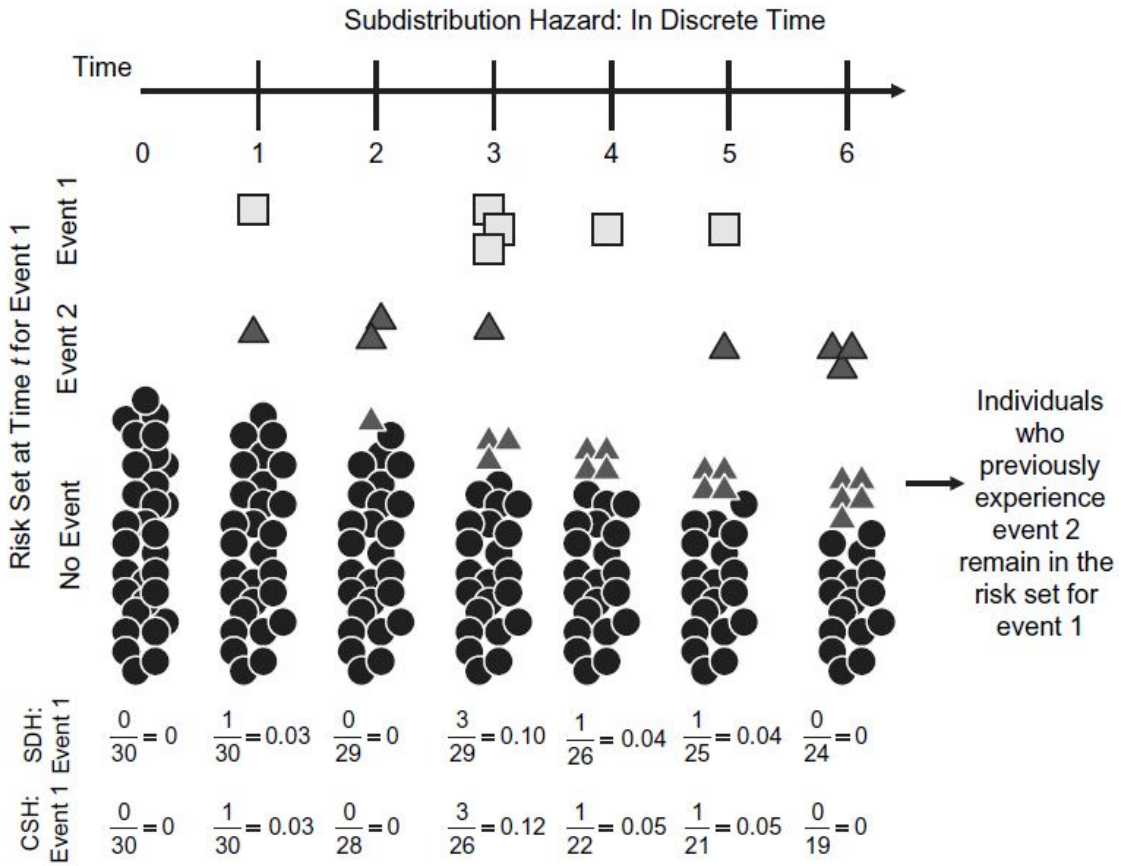

Figure 2.8: Subdistribution Hazard: In Discrete Time. [Borrowed from Lau et al. 2009 (110).]

Given that individuals who experience event 2 (the competing risk) are retained in the risk-set, survival probabilities for the subdistribution proportional hazards model are either equal to or greater than the survival probabilities for the cause-specific proportional hazards model. This message is portrayed by the comparison of the different hazard calculations that occur at the bottom of Figure 2.8. The cause-specific hazard is increasingly greater than the subdistribution hazard even though the competing events occur at the same time and the same frequency in the example.

The subdistribution proportional hazard model provides a means of estimating cumulative incidence and hazard ratios without assuming independence of competing events. A proportional hazard model can be constructed for the event of interest. Moreover, the interpretation of the subdistribution hazard ratio from this model can be stated as: the relative change in the subdistribution hazard of a given event corresponding to a 1-unit increase in the exposure. This interpretation, given the model assumptions hold, applies to the hazard ratio we would predict to observe in real world in which the competing risks captured are observed (110). This interpretation lends itself to more clinical relevance to consider cost-effectiveness and the allocation of resources.

The subdistribution hazard ratio can be calculated through statistical survival analysis procedures like PROC PHREG (SAS) by means of recently added features in version SAS 9.4 (117) or the R Console package 'cmprsk' (118).

The two modeling approaches treat competing events differently and therefore answer different questions. Cause-specific hazard ratios show the pure effect of an exposure on an outcome while the subdistribution hazard ratios more closely represents the predicted risk of the outcome (110, 117). The presentation of both of these models provides an understanding of how an event such as an induced abortion can act as a competing risk when natural perinatal mortality is the outcome of interest.

2.6 External Validity

Bias due to confounding typically arises when factors that affect both exposure and outcome are not accounted for adequately. This leads to a lack of internal validity, i.e. the exposure-outcome relation is not true for your study sample. While confounding frequently is the most-often cited source of bias in health research, selection bias nearly universally affects studies to various degrees depending both on study design and data collection. Selection bias refers to differential exclusion or inclusion of study participants that leads to

results that incorrectly reflect the exposure-outcome relationship of your target population. To understand selection bias, we first define four key terms: target population, source population, study sample, study sub-sample. The target population refers to the population to which you wish to generalize the results of the study. The source population is the population the investigator chooses to represent the target population. The study sample refers only to those individuals you attempt to recruit into or invite to participate in the study. The study sub-sample refers only to the individuals that actually participate in the study. Study sub-sample can also refer more specifically to individuals for whom you have complete data on covariates needed for analysis. Participation thereby refers to providing a complete record of requested information for the study. To illustrate the difference of these terms, consider the following example. You are part of a regulatory body of the United States government that wishes to understand the effect of hypertension among adults on health-care costs. To investigate the effect on the U.S. population you choose to conduct a study in the state of Colorado. You randomly select 20,000 addresses obtained from driver license records to which you send an invitation to participate in a survey on your study topic. Participants are told they will receive an incentive of $20 for completing the survey. Five thousand individuals complete and return the survey. In this example, the target population is the entire adult U.S. population. The source population is residents of Colorado. The study sample is all Colorado residents that receive a survey (20,000; residents that have a current Colorado driver's license and up to date mailing address). The sub-sample is the residents that completed and returned the survey (5,000).

At the core of epidemiologic research is the idea that there is a well-defined population of interest, to which the results are intended to be generalized. The ideal is to be able to generalize our results to the target population. This requires external validity, i.e. a lack of selection bias. Selection biased results do not accurately represent the population of interest and therefore are not generalizable to the target population. Informally, addressing

selection bias requires answering the following question: why do we have data from some of the study sample and others not? (119) Selection bias causes a disconnect between our results and results that would be seen in the population of interest (i.e. the target population).

Selection bias is important to avoid or control for because there is some well-defined population of interest we wish to generalize results to. Selection bias can occur at multiple levels. Results from our sub-sample may well represent the sample and the sample may well represent the source population, but the selection of the source population is not representative of the target population. Likes wise, results from our sub-sample may well represent the sample, but the sample may poorly represent the source population. Lastly, results from the sub-sample may not represent the sample and therefore selection bias is present and the result cannot be generalized to the target population. The situation that is of concern in this study is the last. Results obtained from the sub-sample of participants have not been investigated to provide evidence whether the study sub-sample is representative of the study sample.

2.6.1 Appropriate Incorporation of Non-participant Data

The composition of the study sample is all invited mothers of children born with Spina Bifida. The exhaustive and exclusive groups in the study sample are the non-participants and participants. To check that complete data available for participants reflects the data from the non-participants as well, a few strategies can be used. First, available covariate from both groups can be compared, this includes distribution of covariate values as well as exposure and outcome. For survival analysis, Kaplan-Meier curves can be compared. Ideally, group estimates would be the same. If group estimates for key covariates are reasonably similar, results using data from both groups combined can be presented in addition to results for the two groups separately.

Next, in Chapter 3, we describe in detail the study design and methodology employed in this study.

CHAPTER 3: STUDY DESIGN AND METHODS

3.1 National Birth Defects Prevention Study (NBDPS)

Due to the rarity of most birth defects, case-control study designs are commonly employed to study exposure-outcome relationships for birth defects. This study was conducted using data from the National Birth Defects Prevention Study (NBDPS),(120) a ten state, population-based, case-control study of more than 30 major birth defects (Figure 3.1). Infants in the NBDPS had an estimated date of delivery between October 1, 1997 and December 31, 2011.

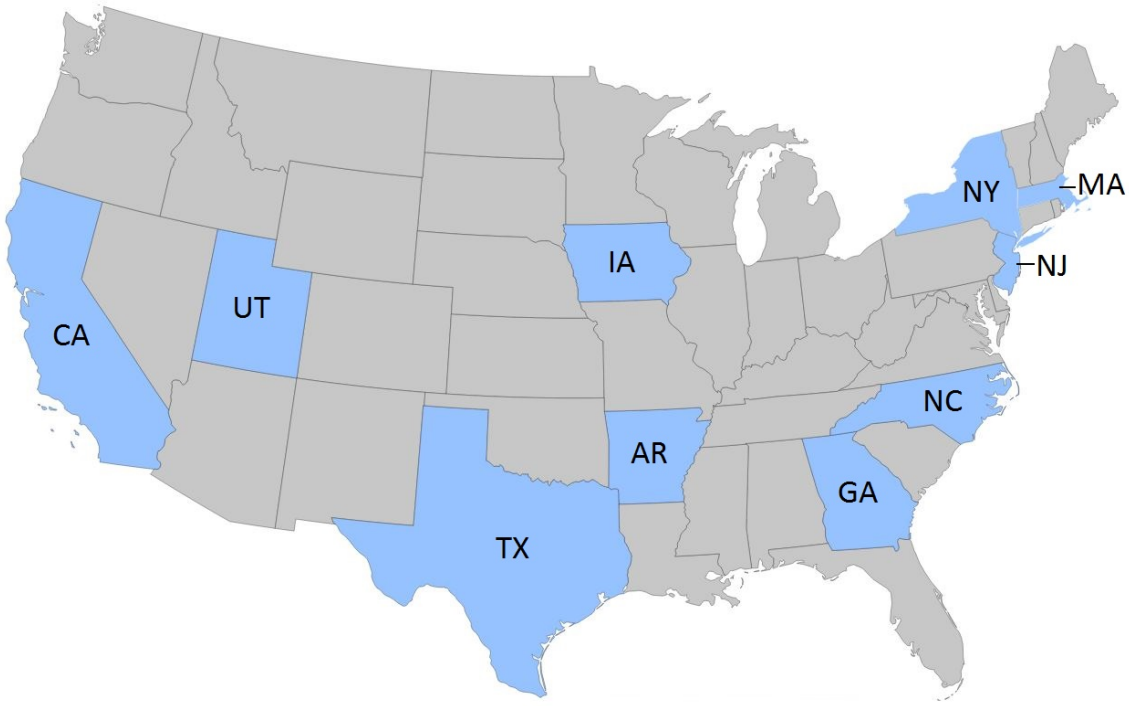

Figure 3.1: States participating in the National Birth Defects Prevention Study.(121)

NBDPS captured birth defects occurring among live births and, in the majority of participating states, birth defects among fetal deaths (stillborn) and induced abortions as well. Figure 3.2 from Reefhuis et al., provides a visual representation of how states participated in identifying birth defects over the length of the study. For simplicity, the term infant used

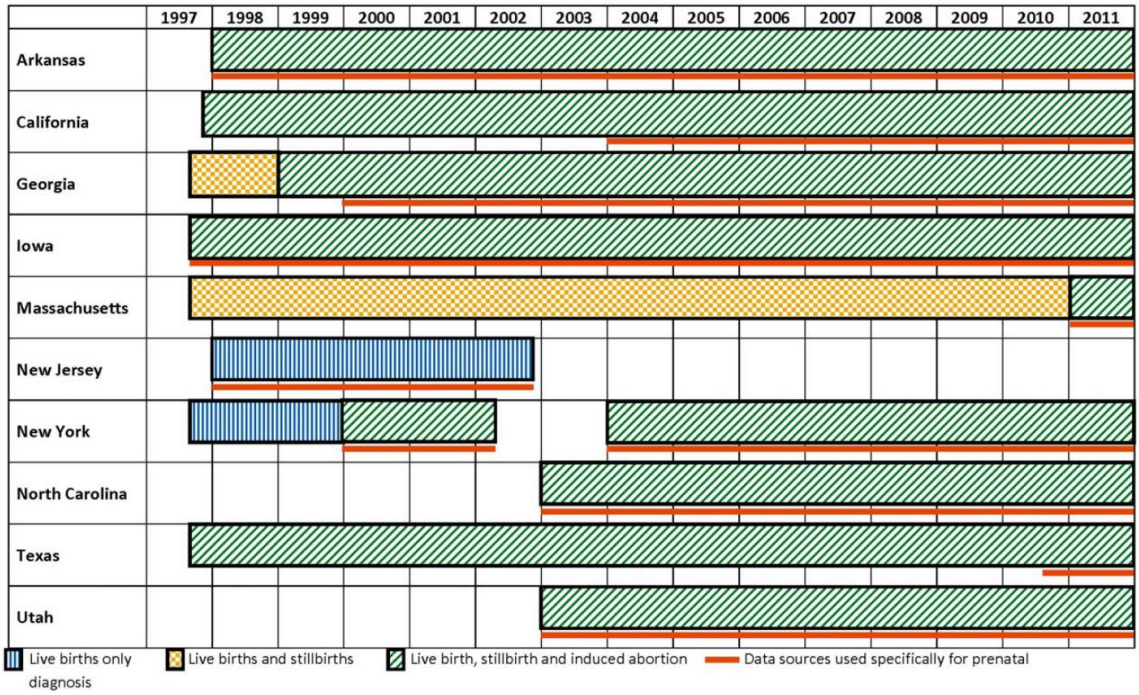

Figure 3.2: Inclusion of birth defects among live births, stillbirths, and induced abortions, and data sources used specifically to ascertain prenatal diagnoses, by estimated year of delivery and study center. [Borrowed from Reefhuis et al. 2015 (120)]

in regard to cases encompasses all three categories (live birth, stillbirth, induced abortion) unless otherwise specified. Active population-based surveillance programs in each participating state (Arkansas, California, Georgia, Iowa, Massachusetts, North Carolina, New Jersey, New York, Texas, and Utah) were used to identify cases. Cases included infants with 1 or more of the major birth defects captured in this study. Infants recognized or strongly suspected to have defects directly related to a single-gene defect or chromosomal abnormalities were excluded from the NBDPS as the purpose of the study was to determine unknown causes of birth defects. Controls were only recruited from among live born

infants and were not matched to cases by design. Controls were sampled at random from birth certificate records and hospital birth records to represent the underlying population from which cases came. Additional details on the study design, sampling framework, and the birth defect surveillance systems for NBDPS have been previously published (120).

The NBDPS study was approved by the institutional review boards of the University of North Carolina at Chapel Hill, the Centers for Disease Control and Prevention, as well as those of the other collaborating study centers.

3.2 Data Sources

This study used data from the following three sources.

CATI, DAT10. These analyses used data from the NBDPS Computer Assisted Telephone Interview (CATI) questionnaire for exposure and covariate information (e.g. age, education, race/ethnicity), the food frequency questionnaire section, the cereal and supplement sections, and beverage/soda questions. For our analysis, we used the NBDPS analytic data file version 10.0 (DAT10) corresponding to a study period of October 1, 1997 to December 31, 2011 which is the entire length of the NBDPS.

Infant mortality data. After active data collection ended for NBDPS in 2011, each study center (except New Jersey) submitted state-level vital records data on infant mortality corresponding to all infants eligible to participate in the NBDPS. This data included key mortality information (date of death, underlying cause of death, etc.) for both interviewed and non-interviewed cases. Among all infants with spina bifida, 1854 mothers (58%) participated and completed the interview compared to 1334 mothers (42%) who did not participate in the NBDPS.

Birth defect case classification data. In the process of determining whether an infant was eligible for the NBDPS, infants were first identified as having a birth defect by their health care provider. An initial classification was then done by study personnel for

eligible cases. An additional and more extensive birth defect classification was done for infants of mothers that chose to participate in the NBDPS. Those mothers then went on to be interviewed for additional data. The infants corresponding to non-interviewed mothers were later reviewed for the same level of birth defect classification using available medical record data. This was done so that interviewed and non-interviewed cases could be compared with the information available for both groups.

Birth defect case classification and covariate data was used in conjunction with linked infant mortality data that had already been provided by all study centers (NBDPS project #9547). Survival analysis, using nonparametric Kaplan-Meier survival functions and Cox proportional hazards models, was performed with SAS 9.4 to produce survival and cumulative incidence curves and calculate the overall infant mortality rate, parameter estimates, and corresponding hazard ratios.

3.3 Outcome Assessment

As infants were initially identified by their health care provider, they were further reviewed by clinical geneticists who collaborated on the NBDPS. Cases at this stage are classified into one of the following categories: isolated (infants with only 1 defect or with 2 or more defects that are developmentally related), multiple (infants with $\geq$2 major unrelated birth defects), or complex (infants with $\geq$2 birth defects that are suspected to be pathogenetically related yet for which the underlying defect is not clear) (44, 122). Detailed guidelines for case classification can be found in Rasmussen et al. 2003. Three primary NBDPS categories of neural tube defects were captured in this study: anencephaly, encephalocele, spina bifida. The hierarchical classification was first anencephaly, second encephalocele, and third spina bifida. In other words, if an infant was born with all three defects, they would be classified as an anencephaly case only. If an infant was born with encephalocele and spina bifida, the infant was be classified as an encephalocele case only.

Spina bifida cases were infants in which spina bifida was the only neural tube defect.

Outcomes of Interest: Mortality (for calculating the overall infant mortality), Time to Infant Mortality (for survival analysis)

Our primary outcome of interest was time to infant mortality among infants with spina bifida. Mortality of cases is specific to the day of death as recorded by state death records.

3.4 Exposure Assessment

Demographic, behavioral, and dietary data were all gathered through an interview with the case infant's mother. Maternal interviews were conducted primarily via telephone using a standardized computer-based interview available in either English or Spanish. Anticipated length of an interview was less than 1 hour. Interviews were administered after 6 weeks or more had passed since the infant's estimated date of delivery (EDD) and no later than 2 years after the EDD. Average time to interview for all infants with a birth defect cases was 11.7 months (11.0 months for infants with spina bifida).

Exposure 1 of Interest: Maternal Pre-pregnancy Body Mass Index

Body mass index was calculated from self-reported pre-pregnancy height and weight as mass (kilograms, kg) divided by height (meters, m) squared (kg/m^2) (123). Height was recorded in either feet and inches or centimeters according to the preference of the mother. The interview question for height was: What is your height without shoes? Weight was recorded in either pounds (lbs) or kilograms (kg) according to the preference of the mother. The interview question for weight was: How much did you weigh before your pregnancy? Appropriate unit conversions (U.S. standard units to metric units) were done as needed. BMI was then calculated as a continuous number. BMI was categorized into four groups used by the National Heart Lung and Blood Institute: Underweight (BMI$<$18.5), Normal weight (18.5$\leq$BMI$<$25), Overweight (25$\leq$BMI$<$30) and Obese (BMI$\geq$30) (123). Both categorical and continuous measures of BMI were used in our statistical analysis though

our primary analyses identified results based on categorized BMI.

