The Pennsylvania State University

The Graduate School

Department of Nutritional Sciences

BIRTH WEIGHT, SEX HORMONES AND SEXUAL MATURATION IN GIRLS

A Dissertation in

Nutrition
by

Elizabeth Hill Ruder

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

August 2008

The dissertation of Elizabeth Hill Ruder was reviewed and approved* by the following:

Terryl J. Hartman
Associate Professor of Nutritional Sciences
Dissertation Advisor
Co-Chair of Committee

Joanne F. Dorgan
Special Member
Population Sciences Division, Fox Chase Cancer Center

Sibylle Kranz
Assistant Professor of Nutritional Sciences
Co-Chair of Committee

Penny Kris-Etherton
Distinguished Professor of Nutritional Sciences

Michael J. Rovine
Professor of Human Development

Gordon L. Jensen
Professor of Nutritional Sciences
Head of Nutritional Sciences Department

*Signatures are on file in the Graduate School

ABSTRACT

Breast cancer is the most frequently diagnosed cancer among American women with over 200,000 new cases diagnosed annually. There is growing interest in the relationship between early life exposures as an antecedent for disease later in life. Birth weight, which is associated with maternal hormone levels during pregnancy, is related to timing of puberty and incidence of hormone dependent cancers in adulthood. The Dietary Intervention Study in Children (DISC) and Hormone Ancillary Study (HAS) was a multi-center, randomized controlled clinical trial that evaluated the effect of a reduced fat diet intervention during puberty on serum lipids, growth, maturation, and sex hormone concentrations in 301 girls enrolled at 8-10 years of age and followed for a median of 7 years. The present investigation allows us to use the longitudinal data from the DISC in combination with birth weight and sex hormone concentrations available for a subset of DISC participants to further understand the role of early life exposures on known and suspected breast cancer risk factors.

Early age at menarche is a known risk factor for breast cancer. The purpose of the first study was to test the hypothesis that birth weight is associated with age at menarche after controlling for potential confounders such as physical activity, body size, and mother's age at menarche. Birth weight data was obtained via maternal / guardian report and was available for 203 girls (mean= 3399 g, standard deviation= 508, range 1899 – 5103). Information regarding onset of first menses was ascertained annually until menarche. For the girls with a documented onset of menses during the study period, the mean age at menarche was 12.79 y (range 9.75 y – 15.71 y). Cox regression models were

used to determine the independent effect of birth weight on age at menarche. Follow-up or survival time was calculated in days from date of birth until date of menarche. Low birth weight (defined as birth weight < 1 standard deviation below sample mean) was nearly significantly associated with age at menarche (HR= 1.53, 95% CI 0.96, 2.45, p = 0.07) after controlling for BMI-for-age percentile, physical activity, race and treatment group. Among the 130 individuals for whom both birth weight and mother's age at menarche data were available, the relationship of earlier menarche among girls belonging to the low birth weight group was strengthened (HR= 1.92, 95% CI 1.07, 3.43, p = 0.03).

The purpose of the second study was to evaluate the association between birth weight and levels of serum hormones (estrone, estradiol, estrone sulfate, progesterone, testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEAS)) and sex hormone binding globulin (SHBG). Exposure to elevated estrogens throughout life is thought to play an important role in increasing breast cancer risk. Serum hormone concentrations were collected at 5 points during the study, and capture the participants' hormone levels before, during, and after puberty. Of the 301 girls randomized into one of two study arms of DISC, 286 participated in the HAS at one of more visits. Girls were not eligible to participate in the HAS if they were pregnant or had used oral contraceptives within three months of blood collection, if they were postmenarcheal and had missing data on the date they started menses after blood collection, or if their next menses started more than 33 days after blood collection. A total of 203/286 girls (71%) who participated in the HAS also provided birthweight data. Mean birth weight for the sample was 3399 g (standard deviation = 508, range 1899 – 5103). Birth weight was

associated with DHEAS concentration throughout the 7 years of follow-up and androstenedione concentration prior to menarche. Low birth weight girls exhibited higher concentrations of these hormones compared to average birth weight girls. Birth weight was also associated with post-menarche SHBG concentrations. Low birth weight girls presented decreased SHBG concentrations compared to high birth weight girls. In addition, certain luteal phase estrogen concentrations were positively associated with birth weight. For pre-menarche DHEAS and androstenedione concentrations, the association with birth weight may have occurred via birth weight's effect on age at menarche. Conversely, the relationship between post-menarche DHEAS, post-menarche SHBG, and luteal phase estradiol, non-SHBG estradiol and estrone appeared to be independent of the effect of birth weight on age at menarche.

These studies provide evidence for the association of birth weight with age at menarache (a documented risk factor for breast cancer); and pubertal concentrations of sex hormones. In a secondary analyses, we the previsouly documented association of body composition with age at menarche was confirmed. This research provides insight to understanding risk factors for an etiologically complex disease. As the biologic mechanisms underlying these associations become better understood, there is potential for the increased development of breast cancer prevention strategies in early life and adolescence.

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LIST OF ABBREVIATIONS

AGA	Average for Gestational Age
BCA	breast cancer
BMI	Body Mass Index
BPA	Bisphenol A
CI	Confidence Interval
CV	Coefficients of Variation
DHEAS	Dehydroepiandrosterone sulfate
DISC	Dietary Intervention Study in Children
FFQ	Food Frequency Questionnaire
g	grams
HAS	Hormone Ancillary Study
HPO	Hypothalmic-Pituitary-Ovarian
HR	Hazard Ratio
IGF	Insulin-like Growth Factor
kcals	kilocalories
kg	kilograms
LDL	Low Density Lipoprotein
m	meters
MET	metabolic equivalent
MRC	Medical Research Council
NHLBI	National Heart Lung and Blood Institute

NHS	Nurses' Health Study
OR	Odds Ratio
PCOS	Polycystic Ovary Syndrome
PHV	Peak Height Velocity
RR	Relative Risk
SD	Standard Deviation
SGA	Small for Gestational Age
SHBG	Sex Hormone Binding Globulin
SIR	Standardized Incidence Ratio
y	years

ACKNOWLEDGEMENTS

It is with sincere gratitude that I thank my advisor, Terry Hartman, for her support and belief in my potential. She has served as a mentor, problem-solver and source of inspiration. I am forever impressed by her unassuming ability to lead and motivate. Joanne Dorgan, the Principle Investigator on the Hormone Ancillary Study of the Dietary Intervention Study in Children also deserves special recognition. Joanne bravely and warmly welcomed me to work on the DISC/HAS study, and her generosity with both time and expertise has been critical in my development. Heartfelt thanks are given to Drs. Kranz, Kris-Etherton, and Rovine for offering guidance and perspective along the way. Thank you to my family and friends; in particular thanks to Paige Miller for her editorial input. Teague deserves special appreciation for providing emotional and technical support at all the right occasions. Thank you.

Chapter 1

INTRODUCTION

Background and Significance

Breast cancer (BCA) is the most frequently diagnosed cancer among American women, with approximately 240,000 new cases and an estimated 40,460 deaths expected in 2007 (American Cancer Society, 2007). Although risk factors for BCA have been identified, the relative importance of certain risk factors varies with chronological age and life stage. Historically, the majority of research on BCA risk factors focused on adult characteristics, but there is growing interest in the relationship between early life exposures as an antecedent for disease later in life.

In 1990, Trichopoulos (Trichopoulos, 1990) was the first to publish a fetal origins of BCA hypothesis. The hypothesis was based on four assumptions: 1.) estrogens are an important component in breast carcinogenesis, 2.) factors which increase the risk of cancer post-natally also may increase the risk of cancer when they act *in utero*, 3.) estrogen concentrations are at least ten times higher during pregnancy than they are during other periods in adult life 4.) estrogen concentration and secretion rates vary widely between individuals during pregnancy, and this variability is partly accounted for by exogenous factors.

Birth weight varies directly with maternal sex hormones in pregnancy (Petridou *et al.*, 1990; Kaijser *et al.*, 2000; Mucci *et al.*, 2003), and is a commonly used proxy measure for fetal hormone exposure. However, some concern exists whether birth weight represents fetal hormone exposure or rather some other unidentified characteristic (Troisi et al., 2003a; Michels & Xue, 2006). In addition, maternal weight and body mass index

(BMI) in pregnancy are strongly linearly associated with infant's ponderal index at birth (a measure of fetal growth status, calculated as birth weight/birth length^{3}x100), suggesting that birth weight and ponderal index are influenced by mother's nutritional status during pregnancy (Forsen *et al.*, 1997)

Birth weight has been related to timing of puberty and incidence of BCA, particularly pre-menopausal BCA, in adulthood (Forman et al., 2005; Michels & Xue, 2006). The mechanism underlying the association of birth weight with BCA is unknown, however endogenous estrogen exposure is highest *in utero* and lifetime steroid hormonal exposures, both endogenous and exogenous, are associated with breast cancer risk (Pike et al., 1993). Steroid hormones promote growth and induce breast cell proliferation by binding to receptors (ie. estrogen receptors) and regulating gene transcription (Tsai & O'Malley, 1994; Russo & Russo, 1997). Individual variation in the level of endogenous hormones may be influenced by environmental factors such as diet, and genetic polymorphisms which effect endogenous production and / or availability. Variation in sex hormone concentration in adolescence may modify BCA risk through altered breast morphology, decreased cell turn over and proliferation, or decreased exposure of the breast to carcinogenic estrogen metabolites (Cavalieri et al., 1997; Sutherland et al., 1998a; Plaut et al., 1999; Atwood et al., 2000; Bocchinfuso et al., 2000; Dorgan et al., 2003a). Thus, examining sex-steroid hormone levels (including available proxy measures of steroid hormone exposure) *in utero*, in early life, and adolescence may unveil associations with BCA risk.

Specific Aims and Hypotheses

Specific Aim 1

The purpose of Study #1, "Birth weight and age at menarche", was to determine whether birth weight was independently associated with age at menarche in a population of well-nourished girls.

Primary Hypothesis**:** Girls with birth weight < 1 standard deviation below the sample mean for birth weight experience menarche at a younger age compared with girls weighing > 1 standard deviation above the sample mean at birth.

Secondary Hypothesis: High physical activity, lower BMI, and later mother's age at menarche also have significant association with later age at menarche.

Specific Aim 2

The purpose of Study #2, "Birth weight and sex hormone concentration during female adolescence", was to characterize the effect of birth weight on sex steroid hormones and SHBG in light of the evidence suggesting that higher sex hormone and lower SHBG concentrations are associated with increased risk of BCA in adulthood.

Primary Hypothesis: Girls with either low birth weight (<1 SD below the sample mean) or high birth weight (> 1 SD above sample mean) have higher sex hormone concentrations and lower SHBG compared with girls born within 1 SD of sample mean for birth weight.

Secondary Hypothesis: The effect of birth weight on hormone concentrations will vary depending on menarche status and post-menarche menstrual cycle phase.

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Chapter 2

REVIEW OF LITERATURE

This chapter will address the literature relevant to early life characteristics and adult breast cancer risk by discussing 1) gestational age, 2) birth weight, 3) birth length 4) height / linear growth 5) childhood and adolescent diet and 6) body mass index. These topics underlie the dissertation studies aimed at further elucidating fetal and early life characteristics and breast cancer risk.

Introduction

The risk of breast cancer (BCA) increases with age with approximately 80% of BCA diagnosed in women > 50 years of age (American Cancer Society, 2007). Exposure to estrogens throughout life plays an important role in increasing BCA risk (Key et al., 2002; Eliassen et al., 2006). Endogenous exposure per unit of body weight to sex steroid hormones is highest *in utero*, surpassing even that experienced in puberty and during pregnancy (Dorgan et al., 2003b; Troisi et al., 2003a). Inasmuch, there is growing interest in the relationship of fetal and early life characteristics, including diet and growth, as an antecedent for later development of BCA. Early life and adolescence are critical times for maturation of the hypothalamic pituitary ovarian (HPO) axis, which regulates production of ovarian hormones including estrogen, progesterone, testosterone and androstenedione (Winter et al., 1995). Unlike most organs, the majority of breast development occurs during puberty under the influence of these hormones. Some evidence suggests that breast tissue is not fully differentiated until after the first full-term pregnancy (Russo & Russo, 1997), and therefore early life may be a period in which the breast is more highly susceptible to carcinogenic influences. This is further supported by

evidence that the younger the age of exposure to ionizing radiation, the greater the increase in BCA incidence (Hildreth et al., 1989; Land, 1995). Infant birth size is positively associated with maternal hormone levels during pregnancy (McFadyen et al., 1982; Petridou et al., 1990; Kaijser et al., 2000; Mucci et al., 2003), maternal diet (Petridou et al., 1998), pubertal timing (Adair, 2001), and incidence of BCA later in life (Sanderson et al., 1996; McCormack et al., 2003a; De Stavola et al., 2005). Other childhood and adolescent characteristics related to growth also are likely to have an influence. The interactions of the intrauterine hormonal milieu, postnatal development and later disease risk are clearly complex and the biological mechanisms underlying the relationship are not fully understood. This review summarizes the literature examining the influence of gestational age, birth weight, birth length, childhood growth, diet, and body mass index (BMI) on later development of adult BCA risk.

Breast Cancer Risk Factors in the Prenatal, Early Childhood, and Adolescent Periods

Gestational Age

Infants born at both extremes of gestational age have been hypothesized to be at increased risk of BCA due to increased exposure to hormones *in utero*. Whereas infants born >40 weeks gestation experience a prolonged exposure to pregnancy hormones, available data suggest that maternal hormones are higher in pregnancies that end prematurely (Table 2.1). Wuu and colleagues (Wuu et al., 2002) reported significant inverse correlations between maternal serum estradiol and estriol at 16 and 27 weeks gestation with ultimate duration of the pregnancy in Caucasian and Chinese women ($p<$ 0.01 for all comparisons) (Wuu et al., 2002). Mazor and colleagues (Mazor et al., 1994)

similarly reported higher maternal plasma and amniotic fluid estradiol concentrations at 32-36 weeks among women who delivered prematurely. However, concerns have been raised about the validity of maternal serum estrogen measures as a proxy for fetal exposure because of lack of correlation with umbilical cord levels (Troisi et al., 2003a; Troisi et al., 2003b) and variation in plasma volume expansion in pregnancy (Faupel-Badger et al., 2007). Still, female infants' urinary estrogen excretion on the day of birth decreases with increasing gestational age from 28 to 41 weeks (Robine et al., 1988). Thus, although imperfect, available data indicate that while a longer gestation results in prolonged exposure of the fetus to hormones of pregnancy including elevated estrogens, maternal (and possibly fetal) estrogen concentrations are higher in pregnancies that are shorter duration.

Linked birth-cancer registry data generally suggest an increased risk of BCA among preterm infants, although results are not totally consistent across all studies. In the prospective Uppsala (Sweden) Academic Hospital birth cohort 1915-29, infants of a given birth size who were born 30-38 weeks (the shortest gestation category) were at a significantly increased BCA risk before age 50 years (Rate Ratio = 2.10, 95% CI = 1.05, 4.21) compared with those born ≥ 41 weeks gestation (the longest gestation category (p-trend 0.03)) (McCormack et al., 2003a). Similarly, in a large age- matched population-based case-control study in Sweden that linked birth records with cancer registry data, BCA risk was inversely associated with gestational age and stratification by menopausal status did not substantially alter the results (Ekbom et al., 1997). Women born at ≤ 33 weeks gestation were almost 4 times more likely to develop BCA compared with those born at > 33 weeks (OR = 3.96; 95% CI = 1.45, 10.81) (Ekbom et al., 1997).

Comparable results were reported in a smaller study of women born in Stockholm from 1925-1934 (Ekbom et al., 2000). However, in another small record linkage study conducted in Sweden, no association of gestational age (≤ 32 weeks or 33-34 weeks compared with > 35 weeks) with BCA was seen (Kaijser et al., 2003). Moreover, a protective effect of prematurity (gestational age < 33 weeks vs. ≥ 37 weeks, adjusted OR = 0.11, 95% CI = 0.16, 0.79) was reported in a in a record-linkage study of American women (Innes et al., 2000). Cases in the American study were < 37 years of age at diagnosis, which may suggest an etiology different from most BCA. A protective effect of short gestation is also supported by a case-control study that compared birth records of 87 Swedish female twin pairs where one twin developed BCA with matched with twin pairs where neither twin developed BCA (Hubinette et al., 2001). However, the analysis was not stratified by zygosity, and since dizygotic twins are exposed to two placentas *in utero* their hormone exposure is higher compared monozygotic twins or singletons (TambyRaja & Ratnam, 1981). In addition, monozygotic twins typically have a shorter gestation than dizygotic twins (Carroll et al., 2005), and pairs in which one twin develops BCA may be less likely to be monozygotic compared to pairs in which neither twin develops BCA. Stratifying the analyses by zygosity could aid in the interpretation of results and understanding the relationship of gestational age and BCA risk.

Studies based on mothers' reports of birth characteristics generally report no association of preterm delivery with BCA risk. No association was found between BCA risk and mother's recall of the index daughter's gestational age among daughters < 45 years of age at diagnosis living in Washington state (Sanderson et al., 1998). A case control study nested within the Nurses' Health Studies (NHS) I and II also obtained birth

characteristics retrospectively from subjects' mothers (Michels et al., 1996). Prematurity was ascertained by asking if the daughter was premature and how early the birth had been (< 2 weeks, 2-4 weeks, >4 weeks). Prematurity of any degree was not associated with BCA risk. The analysis was adjusted for current age, but did not stratify by menopausal status at BCA diagnosis due to small numbers and inadequate statistical power.

In summary, three cohort studies with record linkage (Ekbom et al., 1997; Ekbom et al., 2000; McCormack et al., 2003a) suggest an inverse relationship between gestational age and BCA risk, although case-control studies that rely on recall of gestational age (Michels et al., 1996; Sanderson et al., 1998), a record-linkage study with women diagnosed at a very young age (Innes et al., 2000) or who were twin births (Hubinette et al., 2001), and one small cohort study (Kaijser et al., 2003) found either a null relationship (Michels et al., 1996; Sanderson et al., 1998; Kaijser et al., 2003) or a positive association of gestational age and BCA risk (Innes et al., 2000; Hubinette et al., 2001). Numerous possible explanations for the dissimilar results exist. Foremost is questionable accuracy of self-reported and maternal recall of gestational age (Olson et al., 1997; Yawn et al., 1998; Buka et al., 2004) This potential error leads to misclassification of exposure which when non-differential between cases and controls generally biases results to the null. Recall accuracy declines with time since index birth (Olson et al., 1997), although mothers' recall may be more accurate in their recall if the index infant was born < 36 weeks gestation compared with infants born full term (Buka et al., 2004). Another issue is the wide variety of categorization schemes of gestational age and prematurity. Furthermore, few studies ascertain other health conditions during the index pregnancy, such as pre-eclampsia, which is associated with shorter gestation

(Roberts & Redman, 1993) but lower *in utero* estrogen exposure (Rosing & Carlstrom, 1984). Moreover, the low survival rate of pre-term infants prior to the creation of neonatal intensive care units may differentiate the pre-term infants who survived to adulthood and were at risk of developing BCA. Given the inconsistent results and disparate characteristics of the study populations (including very early BCA diagnosis and twin births) it is not possible to draw strong conclusions about the association of gestational age with BCA risk. Nonetheless, the better designed studies support an inverse relationship between gestational age and BCA risk. Additional work is needed to determine whether the inverse relationship of gestational age and BCA risk is strongest among premature births versus those near term but of comparatively shorter gestation.