Pre-pregnancy BMI, calculated from self-reported recall of height and weight, has been shown to be a valid measure of BMI; prior research has shown that pre-pregnancy weight by recall is highly correlated with weight recorded in clinical records (124). Height typically remains constant from the start of pregnancy to the time of interview, so we anticipate recalled height to be prone to very little measurement error. This measure while not a perfect proxy for body fatness was inexpensive, easily obtainable, and consistently predicts body fat percentage well on average (35).

Exposure 2 of Interest: Maternal Diet Quality

The primary exposure of interest, maternal diet quality, was evaluated in three ways using data collected from the food frequency questionnaire as well as questions from the cereal and supplement sections, and beverage/soda questions. Next we explain how the three methods of measuring diet quality were implemented using the NBDPS data.

3.4.1 Method 1: Diet Quality Index for Pregnancy (DQI-P).

The DQI-P, developed by Bodnar and Siega-Riz, is an ordinal score based on 8 dietary components (60). Our intention was to follow as closely as possible the original scoring method for the DQI-P restricting changes to those required by data limitations. In doing so we follow the same DQI-P calculation presented in Carmichael et al (22). Notably, the original DQI-P includes a component capturing the frequency of meals and snacks. Since such data were not requested in the NBDPS questionnaire, we excluded this part of the DQI-P from our assessment. We added intake of sweets as an additional component as was done in by Carmichael et al. and Feldkamp et al. (22, 68). Sugary foods and sugar sweetened beverages (SSB) were recommended for limited consumption in the 2000 and 2005 Dietary Guidelines for Americans (71, 125).

Each of the 8 components has a minimum score of 0 and a maximum score of 3. Total

scores can therefore range from 0 to 24, a higher score indicating a healthier diet for pregnancy. The first six components listed (grains, vegetables, fruits, folate, iron, and calcium) were scored by quartile of consumption compared to other mothers. Participants who consumed an amount in the lowest quartile of consumption received a score of 0, while participants who consumed an amount in the highest quartile of consumption received a score of 3. This scoring method exactly follows the method presented in Carmichael et al.(22) and results in a range of scores (0-24) close to one-third the score range (0-80) of the original DQI-P.

For the three food groups (grains, vegetables, and fruits), we will use the mapping of food items from the FFQ items to specific food groups in the DQI-P given by Carmichael et al (126). See Appendix 1 for a reproduced copy of this table. To calculate servings per day for these components, we used the following formula.

$$\frac{\sum(\text{servings/day} \times \text{gm/serving for each food item in the group})}{(\text{mean gm/serving of all the foods in the component})}$$

For the sweets component, a slight modification is made to that formula to incorporate soda.

$$\frac{\sum(\text{servings/day} \times \text{gm/serving for each non-soda sweet food item})}{(\text{mean gm/serving of all foods in the component}) + \text{servings/day of soda}}$$

Dividing the summed grams per day by mean grams per serving sizes of all food items in each component provides a way to account for variability in portion sizes of various food items within each component (e.g. cookie vs. cake). Nutrient values, for folate, iron, and calcium, were previously calculated by NBDPS researchers using the USDA nutrient database, version 19 (127). Dietary folate intake takes into account folic acid from fortified foods by calculating dietary folate equivalents (DFEs). Folic acid from fortified foods is multiplied by 1.67 in order to account for its greater bioavailability when compared to

folate[1](130).

Percentage of calories from fat was scored in a categorical manner. Percent of calories from fat has four categories: $\leq 30\%$, $> 30\%$ and $\leq 35\%$, $> 35\%$ and $\leq 40\%$, $> 40\%$ with corresponding scores 3, 2, 1, and 0 points respectively. Sugar intake was ranked by quartile, based on the distribution among participants. Lowest to highest quartiles corresponded to scores of 3 and 0 respectively. This rank order of scores based on consumption is just the opposite of the other food groups. Therefore those in the lowest quartile of sweets consumption will receive a score of 3 and the highest quartile a score of 0. We next calculated DQI-P total scores by summing the component scores and then grouped participants into three score categories (low/medium/high) to compare the high to the low scoring DQI-P groups.

3.4.2 Method 2: Health Eating Index 2010 (HEI-2010).

The HEI-2010 is a measure of diet quality that assesses adherence to the 2010 Dietary Guidelines for Americans. It consists of 12 component scores. Possible total HEI-2010 scores range from 0-100. Our implementation of the HEI-2010 was similar to the approach taken by the University of Minnesota: Nutrition Data System for Research (131) and followed the guidance provided by the National Cancer Institute (132). The steps to calculating the HEI-2010 were:

1. Map each of the 63 food items from the FFQ to their corresponding component in the HEI-2010.
2. Convert all food items from the serving units used in FFQ to the servings units used by the HEI-2010 (e.g. 2 carrots per day converted to 1.6 cup equivalents). We used the Food Patterns Equivalents Database (FPED) 2011-12 produced by the USDA to

[1]When consumed with food, as is the case with fortified foods, 85% or more of the synthetic vitamin, folic acid is estimated to be bioavailable, while approximately only 50% of naturally occurring folate in food is bioavailable.(128, 129)

accurately preform these conversions (133). This database translates the amounts of foods, as eaten (which is how the FFQ asks for the information), into cup and ounce equivalents, the measurement units used for the HEI scoring system (132).

3. Calculate the cumulative servings for each component by summing the servings from each of the corresponding FFQ items.
4. Determine the portion of the maximum score obtained by each component to assign a component score.
5. Sum the 12 component scores to calculate the HEI-2010 overall score.

For example, if a subject recorded on the FFQ eating 3 apples per week, 2 bananas per week, and 1 small glass of orange juice per day for all of her consumption of fruit items, the "Whole Fruit" component score would be calculated as follows.

1. For calculating the score for "Whole Fruit" component, the 3 apples and 2 bananas would be mapped to this component.
2. Using the FPED, we convert 3 apples per week to $(0.91$ cup/equivalents $\times\ 3)/7 =$ 0.39 cup/equiv per day and 2 bananas per week to 0.67 cup/equiv $\times 2)/7 = 0.19$ cup/equiv per day.
3. Sum the servings: $0.39 + 0.19 = 0.58$ cup/equiv. per day
4. Since 0.58 cup/equiv. exceeds the 0.40 cup/equiv. for the maximum score of 5. This subject then receives a maximum score of 5 for the "Whole Fruit" component.[2]
5. Assuming the subject's other eleven components score sum to 80. The HEI-2010 score for this subject would be 85.

3.4.3 Method 3: Latent Class Analysis (LCA).

LCA was used to assign mother-infant pairs into exclusive data-driven and derived dietary patterns, called classes, based on a mother's consumption of food items adjusted for caloric intake. Infants within each dietary class have similar maternal food intakes while

[2]If the subject only ate bananas in the same amount but no apples so the sum of servings was 0.19 rather than 0.58, the score would be $(0.19/0.40) \times 5 = 2.38$ for the "Whole Fruit" component.

variability in maternal diet is greatest when compared across the different dietary classes. This study follows closely the implementation of LCA by Sotres-Alvarez et al. (23), a prior LCA conducted examining the risk of neural tube defects by dietary class. Briefly, Sotres-Alvarez et al. used LCA to form classes based on dietary patterns of 64 food items using data solely from controls. Intake of each food item (gram/day) was divided by the total dietary consumption in grams per day in order to quantify the amount consumed of the food item relative to the total dietary consumption. The relative food item consumption was categorized into 4 levels to address statistical challenges presented by nonconsumption. The four categories were no consumption and then tertiles of nonzero consumption. Rare and less commonly consumed foods had a binary categorization (consumed and nonconsumed). Ubiquitously consumed foods lacked a nonconsumption category and had quartiles of nonzero consumption. They interpreted and named the dietary classes based on the conditional food intake probabilities. The number of classes was specified to four after assessing feedback from trial and error using statistics from fit statistics such as the Bayesian information criteria (BIC) and the Lo-Mendel-Rubin likelihood ratio test. While not truly a measure of diet quality rather a grouping of dietary patterns, we anticipated that the patterns would reflect diets of varying quality and that survival would be better among infants with mothers that had high quality diets.

Using a variety of methods allowed for us to see if a particular type of dietary index (Method 1 or 2) or data-presented pattern (Method 3) was associated with improved survival. Methods 1 and 2 consider comparing categories-levels of exposure. While a specific categorization was given, this is the anticipated use of categories. Choice of the number of categories (2, 3, 4, etc.) used when comparing highest exposure category to lowest exposure for Methods 1 and 2 was based on what best represents the actual distribution of the exposure and deaths so as to maintain sufficient statistical power. Continuous forms of DQI-P and HEI-2010 as exposures were also assessed.

3.4.4 Additional exposures.

In addition to assessing the impact of maternal diet on survival, we also examined a number of exposures many of which have been previously referenced in prior literature on the topic of birth defect mortality. The following is a list of secondary exposures that we examined but with limited depth in comparison to the primary exposures, pre-pregnancy BMI and maternal diet.

Secondary Exposures of Interest: See list below.

- Maternal age
- Maternal race/ethnicity (Black, Hispanic, White, Other)
- Maternal education (<high school, completed high school, attended college)
- Maternal use of alcohol or cigarettes (B1-P3)
- Maternal nativity (US born, non-US born)
- Infant sex (male, female)
- Birth weight
- Region (by study center)
- Gestational age at birth
- Plurality (singleton vs. multiples)
- Isolated, multiple, all defects
- Time period (1998-2000, 2001-2003, 2004-2006, and 2007-2011)

Secondary exposures were investigated simply by calculating survival estimates stratified by the different levels of exposures. Year periods selected above where determined to provide three or more years of data for each time segment. Some stratified survival curves were produced.

3.5 Statistical Analysis

3.5.1 Aim 1. Infant mortality overall and by pre-pregnancy BMI

Infant mortality among babies born with spina bifida was calculated as one minus the Kaplan-Meier survival probability at one year of life since birth. Early neonatal and neonatal mortality was calculated by the same method for seven days and twenty-eight days since birth respectively. The standard error and corresponding pointwise confidence intervals for the Kaplan-Meier survival probabilities were estimated using Greenwood's formula (134). We further constructed and plotted overall Kaplan-Meier survival curves (S_{KM}) (see example Figure 2.5) and cumulative incidence curves (1-S_{KM}) (see example Figure 2.6). Confidence bands were constructed for the survival and cumulative incidence curves.

Using the same methods as above, stratified survival estimates and cumulative incidence curves were presented by pre-pregnancy BMI and exposure status for the secondary exposures and by variations of spina bifida clinical classification (i.e. spina bifida subtypes and anatomical location). Survival was compared by visual inspection of cumulative incidence curves and by use of the log-rank test. To consider more extensively the effect of pre-pregnancy BMI on survival we used Cox proportional hazards models adjusted for potential confounding and reported the corresponding hazard ratios and 95% confidence intervals for our results.

3.5.2 Aim 2. Examine maternal diet quality and infant survival

The three methods for measuring maternal diet quality were implemented as explained in the exposure assessment section. Method 3 required a unique statistical approach, latent class analysis (LCA). LCA was implemented using MPlus version 7.4 (135). The number of latent classes was determined after reviewing goodness of fit statistics such as Bayesian information criteria (BIC) and the Lo-Mendel-Rubin likelihood ratio test.

To consider the effect of maternal dietary patterns on the survival we used Cox proportional hazards models and report the corresponding hazard ratios and 95% confidence intervals for our results. The proportional hazards assumption for adjusted models was assessed by visual inspection of log-log cumulative hazard.

Models included a minimally sufficient set of covariates (e.g. confounders) to allow for theoretically unbiased estimation of hazard ratios representing the exposures effect on survival dependent on the validity of our assumptions. This set of covariates waw determined using a Directed Acyclic Graph (DAG) (136, 137) constructed based on evidence from background scientific literature and subject matter expertise. A preliminary DAG is represented in Figure 3.3.

A possible minimally sufficient set (MSS) of covariates would be access to care, drug use, income, race/ethnicity, and prenatal care. Selection of what MSS was used corresponded to the available data and its quality (e.g. missingness). For instance, we anticipated data challenges such as non-response on questions related to household income.

3.5.3 Aim 3. Investigate the potential effect of bias on survival estimates

Subaim 1. Appropriate Incorporation of Non-participant Data.

During the data collection phase of the NBDPS, mothers whose child had a birth defect were routinely identified and invited to participate in the study. Some mothers were not able to be contacted or chose not to participate (32%). Reasons for not participating in the study were not collected; however medical record data and some vital records data from birth certificates are available for the non-participants. Detailed birth defect classification for these infants has also been done. Using the information available on non-participating cases linked with the infant mortality data, we compared survival among participants and non-participants. Comparing the two groups presented a relevant and generalizable picture

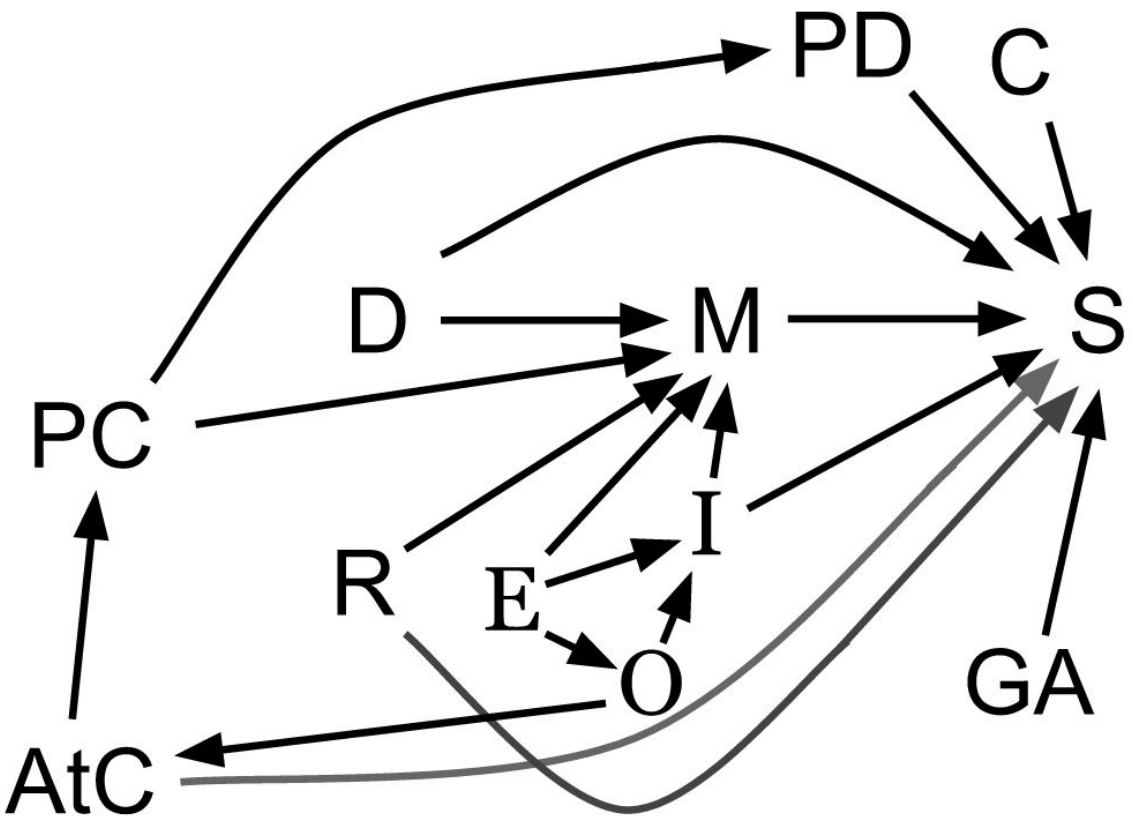

Figure 3.3: Direct Acyclic Graph showing the relation of maternal diet (M) to survival (S) for infants born with spina bifida. Other variables represented are: AtC: access to care, C: classification type of neural tube defect, D: drug use (specifically alcohol and tobacco), E: maternal education, I: income, GA: gestational age at birth, O: occupation, PC: prenatal care, PD: prenatal diagnosis, R: race/ethnicity.

of survival among infants with spina bifida. It also allowed us to detect whether differences between these populations could cause results from study participant only data to not be generalizable to the overall population from which they were sampled.

Substantial missingness due to non-participation merits investigation of the representativeness of the sample and for potential selection bias (119). We inspected the data in two stages. First, we will present a cumulative incidence curve with: (1) participant and non-participant data combined, (2) participant-only data, (3) non-participant-only data to allow for visual comparison. Cumulative incidence curves (2) and (3) were then tested for equivalence via a log-rank test. If curves (2) and (3) appear equivalent by visual inspection and via the log-rank test, we would, secondly, compare available maternal and infant characteristics (e.g. maternal age, race, infant sex, etc.) to see whether values of these variables had similar distributions in both groups. We would test whether there were statistically significant (p-value$<$0.05) differences in two proportions between the two samples (participants and non-participants) for binary variables (t-test), and Cochran-Mantel-Haenszel test statistics of general association for nominal variables and mean score differences for ordinal variables. If variable distributions were similar, non-participant data will be combined with the participant data to the extent possible. Further, when analyses incorporate data unavailable for non-participants, only study participant data will be used (i.e. Aim 1: subaim 1 and Aim 2). When inspection at either the first or second stage yielded results that indicated non-participant mother-infant pairs were different from participants, we would incorporate non-participant and participant data to the extent possible (i.e. when analyses use variables available in both groups). For Aim 2, where exposure data on diet was unavailable for non-participants, we would weight participant data to reflect the combined sample of both non-participants and participants. Weighting would involve the use of inverse probability weights (IPW) to make study participants reflect the overall population based on variables observed in both populations from which they were sampled. To check

the weighting process, we compared survival curves for participants and non-participants combined to the weighted participant data.

Subaim 2. Adjustment for competing events.

Note: While the work for this subaim was planned, it was not completed and upon agreement from all dissertation committee members this subaim was considered a separate project that could be completed after the dissertation itself.

Induced abortions are considered a competing event for studies of perinatal mortality with time understudy beginning at 20 weeks of completed gestation.

Non-parametric handling of competing events.

The cumulative incidence function is an estimate of the cumulative probability of failure from a specific cause over time. The SAS macro %CIF can be used to implement accurate methods for nonparametric survival analysis to estimate cumulative incidence functions in the presence of competing risks as well as in situations with no competing risks. We will use %CIF to construct cumulative incidence curves for perinatal mortality among spina bifida cases. To test whether differences between cause-specific cumulative incidence curves are significant, we will apply Gray's method (114) available through the SAS macro %CIF. Additional details in on implementation of the macro to address competing risks can be found in Lin et al. 2012 (109). Further we will present cumulative incidence curves of perinatal mortality, based on Kaplan-Meier survival estimates, with time starting at 20 weeks gestation to demonstrate the role of fetal death and induced abortions on infant survival.

Semi-parametric handling of competing events.

Using Fine and Gray's methodology (115), we will construct subdistribution proportional hazard models and report corresponding hazard ratios for the Aim 2 analysis to represents the predicted risk of the outcome to complement the more etiologically-focused cause-specific proportional hazards model presented as part of Aim 2. The subdistribution hazard ratio will be calculated through PROC PHREG (SAS) by means of recently added

features in SAS version 9.4 (117).

CHAPTER 4: SURVIVAL OF INFANTS WITH SPINA BIFIDA AND THE ROLE OF MATERNAL PRE-PREGNANCY BODY MASS INDEX

Objective: To investigate first-year survival of infants born with spina bifida, and examine the association of maternal pre-pregnancy body mass index (BMI) on infant mortality.

Methods: This is a retrospective cohort study of 1,533 infants born with non-syndromic spina bifida with estimated dates of delivery from 1998-2011 whose mothers were eligible for participation in the National Birth Defects Prevention Study (NBDPS). Via maternal interview, pre-pregnancy body mass index (BMI, kg/m^2) was calculated from self-reported height and weight. Inverse probability weights were used to make data from study participants representative of the eligible base population of infants with spina bifida regardless of participation in the NBDPS. Data from NBDPS was linked to death certificates to conduct survival analyses. Kaplan-Meier survival functions were fit to estimate one-day, early neonatal ($<$7 days), neonatal ($<$28 days), and infant ($<$1 year) mortality risk. Cox Proportional Hazards models were used to estimate hazard ratios (HRs) for maternal pre-pregnancy BMI categorized as underweight ($<$18.5), normal (18.5-24.9), overweight (25-29.9), and obese ($\geq$30) adjusted for potential confounding by maternal age, education, race/ethnicity, and periconceptional folic acid supplementation.

Results: Overall infant mortality risk among infants with spina bifida was 4.4% (95% CI: 3.52, 5.60%). Infants with multiple co-occurring defects, very preterm delivery, multiples, high-level spina bifida lesions, or non-Hispanic Black mothers were at highest risk of infant mortality. The distribution of BMI in our sample was 3.3% underweight, 39.9% normal weight, 24.1% overweight, and 25.4% obese. Maternal pre-pregnancy underweight

and obesity were associated with higher infant mortality risk (15.7% (95% CI: 7.20, 32.30%) and 5.82% (95% CI: 3.60, 9.35%), respectively). Hazard ratio estimates adjusted for potential confounding showed underweight and obese mothers had greater hazard of infant mortality compared to normal weight mothers (HR: 4.5 (1.08, 16.72) and 2.6 (1.36, 8.02), respectively).

Conclusion: The overall risk of infant mortality for infants born with spina bifida was lower than most prior estimates in the literature. Infants born with spina bifida to mothers who were underweight or obese pre-pregnancy were at higher risk of infant mortality. This study provides additional evidence of the importance of healthy maternal weight prior to pregnancy. It also highlights indicators of elevated mortality risk among infants born with spina bifida including early gestational age at delivery and the existence of multiple co-occurring defects.

4.1 Introduction

Birth defects are a leading cause of infant mortality in the U.S. accounting for 1 in every 5 infant deaths (138). Spina bifida is a congenital anomaly characterized by the protrusion of the spinal cord through a boney defect in the vertebral column. It is the most common neural tube defect (NTD), occurring in approximately 1 out of every 3,000 live births (3). Over time, mortality among infants with spina bifida has greatly declined; however, recent estimates of approximately 8% (4) are still thirteen times higher than the national average for all U.S. births (139).

Identified risk factors for infant mortality among infants born with spina bifida include young maternal age, non-Hispanic Black race, low birth weight for gestational age, existence of multiple co-occurring birth defects (non-isolated defects), nativity, and parity (17, 25, 29–31). Further, lesions located higher on the spine are associated with higher mortality compared to lower lesions (29).

Recent estimates indicate that among U.S. women ages 20-39, 24% are overweight and

32% are obese (37). Pre-pregnancy obesity, defined as a body mass index (BMI) greater than or equal to 30 at the start of pregnancy, is common, occurring in more than 1 in 5 pregnant women in the U.S. (21% in 2009) with increases in prevalence during the last decade (38). Overweight and obese pre-pregnancy BMI has been associated with infant mortality, preterm birth, stillbirth, and longer duration in neonatal intensive care (40). A recent meta-analysis showed a 42% increased odds of infant mortality among infants born to mother who were obese relative to normal weight women (95% CI: 1.24-1.63) with an even greater elevated odds among the most obese category (>35 BMI) (odds ratio (OR): 2.02, 95% CI: 1.61-2.56). Underweight pre-pregnancy BMI (<18.5) has also been associated with negative infant outcomes (e.g. indicated preterm delivery, neonatal intensive care, infant mortality) (40, 41). One large study of the association of maternal pre-pregnancy BMI in women from 38 U.S. states demonstrated a "J"-shaped pattern, with infants of underweight mothers having births with higher infant mortality (5.4/1,000 live births) compared to births of mothers with normal pre-pregnancy weight (4.2/1,000 live births) and infant mortality rapidly increasing with severity of maternal pre-pregnancy obesity: (5.9/1,000 for $30 \leq BMI < 35$; 6.8/1,000 for $35 \leq BMI < 40$; 8.2/1,000 for $BMI \geq 40$ among live births) (41).

Prior research suggests that maternal pre-pregnancy obesity is associated with increased risk of spina bifida (42–46). However, no prior studies, to our knowledge, have examined the influence of maternal pre-pregnancy BMI in relation to infant mortality among infants with spina bifida. The purpose of this study is to investigate the first-year survival of infants born with spina bifida and examine the association of pre-pregnancy BMI with infant mortality.

4.2 Materials and Methods

We conducted a retrospective cohort study using data on live born infants with spina bifida from the National Birth Defects Prevention Study (NBDPS), a multi-state, population-based, case-control study of more than 30 major structural birth defects to define our cohort. Population-based surveillance programs in each participating site (entire state: Arkansas, Iowa, and Utah; selected counties: California, Georgia, Massachusetts, North Carolina, New York, and Texas) were used to identify eligible cases of spina bifida among livebirths, stillbirths, and induced abortions. Details of the NBDPS design and data collection protocol are published elsewhere (120). This analysis used only data from liveborn infants with spina bifida born between January 1, 1998 and December 31, 2011.

In the NBDPS, maternal interviews were conducted via telephone using a standardized computer-assisted interview available in either English or Spanish. The interview collected self-reported socio-demographic, health, and dietary information, among other exposures, before and during pregnancy. Interviews were administered after 6 weeks or more had passed since the infant's estimated date of delivery and no later than 2 years after the estimated date of delivery. Of all invited/eligible mothers of infants with spina bifida, 68% of mothers participated. Average time to interview for these mothers was 11.0 months postpartum.

4.2.1 BMI Assessment

Body mass index was calculated from self-reported pre-pregnancy height and weight as mass (kilograms) divided by height^2 (meters^2) (123). Height was recorded in feet and inches or in centimeters in response to the interview question: What is your height without shoes? Weight was recorded in pounds or kilograms in response to the question: How much did you weigh before your pregnancy? Appropriate unit conversions were done to then calculate BMI. BMI was categorized into four groups: Underweight (BMI$<$18.5),

Normal weight (18.5 $\leq$ BMI $<$25), Overweight (25$\leq$ BMI$<$30) and Obese (BMI$\geq$30) (123). Both categorical and continuous measures of BMI were used in the analysis. Only 7% of infants born with spina bifida had mothers missing responses to one or both of the height and weight questions.

4.2.2 Spina Bifida Classification

Potential cases of spina bifida were ascertained by the population-based birth defect surveillance registry of each NBDPS center. Potential cases were screened for eligibility by clinical geneticists at each center. Data collection for eligible cases involved comprehensive abstraction of medical records to capture detailed clinical information including spina bifida phenotype and diagnostic test results. Eligible cases were then recruited to participate in the NBDPS. Following recruitment, spina bifida cases from all centers were systematically reviewed a second time by a team of study-wide clinical geneticists to ensure consistency across study sites and to provide a detailed clinical classification. Each spina bifida case was assigned to one of the following categories: isolated defect (1 major birth defect), multiple ($\geq$2 major yet unrelated birth defects), or complex ($\geq$2 major birth defects which are suspected to be related) (122). For spina bifida co-occurring with other NTDs, there was an established hierarchy in which each infant was only classified under the highest ranking NTD. The hierarchy was, highest to lowest: anencephaly, encephalocele, and spina bifida. Infants with spina bifida in this study did not have co-occurring anencephaly or encephalocele. Details were also provided on anatomical location of the lesion along the vertebral column: cervical, thoracic, lumbar, and sacral. Cases with chromosomal abnormalities or with recognized or strongly suspected single-gene disorders or syndromes were excluded from the NBDPS by design (120).

4.2.3 Infant Death Ascertainment

Our primary outcome of interest was time to infant mortality, defined as death in the first year of life, among infants with spina bifida. We also assessed neonatal mortality and early neonatal mortality, defined as death before 28 days of age and before 7 days of age, respectively, as well as death within the first 24 hours. NBDPS data and accompanying clinical files containing medical record data were linked to vital statistics data from each participating study center to provide infant mortality data. When the medical record, NBDPS, or vital statistics data were discordant in regard to the primary outcome, that record was examined carefully to determine whether death did occur and if so when. In situations in which two sources agreed on this information, the conclusion shared by the two sources was considered the correct information in the analytic dataset. For most discordant information, the correct conclusion was obvious (i.e. the discordant record indicated the date of death was prior to the date of birth). Cause of death was recorded for some infants though this information was missing for most records and therefore infant mortality in this analysis was defined as all-cause mortality during the first year of life. There were two infants for whom records indicated that the infant had died though no exact date of death was recorded. For these two observations the date of death was imputed using fully conditional specification (140) which has been shown to be generally less subject to bias than complete-case analysis (141).

4.2.4 Statistical Analysis

Infant mortality among infants with spina bifida was calculated as the complement of the Kaplan-Meier survival probability at one year of life. Twenty-four hour, early neonatal ($<$7 days) and neonatal ($<$28 days) mortality was calculated by the same method. We further constructed and plotted cumulative incidence (1-S_{KM}) curves from the Kaplan-Meier survival estimate (S_{KM}) for the entire study sample as well as by interview status

and, among women who were interviewed, prepregnancy BMI. Stratified infant mortality estimates are presented by isolated vs. multiple defect(s), BMI category, gestational age category, maternal race, plurality, and spina bifida anatomical location. The standard error and corresponding pointwise confidence intervals for the Kaplan-Meier survival probabilities were estimated using Greenwoods formula (134). The log-rank test was used to test infant mortality differences across strata.

We used Cox proportional hazards models to adjust for potential confounding. We report corresponding hazard ratios (HRs) and 95% confidence intervals (CIs) for the association of BMI with infant survival at one year. Cox models included a semi-Bayes approach by including a weak Bayesian prior of no association (i.e. a null prior) for BMI with infant survival via data augmentation (142). This approach allowed us to reduce potential sparse-data bias (143). Spare-data bias arises from a lack adequate events (i.e. deaths) for one or more combinations of the exposure (i.e. BMI) and the outcome (i.e. survival) (144). The proportional hazards assumption for all covariates in the model was assessed by visual inspection of log cumulative hazard by BMI categories. The plot showed parallel lines for all the BMI categories suggesting no issues of non-proportional hazards. Cox models included a minimally sufficient set of covariates (i.e. confounders) to reduce the potential for bias in estimation of hazard ratios representing BMIs association with survival. Covariates (maternal age, education, race/ethnicity, smoking, alcohol consumption, periconceptional folic acid consumption, gestational age) were selected using a Directed Acyclic Graph (DAG) (136, 137) constructed based on evidence from the scientific literature and subject matter expertise (Figure S1). To address limited sample size, covariate selection was complemented by backward selectionremoving variables contributing less than a 10% change in the main effect estimate are dropped from the model (145). When determining appropriate covariates for the adjusted Cox model, we examined maternal smoking and binge alcohol consumption. These variables had no substantial impact on the parameter estimate

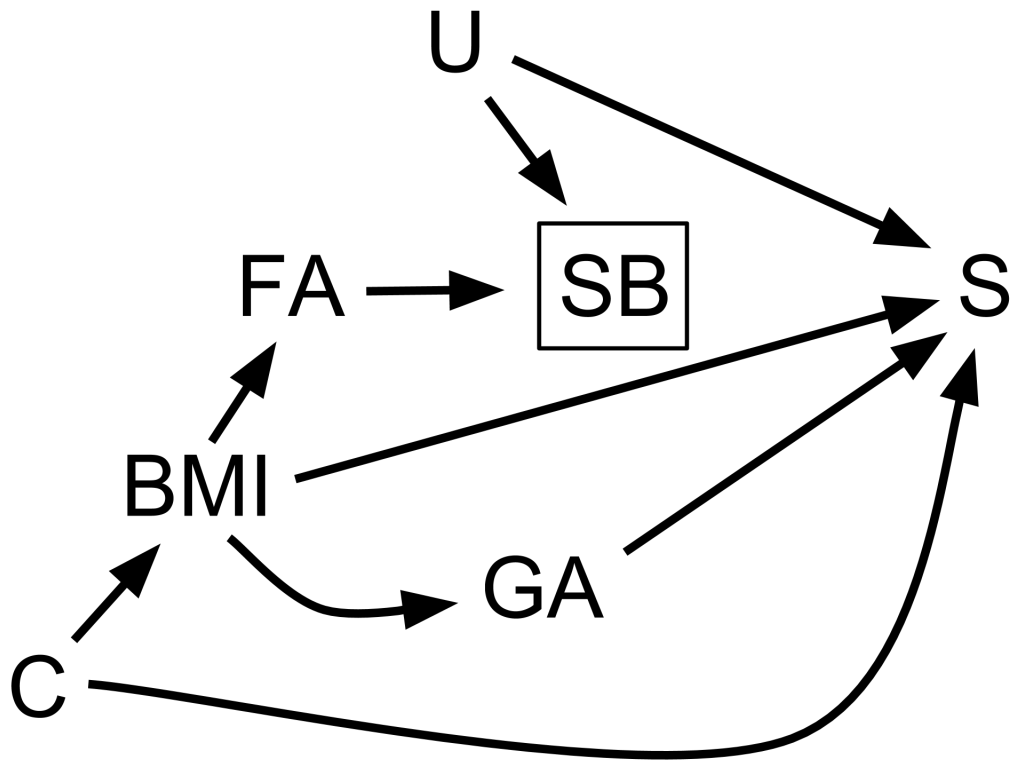

Figure 4.1: Directed Acyclic Graph (DAG) for the effect of maternal pre-pregnancy body mass index (BMI) on infant survival (S). Other variables represented: C: confounders (maternal age, education, race/ethnicity, smoking, alcohol consumption), FA: maternal periconceptional folic acid consumption, GA: infant gestational age, SB: spina bifida, U: potential unmeasured confounders

(change$<$10%) and were therefore excluded from the adjusted model.

As part of a sensitivity analysis to test the robustness of the relation between pre-pregnancy BMI and infant survival, we used Cox proportional hazards models with continuous BMI and allowed for model flexibility with first and second order polynomials as well as cubic splines. We also tested models without the weak null prior to check consistency of results.

Among 1,793 infants with clinically eligible spina bifida whose mothers were recruited for the NBDPS interview, for 31.5% their mothers were either unable to be contacted or declined participation in the interview. Though self-reported information on BMI and other factors collected via interview were thus not available for infants whose mothers were not interviewed, clinical information from abstracted medical records of clinically eligible infants were used to apply the same systematic case classification to infants with spina bifida whose mothers were not interviewed, as was done for infants for whom there was a maternal interview. Likewise, the infants born with spina bifida for whom no maternal interview was conducted were linked to vital statistics data to ascertain first-year deaths. To address the potential for selection bias due to participation status and to produce estimates representative of the underlying population (119), we weighted data from infants with maternal interview data to reflect the combined sample of both subjects with and without maternal interview. Weighting involved the use of inverse probability weights (IPWs) (146) to make interviewed study participants reflect the overall population based on variables available in both groups (i.e. birth defect classification, gestational age, plurality, maternal age, race/ethnicity) (Appendix 1). Corresponding 95% confidence intervals were then generated for weighted model parameter estimates using Efrons nonparametric bootstrap (147).

The National Birth Defects Prevention Study and this analysis, which used data from both interviewed and non-interviewed mothers and their infants, were approved by the institutional review boards of the Centers for Disease Control and Prevention, the University

of North Carolina at Chapel Hill, and all other participating study centers.

4.3 Results

All analyses excluded infants with spina bifida classified as having a complex birth defect (n=9) or not a live birth (n=252; i.e. fetal death, induced abortion, spontaneous abortion, or missing pregnancy outcome information). Based on these restrictions, we reduced our sample size to 1,533 infants (1,080 (70%) with maternal interview and 453 (30%) without maternal interview) from the original sample size of 1,793 infants (1,228 (68%) with maternal interview and 565 (32%) without maternal interview). In our analytical sample, 1,336 (87%) infants had isolated spina bifida and 197 (13%) infants had non-isolated spina bifida. The first aim of this study was to estimate first year mortality among infants with spina bifida. For this aim, we included both interviewed and non-interviewed mothers. The second aim was to assess whether pre-pregnancy BMI is associated with infant mortality. For this aim, data were limited only to interviewed mothers and their infants, but we accounted for possible selection bias using IPW.

Three percent of interviewed mothers were underweight prior to pregnancy, 40% had normal BMI, 24% were overweight, and 25% were obese; seven percent of mothers did not supply information that allowed us to calculate body mass index (Table 4.1) and were excluded from analyses requiring BMI. Slightly more than half of mothers had greater than a high school education and the majority of mothers (82%) took folic acid sometime between two months prior to pregnancy and the first trimester.

Similar distributions of maternal age, sex, gestational age, and plurality were observed for infants with maternal interview data and those without. Interviewed mothers were more likely to be non-Hispanic White and to have infants with isolated spina bifida compared to non-interviewed mothers and their infants. Overall infant mortality at one year was 4.4%,

Table 4.1: Characteristics of infants born with spina bifida eligible for the National Birth Defects Prevention Study by maternal interview status, 1998-2011.