Table 2.1 **Selected studies on the association of gestational age and the risk of breast cancer**

Study	Study Design	Birth Year	*n* of cases	Gestational age categories ‖	OR/HR/SIR (95% CI)	*p*-trend	Covariate adjustment
Ekbom et al., 1997	Population based, case-control nested in cohort **	1867 - 1961	1068§	≤ 33 weeks vs. > 33 weeks	3.96 (1.45, 10.81)		Matched on age and date of birth, adjusted for birth characteris-tics including birth weight and maternal pre-eclampsia
Ekbom et al., 2000	Cohort **	1925 – 1934	7† 5‡	< 31 weeks vs. ≥ 35 weeks	6.7 (1.4, 19.5)		Age, sex and time
Hubinette et al., 2001	Population based, case-control ‡‡	1886 - 1958	87§	40 weeks vs. < 33 weeks	8.4 (1.3, 54.4)		Matched on person-years at risk of developing BCA, and birth year +/- 9 years
Innes et al., 2000	Population based, case-control **	1958 - 1981	484†	< 33 weeks vs. ≥ 37 weeks	0.11 (0.16, 0.79)	0.05	Race, birth weight, and birth characteris-tics including maternal pre-eclampsia
Kaijser et al., 2003#	Cohort **	1925 - 1949	19† 39§	≤ 34 weeks vs. > 35 weeks	1.13 (0.68, 1.77)† 0.99 (0.40, 1.35) §		Standar-dized using age, sex and time specific cancer incidence rates
McCormack et al., 2003a	Cohort **	1915 - 1929	63† 296‡	< 39 weeks vs. ≥ 41 weeks	2.10 (1.05, 4.21)†	0.03	Ponderal Index, proxy measures of adult life risk factors

Michels et al,. 1996	Population-based, case-control nested within two cohorts ††	1921 - 1965	550§	> 4 weeks premature vs. not	1.04 (0.46, 2.38)		Adult life risk factors
Sanderson et al., 1998	Population-based, case-control ††	1945 - 1947	510†	< 37 weeks vs. 37 – 42 weeks	0.9 (0.5,1.8)		Matched on age and county of residence. Adjusted for birth weight and adult life risk factors

menopausal status: * = premenopausal, † = < 50 years, ‡ = ≥ 50 years, § = both age groups
‖ referent listed second, only highest and lowest categories are listed where *p*-trend is indicated
includes cases from Ekbom, 2000, ** data obtained from birth records linked to cancer registry
††data obtained from maternal recall of birth characteristics and medically confirmed BCA diagnosis.
‡‡ data obtained from Swedish Twin Registry

Birth Weight

Birth weight is positively associated with maternal sex hormones during pregnancy, including estriol (McFadyen et al., 1982; Petridou et al., 1990; Kaijser et al., 2000; Mucci et al., 2003) and maternal insulin-like growth factor-1 (IGF-1) (Wang & Chard, 1992), (Lindsay et al., 2007) during pregnancy. Sex hormones and IGF-1 have both been implicated in the initiation and promotion of BCA (Pike et al., 1993; Kazer, 1995; Pollak, 2000; Key et al., 2002; Ibrahim & Yee, 2005; Schernhammer et al., 2005; Eliassen et al., 2006). Birth weight has been related to timing of puberty and incidence of BCA in adulthood (Fall et al., 1995; Sanderson et al., 1996; Innes et al., 2000; Adair, 2001; Hubinette et al., 2001; Sanderson et al., 2002; McCormack et al., 2003a; Michels & Willett, 2004; De Stavola et al., 2005). Therefore, birth weight could be a proxy for *in utero* hormonal exposures that affect BCA risk in adulthood. Although recorded birth weights are preferred, maternal recall of birth weight years after the index birth is

reasonably valid (Olson et al., 1997; Yawn et al., 1998; O'Sullivan et al., 2000; Buka et al., 2004). A recent review and meta-analysis by Michels and Xue (Michels & Xue, 2006) provides a comprehensive review of the literature relating to birth weight and BCA risk, indicating an overall increased relative risk (RR) for BCA (combined pre- and post-menopausal) of 23% (95% CI = 13, 34%) for women with higher birth weights (mostly above 4000g) compared to lower birth weight (mostly below 2500g), and readers should refer to this manuscript for a detailed analysis. We will present only a brief overview of the most salient studies.

Numerous studies describe a positive association of birth weight and BCA risk, particularly among pre-menopausal BCA cases (Michels et al., 1996; Sanderson et al., 1996; Innes et al., 2000; Hubinette et al., 2001; Vatten et al., 2002; Ahlgren et al., 2004; dos Santos Silva et al., 2004; Lof et al., 2007). Pre-menopausal BCA risk linearly increased with increasing birth weight in a nested case-control study within the two cohorts of the NHS (Michels et al., 1996). Birth records of 2176 women (including 59 BCA cases) from the Medical Research Council (MRC) National Survey of Health and Development 1946 Birth Cohort in Great Britain indicated birth weight ≥ 4000g increased age-adjusted pre-menopausal BCA risk (RR = 5.03, 95% CI = 1.13, 22.47) compared to women weighing < 3000g at birth (*p*- trend 0.03) (dos Santos Silva et al., 2004). However, as reflected by the wide confidence interval, the estimate of the magnitude of the association lacked precision due to small numbers of cases. Nevertheless, medical records for 373 Norwegian BCA cases and 1,150 control women (Vatten *et al.*, 2002) also suggested BCA risk was positively associated with birth weight. The odds ratio (OR) for women whose birth weight was in the highest (≥ 3730g) versus

the lowest (< 3090g) quartile was 1.4 (95% CI = 1.1, 1.9; *p*-trend 0.02). A matched case-control study that linked New York State birth and tumor registry data of women < 37 years at diagnosis (*n* cases = 484) (Innes et al., 2000) and a case-control study using cancer-registry data and self-reported birth weight of women < 45 years at diagnosis (*n* cases= 746) (Sanderson et al., 1996) both describe increased BCA risk at both the low and high ends of the birth weight spectrum, but associations were significant only for high birth weight.

Although most of the published work examining the relationship between birth weight and BCA risk suggests a positive association, some studies find no relationship. Ekbom et al. (Ekbom et al., 1997) failed to find a statistically significant association of BCA risk with either birth weight or birth length among members of one case-control study nested within a Swedish cohort; although as noted previously gestational age was inversely associated with BCA risk among cohort members. Two other studies report that the effect of birth weight was no longer apparent after adjustment for either birth length or head circumference (McCormack et al., 2003a; Vatten et al., 2005), suggesting potential multicollinearity. No association of birth weight and pre-menopausal BCA risk was detected in the Shanghai Breast Cancer Study (OR for birth weight > 4000g = 0.7, 95% CI = 0.4, 1.4 compared with birth weight 2500 - 2999g) (Sanderson et al., 2002). Small sample sizes at the extremes of birth weight may have been insufficient to detect an effect. Also, Asian populations exhibit a narrower birth weight distribution compared with non-Hispanic Whites (Singh & Yu, 1994), and this may contribute to the null findings in ethnic-Asian populations (Le Marchand et al., 1988; Sanderson et al., 2002).

In summary, the evidence supporting a positive association of birth weight and BCA risk is moderately strong and it is generally more apparent among pre-menopausal women (Sanderson et al., 1996; Michels & Willett, 2004; De Stavola et al., 2005). As with gestational age, the possibility of a survival bias of the low birth weight infants as well as possible unreliable retrospective reports of birth weight and the combined effects of other birth size variables could influence the results.

Birth length

Birth length is positively associated with adult height (Eide et al., 2005), which in turn has been positively related with adult BCA risk (van den Brandt et al., 2000; Gunnell et al., 2001). Length at birth is a stronger predictor of adult height than weight at birth (Sorensen et al., 1999; Tuvemo et al., 1999), and like higher birth weight, a longer length at birth may be a proxy for greater *in utero* estrogen and growth hormone exposure (Troisi et al., 2003a) which potentially increase future BCA risk (Table 2.2).

Research to date generally supports a positive association of birth length with BCA risk. In the previously discussed Uppsala birth cohort (McCormack et al., 2003a), risk of incident BCA < 50 years of age was more than three times higher among women in the highest compared to the lowest quintile of birth length (RR = 3.40, 95% CI = 1.5, 8.0, *p*-trend < 0.001) following adjustment for gestational age. However, birth length is highly correlated with birth weight and head circumference (Lunde et al., 2007), and further adjustment for these characteristics attenuated the association between birth length and BCA risk such that trends no longer achieved statistical significance (*p*-trend 0.09 and 0.12 for birth weight and head circumference, respectively). Unlike the

association observed for early age at onset of BCA, there was no evidence of an association between birth length and BCA risk in older women.

Two studies conducted in Norway similarly report positive associations between birth length and BCA risk. The first was a case-control study where birth length was abstracted from medical records of cases and age-matched controls (Vatten et al., 2002). Compared with the lowest quartile of birth length (< 50.0 cm), women in the highest quartile (≥ 51.5 cm) at birth were at increased risk for developing BCA (OR = 1.4, 95% CI = 1.1, 1.9, *p*-trend 0.02). Results were adjusted for age at first birth and parity, but not birth weight and were not stratified by age at diagnosis. The second study, conducted by the same author (Vatten et al., 2005), was a prospective study of a 16,016 member Norwegian cohort with birth data abstracted from hospital records. A total of 312 BCA cases were observed over 40 years of follow-up. Women ≥ 53 cm at birth were at a 1.8 fold (95% CI = 1.2, 2.6) increased risk of developing BCA compared to women < 50 cm at birth (*p*-trend 0.02). Adjustments were made for birth year, length of gestation, birth order, maternal age, maternal marital status, and maternal socioeconomic status at childbearing. After stratification by age at diagnosis (< 50 or > 50 years) and adjustment for the aforementioned variables plus birth weight and head circumference the RR of BCA < 50 years = 1.5 (95% CI= 0.8, 2.9) for women with birth length ≥ 53 cm compared to < 50 cm at birth. BCA risk among women ≥ 50 years at diagnosis also was increased among the group with the longest length at birth, but not significantly (RR= 2.1; 95% CI= 0.9, 5.0).

The evidence for a positive relationship between length at birth and BCA risk appears strong. Potential reasons for some of the discrepant findings could arise from the

difficulty in obtaining accurate length measurements in infants. In addition, measurements are frequently recorded to the nearest 0.5 cm, thus reducing precision and variability. In addition to racial/ethnic differences, the impact of genetics must be considered in future investigations. A recent analysis of genetic effects on birth weight, length and gestational age concluded that 31% of normal variation in birth weight and birth length and 11% of normal variation in gestational age was explained by inherited genetic factors (Lunde et al., 2007). It is also possible that polygenic factors control both birth size and disease risk (Meyre et al., 2005), and this interaction deserves further attention. Recent work to identify the Quantitative Trait Loci for birth weight and birth length may help to identify specific genetic variants and further refine this relationship (Fradin et al., 2006). Additionally, at least one analysis (Adair, 2001) suggests that girls born short-heavy reach menarche later compared with peers born long-light. This finding, in addition to supporting the importance of characterizing birth size in more than one dimension to capture the collective effect of birth size, highlights the importance of understanding intermediate risk factors for potential incongruence over the life course. In this example, high birth weight is associated with later menarche, although early menarche is an established BCA risk factor. Although beyond the scope of this review, it is thought that growth and body weight mediate the relationship (Cooper et al., 1996; dos Santos Silva et al., 2002).