Characteristic[a]		Interviewed (n = 1080) n (%)	Non-interviewed (n = 453) n (%)
Body Mass Index (kg/m^2)[b]			
	Underweight (<18.5)	36 (3.3)	-
	Normal (18.5–24.9)	431 (39.9)	-
	Overweight (25-29.9)	260 (24.1)	-
	Obese (≥30)	274 (25.4)	-
	missing	79 (7.3)	
Maternal Education[b]			
	0-12 years	487 (45.1)	-
	>12 years	557 (51.6)	-
Folic Acid supplementation[b, c]			
	Yes	885 (81.9)	-
	No	181 (16.8)	-
Maternal Age, *mean (sd)*		27.3 (6.0)	26.9 (6.3)
Male Sex		557 (51.6)	239 (52.8)
Race	Non-Hispanic White	590 (54.6)	212 (46.8)
	Non-Hispanic Black	92 (8.5)	51 (11.3)
	Hispanic	379 (35.1)	160 (35.3)
	Other	19 (1.8)	22 (4.9)
Center	Arkansas	130 (12.0)	47 (10.4)
	California	200 (18.5)	90 (19.9)
	Iowa	120 (11.1)	58 (12.8)
	Massachusetts	73 (6.8)	55 (12.1)
	New York	70 (6.5)	44 (9.7)
	Texas	141 (13.1)	66 (14.6)
	CDC/Atlanta	126 (11.7)	37 (8.2)
	North Carolina	94 (8.7)	33 (7.3)
	Utah	126 (11.7)	23 (5.1)
Gestational age, *mean (sd)*		38.0 (3.6)	37.9 (3.9)
Plurality			
	Singleton	1044 (96.7)	433 (95.6)
	Twin	30 (2.8)	16 (3.5)
Defects Classification			
	Isolated	961 (89.0)	375 (82.8)
	Multiple	119 (11.0)	78 (17.2)
Anatomical Location			
	Cervical	11 (1.0)	5 (1.1)
	Thoracic	65 (6.0)	50 (11.0)
	Lumbar	835 (77.3)	313 (69.1)
	Sacral	133 (12.3)	62 (13.7)
Infant Deaths (<1 yr)		40 (3.7)	28 (6.2)

Abbreviation: sd, standard deviation

a: small discrepancies between totals cases and summed categories are a result of missing data

b: BMI, maternal education, and folic acid supplementation were not recorded for non-interviewed cases

c: any periconceptional use (2 months prior to conception through the 1st trimester) of folic acid supplements

though infant mortality was 3.7% among infants whose mothers were interviewed compared to 6.2% among infants whose mothers were not interviewed (log-rank test: p=0.03, Figure 4.2).

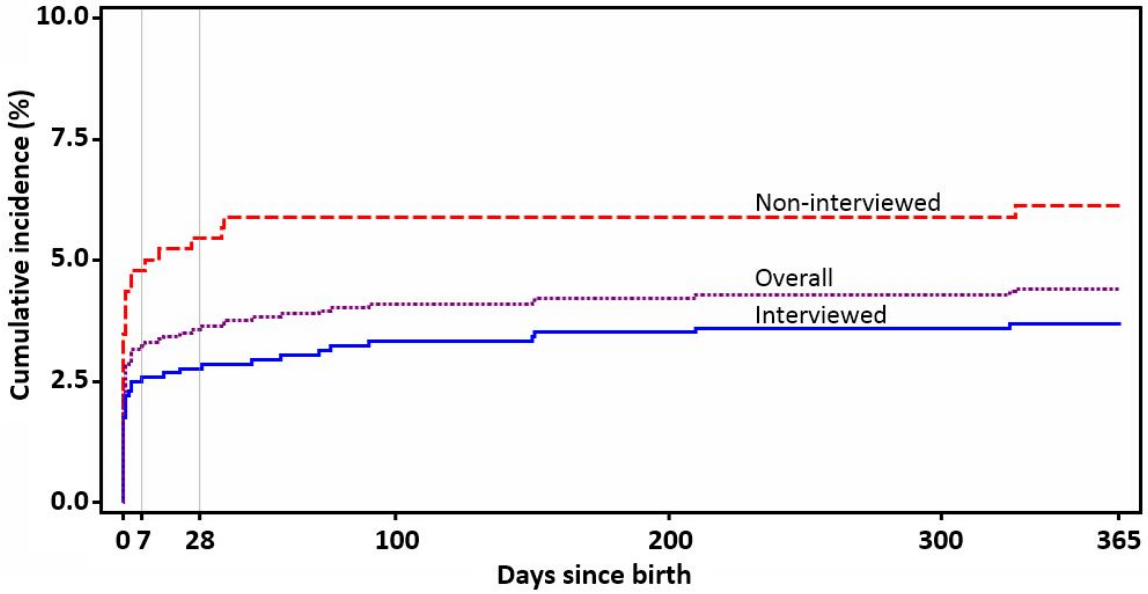

Figure 4.2: Crude risk of infant mortality during the first year of life among infants born with spina bifida by interview status (National Birth Defects Prevention Study, 1998-2011)

There were 68 infant deaths among infants with and without maternal interview data; among infants with maternal interview data there were 40 infant deaths. We observed that approximately half of infant deaths among infants born with spina bifida occurred within the first 24 hours after birth (n=35). Infant mortality differed by maternal pre-pregnancy BMI. Compared to normal weight mothers, underweight and obese mothers had 7 times and 2.6 times the risk of infant mortality, respectively (Table 4.2). A less distinct but also elevated risk for infant mortality was seen in overweight mothers. Cumulative incidence curves showed clearly different mortality risk by BMI category (Figure 4.3; log-rank test, $p< 00.01$). The greatest difference in survival between maternal BMI categories was observed for 24-hour survival while afterwards similar incidence curves were followed (see Figure 4.3). Infants with spina bifida with another co-occurring defect (multiple/non-isolated defects) had higher infant mortality (17.9%; 95% CI: 13.23, 24.09%) than infants

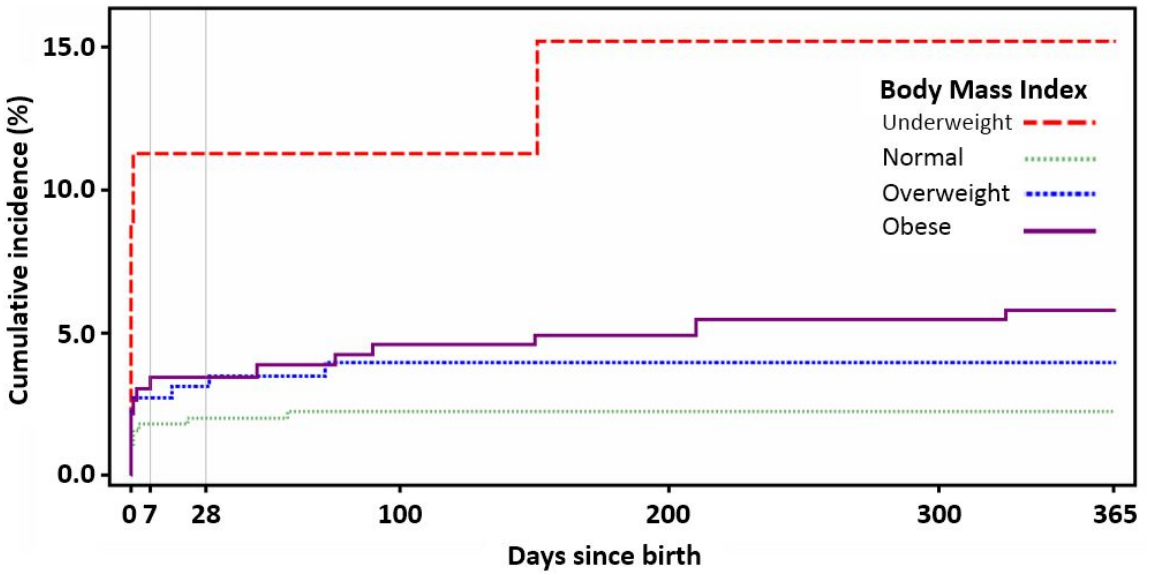

Figure 4.3: Crude risk of infant mortality during the first year of life among infants born with spina bifida by maternal category of pre-pregnancy body mass index (National Birth Defects Prevention Study, 1998-2011)

with isolated spina bifida (2.5%; 95% CI: 1.76, 3.46%), more than seven times the risk of death at 1 year. Infant mortality was much greater among very preterm (<32 weeks) infants (38.0%; 95% CI: 27.89, 50.35%) compared to preterm (32-36 weeks) and term (≥37 weeks) infants (7.2%; 95% CI: 4.17, 12.38% and 1.6%; 95% CI: 1.06, 2.52%, respectively). Among race/ethnicity groups, infants of non-Hispanic Black mothers had the highest infant mortality (7.7%; 95% CI: 4.34, 13.46%) and infants of non-Hispanic White mothers had the lowest (3.3%; 95% CI: 2.22, 4.74%). This racial/ethnic disparity was evident in the first day and week of life and gradually grew over the first year. Twins had infant mortality four times that of singletons after one day (8.7% vs. 2.1%) though this difference narrowed at one year after birth to slightly more than double the risk. Anatomical location of the lesion was strongly related to morality risk with infants with thoracic lesions having notably elevated risk of infant mortality compared to lumbar and sacral lesions; there was only one infant death among those with a cervical lesion. We also noted temporal trends of improving survival. Infants with an estimated day of delivery within the following year

categories, 1998-2000, 2001-2003, 2004-2006, 2007-2011, experienced 5.5, 4.3, 4.0, and 3.0% infant mortality respectively (data not shown).

Table 4.2: Number of deaths (n) and infant mortality (IM) estimates (IM=1-S_{KM}) with 95% CIs by selected maternal and infant characteristics among infants born with spina bifida, National Birth Defects Prevention Study, 1998-2011.

	<1 day				<7 day				<28 days				<1 year			
Characteristic[a,b]	**n**	IM (%)	95% CI		**n**	IM (%)	95% CI		**n**	IM (%)	95% CI		**n**	IM (%)	95% CI	
All infants with spina bifida[c]	35	2.3	1.65	3.17	49	3.2	2.43	4.21	55	3.6	2.77	4.65	68	4.4	3.52	5.60
Body Mass Index (kg/m^2)[d]																
Underweight (<18.5)	3	9.3	3.30	24.84	4	11.7	4.67	27.66	4	11.7	4.67	27.66	6	15.7	7.20	32.30
Normal (18.5–24.9)	4	1.0	0.41	2.63	8	1.8	0.90	3.64	9	2.0	1.04	3.91	9	2.2	1.19	4.18
Overweight (25-29.9)	6	2.3	1.01	5.10	7	2.7	1.30	5.69	8	3.2	1.59	6.24	10	4.0	2.16	7.27
Obese (≥30)	6	2.2	0.97	4.79	8	3.1	1.56	5.93	9	3.5	1.85	6.47	16	5.8	3.60	9.35
Defect Classification																
Isolated	11	0.8	0.46	1.48	19	1.4	0.91	2.22	24	1.7	1.15	2.58	33	2.5	1.76	3.46
Multiple	24	12.3	8.42	17.80	30	15.4	11.02	21.26	31	16.4	11.90	22.40	35	17.9	13.23	24.09
Gestational Age (weeks)																
<32	13	18.3	11.07	29.43	19	26.8	17.99	38.69	23	32.4	22.85	44.60	27	38.0	27.89	50.35
32-36	8	4.8	2.44	9.41	10	6.0	3.29	10.91	10	6.0	3.29	10.91	12	7.2	4.17	12.38
≥ 37	8	0.7	0.33	1.30	12	1.0	0.56	1.72	13	1.1	0.62	1.82	20	1.6	1.06	2.52
Race																
Non-Hispanic White	13	1.6	0.95	2.78	16	2.0	1.23	3.24	19	2.4	1.52	3.70	26	3.3	2.22	4.74
Non-Hispanic Black	5	3.5	1.47	8.20	7	4.9	2.36	9.99	7	4.9	2.36	9.99	11	7.7	4.34	13.46
Hispanic	16	3.0	1.83	4.80	24	4.5	3.01	6.57	26	4.8	3.31	7.00	28	5.2	3.62	7.44
Plurality																
Singleton	31	2.1	1.48	2.98	45	3.1	2.29	4.07	50	3.4	2.58	4.45	63	4.3	3.35	5.44
Twin	4	8.7	3.36	21.53	4	8.7	3.36	21.53	5	10.9	4.67	24.16	5	10.9	4.67	24.16
Spina Bifida location																
Cervical	0	0.0	0.00	0.00	0	0.0	0.00	0.00	0	0.0	0.00	0.00	1	6.3	0.91	36.77
Thoracic	5	4.3	1.83	10.13	8	7.0	3.54	13.43	10	8.7	4.78	15.56	13	11.3	6.73	18.67
Lumbar	20	1.7	1.13	2.69	29	2.5	1.77	3.62	32	2.8	1.98	3.93	40	3.5	2.57	4.73
Sacral	6	3.1	1.39	6.72	7	3.6	1.73	7.38	7	3.6	1.73	7.38	7	3.6	1.73	7.38

Abbreviations: IM: infant mortality, S_{KM}: Kaplan-Meier Survival estimate, CI: confidence interval

a: All characteristcs, apart from BMI, include all interviewed and non-interviewed mother-infants pairs collectively.

b: Log-rank test of differences in survival across strata was conducted for each characteristic and yielded p<0.05 for all characteristics.

c: Excludes spina bifida cases with co-occuring anencephaly, encephalocele, but with or without hydrocephalus

d: BMI data are only available for interviewed mothers. Estimates and number of deaths are weighted to reflect the entire sample population.

Among all infants with spina bifida for whom maternal interview data were available, unadjusted estimates indicated that infants with underweight mothers had a hazard of infant mortality 7.6 times (95% CI: 2.20, 21.80) that of infants with normal weight mothers during the first year of life (Table 4.3). Infants born to obese mothers also had a hazard of infant mortality significantly greater (HR: 2.6; 95% CI: 1.25, 6.94) than normal weight mothers. After adjusting estimates for potential confounding by maternal age, education, race/ethnicity, and periconceptional folic acid supplementation, infants with spina bifida born to underweight and obese mothers still had significantly greater hazard of infant mortality compared to infants with spina bifida born to normal weight mothers (HR: 4.5; 95% CI: 1.08, 16.72 and HR: 2.6; 95% CI: 1.36, 8.02, respectively). The estimates were imprecise as indicated by the wide confidence intervals. Use of a cubic spline modeling of BMI showed a similar pattern of exposure-outcome relation (Figure 4.4) with a sharp increase of the hazard ratio with greater severity of underweight and a progressively more gradual increase in the hazard ratio as BMI exceeds 22, although the lower bound included the null value of 1 across most BMI values over 20. When looking at infants with isolated spina bifida only, we found a similar pattern of results, though with less precision due to decreased sample size. Infants with isolated defects in underweight mothers had 4.7 times the hazard of infant mortality compared to normal weight mothers (HR: 4.7; 95% CI: 1.21, 29.48). We do not report hazard ratios and confidence intervals for infants with non-isolated spina bifida (multiple defects) due to model non-convergence because of a limited sample size.

4.4 Discussion

Mortality in the first year of life among infants with spina bifida has greatly improved in the past several decades. Lorber et al. observed a 50% two-year infant mortality from 1959-1963, a 36% two-year infant mortality from 1967-1968(24). A study from 1999-2007 showed an infant mortality of babies with spina bifida of 8.1% (4). Improvements

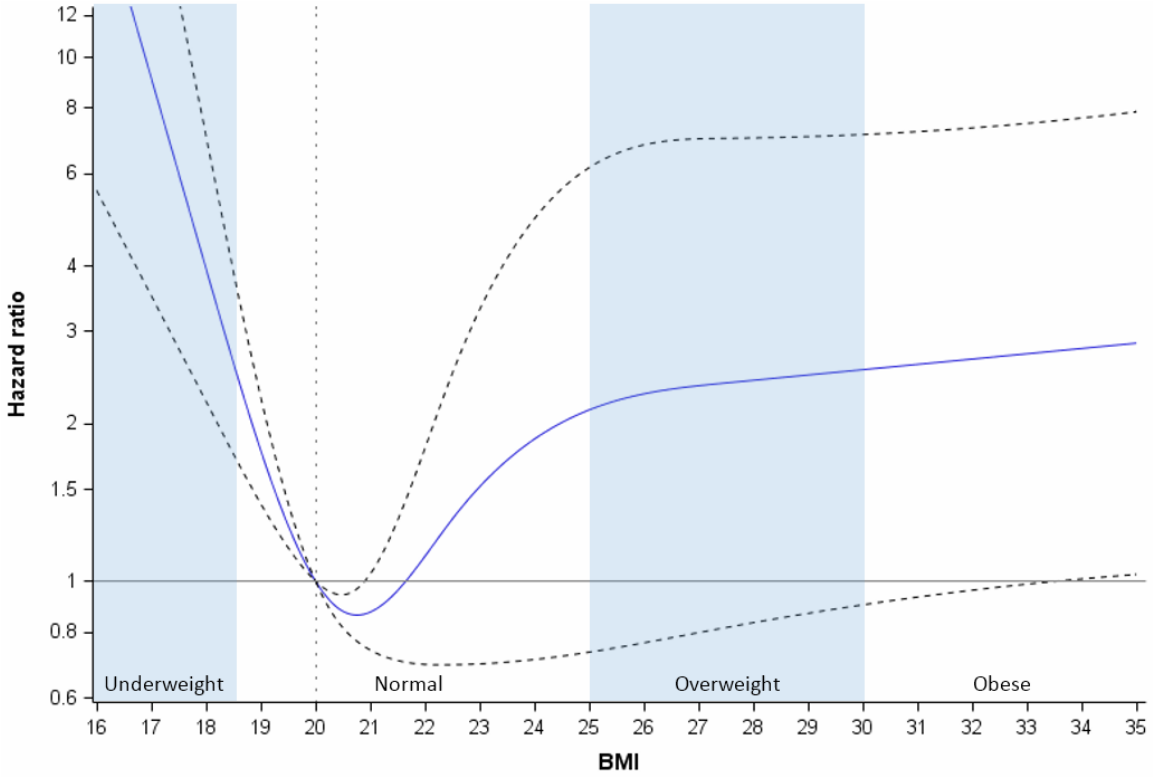

Figure 4.4: Hazard ratio and 95% confidence intervals for infant mortality by pre-pregnancy body mass index compared relative to a body mass index of 20 (National Birth Defects Prevention Study, 1998-2011)

Table 4.3: Hazard ratios and 95% CIs for mortality of infants born with spina bifida by category of pre-pregnancy maternal body mass index in the National Birth Defects Prevention Study, 1998-2011.

		All Spina Bifida (n= 961)			Isolated Spina Bifida (n=854)		
Model		**HR**	**95% CI**		**HR**	**95% CI**	
Unadjusted model							
BMI	Underweight	7.6	2.20	21.80	4.1	1.30	25.50
	Normal	Reference			Reference		
	Overweight	1.8	0.62	4.71	0.9	0.19	3.89
	Obese	2.6	1.25	6.94	2.0	0.65	7.83
Adjusted model[a]							
BMI	Underweight	4.5	1.08	16.72	4.7	1.21	29.48
	Normal	Reference			Reference		
	Overweight	1.9	0.68	5.44	1.1	0.24	4.76
	Obese	2.6	1.36	8.02	2.1	0.59	8.19

Abbreviations: CI: confidence intervals, BMI: body mass index

a: Adjusted for maternal age, education, race/ethnicity, and periconeptional folic acid use

in clinical care and medical technology have paved the way for this reduction in mortality (26). In this study a temporal trend of reduced infant mortality was also seen. Overall infant mortality in this study of 4.4% (95% CI: 3.52, 5.60%) represents one of the lowest infant mortality risks reported to date. The exclusion of infants with chromosomal anomalies or with recognized or strongly suspected single-gene disorders or syndromes may relate to the differences in infant mortality estimates between this study and others covering similar time periods without such exclusions (4, 30, 124). We found that infants with spina bifida born to mothers with pre-pregnancy underweight or obesity had poorer survival trajectories than infants of normal weight mothers. This association was strongest for underweight mothers.

If this relation where found to be causal, the mechanism by which pre-pregnancy BMI could alter the risk of infant mortality in infants born with spina bifida is uncertain and may be different for underweight versus obese women. If the association we observed is of a causal nature, one pathway by which maternal adiposity might increase mortality risk is through stored yet less accessible nutrients in obese mothers or deficient nutrients in underweight mothers, such as reduced folate or iron levels among women at extreme BMI categories (148, 149). Both extremes of BMI would be related to greater risk of nutritional deficiency increasing both the risk of certain birth defects and setting up an infant for poorer chance of survival. Other mechanisms may include compromised immune system functioning or inflammation related to maternal adiposity that thereby also effects the health of the developing fetus. The effect of maternal prepregnancy BMI on survival among infants with spina bifida may be mediated by higher risk of preterm birth. Underweight mothers are at higher risk of spontaneous preterm birth (Liu 2016) and obese women are at higher risk for indicated preterm birth due to co-morbidities such as gestational hypertension and diabetes (150). A basic mediation analysis, in our data, examining mediation by length of gestation showed only minor attenuation of the hazard ratio which may suggest only some of the association of BMI with survival is attributable to gestational age at birth (data not shown).

Type 2 diabetes is strongly associated with obesity (151). The incidence of type 2 diabetes often follows weight gain (152–154) and can be thought of as another potential mediator in this analysis. Using the same mediation technique for diabetes resulted in a slight increase in the hazard ratio estimates and similar precision of confidence intervals (data not shown). This suggests diabetes may not mediate the association of BMI with survival.

This analysis had several limitations, including the self-reported nature of the questionnaire, which may have led to misclassification due to inaccurate recall. The exposure, pre-pregnancy BMI, calculated from self-reported recall of height and weight, has been shown to be a valid measure of BMI; prior research has shown that pre-pregnancy weight by recall was highly correlated with weight recorded in clinical records (124). Also, while BMI is used as a proxy for body fatness, it more accurately represents excess weight given ones height (34). That said, this measure is inexpensive, easily obtainable, and predicts body fat percentage well (35). BMI was missing for 7.3% of mothers and as BMI is more likely to be missing for Hispanic women, underrepresentation of Hispanic mothers may have resulted (155). Residual confounding may also be present due to imperfect covariate measures and unknown confounders. Induced abortions could impact our analysis. For instance, one might anticipate that fetuses with more severe spina bifida were more likely to be aborted than those with less severe spina bifida. In addition, prenatal detection of spina bifida by ultrasound could be more difficult in obese women (156), though obese women are at greater risk of an affected pregnancy and may therefore be more likely to receive prenatal testing. We recognize the potential impact on survival if BMI is associated with prenatal ascertainment of spina bifida and subsequent pregnancy termination. That said, prior research suggests that the impact on infant survival would likely be minimal if this were truly the underlying relation (157).

Some of our estimates were imprecise and limited sample size prevented us from examining mortality by subtype (meningocele, myelocele, myelomeningocele lipomeningocele,

and lipomyelomeningocele). Lastly, due to the fairly large number of models that were fit, the chance of a type 1 error owing to multiple comparisons is elevated above 5%, however, the consistent trend of association we saw of increasing risk and hazard as BMI goes from normal weight to overweight to obese would not be likely if significant associations were due to chance.

This study had several strengths. NBDPS data, which combine data from population-based birth defects surveillance and a comprehensive maternal questionnaire, allow for pre-pregnancy BMI to be examined in relation to the risk of infant mortality among infants with spina bifida. NBDPS data used in this analysis are from sites covering 9 distinct regions of the U.S., increasing the generalizability of results to multiple regions of the U.S.

Second, inverse probability weighting applied to the interviewed sample corrected for some of the bias due to non-participationmaking the results more representative of the source population (e.g. non-Hispanic Blacks were underrepresented among participating mothers though IPWs made estimates more accurately representative). While IPW allowed for more representative results, an accompanying assumption was made that known characteristics about non-participating mothers were sufficient to weight participating mothers to accurately represent them. Clear differences in key characteristics between infants with and without maternal interview substantiated this approach.

Third, the spina bifida case definition was based on strict inclusion or exclusion criteria. Individual case review by a clinical geneticist limits the potential for outcome misclassification. Exclusion of infants with recognized or strongly suspected single gene disorders or syndromes makes our study sample more homogeneous with respect to underlying etiology and presence of major comorbid conditions.

Analyses in which our models included a continuous measure of BMI allowed for additional flexibility of exposure modelling, demonstrating stability of the exposure-outcome

relation. When the null prior was removed from the proportional hazards model, conclusions remained the same. Only in one case did the determination of a statistically significant relation change (i.e. the hazard ratio for underweight compared to normal weight among infants with isolated spina bifida no longer included the null value of one) suggesting very minor sparse data bias if results omitted the inclusion of a null prior.

Other results from our analysis support previous observations. The disparities in infant mortality among infants born with spina bifida by race and ethnicity followed patterns recorded in prior literature (4) with non-Hispanic White having the lowest infant mortality, followed by Hispanics, and with non-Hispanic Blacks having the highest infant mortality. Prior literature also indicated an increased risk of mortality with higher anatomical location (29). To our knowledge, information on infant mortality of spina bifida cases has not previously been presented by plurality.

Currently maternal obesity is the number one obstetrical risk factor for a multitude of negative maternal and infant conditions (39). Given that the risk of spina bifida is increased among obese women (42–46) as well as an increased risk of many other negative health outcomes (150), there is a critical need to promote at a population level a shift towards healthier weight for women of reproductive age, and while underweight women represent a small fraction of the population with prevalence around 3%, the benefits of achieving a healthy weight may be greatest in this group. Shifts towards a healthier pre-pregnancy BMI may both prevent spina bifida incidence (158) as well as reduce mortality among infants born with spina bifida.

In conclusion, findings from this analysis suggest that infants born with spina bifida to mothers considered underweight or obese prior to pregnancy have an elevated risk of infant mortality, particularly for underweight mothers. These findings add further evidence to the importance of a womens periconceptional health in reducing the occurrence of poor neonatal and infant outcomes. Further investigation of potential causal mechanisms by

which maternal pre-pregnancy BMI may increase mortality risk in infants with spina bifida is warranted. We recommend replication of this research in other studies to examine the consistency of results.

CHAPTER 5: MATERNAL DIETARY PATTERNS AND FIRST-YEAR MORTALITY AMONG INFANTS BORN WITH SPINA BIFIDA

Objective: To investigate the potential impact of pre-pregnancy maternal dietary patterns on one-year survival among infants born with spina bifida.

Methods: We analyzed data from mothers and their infants with spina bifida (n=1,487) in the National Birth Defects Prevention Study (1999-2011) linked to vital records for infant mortality. Using data from self-reported food frequency questionnaires (completed on average 11 months after delivery) that referred to consumption one year prior to pregnancy, we examined maternal dietary patterns via three methods: the Healthy Eating Index (HEI), the Diet Quality Index for Pregnancy (DQI-P), and latent class analysis (LCA). Kaplan-Meier survival functions were fit to estimate infant mortality by dietary patterns. Cox proportional hazards models were used to estimate hazard ratios by three levels of the HEI and DQI-P scores (low/medium/high) and four latent classes adjusted for potential confounding.

Results: Among 1,487 infants born with spina bifida, 66 died during the first year of life (4.43%). One year risk of death for infants born with spina bifida did not vary significantly across maternal dietary patterns. After adjusting estimates for potential confounding by maternal age, education, race/ethnicity, and periconceptional folic acid supplementation, mothers that scored low (poorer diet quality) in both the HEI and DQI-P had higher hazard of infant mortality compared to mothers with high (better diet quality) scores (HR: 1.44 (0.54, 4.33) and 2.36 (0.93, 5.78) for HEI and DQI-P, respectively) though the estimates were imprecise.

Conclusion: This study provides suggestive evidence that maternal pre-pregnancy diet is associated with infant survival among babies born with spina bifida. Nonetheless, adherence to dietary guidelines and healthy eating patterns have been associated with reduced risks of adverse pregnancy outcomes such as birth defects and improved maternal health.

5.1 Introduction

Pregnancy is a critical period of rapid development and heightened metabolic activity for a growing fetus. Imbalances in specific nutrient and overall energy intakes for the mother prior to and during pregnancy have been associated with adverse pregnancy outcomes (47–55). Further, certain dietary patterns and poor diet quality during preconception and prenatal periods have been associated with several adverse birth outcomes (159–164) including neural tube defects and other birth defects (22, 68, 74). Notably, in the U.S., the average childbearing woman has a diet that is considered poor quality (165) (HEI-2010 avg. score=49.3, out of 100 possible points. For example, womens consumption of greens, beans, and whole grains are markedly lower than national recommendations (15-20% of recommended amount) (165).

One out of every 5 infant deaths in the U.S. can be attributed to birth defects, a leading cause of infant mortality(166). Spina bifida, a congenital anomaly marked by the spinal cord and meninges protruding from a defect of the vertebral column, is the most common neural tube defect, and occurs in 1 out of every 3,000 live births in the U.S. (3). Despite incredible improvements in infant survival among those born with spina bifida in the last 60 years when infant mortality was 90% (24), recent infant mortality estimates range from 4.4 to 8.0% (4, 167), seven to thirteen times higher than the national average for all U.S. births of 0.6% (139).

Identified risk factors of infant mortality among infants born with spina bifida include maternal age, race, ethnicity, low birth weight for gestational age, existence of multiple co-occurring birth defects, nativity (U.S. born or non-U.S. born), and parity (17, 25, 29–31).

Further, lesions high on the spine showed significantly lower survival probability compared to lower lesions (29).

Maternal diet and nutrition play a key role in embryonic and fetal development. Folic acid supplementation and fortification in the periconceptional period has led to a decline in the prevalence of spina bifida and neural tube defects overall (15, 17–21). Neural tube defects occur with less frequency among mothers with prudent preconception dietary patterns compared to other dietary patterns (Western, low-calorie Western, and Mexican) (23). However, no prior studies have examined the influence of maternal dietary patterns in relation to infant mortality among infants with spina bifida. The purpose of this study is to investigate the potential association of maternal dietary patterns on one-year survival of infants born with spina bifida.

5.2 Materials and Methods

5.2.1 Study Design

This analysis used data from the National Birth Defects Prevention Study (NBDPS), a multi-state, population-based, case-control study of more than 30 major structural birth defects. Active population-based surveillance programs in each participating state (entire state: Arkansas, Iowa, New Jersey, Utah; selected counties: California, Georgia, Massachusetts, North Carolina, New York, Texas) were used to identify eligible cases of birth defects as well as liveborn infants without a birth defect (controls). Details of the NBDPS design and data collection protocol are published elsewhere (120). Infants in the NBDPS had an estimated date of delivery between October 1, 1997 and December 31, 2011. Cases were eligible whether born as live births, stillbirths, or prenatally diagnosed terminations. This study uses the case infants only from the original case-control study (i.e. spina bifida cases) that were liveborn between 1998 and 2011 from all centers except New Jersey.

5.2.2 Maternal Interview

Maternal interviews were conducted via telephone using a standardized computer-assisted interview available in either English or Spanish. The interview collected socio-demographic, health, dietary information and other data by maternal recall. Interviews were administered after 6 weeks or more had passed since the infant's estimated date of delivery and no later than 2 years after the estimated date of delivery. Average time to interview for mothers of infants with spina bifida was 11.0 months. Among all NBDPS cases, 67% of eligible mothers participated (120).

5.2.3 Food Frequency Questionnaire

As part of the maternal interview, dietary information was collected. Average intake of foods was captured using a semi-quantitative food frequency questionnaire (FFQ) developed by Willett et al. for The Nurses Health Study (168, 169). The FFQ was shortened from 153 to 63 items for purposes of the NBDPS. For each of the food items, mothers reported average frequency of consumption for food items in the year prior to becoming pregnant (6 months prior for seasonal foods). For example, one question would read, "How often, on average, did you eat fresh apples or pears? followed by 16 frequency options ranging from "Never or less than once a month to "6 or more times per day. In addition to the FFQ, data on breakfast cereals, sodas, food supplements, and caffeinated tea and coffee consumption were captured via a separate series of detailed question asking about intake during the 3 months prior to pregnancy. Nutrient intake estimates were calculated after dietary data was collected using the U.S. Department of Agriculture (USDA) release 25 nutrient database (170). Vitamin supplements and food supplements (e.g. meal replacement bars or shakes) were not included in nutrient calculations (with the exception of folic acid) because consumption among participating mothers was rare and nutrient data for many products were not available. Dietary folate equivalents (DFEs), calculated as DFE = [(folic acid from

fortified foods)*1.7 + (total natural folate from foods)], were used to estimate folate intake.

5.2.4 Maternal Dietary Patterns

Measures of overall dietary patterns fall generally into two approaches: (1) dietary pattern indexes where maternal diet is assessed against some pre-determined scale of diet quality, typically conveyed as a score, and (2) data-driven approaches where an automated statistical method identifies exclusive dietary pattern groups based on the foods consumed by their frequency of consumption. The primary exposure of interest, maternal dietary patterns, was evaluated in three ways using data collected during the maternal interview from the FFQ as well as questions from the cereal and supplement sections, and beverage/soda questions: two diet indices, the Healthy Eating Index (171) and the Diet Quality Index for Pregnancy (60), and one data-driven approach, latent class analysis (LCA) (172, 173). All three of these methods were used to quantify maternal dietary patterns well after the initial dietary data was collected via maternal interview questionnaires.

5.2.4.1 Method 1: Healthy Eating Index 2010 (HEI-2010)

The HEI-2010 is a measure of diet quality that assesses adherence to the 2010 Dietary Guidelines for Americans (75). It consists of 12 component scores with a possible total HEI-2010 score ranging from 0-100. The HEI-2010 had not previously been implemented using the NBDPS data. Our implementation was similar to the overall approach taken by the University of Minnesota: Nutrition Data System for Research (ref: NDSR) and follows the guidance provided by the National Cancer Institute (ref: NCI).

The HEI-2010 was calculated using the following steps. (1) Each of the 63 FFQ food items was mapped to their corresponding component(s) in the HEI-2010. (2) All food items were converted from the common serving units used in FFQ to the servings units used by the HEI-2010 (e.g. 2 carrots per day converted to 1.6 cup equivalents). We used the USDAs Food Patterns Equivalents Database (FPED) 2011-12 to perform these conversions (133).

(3) Cumulative servings were calculated for each component by summing the servings from each of the FFQ items corresponding to that component. (4) Component scores were determined by calculating the portion of the maximum component score obtained. (5) The 12 component scores were then summed to calculate the HEI-2010 overall score.

Once calculated, HEI scores were compared using low ($\leq$61), medium (62-78), and high ($\geq$79) HEI score categories with low to high scores indicating poorer to better diet quality, respectively. Category thresholds were decided upon by 1), allocating a similar number of mortality events in to each category to allow for greater statistical power and, 2), making differences in categories substantively meaningful (i.e. a difference of 2 between categories would not be substantively meaningful).

5.2.4.2 Method 2: Diet Quality Index for Pregnancy (DQI-P)

The DQI-P, developed by Bodnar and Siega-Riz, is an ordinal score based on 8 dietary components (60). The DQI-P has been implemented by NBDPS collaborators (22, 68) but with modifications due to data limitations. These include removal of the meal pattern component (no data on this was captured in the NBDPS) and the addition of a sweets component. The DQI-P score implemented in the NBDPS then consists of 6 positively scored components (grains, vegetables, fruits, folate, iron, and calcium) and 2 negatively scored components (percentage of calories from fat and sweets). Scores for each component were based on quartiles of consumption/intake (differently from the original DQI-P which based scores on absolute value). Consumption was estimated from FFQ information as well as responses to a cereal and beverage supplemental questionnaire in the NBDPS. Component scores were assigned 0, 1, 2, or 3 based on quartile of consumption and the cumulative score then ranges from 0 to 24, poorest diet to best diet (22).

Once calculated, DQI-P scores were compared using low ($\leq$8), medium (9-13), and high ($\geq$14) DQI-P score categories. Category thresholds were decided upon by based on the same principles as the HEI score categories, namely, a similar number of mortality

events occurred in each category and differences in categories were substantively meaningful.

5.2.4.3 Method 3: Latent Class Analysis (LCA)

LCA was used to assign cases into exclusive data-driven and derived dietary patterns, called classes, based on a participants consumption of food items adjusted for caloric intake. Mothers within each dietary class have similar food intakes while variability in maternal diet is greatest making comparisons between different dietary classes. This studys implementation of LCA mirrored that done by Sotres-Alvarez et al. (23), a prior LCA that examined the risk of neural tube defects by dietary class in the NBDPS.

Briefly, we used LCA to form classes based on dietary patterns from 58 FFQ items available from the interviewed mothers of infants with spina bifida. Intake of each food item (grams/day) was divided by the total dietary consumption in grams per day in order to quantify the amount consumed of the food item relative to the total dietary consumption. The relative food item consumption was categorized into 4 levels to address statistical challenges presented by nonconsumption. The four categories were no consumption and then tertiles of nonzero consumption. Rare and less commonly consumed foods had a binary categorization (consumed and nonconsumed). Ubiquitously consumed foods lacked a category solely for nonconsumption but rather had categories based on quartiles of consumption.

LCA was implemented using Mplus version 7.4 (135). The number of latent classes was determined using goodness of fit statistics: Bayesian information criteria (BIC) and the Lo-Mendel-Rubin likelihood ratio test. Most probable class assignment was then extracted from Mplus output and implemented as an exposure with all other analyses in SAS software version 9.4 (Cary, NC).

[0]Only 58 FFQ items used in this analysis method because we have data on all 58 of these items for the vast majority of participants. The FFQ originally had 58 questions and then at a later year of the study 5 more items were added.

5.2.5 Spina Bifida Classification

Infants born with spina bifida were first identified by population-based birth defect surveillance registries at each NBDPS site. An initial eligibility screening was done by clinical geneticists at their respective site for each potential participant. Detailed clinical information including spina bifida phenotype and diagnostic test results were obtained for eligible infants through comprehensive abstraction of medical records. Mothers of eligible infants were then invited to participate in the NBDPS. After recruitment, spina bifida diagnoses from all centers were systematically reviewed a second time by a study-wide team of clinical geneticists to ensure consistency across study sites and to provide a detailed clinical classification. The classification included information such as lesion level (defined as the upper most level of the disrupted portion of the vertebral column): cervical, thoracic, lumbar, and sacral vertebra. This detailed classification was also completed for infant spina bifida diagnoses corresponding to mothers who did not complete the maternal interview (i.e. non-interviewed cases). As part of the clinical classification, birth defects were grouped in one of the following categories: isolated defect (1 major birth defect), multiple ($\geq$2 major yet unrelated birth defects), or complex ($\geq$2 major birth defects which are suspected to be related) (Rasmussen 2003). For spina bifida co-occurring with another NTD, there was an established hierarchy in which each infant was only considered a case under the highest ranking NTD (ranked highest to lowest: anencephaly, encephalocele, spina bifida). Therefore, infants with spina bifida in this study, by definition, did not have co-occurring anencephaly or encephalocele. By design, birth defects suspected to arise from chromosomal abnormalities or with recognized or strongly suspected single-gene disorders or syndromes were excluded from the NBDPS (120).