Table 2.2 **Selected studies on the association of birth length and the risk of breast cancer**

Study	**Study Design**	**Birth Year**	***n* of cases**	**Birth length categories‖ (cm) (referent listed second)**	**OR/HR/SIR (95% CI)**	***p*- trend**	**Covariate adjustment**
McCormack et al., 2003a	Cohort[#]	1915-1929	63[†] 296[‡]	≥ 52.5 vs. ≤ 49.0	2.59 (0.90-7.47)[†] OR for postmeno-pausal risk n.s., but values not reported	0.09	Gestational age, birth weight, proxy measures of adult life risk factors
Vatten et al., 2002	Popula-tion based case-control [#]	1910-1970	373[§]	≥ 51.5 vs. ≤ 50.0	1.3 (1.0- 1.8)	0.02	Matched on age, birth date, city of residence at the time of diagnosis. Adjusted for parity and age at first birth
Vatten et al., 2005	Cohort [#]	1920-1958	167[†] 145‡	≥ 53 vs. ≤ 50.0	1.8 (1.2- 2.6) [§]	0.02	Year of birth, length of gestation, birth order, maternal age at childbearing, marital status and maternal socioeconomic status

[*] = premenopausal, [†] = < 50 years, [‡] = ≥ 50 years, [§] = both age groups
‖ only highest and lowest categories are listed
[#] data obtained from birth records linked to cancer registry

Childhood Height / Linear Growth

The positive association of birth length and adult height have been referenced previously (Eide et al., 2005), but growth through childhood to final adult height deserves further attention. Adult height is positively associated with BCA risk (Gunnell et al., 2001). Adult height is also positively associated with age at menarche, or otherwise stated: girls who have menarche late are on average taller adults (Luo et al., 2003). This association appears incongruent with evidence that early menarche is also an established risk factor for BCA (Hsieh et al., 1990). The biologic rationale for this late menarche-taller adult height relationship lies in that skeletal growth typically reaches completion approximately two years after menarche (Tanner, 1989). On average, girls who are taller than their peers as children tend to reach menarche earlier (Bogin, 1999; Hilakivi-Clarke et al., 2001), but stop linear growth earlier, leading to a post-pubertal crossover in average height for different age-at-menarche groups (De Stavola et al., 2004). Another key to understanding this complex relationship with BCA risk may rest in childhood growth velocity and the timing of childhood growth spurts. This suggests a multifaceted connection, and growth velocity may be central to understanding the seemingly contradictory relationship between age at menarche and adult height in relation to BCA risk (Table 2.3).

Ahlgren *et al.* (Ahlgren et al., 2004) modeled individual growth curves from 8 to 14 years of age for 117,415 Danish women with information on birth weight and annual height and weight measurements linked to the Danish Cancer Registry. After mutual adjustment for birth weight, height at age 8 years, BMI at age 14, and age at menarche, age at peak growth was inversely associated with BCA risk. Each year delay in age at

peak growth was associated with a decrease in RR= 0.94 (95% CI= 0.91, 0.97), and results did not differ materially when analyses were stratified by age at diagnosis. Moreover, regardless of age at diagnosis, each 5-cm increase in height from age 8 to age 14 years was associated with increased BCA risk (RR = 1.17, 95% CI = 1.09, 1.25).

Analyses from the prospective MRC National Survey of Health and Human Development birth cohort (De Stavola et al., 2004) suggest that BCA cases are consistently taller than non-cases during childhood. Furthermore, after mutually adjusting for other components of growth, each one standard deviation (SD) increase in height velocity at ages 4-7 years increased the BCA OR to 1.54 (95% CI = 1.13, 2.09), while a comparable increase at ages 11-15 years increased the OR to 1.29 (95% CI = 0.97, 1.71). Associations of growth velocity were stronger for women with earlier menarche. When restricted to women with menarche <12.5 years, each one SD increase in height velocity at ages 4-7 years and 11-15 years increased the BCA OR to 1.95 (95% CI 1.25, 3.04) and 1.66 (95% CI = 1.00, 2.78), respectively. Thus the adverse effect of fast growth may be stronger among women with an early age at menarche.

Berkey and colleagues (Berkey et al., 1999) also observed a positive association between adolescent peak height velocity (PHV) and both pre- and post-menopausal BCA risk in an analysis of the NHS I. Annual height measurements were not available, but PHV was estimated using age at menarche, adiposity at age 10 y (assessed retrospectively with somatotype pictograph) and adult height. Among women in the highest quintile of estimated PHV (8.9 cm/y) risk of BCA was 1.31 compared to those in the lowest quintile (≤ 7.6 cm /y) with a significant trend observed across increasing categories of PHV (*p*-

trend 0.001). Results were similar for post-menopausal BCA cases, the RR for PHV > 8.9 cm / y was 1.40 (*p*-trend 0.001).

In contrast, a case control study nested in a Swedish cohort that was constructed from birth and school health records in childhood with linkage to the National Hospital Discharge Registry and Death Registry (Hilakivi-Clarke et al., 2001) failed to detect an association of PHV with pre- or post-menopausal BCA risk. Although BCA cases were significantly taller on average than controls at each age from age 7 to age 15 years, the Z-score (a normalized measure of growth velocity) for change in height for each age from 7 – 15 y did not differ by case status. This finding contradicts that of the previously discussed Berkey et al. (Berkey et al., 1999) study which found a positive association of PHV with BCA risk. However, several differences in the studies exist which make a direct comparison difficult, including that Berkey et al.'s characteristics were extrapolations on available data. Additional research examining growth velocity using prospectively collected data and adjustment for potential BCA confounders is warranted, and widespread calculation of Z-scores or other normalized measure would aid in comparison across studies (Forman et al., 2005).

Overall, the available data suggest that growth patterns appear less influential than maximal height attainment on BCA risk. However, growth velocity and patterns at different ages during childhood should not be disregarded. Growth patterns in relation to BCA risk remain a new area of inquiry and further developments will help to clarify the relationship, such as the relative importance of increased height velocity at specific ages. These factors may be useful in understanding the pathway to risk and offer important contributions to understanding a complex disease process.

Table 2.3 **Selected studies on the association of height and linear growth and the risk of breast cancer**

Study	Study Design	Birth Years	*n* of cases	Relationship measured	OR/HR/SIR (95% CI)	*p*- value	Covariate adjustment
Algren et al., 2004	Cohort[††]	1930 - 1975	2074[§]	Risk of BCA with each year delay in age at peak growth	0.94 (0.91, 0.97)[§] 0.90 (0.86, 0.95)[†] 0.98 (0.93, 1.03)[‡]	0.03[‖] 0.74[‖]	Birth weight, height at age 8 years, BMI at 14 years, adjustment for age at menarche did not appreciable change the estimates
				Risk of BCA with each 5 cm increase in height from age 8 to 14 years	1.17 (1.09, 1.25)[§] 1.15 (1.05, 1.27)[†] 0.94 (0.92, 0.97)[‡]		
Berkey et al., 1999	Cohort[**]	1921 - 1946	806[†]	Risk of peak height velocity > 8.9 cm	1.31 (CI not reported)[*]	0.001[#]	Age in 1976 or age at menopause, peak height velocity, adiposity at ages 5,10, and 20 years, other adult life risk factors
			1485[‡]		1.40 (CI not reported)[†]	0.001[#]	
De Stavola et al., 2004	Prospec -tive Cohort	1946	59[§]	Odds of developing BCA with each one standard deviation increase in height velocity:			Height, height velocity at other ages, BMI, BMI velocity
				From age 4 years to age 7 years	1.54 (1.13, 2.09)		Age at first birth, interval between menarche and first birth, parity, social class, and BMI did not substantially alter results
				From age 11 years to age 15 years	1.29 (0.97, 1.71)		

Hilakivi-Clarke et al., 2001	Cohort [§]	1924 - 1933	42[2] 35[3]	HR of BCA for height in highest quintile at ages: 7 years 15 years	1.9 (1.1-3.1)[§] 1.9 (1.2-3.2)[§]	0.01 [#] 0.005 [#]	Unadjusted
				Mean height of BCA cases taller than mean height of controls at each age from 7 to 15		< 0.05	
				Z-score change in height for each age from 7 to 15 years not significantly different		n.s., *p*-value not reported	

[*] = premenopausal, [†] = < 50 years, [‡] = ≥ 50 years, [§] = both age groups;
‖ *p*-value represents difference in RR according to attained ages
[#] *p*-trend across quintiles of increasing weight or growth
[**] growth data obtained from self-reported retrospective report and medically confirmed BCA diagnosis
[††] height data obtained from health records linked to cancer registry

Diet in Early Life and Adolescence

In animal models, maternal high fat diet during pregnancy is associated with increased estradiol in the mother and increased incidence of induced mammary tumors in first generation offspring (Hilakivi-Clarke et al., 1994). To date, no published investigations of maternal diet and subsequent BCA risk in human offspring are available although several investigations of childhood and adolescent diet have been conducted. A recent meta-analysis of eleven studies on the history of having been breastfed concluded no effect on BCA risk (Martin et al., 2005) and will not be reviewed in detail.

In a case-control study nested in the NHS I and II, Michels and colleagues (Michels et al., 2006) investigated the relationship between pre-school diet and BCA risk. Nurses' mothers completed a 30-item Food Frequency Questionnaire (FFQ) describing their daughter's diet at ages 3-5 years. After adjustment for numerous confounders, regular consumption of French fries was associated with increased risk of BCA (OR =

1.27 for one additional serving / week, 95% CI = 1.12 – 1.44). Although recall bias is possible, the authors note that if less healthful items were recalled more often by the case mothers, it would be expected that other foods generally deemed unhealthy also would have been associated with an increased risk of BCA. Future research of preschool diet patterns with BCA risk among a cohort of children followed prospectively could help identify the true nature of the relationship.