5.2.6 Infant Death Ascertainment

In this study of infants born with spina bifida, our outcome of interest was time to infant death. Infant death and its timing were determined by linking data from three sources: maternal interview data (for mothers that participated), clinical data from the comprehensive medical record abstraction, and vital statistics (birth and infant death) records from each participating study site. Records were matched by staff at each of the NBDPS study sites and transmitted to the Centers for Disease Control and Prevention. Matched records provided information on (1) whether the baby had died (from medical records and maternal interview, yes/no), the exact date of death (from vital records), (3) approximately how old the baby was when he/she died (from maternal interview). Cause of death was recorded for some infants though this information was missing or very limited for most records and therefore infant mortality in this analysis was defined as all-cause mortality during the first year of life. When the three sources were conflicting in regard to the occurrence of or timing of infant death, that infants record was inspected to ascertain whether infant death did occur and if so when. In situations in which two of the three sources agreed on this information, the conclusion shared by the two sources was considered correct for our analysis. The correct conclusion was also often obvious (i.e. the discordant record indicated a date of death prior to the date of birth). For two occurrences of infant death, sources indicated the infant died though the date of death was missing. The date of death was imputed for these records using fully conditional specification (140), an imputation method which on average is less subject to bias than complete-case analysis (141).

5.2.7 Statistical Analysis

Infant mortality among babies born with spina bifida cases is the cumulative incidence of death (one minus the Kaplan-Meier survival probability (S_{KM}) at one year of life since birth). Using the same calculation method, early neonatal (7 days) and neonatal mortality

(28 days) were reported. Corresponding point-wise confidence intervals were estimated from standard errors based on Greenwoods formula (134). Plotted cumulative incidence curves (1-S_{KM}) were used to depict mortality incidence over time. The stratified infant mortality estimates and cumulative incidence curves were presented by HEI and DQI-P score categories (low/medium/high) as well as by latent class. The log-rank test was used to test for statistically significant differences of infant mortality across strata of maternal diet categories.

To investigate the association of maternal dietary pattern with survival and adjust for potential confounding, we used Cox proportional hazards models and reported corresponding hazard ratios (HRs) and 95% confidence intervals (CIs). Sparse-data bias is of concern due to the small number of events (i.e. deaths) for one or more combinations of the exposure (i.e. maternal diet) and the outcome (i.e. time to infant mortality) (144). To reduce sparse data bias (143), we took a semi-Bayes approach by adding a weak Bayesian prior via data augmentation (142). This prior was one of no association (i.e. a null prior). The proportional hazards assumption for the adjusted Cox model was assessed by visual inspection of the log cumulative hazard by maternal diet classification. The plot showed approximately parallel lines for all the maternal diet categories suggesting no major issues due to non-proportional hazards.

To reduce confounding, hazard ratios were estimated from models adjusted for a set of covariates. This set of covariates were determined using a Directed Acyclic Graph (DAG) (136, 137) constructed based on evidence from background scientific literature and subject matter expertise. This DAG is represented in Figure 5.1. To address limited sample size, covariate selection through the use of a DAG was complemented by backward selection to where variables with less than a 10% change in main effect estimate are dropped from the model (145). From the DAG, the following variables were considered: maternal age at

conception (continuous), education (completed high school, yes/no), race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Hispanic, other), prenatal care (yes/no), any use of supplements containing folic acid (2 months prior to pregnancy through the first trimester), maternal smoking (any smoking 1 month prior to conception through the first trimester, yes/no), and maternal alcohol consumption (no alcohol, drinking but no binge drinking, or binge drinking of five or more alcohol drinks on one or more days). As part of a sensitivity analysis, we ran Cox models with continuous measures of the diet indices (linear, quadratic, cubic splines).

During the data collection phase of the NBDPS, mothers whose child had a birth defect were identified and invited to participate in the study. Some mothers did not participate (32% of mothers of infants with spina bifida). Reasons for not participating in the study were not collected; however abstracted medical record data and vital records mortality data were available for the non-interviewed mother-infant pairs, as were detailed spina bifida classifications. Maternal diet and other information gathered during the interview were not available for these mother-infants pairs. To avoid potential selection bias and to produce estimates representative of the underlying population (119), we weighted participant data to reflect the combined sample of both those interviewed and those not interviewed. Weighting involved the use of inverse probability weights (IPWs) (146) that, in effect, made interviewed participants reflect the underlying population based on variables available in both groups (i.e. birth defect classification, gestational age, plurality, maternal age, race/ethnicity). Efrons nonparametric bootstrap method (147) was used to calculate appropriate 95% CIs for weighted model parameter estimates.

The National Birth Defects Prevention Study and this analysis, using data from both interviewed and non-interviewed mothers and their infants, were approved by the institutional review boards of the Centers for Disease Control and Prevention, the University of North Carolina at Chapel Hill, and all other participating study centers.

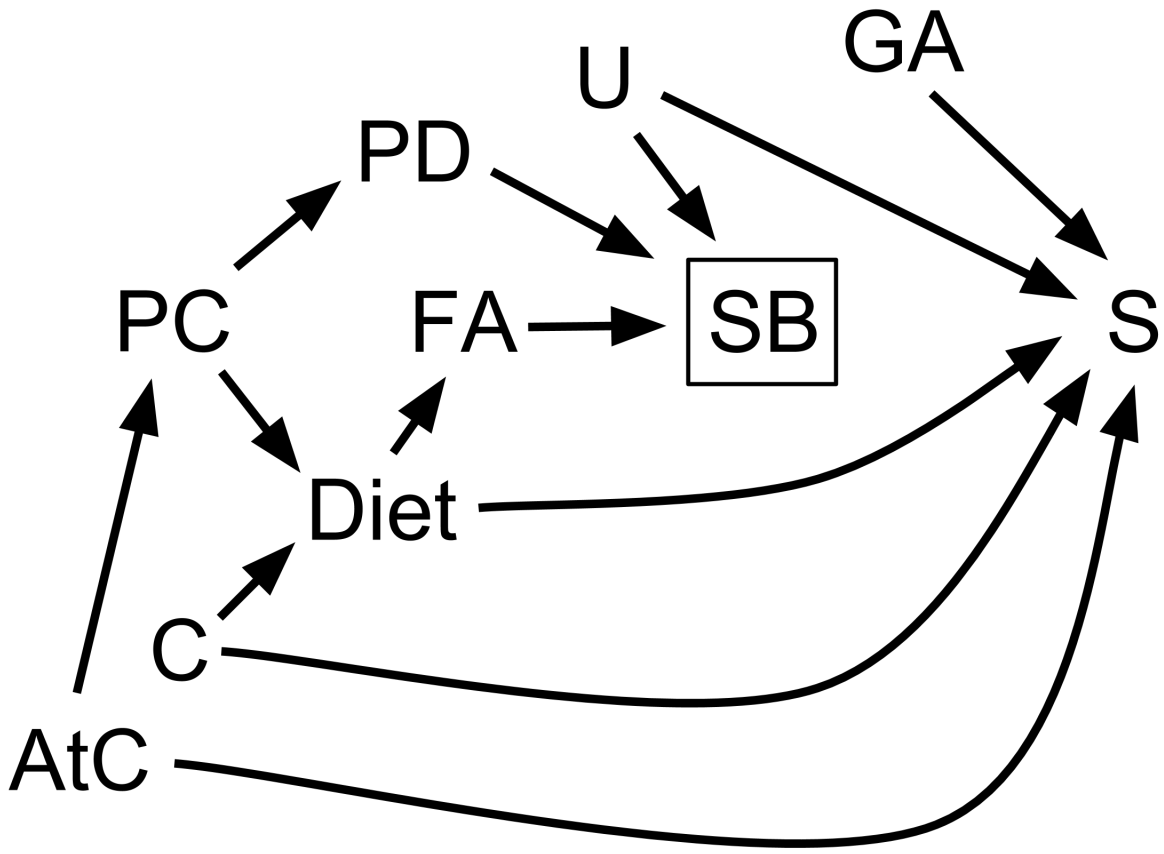

Figure 5.1: Directed Acyclic Graph (DAG) for the potential effect of maternal dietary patterns (Diet) on infant survival (S). Other variables represented: AtC: access to health care, C: confounders (maternal age, education, race/ethnicity, smoking, alcohol consumption), FA: maternal periconceptional folic acid consumption, GA: infant gestational age, PC: prenatal care, PD: Prenatal diagnosis of spina bifida, SB: spina bifida, U: potential unmeasured confounders

5.3 Results

All analyses excluded observations classified as a complex birth defect (n=9) or if the delivery was not a live birth (n=252; i.e. fetal death, induced abortion, spontaneous abortion, missing). We further restricted analyses to mothers who had little to no missing data on food items (4 items, 32 excluded) and had caloric intake greater than the 1^{st} percentile and lower than the 99^{th} percentile (40 excluded). Based on the following restrictions, we reduced our sample size to 1,487 liveborn infants with spina bifida (1,034 (70%) interviewed and 453 (30%) non-interviewed) from the original sample size of 1,793. In our restricted sample, 1,298 (87%) infants had isolated spina bifida and 189 (13%) infants have non-isolated spina bifida (i.e. cases of spina bifida with additional co-occurring birth defects).

Interviewed and non-interviewed mothers had similar distributions of maternal age, gestational age, and singletons to multiples ratio (Table 5.1). Interviewed mothers had a smaller percent of non-Hispanic Blacks and multiple defect cases compared to non-interviewed mothers. Among interviewed cases, slightly more than 50% of mothers had greater than a high school education and the majority of mothers took folic acid starting during the periconceptional window, defined as two months prior to pregnancy through the first trimester.

The mean HEI score was 70.2 ($\pm$11.2). Low HEI scores ($\leq$61) were reported by 20.8% of the sample while high HEI scores ($\geq$79) were reported by 23.5% of mothers. Scores were high across all individual HEI components though no one had a perfect score of 100 (maximum=93.6) and the minimum score earned was 27.1. More than 75% of all participants had a maximum score for the total protein component. The lowest component scores were seen for the fatty acid component (ratio of healthy to unhealthy fats) and the whole grains component (data not shown).

The mean DQI-P score was 11.6 ($\pm$5.15). Low DQI-P scores ($\leq$8) made up 29.0 % of

participants while high scores ($\geq$14) were 36.9% of mothers. Only one participant had the minimum score of 0, and only 2 participants scored the maximum score of 24.

Four latent classes were derived with 14.9 to 34.8% of mothers in each class. Probability of high intake for certain food items for each class can be seen in (Figure 5.2) as the percent of class members consuming the highest quantile of consumption for a given food item. Of note, class one had the highest consumption of hot dogs, hamburgers, French fries and potato chips; class two had the highest relative consumption of carrots, wheat bread, and nuts and high consumption of fish; class three had the highest consumption of avocadoes, chile peppers, tortillas, and refried beans; class four had the highest consumption of rice/pasta and comparable consumption to classes 2 and 3 for many items (e.g. apples, tomatoes, broccoli). In regards to estimated energy intake, class 3 had the highest mean energy intake (1987.7 kcal) then class 2 (1573.7 kcal) and classes 2 and 4 had similar intake (1484.1 and 1463.8 respectively). Nutrient intake also differed between classes. Class 1 had the lowest intake of calcium and iron and the highest sugar intake, while class 2 had the highest intake of calcium, iron, and mono/poly-unsaturated fatty acids. Class 3 had the lowest sugar consumption but sodium consumption more than 33% greater than all other classes. Class 4 had moderate levels of most nutrients and the lowest levels of mono/poly-unsaturated fatty acids (data not shown).

Overall infant mortality was 4.4% with 66 deaths total. Infant mortality was 3.7% among interviewed cases compared to 6.2% among non-interviewed cases. A log-rank test of different survival curves was statistically significant (p=0.03) indicating that the interviewed mothers and their infants did not adequately represent the intended study sample. Analyses limited to interviewed data only were therefore weighted to represent the characteristics of the entire sample of eligible cases.

Infant mortality estimates by maternal dietary pattern are presented by 1 day, early neonatal (7 days), neonatal (28 days), and infant mortality (1 year) time points (Table 5.2).

For all three measures of dietary pattern, the poorest diets (low HEI, low DQI-P, class 1 of LCA) had the highest infant mortality. Mortality estimates as well as cumulative incidence curves (Figure 5.3 and Figure 5.4) seemed to indicate that improved survival was aligned with higher diet quality, but a closer look at confidence intervals and statistical tests yielded no clear differences in mortality between high, medium, and low scoring categories for both the HEI and DQI-P measures (log-rank: p 0.10). Cumulative incidence curves by latent classes were also found not to differ (log rank: p 0.10).

We calculated the hazard of infant mortality by maternal dietary pattern. Mothers with low HEI scores had a 42% increased hazard (HR: 1.42; 95% CI: 0.74, 3.00) of their infant dying in the first year of life compared to mothers with a high score. Low DQI-P score was also associated with a 95% increased hazard (HR: 1.95; 95% CI: 0.78, 5.60) of infant mortality compared to a high score. Infant of mothers in latent class 1 had a 54% increased hazard (HR: 1.54; 95% CI: 0.70, 3.63) of infant mortality compared to infants of mothers in latent class 2. All unadjusted HRs included the null value of 1. The Cox proportional hazards model adjusted for the following factors: maternal age, education, race/ethnicity, prenatal care, folic acid supplement use and maternal smoking. Alcohol consumption, though considered a potential confounder, had no substantial impact on the parameter estimate (change10%) and was therefore excluded from the adjusted model. After adjustment for potential confounding, mothers that scored low in both the HEI and DQI-P had a higher hazard of infant mortality compared to mothers with high scores (HR: 1.44 (0.54, 4.33) and 2.36 (0.93, 5.78) for HEI and DQI-P respectively) though confidence intervals were wide and included the null. Adjusted models examining the association between latent classes and survival did not provide significant evidence to indicate that latent classes were associated with differences in survival and estimates lacked precision. Sensitivity analyses using cubic splines of continuous measures of HEI and DQI yielded results consistent with those in Table 5.3 (data not shown).

When stratifying infants by defect classification group (isolated defect v. non-isolated (multiple) defects), we found similar results, i.e., mothers with medium scoring diets had HRs similar to high scoring diet mothers and mothers with low scoring diets had more striking differences when compared to mothers with high scoring diets (Table 5.3) though again there was a lack of precision in these estimates. Latent classes provided no evidence for relative differences in hazard ratios. These estimates were very imprecise as evidenced by the wide confidence intervals.

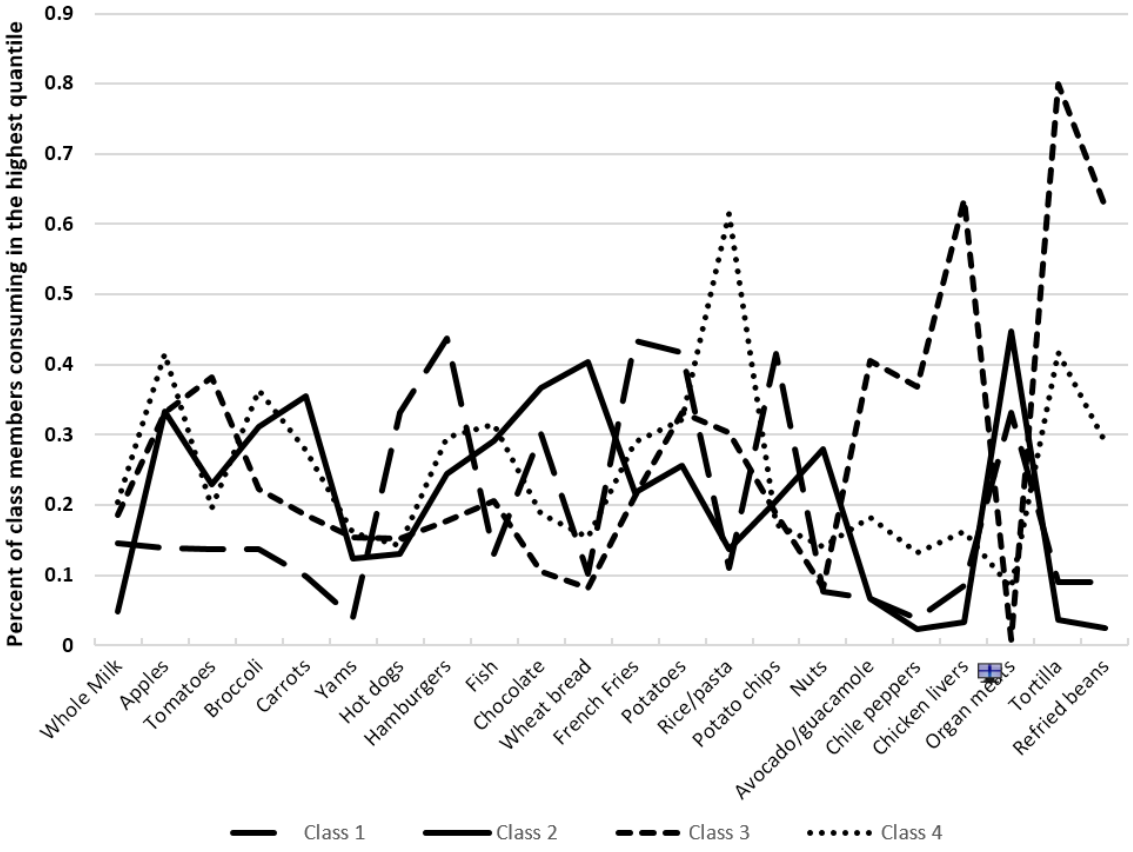

Figure 5.2: Probability of highest level of consumption for selected foods by latent class.

Table 5.1: Characteristics of infants born with spina bifida eligible for the National Birth Defects Prevention Study by interview status, 1998-2011.[a]

Characteristic		Interviewed (n = 1034)	Non-interviewed (n = 453)
		n (%)	n (%)
HEI score, *mean (sd)*		70.2 (11.2)	-
	Low (≤61)	215 (20.8)	-
	Mid (62–78)	576 (55.7)	-
	High (≥79)	243 (23.5)	-
DQI-P, *mean (sd)*		11.6 (5.2)	-
	Low (≤8)	300 (29.0)	-
	Mid (9-13)	335 (32.4)	-
	High (≥14)	381 (36.9)	-
LCA	Class 1	154 (14.9)	-
	Class 2	215 (20.8)	-
	Class 3	360 (34.8)	-
	Class 4	305 (29.5)	-
Maternal Education[b]			
	0-12 years	471 (45.6)	-
	>12 years	549 (53.1)	-
Folic acid supplementation[c]			
	Yes	857 (82.9)	-
	No	177 (17.1)	-
Maternal age, *mean (sd)*		27.3 (5.9)	26.9 (6.3)
Male Sex		531 (51.4)	239 (52.8)
Race	Non-Hispanic White	571 (55.2)	212 (46.8)
	Non-Hispanic Black	83 (8.0)	51 (11.3)
	Hispanic	361 (34.9)	160 (35.3)
	Other	19 (1.8)	22 (4.9)
Center	Arkansas	128 (12.4)	47 (10.4)
	California	197 (19.1)	90 (19.9)
	Iowa	117 (11.3)	58 (12.8)
	Massachusetts	68 (6.6)	55 (12.1)
	New York	67 (6.5)	44 (9.7)
	Texas	126 (12.2)	66 (14.6)
	CDC/Atlanta	118 (11.4)	37 (8.2)
	North Carolina	89 (8.6)	33 (7.3)
	Utah	124 (12.0)	23 (5.1)
Gestational age, mean (sd)		38.0 (3.6)	37.9 (3.9)
Plurality			
	Singleton	1001 (96.8)	433 (95.6)
	Twin	27 (2.6)	16 (3.5)
Defects Classification			
	Isolated	923 (89.3)	375 (82.8)
	Multiple	111 (10.7)	78 (17.2)
Infant Mortality		38 (3.7)	28 (6.2)

Abbreviation: SD, standard deviation

a: discrepancy between totals cases and summed categories are a result of missing data

b: BMI, maternal education, and folic acid supplementation were not recorded for non-interviewed cases

c: any periconceptional use (2 months prior to conception through the 1st trimester) of folic acid supplements

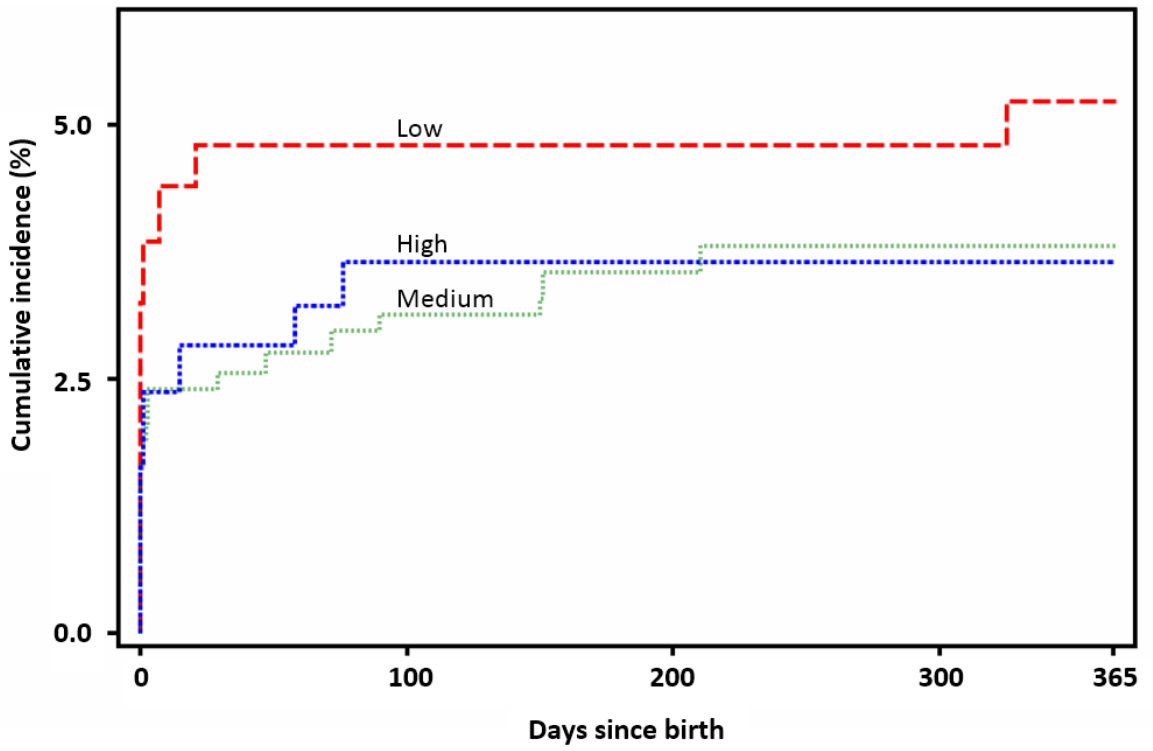

Figure 5.3: Crude risk of mortality among infants with spina bifida by HEI category during the first year of life, National Birth Defects Prevention Study (1998-2011).

Table 5.2: Cumulative number of deaths (n) among infants born with spina bifida and Kaplan-Meier infant mortality estimates (IM=1-S_{KM}) with 95% CIs presented by maternal dietary pattern measures during the first year of life, National Birth Defects Prevention Study (1998-2011).

		<1 day				<7 day				<28 days				<1 year			
Dietary Pattern Measure		**n**	IM (%)	95% CI		**n**	IM (%)	95% CI		**n**	IM (%)	95% CI		**n**	IM (%)	95% CI	
HEI score	Low (≤61)	7	3.30	1.60	6.77	8	3.91	2.01	7.54	10	4.85	2.67	8.72	11	5.28	2.99	9.26
	Mid (62–78)	9	1.68	0.89	3.14	14	2.42	1.43	4.07	14	2.42	1.43	4.07	22	3.82	2.53	5.77
	High (≥79)	4	1.64	0.61	4.42	6	2.39	1.05	5.40	7	2.85	1.34	6.01	9	3.67	1.89	7.05
DQI-P	Low (≤8)	8	2.82	1.44	5.50	11	3.60	1.99	6.47	12	3.99	2.27	6.96	15	5.09	3.10	8.30
	Mid (9-13)	7	2.08	0.99	4.35	10	2.98	1.61	5.50	11	3.31	1.84	5.91	15	4.48	2.72	7.35
	High (≥14)	4	1.08	0.41	2.84	6	1.61	0.73	3.55	7	1.85	0.88	3.86	10	2.59	1.38	4.81
LCA	Class 1	6	2.12	0.98	4.53	8	2.91	1.52	5.55	10	3.29	1.78	6.03	16	5.34	3.32	8.54
	Class 2	6	1.79	0.82	3.89	8	2.29	1.15	4.54	9	2.60	1.36	4.94	12	3.48	2.00	6.05
	Class 3	5	2.15	0.87	5.30	7	3.15	1.49	6.61	8	3.57	1.76	7.14	9	4.27	2.25	8.04
	Class 4	3	2.11	0.72	6.15	4	2.77	1.08	7.01	4	2.77	1.08	7.01	4	2.77	1.08	7.01

Abbreviations: IM: infant mortality, S_{KM}: Kaplan-Meier Survival estimate, CI: confidence interval

°Excluding spina bifida cases with co-occuring anencephaly, encephalocele, but with or without hydrocephalus.

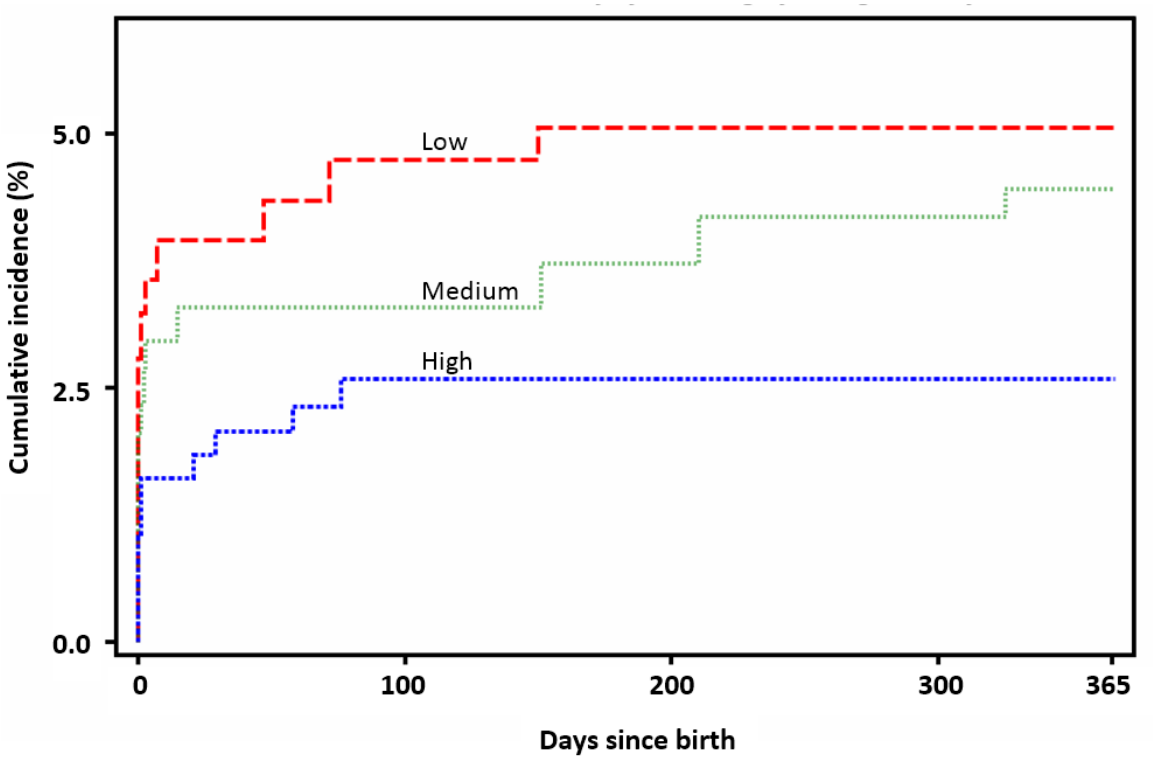

Figure 5.4: Crude risk of death among infants with spina bifida by DQI-P category during the first year of life, National Birth Defects Prevention Study (1998-2011).

5.4 Discussion

Spina bifida, a defect once associated with high infant mortality (90%) as recently as the 1960s (24), is now survived by the vast majority with the condition (8.0% infant mortality)(4). This drastic improvement has paralleled advances in clinical care and medical technology (26) such as the use of antibiotics, shunts, and the standard practice of early surgery for nearly all cases of spina bifida. Overall infant mortality in this study of 4.4% (95% CI: 3.52, 5.60) is similar to an estimate reported previously (167) that used this dataset with slightly different exclusion criteria. Differences between this estimate and others from different studies covering similar time periods (4, 30, 124) may have been due to the exclusion of syndromic cases, including those with chromosomal anomalies, of spina bifida.

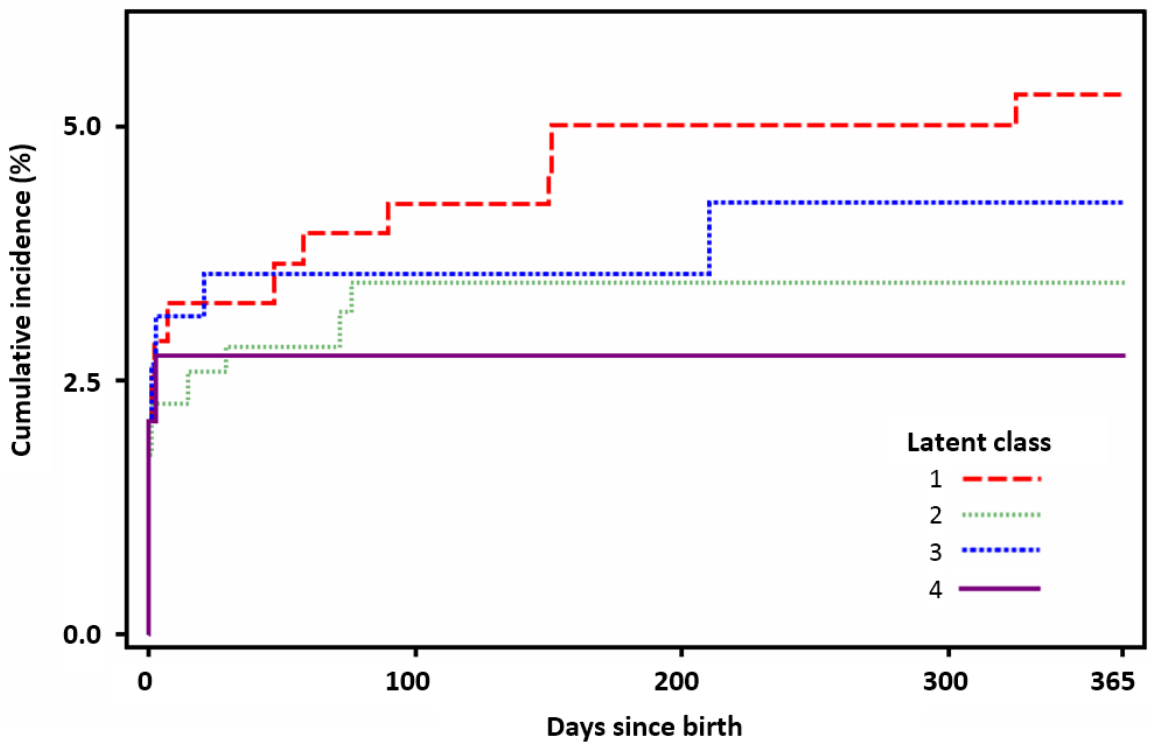

Figure 5.5: Crude risk of death among infants with spina bifida during the first year of life by latent class, National Birth Defects Prevention Study (1998-2011).

Table 5.3: Hazard Ratios and 95% CIs for survival of infants born with spina bifida by HEI, DQI-P, and LCA categories in the National Birth Defects Prevention Study, 1998-2011.

		All Infants (n=1007)			Isolated (n=923)			Multiple[a] (n=109)		
Model		**HR**	**95% CI**		**HR**	**95% CI**		**HR**	**95% CI**	
Healthy Eating Index										
Unadjusted model										
HEI	Low	1.42	0.74	3.00	1.16	0.33	4.10	1.63	0.46	5.77
	Medium	1.04	0.49	2.38	0.89	0.30	2.66	1.02	0.32	3.23
	High	Reference			Reference			Reference		
Adjusted model[b]										
HEI	Low	1.44	0.54	4.33	1.38	0.34	5.60	2.49	0.47	13.24
	Medium	1.05	0.44	2.86	0.98	0.30	3.20	1.15	0.27	4.95
	High	Reference			Reference			Reference		
Diet Quality Index for Pregnancy										
Unadjusted model										
DQI	Low	1.95	0.78	5.60	2.57	0.83	7.96	1.60	0.49	5.23
	Medium	1.70	0.74	4.28	1.30	0.36	4.64	1.79	0.62	5.22
	High	Reference			Reference			Reference		
Adjusted model[b]										
DQI	Low	2.36	0.93	5.78	2.56	0.80	8.15	3.52	0.69	18.00
	Medium	1.98	0.83	5.74	1.26	0.35	4.60	4.11	0.90	18.84
	High	Reference			Reference			Reference		
Latent Class Analysis										
Unadjusted model										
Class	1	1.54	0.70	3.63	1.30	0.47	3.62	1.86	0.62	5.55
	2	Reference			Reference			Reference		
	3	1.23	0.42	3.29	0.44	0.09	2.20	2.68	0.87	8.30
	4	0.80	0.24	2.32	0.97	0.25	3.78	0.60	0.09	4.26
Adjusted model[b]										
Class	1	1.15	0.48	2.74	1.02	0.33	3.14	1.42	0.37	5.46
	2	Reference			Reference			Reference		
	3	0.72	0.21	2.68	0.37	0.05	2.67	1.39	0.29	6.71
	4	0.45	0.10	1.38	0.73	0.16	3.37	-		

Abbreviations: HR: hazard ratio, CI: confidence interval, HEI: Healthy Eating Index, DQI-P: Diet Quality Index for Pregnancy, LCA: Latent Class Analysis

a: Limited sample size resulted in wide confidence intervals

b: Adjusted for maternal age, education, race/ethnicity, prenatal care, smoking, and periconeptional folic acid supplementation

Infants born with a major congenital anomaly face an inherently elevated risk of infant mortality, especially during the first day, week, and month of life. Spina bifida poses risk through infection at the site of the defect or in the placement of a shunt, respiratory failure with infants that have co-occurring Arnold-Chiari II malformation, premature birth, renal failure (174, 175), and complications resulting from other co-occurring defects. All these risks can be fatal. We aimed in this research to further improve infant survival by considering lifestyle factors such as diet.

Healthy maternal dietary patterns are thought to prevent spina bifida through the folate pathway (15). When healthy maternal diet alone might not be sufficient to prevent spina bifida or when another determinant might be the cause of spina bifida, we hypothesized that healthier maternal dietary patterns could set up an infant for better health overall once born. A superior level of health at birth, we suspected, would thereby enable the child to better overcome the inherent risks that accompany spina bifida. Results suggest that infants born with spina bifida whose mothers had higher diet quality had better survival trajectories than infants of mothers with lower diet quality though these effect estimates were imprecise. Further, healthier dietary patterns derived by latent class analysis showed no clear difference in mortality from less healthy dietary patterns. Because our results suggest no clear association, it is questionable whether maternal diet has any meaningful impact on first-year survival among infants born with spina bifida.

The role of maternal diet in infant mortality among babies with spina bifida had not been investigated. This study examined the relation between maternal dietary patterns and mortality of infants born with spina bifida. However, it has limitations. HEI scores are typically calculated using one or two 24-hour food recalls as had been done for national HEI estimates (171) though this study was limited to data from a shortened FFQ. Scores in this study were 18 points higher on average than national estimates. However, the HEI here follows patterns as seen in similar research where HEI scores were calculated with

greater accuracy (171). The HEI scores vary by age (greater maternal age is correlated with a higher score), by education (higher education associated with higher score), smokers have significantly lower scores than non-smokers, and scores vary by geographic region being higher in northeast and lower in the south. We therefore find it reasonable to assume that scores estimated in this study accurately differentiate healthier from poorer dietary patterns (internal validity for within study comparison), but the actual magnitude of the scores themselves cannot be compared to other studies (i.e. lack external validity). This is most likely due to the limited nature of the shortened FFQ used to estimate HEI scores which led to a systematic overestimation of scores and their variability.

The self-report of maternal characteristics and food consumption may have led to non-differential misclassification due to inaccurate recall. The average time from delivery to interview completion was 11 months. Because mothers were providing dietary information the year prior to pregnancy, a substantial amount of time had elapsed. Dietary recall research has shown correlated results comparing diets records with values from a questionnaire administer three to four years later about food intake during the same period (176). Further research has shown recall for close to 20 years previous to be suitable for ranking individuals by intake of overall food groups (177). Based on this work we believe that maternal recall from this study is sufficiently accurate to capture overall diet and dietary behaviors.

Other limitations include limited sample size (number of infant deaths among interviewed mothers, 38). This resulted in imprecise effect estimates. Further, it is difficult to fully tease apart maternal diet from other factors that may influence infant survival. For instance, one could imagine that it is not maternal diet as a causal factor but that those who are more health conscious in regard to their diet might also be health conscious in newborn care pursuing and providing the best care possible. Though we expect our adjustment for socioeconomic variables (e.g. age, education) to reduce possible residual confounding.

Our ability to account for clinical care was limited by our collected data. Models adjusted for prenatal care (yes/no). We also could not to account for important post-natal factors such as access to care though we do not consider these factors to confound our results but rather predict survival.