Analyses from the NHS cohort I similarly indicated a possible relationship between adolescent diet and adult BCA risk (Frazier et al., 2003). Cases and controls selected from the cohort completed a 24-item FFQ reflecting their dietary intakes at ages 12 - 18 years. Higher consumption of eggs (RR = 0.82 per increase of one egg/day, 95% CI = 0.67 - 0.99) and vegetable fat (RR = 0.85 for highest quartile compared to lowest quartile, CI not reported, *p*-trend 0.05) were associated with a reduced BCA risk following multivariate adjustment, whereas consumption of butter was positively associated with risk (RR = 1.06 per increase of one pat/ day, 95% CI = 1.00, 1.13). Little difference in the RR was detected following stratification by menopausal status at diagnosis. Incident BCA was diagnosed between 1976 and 1986, and adolescent diet was retrospectively assessed in 1986 when participants were 40-65 years old; thus bias may have been introduced by inaccurate recall, particularly if it was differential in cases and controls. In a related study, 47,355 participants in the NHS II aged 34-51 completed a 131-item FFQ regarding their diets during ages 12-18 years (Frazier et al., 2004). At the time of diet recall, BCA cases were post-diagnosis resulting in potential for recall bias as with the earlier NHS study. Albeit, increased intake of vegetable fat and vitamin E were both found to be protective against BCA diagnosis, but when vitamin E and vegetable fat

were entered simultaneously in the model, only vitamin E upheld its significance. In vitro evidence indicates that vitamin E succinate (an ester of vitamin E) induces apoptosis in BCA cells (Malafa & Neitzel, 2000) and the study authors (Frazier et al., 2004) speculate that similar activity may be important during remodeling of the terminal end buds during adolescence. Higher glycemic index diets during adolescence were also positively associated with higher BCA risk (RR =1.47 for highest vs. lowest quintile, 95% CI = 1.04, 2.08, *p*- trend 0.01) in the present analysis. High glycemic index diets may increase serum glucose and insulin which in turn increase late post-prandial secretion of IGF-1 (Pollak, 2000). IGF-1 concentration has been positively associated with premenopausal BCA risk in the NHS Cohort I (Schernhammer et al., 2005), but no association was detected in the NHS Cohort II with no obvious explanation for the discrepancy (Schernhammer et al., 2006). Issues related to the retrospective diet recall and possible recall bias, as with the earlier NHS diet investigation remain (Frazier et al., 2003). One smaller (*n* cases =172) (Pryor et al., 1989) and one larger (*n* cases =1647) (Potischman et al., 1998) U.S. based case-control study also failed to detect a clear relationship of retrospectively recalled adolescent diet and adult BCA risk, including early onset BCA (Potischman et al., 1998).

Soy isoflavones genistein and daidzein are highly concentrated in soy foods and have possible cancer protective effects (Messina et al., 2006). Shu and colleagues (Shu et al., 2001) analyzed retrospective dietary data from the population based Shanghai Breast Cancer Study in which soy intake at ages 13-15 years was ascertained through a 17-item FFQ. BCA cases reported lower soy intake during adolescence (6.45 g soy protein / day for cases versus 7.23 g / day for controls, $p = 0.002$); differences were highly significant

for both pre- and postmenopausal women. The authors suggest that soy food intake in adolescence may explain some variability in BCA incidence among Caucasian and Asian populations, as well as explain increasing incidence among Asian-Americans who adopt a Western-style diet. For comparison, it is estimated that soy intake is 13 to 80 times higher in China than in select European countries and the United States (Horn-Ross et al., 2000; de Kleijn et al., 2001; Keinan-Boker et al., 2002). However in the present study, similar to the NHS that relied on recall of adolescent diet after BCA diagnosis, there is potential for bias.

Because of difficulties inherent in directly evaluating associations of childhood and adolescent diet with BCA in adulthood, Dorgan et al. (Dorgan et al., 2003b) evaluated the effect of adolescent diet on serum hormones that influence breast development and are associated with BCA in adulthood. In that study, which was a randomized controlled trial to evaluate the effects of a reduced fat diet at 8-17 years of age, participants in the intervention arm had lower serum estrogens and progesterone during follow-up compared to those in the usual care arm. These findings are consistent with a possible influence of adolescent diet on BCA risk. Thus, although direct evidence for an association of early life or adolescent diet with BCA risk is weak, indirect evidence is consistent with such an association. Lack of clarity from studies that have attempted to directly assess the association may be due to errors in recall of adolescent or early life diet decades later. Prospective studies are needed to clarify the relationship.

Body Mass Index (BMI)

In adults, pooled analyses indicate BMI is inversely associated with BCA risk among pre-menopausal women, but positively associated with post-menopausal BCA risk (van den Brandt et al., 2000). It has been suggested that weight gain, particularly weight gain since age 20 years, may be a stronger determinant of post-menopausal BCA risk than absolute BMI at a single point in time (Trentham-Dietz et al., 2000). The relationship of childhood and adolescent BMI with adult BCA risk is similarly complex.

Ahlgren et al. (Ahlgren et al., 2004) investigated BMI among 141,393 Danish girls, born between 1930 -1975 with school health records linked to the Danish Cancer Registry. BMI was inversely associated with BCA risk, with each 1-unit increase in BMI from ages 8 to 14 corresponding with a RR = 0.96 (95% CI = 0.93, 0.99) after adjusting for attained age. At age 14 years, individuals in the highest quintile of BMI (median =22.4) had a BCA RR = 0.84 (95% CI = 0.75, 0.94) compared to those in the lowest quintile (median= 16.7). However, the median BMI in the highest quintile may not be reflective of the adiposity in contemporary populations. The inverse association of BMI at 14 years of age was independent of birth weight, age at peak growth, age at menarche, height at age 8, and height increase between ages 8-14 years and did not differ by menopausal status at diagnosis. Thus the effect of childhood obesity on BCA does not appear to be due to acceleration of puberty brought about by adiposity.

An inverse association of childhood and adolescent BMI with BCA (predominantly pre-menopausal) also was observed in the 1946 MRC National Survey of Health and Development British birth cohort (De Stavola et al., 2004). In that cohort,

women who subsequently developed BCA were consistently thinner from ages 2–15 years than women who remained BCA free. Cases also experienced a faster decrease in BMI between ages 2 and 4 compared to non-cases. After adjusting for BMI at the time of mammography women with more dense breasts were at an increased risk of BCA. In addition, childhood BMI was inversely associated with mammographic density in this cohort (McCormack et al., 2003b). Thus, one mechanism by which childhood BMI could potentially influence BCA risk is via effects on mammographic density.

In contrast, perceived weight at 15 years of age was not associated with BCA risk in the Shanghai Breast Cancer Study. However, the Shanghai study used a retrospective self-report of adiposity whereas the previous studies had prospective measurements of weight and height. Additionally, the previous studies were conducted among predominantly Caucasian populations living in Europe and the BMI distribution among Asians is different from that of Caucasians, which may be a factor in the discrepant findings.

In general, the evidence indicates increased adiposity in childhood may protect against later pre- and post-menopausal BCA risk. However, in the studies reviewed, adiposity typically refers to the highest category of the study population. Given that many of the cohorts were born in the 1940's or earlier, the degree of adiposity was likely less severe than the obesity observed in upper percentiles of contemporary pediatric populations. Future work should examine the existence of BMI threshold values for BCA risk. Caution should be heeded before using such findings to construct a public health message.

Summary and Conclusions

Breast cancer is an etiologically complex disease. Additional work is needed to clarify how known and proposed BCA risk factors influence risk. Although there are inconsistencies in the literature, gestational age appears to be inversely related with BCA risk, whereas birth weight and birth length appear to be positively associated. Adult height is positively associated with BCA risk, but the relationship may be mediated by height velocity with fast growth early in life increasing overall BCA risk. The evidence regarding childhood diet is limited and inconclusive, but child and adolescent BMI appear to be inversely related with BCA risk.

Birth characteristics tend to be highly correlated, and multicollinearity may hinder the ability to detect independent effects. Other issues in the current literature include lack of conformity in controlling for covariates and potential confounders, including familial BCA history and parity. In addition, the majority of work has been conducted in non-Hispanic Caucasian and Asian populations, with little available evidence from other racial/ethnic groups. Furthermore, much of the prospectively collected data is derived from cohorts born in an environment with different lifestyle trends than experienced today. These issues could impact the relevance of the research in the current environment.

Most of the available evidence supporting a relationship between birth characteristics and *in utero* hormone exposure is based on measurements of maternal plasma hormone concentrations which may not be reflective of fetal concentration in the cord blood (Troisi et al., 2003a; Troisi et al., 2003b), similarly, the stress of labor may alter cord blood concentrations differently than maternal serum concentrations.

Additional work is needed to clarify the true relationship. In the interim, the underlying hypothesis that the associations of birth size and gestational age with BCA risk manifest from in utero estrogen exposure should be considered speculative while the research continues and other explanations for the relationship are pursued.

Directions for future work should aim to connect the relevance of risk factors over the developmental spectrum. The association of adolescent diet and BCA, specifically within the context of BMI and dietary quality, has not been extensively investigated. This is an area of particular public health interest given that diet is amenable to change. Furthermore, research aimed at clarifying apparently contradictory relationships of early life and adolescent characteristics with BCA risk could provide insights into the underlying cause of the disease. Prospective studies beginning in utero with the outcome of BCA are the ideal study design for clarifying these relationships. As early life risk factors become better understood, individuals and families may make lifestyle choices to minimize risk. Furthermore, given the etiologic complexity of BCA, understanding early life risk factor plays an important role in unraveling disease development.

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Chapter 3

BIRTH WEIGHT AND AGE AT MENARCHE

Introduction

Age at menarche (first menses) has declined in the United States and internationally (Parent et al., 2003; Anderson & Must, 2005; Herman-Giddens, 2007). In addition to the short-term risks of early pubertal maturation, including increased risk of adolescent substance abuse (Deardorff et al., 2005), psychological distress (Ge et al., 1996), and early sexual intimacy (Kaltiala-Heino et al., 2003; Deardorff et al., 2005), earlier puberty is associated with increased risk of adult chronic disease including breast cancer (BCA) (Hamilton & Mack, 2003). Early age at menarche is an established risk factor for BCA (American Cancer Society, 2007) with each one-year delay of the onset of menses associated with an estimated BCA risk reduction of 4 to 20 percent (Kvale & Heuch, 1988; Henderson et al., 1991; Harris et al., 1992; Ursin et al., 1994). Earlier menarche is associated with an increased number of lifetime ovulatory menstrual cycles (Kvale & Heuch, 1988; Ursin et al., 2000). Endogenous estradiol and progesterone concentrations peak with ovulation-- thus the more ovulatory menstrual cycles a woman experiences the greater her lifetime exposure to these hormones. Accordingly, this is one suspected causal pathway linking early menarche to increased BCA risk.

A growing body of literature has documented a positive association between birth weight and BCA, with the evidence being stronger for pre-menopausal BCA (Michels & Xue, 2006). Much of this literature is framed in the context of the four assumptions of the original fetal origins of BCA hypothesis: 1.) estrogens are an important component in breast carcinogenesis, 2.) factors which increase the risk of cancer post-natally may also increase the risk of cancer when they act *in utero*, 3.) estrogen concentrations are at least ten times higher during pregnancy than they are during other periods in adult life 4.)

estrogen concentration and secretion rates vary widely between individuals during pregnancy, and this variability is partly accounted for by exogenous factors (Trichopoulos, 1990). In addition, the observed positive associations between maternal circulating estrogens during pregnancy and birth weight (Petridou et al., 1990; Kaijser et al., 2000; Mucci et al., 2003) have led many to hypothesize that the association between birth weight and BCA risk is attributable to increased *in utero* exposure to estrogens. Substantially less work has examined birth weight and intermediary risk factors, such as age at menarche. The limited work that has been conducted suggests that birth weight is positively related to age at menarche (Cooper et al., 1996; Persson et al., 1999; Romundstad et al., 2003), but that this relationship may be mediated by fast growth in early childhood (Adair, 2001; dos Santos Silva et al., 2002). If true, then growth mediation offers an explanation to the initially contradictory evidence that high birth weight is positively related to both BCA risk and later age of menarche. The relationship between the prenatal environment and age at menarche deserves further attention to better understand BCA risk factors over the lifecourse. To that end, we assessed the relationship between birth weight and age at menarche after controlling for potential confounders such as physical activity, body fat and mother's age at menarche in the Dietary Intervention Study in Children (DISC).

Subjects and Methods

DISC Study Design

DISC was a two-armed, multi-center, randomized clinical trial sponsored by the National Heart Lung and Blood Institute (NHLBI) and conducted at six clinical centers (Children's' Hospital, New Orleans, LA; Johns Hopkins Hospital, Baltimore, MD; Kaiser Permanente Center for Health Research, Portland, OR; Univ. of Medicine and Dentistry of New Jersey, Newark, NJ; Northwestern Medical School, Chicago, IL; and Univ. of Iowa Hospital, Iowa City, IA). Data management was performed by the coordinating center at Maryland Medical Research Institute, Baltimore, MD. Assent was obtained from DISC participants and written informed consent was obtained from their parents or guardians. Institutional Review Boards at all participating centers approved the DISC protocol, and an NHLBI-appointed independent data and safety monitoring committee provided oversight.

A total of 663 participants (362 boys and 301 girls), 8-10 y old with elevated LDL-cholesterol levels were randomly assigned to either dietary intervention (n = 334) or usual care (n= 329) between 1988 and 1990. Overall trial results have been published (Van Horn et al., 2003). Age at menarche and Tanner stage progression did not differ at any visit by treatment group (Dorgan et al., 2003b). The initial DISC protocol was designed for 3 years of intervention and was subsequently extended with planned intervention and follow-up until participants reached 18 years of age. However, DISC was terminated in 1997, when the mean age of participants was 16.7 y, because the participants in the two treatment groups did not have statistically significantly different

serum LDL-cholesterol levels (Obarzanek et al., 2001). In total, five visits for the female participants are included in the present analysis: baseline, Year-1, Year-3, Year-5 and last visit (median duration 7.0 y, range = 6.4-9.1 y).

Female Participants in the DISC

Subjects were recruited through schools, health maintenance organizations and pediatric practices. Female eligibility requirements for the overall DISC trial included baseline age of 8 to 10 years, a serum LDL cholesterol level in the 80-90th percentiles (Department of Health and Human Services, 1980), no major illnesses, and no medications that could affect blood lipid levels or growth. In addition, participants had to be at or above the 5th percentile for height and in the 5-90th percentiles for weight-for-height according to growth data from the Bogalusa Heart Study (Dorgan et al., 2003b), be Tanner Stage 1 for breast and public hair development (Tanner, 1962), and have normal cognitive and psycho-social development as assessed by use of the Achenbach Child Behavior Checklist (Achenbach & Edelbrook, 1983). Girls whose families or themselves were already following a low-fat diet, with a parent with early heart disease, with a planned family move in the next three years or with known behavioral problems were excluded. Subjects remained under the care of their primary care physicians during the study and the LDL-cholesterol criteria were set so that children with severe hypercholesterolemia in need of possible pharmaceutical intervention were excluded. Of the female participants, 203 had recorded birth weight as reported by their parent or guardian at the year-3 visit.

Diet Intervention

The DISC dietary intervention featured a food pattern designed to reduce intake of total fat to ≤28% calories and saturated fat to ≤8% calories, increase polyunsaturated fat intake to 9% calories and monounsaturated fat to 11% calories, reduce cholesterol intake to 75 mg per 1000 calories (to a maximum of 150 mg per day), maintain protein intake at 14% calories (2/3 animal protein, 1/3 vegetable protein), and maintain carbohydrate intake at 58% calories. At the year-3 and last visit, the intervention girls achieved 28.7% and 27.4% calories dietary fat, 10.3% and 9.6% calories from saturated fat, 95 mg and 99 mg dietary cholesterol / 1000 calories, respectively (Dorgan et al., 2003b).

Data Collection

Data were collected prior to randomization and annually thereafter by trained staff who were blinded to treatment assignment. Demographic characteristics, medical history, and use of medications were obtained via self-report with parental assistance where necessary. Birth weight was included in the mothers'/guardians' questionnaire and reported at the year-3 visit to the closest ounce or gram. Maternal recalled birth weight is generally very close to the true birth weight, with correlations as high as 0.94 (Tomeo et al., 1999; O'Sullivan et al., 2000; Walton et al., 2000; Buka et al., 2004). Height was measured with stadiometers centrally constructed by the Medical Instruments Unit of the University of Iowa. Weight was measured using a beam balance or digital scales that were calibrated weekly against a range of standard weights ranging between 20 -100 kg. BMI was calculated with measured height and weight (BMI: weight in kg/ height in m^2).

Girls were questioned annually regarding onset of menses. Diet information was obtained through a series of three 24-hour recalls; one in person and two by telephone by means of the Nutrition Data System (version 2.2-2.4, Nutrition Coordinating Center, University of Minnesota). Physical activity was assessed with a questionnaire designed to determine time spent in five intensity levels of physical activity. An estimated-metabolic-equivalent (MET) score was calculated by multiplying the number of hours spent on each level of activity intensity by a MET. MET scores used in this study were the following: sleeping (1 MET), sedentary activity (1.5 METs), light activity (4 METs), moderate activity (6 METs), and intense activity (10 METs).

Statistical Analysis

Statistical analyses were performed using SAS software (SAS System for Windows, version 9.1; SAS Institute, Cary, NC). BMI-for-age percentiles were calculated using the U.S. Centers for Disease Control SAS Program for Growth Charts (U.S. Centers for Disease Control, 2007). One-way ANOVA analyses were used to compare differences in mean birth weight, BMI-for-age percentile at three time points (study entry, visit prior to reaching menarche, and last visit), and mother's age at menarche between early (≤11.75 y), average (11.76-13.74 y), and late menarche (≥ 13.75 y) groups.

Partial likelihood Cox regression survival models (the PHREG procedure in SAS) were used to examine the relationship of birth weight with age at menarche after controlling for potential confounding variables, including BMI-for-age percentile at the visit prior to reaching menarche, physical activity at the visit prior to reaching menarche,

and where available, mother's age at menarche. Since the PHREG procedure drops any observation with missing data and mother's age at menarche was available for a subset of the girls (130 / 203 girls with birth weight), two sets of model specifications were used. The first set of specifications examined treatment group, race, birth weight, and BMI-for-age percentile and physical activity at visit prior to onset of menses. The alternate specification set examined mother's age at menarche in addition to the aforementioned variables. The following potential covariates were tested, but did not have a statistically significant effect and were dropped from the model: percent energy from fat, total fat, total energy / body weight, thiamin, riboflavin, niacin, folic acid, vitamin B6, vitamin B12, soluble fiber, total fiber, and three separate childhood growth velocity variables (as calculated by change in rank order of birth weight to rank order of BMI, weight, and height at study entry). Cigarette smoking prior to menarche was only reported by one individual and was not included in the models. Follow-up was calculated from date of birth to date of first menses or date of last study contact, which ever came first. Girls who left the study prior to reaching menarche, who did not have menarche by study completion, or who were missing age at menarche were treated as censored observations.

Results

Subject Characteristics by Age at Menarche

A total of 191 / 203 (94%) girls with recorded birth weight had a documented age at menarche. The mean age of menarche for this sample was 12.79 y with a standard deviation (SD) of 1.05 y. The minimum age at menarche was 9.75 y and the maximum was 15.71 y. Of the 12 girls without a recorded age at menarche, two were known to have reached menarche during follow-up, seven girls were pre-menarcheal at the last visit (mean age at visit for pre-menarcheal sample at last visit = 16.15 y), and three individuals left the study before menarche was documented.