There were several strengths to this study. Due to the rarity of many birth defects, including spina bifida, extensive surveillance paired with thorough questionnaire data, the NBDPS allows for maternal diet to be examined in relation to the risk of infant mortality for infants with spina bifida which has not been investigated previously. Second, the representative nature of the study through population-based sampling in 9 states lends itself to readily generalizable results for multiple regions of the US. Further, corrective weighting of the interviewed sample provided estimates that more accurately represent the source population. Differences in key characteristics between interviewed and non-interviewed cases substantiated this approach. Third, the spina bifida case definition was based on strict inclusion or exclusion criteria. Individual case review by a clinical geneticist limits the potential for outcome misclassification. The thorough nature of the dietary patterns assessment looked at both diet quality by standardized measures (HEI, DQI-P) as well as patterns and habits identified by data driven methods (LCA). Further, considering maternal diet as a whole rather than with the examination of individual foods or nutrients captures potential synergistic effects that are greater than the sum of individual item effects.

In conclusion, findings from this study do not indicate that infants born with spina bifida to mothers with healthier diets are at lower risk of infant mortality. That said, we did observe some non-significant patterns suggesting that healthier diets could be associated with a better chance of survival. Nonetheless, adherence to dietary guidelines and healthy eating habits in other studies have been associated with reduced risks of adverse pregnancy outcomes and improved maternal health (159–164, 178–180). Further research is warranted, as this is the first study to investigate the potential impact of maternal pre-pregnancy diet

quality on survival of infants with spina bifida.

CHAPTER 6: CONCLUSIONS

The goal of this dissertation was to describe mortality among infants born with spina bifida during the first year of life. In addition to reporting overall mortality, we examined the association of infant mortality with previously identified risk factors, and then preformed a detailed analysis to understand the potential influence of pre-pregnancy BMI and maternal dietary patterns on mortality. This chapter concludes the dissertation by highlighting key findings, important strengths and limitation, public health implications, and future directions for research.

Our sample consisted of mother-infant pairs identified through the National Birth Defects Prevention Study (NBDPS), a multi-state, population-based, case-control study of more than 30 major structural birth defects (120). Infants with spina bifida from any of the 9 participating states between January 1, 1998 and December 31, 2011 were part of our study. Analyses excluded infants with spina bifida classified as having a complex birth defect (n=9) or not a live birth (n=252; i.e. fetal death, induced abortion, spontaneous abortion, or missing pregnancy outcome information). There were 1,533 infants with spina bifida included in our study of which 70% participated in NBDPS interview and its corresponding questionnaires. When studying dietary patterns, we further restricted the analysis to mothers who had little to no missing data on food items and had caloric intake greater than the 1^{st} percentile and lower than the 99^{th} percentile for a reduced sample of 1,487

infants with spina bifida.

6.1 Key Findings

Mothers who participated in the NBDPS interview and their infants were distinct from non-interviewed mothers and their infants. Non-interviewed mothers were more likely to be non-Hispanic Black and to have infants with spina bifida accompanied by other birth defects compared to interviewed mothers and their infants. Overall infant mortality at one year was 4.4% (95% CI: 3.5, 5.6%), though infant mortality was 3.7% among infants whose mothers were interviewed compared to 6.2% among infants whose mothers were not interviewed (log-rank test: $p = 0.03$). Analyses limited to interviewed mother and their infants were weighted to make these mothers-infant pairs representative of entire intended sample (both interviewed and non-interviewed mothers; i.e. all eligible infants born with spina bifida) in regard to known characteristics.

Our first aim was to report survival among infants born with spina bifida overall and by key risk factors and investigate the relation of infant mortality and pre-pregnancy BMI. Overall infant mortality risk among infants with spina bifida was 4.4% which is lower than estimates reported in prior studies. Infants with multiple co-occurring defects, very preterm delivery, multiples, high-level spina bifida lesions, or non-Hispanic Black mothers were the groups at highest risk of infant mortality. Prior to pregnancy, 3% of interviewed mothers were underweight, 40% had normal BMI, 24% were overweight, and 25% were obese; 7% of mothers had missing BMI information. Pre-pregnancy BMI appeared to be associated with infant mortality such that underweight and obese mothers had infants with higher infant mortality risk (15.7% (95% CI: 7.20, 32.30%) and 5.82% (95% CI: 3.60, 9.35%), respectively). After adjustment for confounding, hazard ratio estimates showed underweight, overweight, and obese mothers had a greater hazard of infant mortality compared to normal weight mothers (HR: 4.2 (1.08, 16.72), 1.9 (0.68, 5.44), and 2.6 (1.36, 8.02), respectively).

Our second aim was to examine the relation between maternal dietary patterns and mortality during the first year of life among infants born with spina bifida. We assessed overall diet using three measures. The Healthy Eating Index (HEI-2010) is a measure of diet quality that assesses adherence to the 2010 Dietary Guidelines for Americans (75). It consists of 12 component scores with a possible total HEI-2010 score ranging from 0-100. The Diet Quality Index for Pregnancy (DQI-P), developed by Bodnar and Siega-Riz, is an ordinal score based on 8 dietary components that assess diet quality based on professional dietary guidelines and behaviors (60). The DQI-P was adapted for the NBDPS with scores ranging from 0-24. Latent Class Analysis (LCA) was used to assign mothers into exclusive data-driven and derived dietary patterns, called classes, based on a participants consumption of food items adjusted for caloric intake. Mothers within each dietary class have similar food intakes while variability in maternal diet is greatest making comparisons between different dietary classes.

The mean HEI score was 70.2 ($\pm$11.2). Low HEI scores ($\leq$61) made up 20.8% of the sample while high scores ($\geq$79) were 23.5% of mothers. The mean DQI-P score was 11.6 ($\pm$5.15). Low DQI-P scores ($\leq$8) made up 29.0 % of participants while high scores ($\geq$14) were 36.9% of mothers. Four latent classes were derived with 14.9 to 34.8% of mothers in each class. Class one had the highest consumption of hot dogs, hamburgers, French fries and potato chips; class two had the highest relative consumption of carrots, wheat bread, and nuts and high consumption of fish; class three had the highest consumption of avocadoes, chile peppers, tortillas, and refried beans; class four had the highest consumption of rice/pasta and comparable consumption to classes 2 and 3 for many items (e.g. apples, tomatoes, broccoli).

The one-year risk of death for these infants did not vary significantly across levels of adherence to the Diet Quality Index for Pregnancy (DQI-P) or the Healthy Eating Index (HEI) and was not distinguished by latent classes. After adjusting estimates for potential

confounding, mothers with low scores on the HEI or the DQI-P had a higher hazard of infant mortality compared to mothers with high scores (HR: 1.44 (0.54, 4.33) and 2.36 (0.93, 5.78) for HEI and DQI-P, respectively) though the estimates were imprecise.

Our findings indicate that infants born with spina bifida to underweight or obese mothers are at higher risk of infant mortality compared to infant of normal weight mothers. We did not, however, find clear evidence that maternal diet is associated with infant mortality among babies born with spina bifida. Our results give additional evidence of the importance of healthy maternal weight prior to pregnancy. And even though maternal diet was not associated with survival, adherence to dietary guidelines and healthy eating patterns has been associated with a reduced risk of birth defects and improved maternal health in general.

6.2 Research Limitations

This project had several limitations. First, the self-reported nature of responses to the NBDPS maternal interview, may have led to misclassification of BMI and dietary information due to inaccurate recall. That said, pre-pregnancy BMI, calculated from self-reported recall of height and weight, has been shown to be a valid measure of BMI; prior research has shown that pre-pregnancy weight by recall was highly correlated with weight recorded in clinical records (Shin 2014). Also, while BMI is used as a proxy for body fatness, it more accurately represents excess weight given ones height (34). That said, this measure is inexpensive, easily obtainable, and predicts body fat percentage well (35). In regard to diet measurement, HEI and DQI-P scores are typically calculated using one or more 24 hour recalls but were computed based off data from a shortened FFQ likely leading to inaccurate estimates of absolute intake. Since the calculated scores do follow know patterns and are probably systematically inaccurate, it is reasonable to use the measure for within study comparisons.

Second, residual confounding may also have been present due to imperfect covariate measures and unknown confounders. For instance, household income, a driver of appropriate medical care access and use, was often not reported by choice of interviewee.

Third, induced abortions as a competing event to perinatal morality could have altered the picture of infant mortality we observed. This bias is common in studies of infant mortality. One might anticipate that fetuses with more severe forms of spina bifida, who would also be more likely to die in the first year of life, were more likely to be aborted than those with less severe spina bifida. If this were the case, the risk of infant mortality we observed is deflated, compared to historical estimates, due in part to the practice of elective termination of pregnancy.

Fourth, sample size was limited principally by the number of infant deaths. Many estimates were therefore imprecise, manifest in wide confidence intervals. The rarity of infant death, not only limited study-wide precision but prevented us from examining mortality by spina bifida subtype (meningocele, myelocele, myelomeningocele lipomeningocele, and lipomyelomeningocele) and other less common risk factors.

Lastly, due to the fairly large number of models that were fit, the chance of a type 1 error owing to multiple comparisons is elevated above 5% overall, however, the consistent trend of association we saw of increasing risk and hazard as BMI goes from normal weight to overweight to obese would not be likely if significant associations were due to chance alone.

6.3 Research Strengths

This study is unique in that it was the first to examine the relation between maternal pre-pregnancy BMI and maternal dietary patterns with mortality of infants born with spina bifida. This study had several important strengths. First, the NBDPS combined data from population-based birth defects surveillance and a comprehensive maternal questionnaire,

allowing for pre-pregnancy BMI to be examined in relation to the risk of infant mortality among cases of spina bifida. Also, the study design included 9 sites that covered distinct regions of the U.S., increasing the generalizability of results to multiple regions of the country. Clinical geneticists, further provided individual case review of screened spina bifida cases for further classification and determined eligibility based on strict inclusion or exclusion criteria limiting the potential for outcome misclassification.

Second, inverse probability weighting (IPW) applied to the interviewed sample corrected for some of the bias due to non-participationmade results more representative of the source population (i.e. non-Hispanic Blacks were underrepresented among non-participating mothers though IPWs made estimates more accurately representative). Clear differences in key characteristics between interviewed and non-interviewed cases substantiated this approach.

Lastly, the thorough nature of the dietary patterns assessment explored both diet quality by standardized measures (HEI, DQI-P) as well as patterns and habits identified by data driven methods (LCA). Considering maternal diet as a whole rather than with the examination of individual foods or nutrients captured potential synergistic effects that could be greater than the sum of individual food of nutrient effects.

6.4 Research and Public Health Implications

Pre-pregnancy obesity is common (1 in 5 pregnant women in the U.S.)(38) and has been associated with infant mortality, preterm birth, and stillbirth (40). Underweight pre-pregnancy BMI, though much less common, has also been associated with preterm delivery, neonatal intensive care, and infant mortality (40, 41). Infants born with spina bifida to underweight and obese mothers were at higher risk of infant mortality compared to infants of normal weight mothers. In light of the obesity epidemic as a major public health concern, our results adds further evidence to the importance of healthy maternal weight prior to

pregnancy. Though our results provide little to no evidence that maternal diet is associated with infant mortality among babies born with spina bifida, healthy dietary patterns preconceptionally continue to be associated with a reduced risk of having a child with certain birth defects: high DQI-P scores are associated with a reduced risk of anencephaly and orofacial clefts (22) and prudent diets are associated with a reduced risk of neural tube defects and some congenital heart defects (23).

In planning to investigate the association of pre-pregnancy BMI and maternal diet with infant survival we recognized that both these factors were associated with the prevalence of spina bifida at birth. By examining the association of these factors with infant survival we could potentially find factors that are related to both prevention of birth defects as well as survival of a birth defect. We found this to be the case for pre-pregnancy BMI. Normal weight mothers compared to obese mothers, prior to pregnancy, have a reduced risk of having an infant born with spina bifida and should that baby still have spina bifida, the baby is at a reduced risk of infant death. Should this causal relation mirror the association we have found, both burdens of disease could be reduced through health pre-pregnancy weight.

In comparing participating mother-infant pairs to non-participating pairs, we discovered that participants and non-participants differed significantly. To account for this, we used inverse probability weights based on participation which, when applied to our sample, made results reflect the characteristics of the entire eligible study population. This example reiterates the need for all clinical and public health research to recognize potential sources of selection bias and address that bias to produce results that can be externally valid. This is particularly true for future studies that will use this data source.

6.5 Future Directions

As in all good research, in the pursuit of answering questions new question arise that can expand our knowledge and understanding on a topic. New questions include: What is the impact of elective terminations on infant survival estimates? What is the risk of fetal death among fetuses with spina bifida? Is the potential effect of pre-pregnancy BMI on survival mediated by prenatal diagnosis or gestational age? Limitations of both time and resources have prevented some of these questions from being answered here. Further investigation is needed to understand the etiology of birth defect related mortality, mediators, and the impact of maternal diet on mortality as well as prevention. The following areas of research are promising and can help answer novel, pertinent questions.

6.5.1 Competing Events Analysis

The proposal of this dissertation originally set out, in a third aim, to describe the impact of competing events (i.e. fetal death and induced abortions) on infant mortality. Prior literature has mistakenly assumed the absence of competing events among studies of perinatal mortality (105). In the study of naturally occurring perinatal mortality, induced abortions preclude the observation of a natural perinatal death (i.e fetal death or infant death). While there are 3.5 cases of spina bifida per 10,000 live births in the U.S., the actual prevalence of spina bifida is difficult to determine due to prenatal diagnosis and sometimes subsequent elective termination of pregnancy. The presence of competing events can lead to non-generalizable results and survival estimates. Starting follow-up at 20 weeks gestation would allow us to address competing events. The potential for competing events to affect observed survival estimates needs to be investigated.

6.5.2 Examining survival of other birth defects

This is the first mortality study using data from the National Birth Defects Prevention Study. Subsequent studies will follow. Collaborations have been formed to examine the survival of infants born with congenital diaphragmatic hernia. Infant mortality of critical congenital heart defects will also be examined using this data set. These analyses can glean techniques and anticipate challenges, as well as how to address them, from the research presented.

6.5.3 HEI and Birth Defects Prevention

A large amount of work went into estimating the Health Eating Index (HEI) scores for study participants, and while HEI scores were not associated with infant survival, high scores on a previously created measure of diet quality, the Diet Quality Index for Pregnancy (DQI-P), have been associated reduced risk of some birth defects. The HEI should be used in like manner to see if the measure is related to a reduced risk of birth defects.

6.5.4 Updating the DQI-P

The Diet Quality Index for Pregnancy (DQI-P) since being published in 2002 has been employed in numerous studies. In birth defects research, higher scores have been associated with a reduced risk of several birth defects. That said, this measure needs to reflect our expanded understanding of maternal nutrition that has come since the DQI-P was created more than a decade ago. An update would be advantageous. Many components would remain the same. Others would change. The component for grains should be changed to emphasize whole grains and the component for fat should consider both healthy and unhealthy fats. An updated DQI-P could then be studied in relation to birth defects or other pregnancy outcomes. Comparison of results between the original DQI-P and an updated version could substantiate its use moving forward.

6.5.5 Mediation of Pre-pregnancy BMI and Infant Survival

In understanding the strong association we observed between pre-pregnancy BMI and survival, one must considered what could mediate this potential causal relationship. Mediation by gestational age or diabetes was touched upon in Chapter IV but deserves further investigation and more sophisticated mediation methods. A thorough mediation analysis by these factors and other potential factors could bring to light why extremes of pre-pregnancy BMI are closely tied to elevated risk of infant mortality for babies born with spina bifida.

6.6 Final Remarks

This dissertation has shown the importance of (1) recognizing and accounting for non-participation in studies of birth defects research and (2) pre-pregnancy BMI as a risk factor for first-year mortality among infants with spina bifida. Maternal dietary patterns while not associated with improvements in infant survival probability remains a promising, modifiable factor to prevent the occurrence of birth defects. Future research should further investigate competing events, mediating factors, and other specific birth defects that we may understand how to reduce mortality among this highly vulnerable population.

APPENDIX

Table 1: Mapping of Food Frequency Questionnaire items to DQI-P Food Groups

Food item in Food Frequency Questionnaire	Diet Quality Index food group*
Skim or low fat milk (8 oz. glass)	-
Whole milk (8 oz. glass)	-
Yogurt (1 cup)	-
Ice cream (1/2 cup)	Sw
Cottage or ricotta cheese (1/2 cup)	-
Other cheese e.g., American, cheddar, etc., plain or as part of a dish (1 slice or 1 oz. serving)	-
Margarine (pat), added to food or bread; exclude use in cooking	-
Butter (pat), added to food or bread; exclude use in cooking	-
Fresh apples or pears (1)	F
Oranges (1)	F
Orange juice (small glass)	F
Peaches, apricots, plums, or nectarines (1 fresh or 1/2 cup canned)	F
Bananas (1)	F
Cantaloupe (1/4 melon)	F
Avocado (1) or guacamole (1 cup)	F
Other fruits, fresh, frozen, or canned (1/2 cup)	F
Tomatoes (1) or tomato juice (small glass)	V
String beans (1/2 cup)	V
Broccoli (1/2 cup)	V
Cabbage, cauliflower, or brussel sprouts (1/2 cup)	V
Carrots, raw (1/2 carrot or 2-4 sticks)	V
Carrots, cooked (1/2 cup)	V
Corn (1 ear or 1/2 cup frozen, canned)	V
Peas or lima beans (1/2 cup frozen, canned)	V
Yams or sweet potatoes (1/2 cup)	V
Spinach or collard greens, cooked (1/2 cup)	V
Refried beans (1 cup)	-
Beans or lentils, baked or dried (1/2 cup)	-
Yellow (winter) squash (1/2 cup)	V
Raw Chile peppers, Jalapeno (1)	V
Salsa (1 cup)	V
Eggs (1)	-
Chicken or turkey (4-6 oz.)	-
Bacon (2 slices)	-
Hot dogs (1)	-
Processed meats, e.g., sausage, salami, bologna, chorizo, etc. (piece or slice)	-
Liver (3-4 oz.)	-
Chicken livers (1 oz.)	-
Organ meats Barbacoa, Menudo, sweetbreads, tongue, intestines (3-4 oz.)	-
Hamburger (1 patty)	-
Beef, pork, lamb or cabrito as a sandwich or mixed dish, e.g., stew, casserole, lasagna, etc.	-
Beef, pork, lamb or cabrito as a main dish, e.g., steak, roast, ham, etc. (4-6 oz.)	-
Fish (3-5 ozs.)	-
Chocolate (1 oz.)	Sw
Candy without chocolate (1 oz.)	Sw
Pie (slice)	Sw
Cake (slice)	Sw
Cookies (1)	Sw
White bread (slice), including pita bread	G
Dark bread (slice), including wheat pita bread	G
French fried potatoes (4 ozs.)	-
Potatoe baked, boiled (1) or mashed (1 cup)	V
Rice or pasta e.g., Spanish rice, spaghetti, noodles, etc. (1 cup)	G
Tortilla (1)	G
Potato chips or corn chips (small bag or 1 oz.)	-
Nuts (small packet or 1 oz.)	-
Peanut butter (1 tbs)	-
Oil and vinegar dressing e.g., Italian (1 tbs)	-
Food items not in food frequency questionnaire	
Cereal	G
Non-diet sodas (12 oz)	Sw

*F=Fruits, V=Vegetables, Sw=Sweets, G=Grains

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