Unadjusted subject characteristics are in Table 3.1. For descriptive purposes, age of menarche was categorized as early (≤ 11.75 y), average (11.76 -13.74 y) or late (≥ 13.75 y). These categories are based roughly on the mean age of menarche +/- one SD. Mean birth weight increased with age at menarche categorization (early, average, or late), but the unadjusted between group differences in birth weight were not statistically significant. Nine girls (4%) were of clinical low birth weight (≤ 2500g at birth) and twenty girls (10%) weighed ≥ 4000g. Girls with early menarche (≤ 11.75 y) exhibited a significantly higher BMI-for-age percentile compared to girls with late menarche (≥ 13.75 y) at the pre-puberty baseline visit ($p< 0.001$), at the visit directly before reaching menarche ($p< 0.001$), and at the last visit ($p< 0.001$). Girls with early menarche also had a higher mean BMI-for-age percentile compared to girls with average menarche (11.76 - 13.74 y) at the visit prior to onset of menses ($p= 0.04$), but not at baseline or at the last visit ($p= 0.20$, $p= 0.10$, respectively). Girls with average age at menarche had a significantly higher BMI-for-age percentile compared to girls with late menarche at

baseline (pre-puberty) ($p< 0.001$), at the visit directly before reaching menarche ($p< 0.001$), and at the last visit ($p= 0.005$). Among the 126 girls with reported birth weight, age at menarche and mother's age at menarche (62% of the sample), girls with early menarche also had the earliest mean reported mother's age at menarche, and the group means for mother's age at menarche increased with girls' increasing age at menarche categorization (early, average, or late; $p \leq 0.05$ for all comparisons).

Table 3.1**: Characteristics of DISC Girls with Early, Average or Late Menarche**

	Early (≤ 11.75 y) *n* = 30	**Average (11.76 – 13.74 y) *n* =130**	**Late (≥ 13.75 y) *n* = 31**	***p*-value**
Birth Weight (g) Mean (SD)	3282.89 (526.09)	3384.78 (454.88)	3489.75 (610.16)	0.31[a] 0.29[b] 0.10[c]
BMI-for-age percentile at study baseline [1] Mean (SD)	65.62 (23.62)	58.72 (27.97)	39.42 (24.19)	0.20[a] **<0.001[b]** **< 0.001[c]**
BMI-for-age percentile at visit prior to menarche Mean (SD)	72.27 (20.65)	60.04 (28.56)	38.90 (28.64)	**0.04[a]** **<0.001[b]** **<0.001[c]**
BMI-for-age percentile at last visit[2] Mean (SD)	69.26 (22.86)	60.26 (27.23)	45.03 (28.25)	0.10[a] **0.005[b]** **<0.001[c]**
Mean mother's age at menarche (years)[3] Mean (SD)	12.17 (1.34)	13.02 (1.34)	13.68 (1.34)	**<0.01[a]** **0.05[b]** **<0.001[c]**
White (*n*)	21	119	29	
Black (*n*)	2	9	1	
Asian (*n*)	4	2	0	
Race not reported (*n*)	3	0	1	

SD= standard deviation
[1] mean age at baseline= 9.14 y, range 8.02 y – 10.35 y
[2] mean age at last visit = 16.66 y, range 14.63 y – 19.05 y
[3] mother's age at menarche available for *n*=23, *n*=84, and *n*=19 girls with early, average, or late menarche, respectively
[a] comparing early to average
[b] comparing average to late
[c] comparing early to late

Birth Weight and Age at Menarche

Using the first set of specifications (without mother's age at menarche) there was suggestion of a relationship between birth weight and menarche (Table 3.2). The birth weight effect approached statistical significance for low birth weight group (≤ 1 SD from the sample mean, n= 28, p= 0.07) and high birth weight group (≥ 1 SD from the sample mean, n= 24, p=0.09) compared to the normal birth weight group (within +/- 1 SD from sample mean, n= 151). Subsequent analyses using the median value of each category to calculate a p-trend for birth weight was statistically significant (p-trend <0.01). The Kaplan-Meier curve for the for the three categories of birth weight (Figure 3.1) suggests an increased risk of earlier menarche of girls in the low birth weight category, followed by normal birth weight and high birth weight although the difference between groups was not statistically significant (p for χ^2 Wilcoxon statistic = 0.10). When birth weight was modeled as a continuous variable, the independent effect was significant (p= 0.002), with each 225-gram increase (approximately 8 ounces) in birth weight decreasing the risk of earlier age at menarche (HR= 0.90, 95% CI =0.84, 0.96).

Table 3.2: **Results From Cox Regression Model of Age at Menarche in DISC Girls**[†]

	***n* subjects**[*]	***n* subjects with age at menarche**	**Median age at Menarche**[a] **(y)**	**Hazard Ratio (95% CI)**	***p*-value**	***p*-trend**
Intervention Group	102	95	12.83	1.26 (0.91, 1.74)	0.17	
Usual Care	101	96	12.74	1.00		
Low Birth Weight[b]	28	26	12.62	1.53 (0.96, 2.45)	0.07	< 0.01
Normal Birth[c] **Weight**	151	143	12.75	1.00	--	
High Birth Weight[d]	24	22	13.21	0.65 (0.39, 1.07)	0.09	
BMI-for-age percentile < 50	84	77	13.21	1.00	--	<0.0001
BMI-for-age percentile 50-85	72	67	12.54	1.88 (1.31, 2.70)	<0.001	
BMI-for-age percentile ≥ 85	47	47	12.45	2.89 (1.89, 4.41)	<0.0001	
Quartile 1 Physical Activity (Low)	30	29	12.81	1.00	--	0.37
Quartile 2 Physical Activity	46	46	12.56	1.47 (0.89, 2.41)	0.13	
Quartile 3 Physical Activity	40	39	12.67	1.44 (0.85, 2.42)	0.18	
Quartile 4 Physical Activity (High)	79	70	13.04	0.68 (0.43, 1.09)	0.11	

[a] unadjusted
[b] median weight of category = 2665g, range 1899-2863g
[c] median weight of category = 3402g, range 2892-3884g
[d] median weight of category = 4281g, range 3941-5103g
[*] *n* of subjects may be less than 203 due to missing data
[†] analysis adjusted for race (not shown)

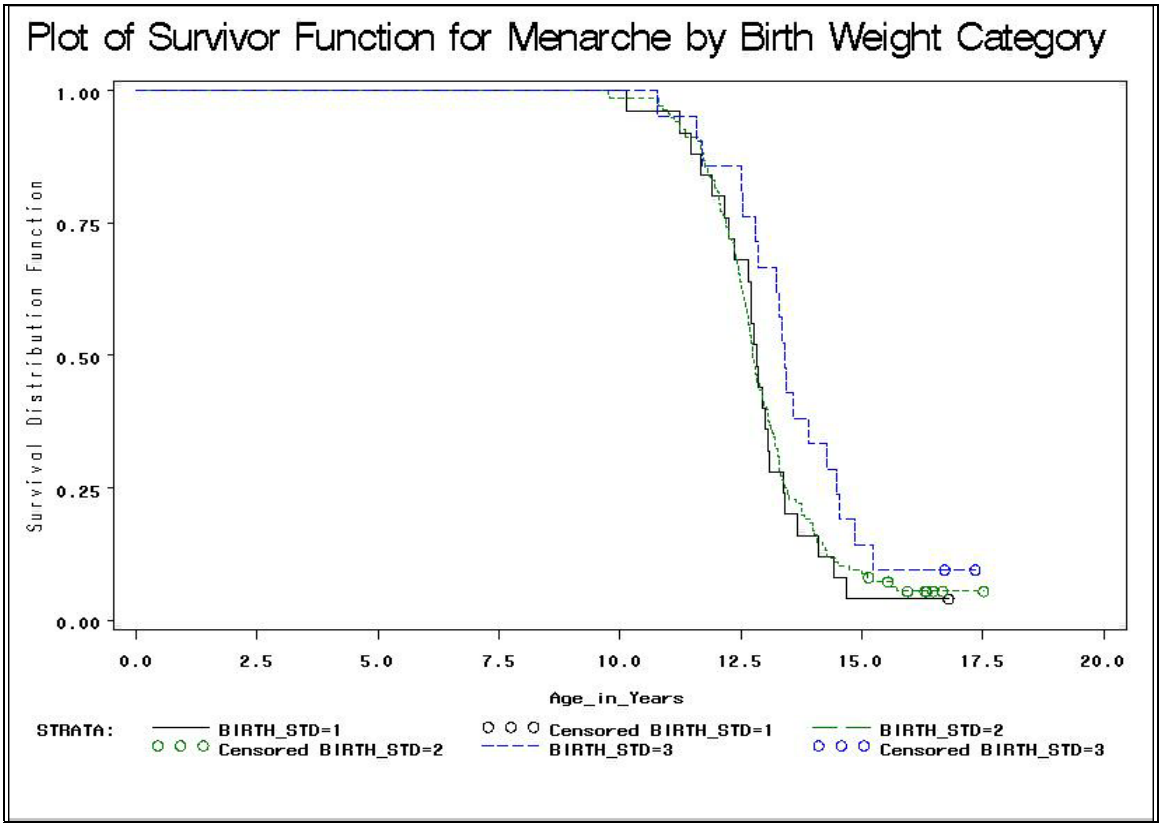

Figure 3.1: Kaplan-Meier curve of survival time until menarche for full sample categorized by birth weight where BIRTH_STD=1 represents birth weights ≤ 1 standard deviation below sample mean, BIRTH_STD=2 represents birth weight +/- 1 standard deviation of sample mean, and BIRTH_STD=3 represents birth weights ≥ 1 standard deviation of mean.

Analysis of the sub-sample which included mother's age at menarche yielded a strengthened association of birth weight with age at menarche. Compared to girls with normal birth weight (*n*= 98), girls with low birth weight (*n*= 16) were at increased risk of an earlier menarche (HR= 1.92, 95% CI = 1.07, 3.43, *p*= 0.03). No independent effect of high birth weight was detected (*p*= 0.51), but the *p*-trend across all three categories was statistically significant (*p*-trend 0.05). As with the analysis of the full sample, modeling birth weight as a continuous variable yielded a statistically significant independent effect

of birth weight (p= 0.01) with each 225-g increase in birth weight decreasing the risk of earlier menarche (HR= 0.88, 95% CI = 0.80, 0.97).

Table 3.3: **Results From Cox Regression Model of Age at Menarche in DISC Girls with Mother's Age at Menarche**[†]

	***n* subjects**[*]	***n* subjects with age at menarche**	**Median Age at Menarche**[a] **(y)**	**Hazard Ratio (95% CI)**	***p*-value**	***p*-trend**
Intervention Group	60	58	12.75	1.17 (0.78, 1.73)	0.45	--
Usual Care	70	68	12.83	1.00		
Mother's Age at Menarche[b]	130	126	12.77	0.75 (0.65, 0.86)	0.0001	--
Low Birth Weight[c]	16	16	12.88	1.92 (1.07, 3.43)	0.03	0.05
Normal Birth Weight[d]	98	95	12.72	1.00	--	
High Birth Weight[e]	16	15	13.23	0.82 (0.46, 1.46)	0.51	
BMI-for-age percentile < 50	52	49	13.29	1.00	--	<0.0001
BMI-for-age percentile 50-85	48	47	12.49	2.56 (1.64, 3.98)	<0.0001	
BMI-for-age percentile ≥ 85	30	30	12.64	3.14 (1.86, 5.32)	<0.0001	
Quartile 1 Physical Activity (Low)	19	18	12.67	1.00	--	0.09
Quartile 2 Physical Activity	31	31	12.47	0.91 (0.48, 1.72)	0.77	
Quartile 3 Physical Activity	29	28	12.82	1.10 (0.58, 2.06)	0.77	
Quartile 4 Physical Activity (High)	50	48	13.01	0.65 (0.37, 1.13)	0.13	

[a] unadjusted
[b] median mother's age at menarche = 13.0 y
[c] median weight of category = 2722g, range 2296-2863g
[d] median weight of category = 3459g, range 2892-3884g
[e] median weight of category = 4196g, range 3941-5103g
[*] *n* of subjects may be less than 203 due to missing data
[†] analysis adjusted for race (not shown)

BMI-for-age and Age at Menarche

To investigate the effect of BMI-for-age associated with age at menarche, we used the individual's BMI-for-age percentile at the visit prior to onset of menses, hence the visit number for the BMI-for-age percentile used varied by individual. A total of 105 / 203 girls indicated for the first time that they were post-menarcheal at the year-5 visit. Thus, the year-3 visit was the most commonly used BMI-for-age percentile. The last available BMI-for-age percentile was used for girls with censored observations. We explored using BMI-for-age percentiles at multiple visits leading up to menarche as well as using the BMI-for-age percentile at visits ≥2 visits prior to reaching menarche, but neither of these tactics proved to be as predictive as BMI-for-age percentile for the visit directly prior to onset of menses.

Analyses using the first set of specifications (without mother's age at menarche) indicated a statistically significant inverse relationship between BMI-for-age percentile and age at menarche (Table 3.2). BMI-for-age percentile was categorized into one of three groups: $< 50^{th}$ percentile (n= 77, median value of category = 25.36), $50\text{-}85^{th}$ percentile (n= 67, median value of category = 67.57), or $\geq 85^{th}$ percentile (n= 47, median value of category =91.17). Compared to having a BMI-for-age percentile < 50, there was an independent effect of BMI-for-age in the $50\text{-}85^{th}$ percentile ($p< 0.001$), indicating an increased risk for earlier menarche with increased body fatness (HR= 1.88, 95% CI = 1.31, 2.70). The increased risk of earlier menarche was further strengthened among girs with a BMI-for-age percentile ≥85, (HR= 2.89, 95% CI= 1.89, 4.41, $p< 0.0001$) compared to individuals with a BMI-for-age percentile <50. The p-trend was highly significant ($p < 0.0001$). In addition, modeling BMI-for-age percentile as a continuous

variable provided a strong indication that increasing body fatness at the visit prior to reaching menarche is an independent predictor of earlier menarche with each 10-point increase in BMI-for-age percentile resulting in a 10% increased risk of earlier menarche (HR= 1.20, 95% CI 1.13, 1.27, p= 0.0001).

The effect of BMI-for-age percentile on age at menarche was an even stronger when the analysis was restricted to the sub-sample which included mother's age at menarche (Table 3.3). Compared to having a BMI-for-age percentile <50, there was a strong independent increased risk of earlier menarche for BMI-for age in the 50-85th percentile (HR= 2.56, 95% CI = 1.64, 3.98, p< 0.0001). An increased risk of earlier menarche was also detected for having a BMI-for-age percentile ≥85, (HR= 3.14, 95% CI = 1.86, 5.32, p< 0.0001) using the same referent. The p-trend was highly significant (p < 0.0001). Modeling BMI-for-age percentile as a continuous variable was also a strong predictor of menarche. Each 10-point increase in BMI-for-age percentile resulted in a 22% increased risk of earlier menarche (HR= 1.22, 95% CI 1.14, 1.31, p< 0.0001).

Physical activity and age at menarche

Using the first set of specifications (without mother's age at menarche) there was no indication that higher levels of physical activity contribute independently to a delay in age at menarche (Table 3.2). Physical activity was quantified in MET scores and categorized into quartiles. Compared to individuals in the lowest quartile of physical activity, no independent effect was found with membership in either the second, third or fourth quartiles of MET score (p= 0.13, 0.18, 0.11 respectively). Restricting the analysis to the sub-sample with mother's age at menarche also yielded no independent effect of

the individual quartiles of MET score compared to membership in the lowest quartile, however the overall *p*-trend approached statistical significance at 0.09 (Table 3.3). Modeling MET score as a continuous variable did not yield a statistically significant results using either set of covariates. In an effort to rule out multicollinearity between BMI-for-age percentile and physical activity, we calculated the Pearson correlation for BMI and physical activity as continuous variables, but the correlation was not statistically significant (r = -0.05, *p*-value= 0.53).

Discussion

The major finding of this research is that birth weight is positively related to age at menarche, independent of recent BMI-for-age percentile, recent physical activity, and mother's age at menarche. In our well-nourished sample, specific dietary components appear to have little influence on age at menarche. The onset of menses requires environmental and genetic factors synchronized with the HPO axis and endocrine organs, including adipose tissue (Goldman et al., 2000; Stavrou et al., 2006). In addition to genetic factors, earlier age at menarche has been associated with increased body weight and fatness (Tanner, 1962) and our data are consistent with previous research. Two diet-related pathways (direct and indirect) have been proposed to influence timing of menarche (Goldman et al., 2000). The direct pathway hypothesizes an influence of diet composition independent of body fatness (Merzenich et al., 1993), whereas the indirect pathway influences menarche through body composition (Moisan et al., 1990). We found no significant effect of specific dietary components independent of BMI-for-age percentile, and as such, our data support the indirect pathway. However, it is possible

that since our subjects were participating in a dietary intervention that their intake lacked sufficient variability to detect an effect.

Results from the birth weight and age at menarche analyses of the present study confirmed those of Medical Research Council (MRC) National Survey of Health and Development Birth Cohort 1946 (Cooper et al., 1996). In that study, the group with the youngest age at menarche (mean= 11.89 years) also had the lowest birth weight (< 2850 g) and were heaviest (> 24.9 kg) at 7 years. When birth weight and weight and height at age 7 were simultaneously included in a survival model, both birth weight ($p< 0.0001$) and weight at age 7 years ($p< 0.0001$) were significantly associated with age of menarche. However, the study did not investigate mother's age at menarche or physical activity, the former of which was influential in our sample. Later analyses of the same cohort (dos Santos Silva et al., 2002) initially suggested that lower birth weight was associated with earlier age at menarche, but the association with birth weight, as well as the effect of growth velocity between birth and 2 years of age, disappeared when growth velocity from 2-7 years was included in the model. This finding suggests that the effects of early life characteristics on timing of menarche are mediated through childhood growth from 2-7 years of age (dos Santos Silva et al., 2002). If such an effect is true, it helps to explain why lower birth weight is associated with earlier age at menarche yet high birth weight is associated with increased BCA risk. In the DISC sample, our proxy of growth velocity between birth and study entry (mean age= 9.14 y) was not significant. Annual height and weight measurements between birth and study entry were not available, but if accessible would have allowed us to better refine the growth velocity approximation, and may have yielded different results. However, similar to the earlier

MRC cohort investigation, mother's age at menarche and physical activity were not controlled for in the latter dos Santos Silva investigation (2002). Given that studies of twins suggest that genetic factors may be more important than environmental characteristics in determining the onset of menarche and estimate that 53 -74% of the variation in age of menarche is genetic (van den Akker et al., 1987; Treloar & Martin, 1990; Kaprio et al., 1995), controlling for mother's age at menarche would likely have a profound effect in the MRC cohort investigation.

Our results are comparable to those of 997 female participants in the Cebu Longitudinal Health and Nutrition Survey (Adair, 2001), which found that girls born long and thin (> 49 cm, < 3 kg) attained menarche ~ 6 months earlier (HR = 1.61, 95% CI, 1.27 – 2.04) than girls born short and heavy (< 49 cm, > 3 kg). However, in this Peruvian population where dietary inadequacy and growth restriction are likely to be more common than in our U.S. population, birth weight alone was not found to be significantly related to menarche. Faster growth created a more pronounced effect of thinness at birth on age of menarche, and was unmodified by BMI and skin-fold thickness at age 8 years. These findings suggest that fetal growth restriction followed by rapid post-natal growth may be predictive of early menarche.

Our results, although similar to those described by other groups, still leaves one to ponder the continuum of the relationship between *in utero* hormone exposure, birth weight, age at menarche, and BCA risk in adulthood. First, we must acknowledge that although the body of evidence linking birth weight and many chronic diseases is robust, our knowledge of the specific biological mechanisms influencing pre-natal programming is meager. In the field of BCA epidemiology, much of the literature uses birth weight as

a proxy measure for *in utero* estrogen exposure. The use of this proxy is based on positive associations between maternal circulating estrogens during pregnancy and offspring's birth weight (Petridou et al., 1990; Kaijser et al., 2000; Mucci et al., 2003). However, the majority of studies that have investigated the correlation between the circulating hormone concentrations in the maternal and fetal compartments have indicated that the correlations for the estrogens are not high and that umbilical cord estrogen concentrations are not associated with birth weight (Simmons et al., 1994; Shibata et al., 2002; Troisi et al., 2003a). Additionally, concern has been raised that levels and variation in plasma volume expansion during pregnancy were not considered in the studies assessing maternal hormone concentration and birth weight (Faupel-Badger et al., 2007). Clearly, additional work is needed to measure hormones in the fetal circulation and describe the association with birth characteristics. This could be accomplished either through measurements of the cord blood at birth or through clinically indicated fetal blood samples (ie. during karotyping). In the interim, research must continue to explore alternate explanations for the association between birth weight, menarche, and BCA. In particular, greater attention is needed on the effects of androgen exposure *in utero*. Fetal androgens are involved in imprinting the hypothalamus for the pulsatile control of gonadotropin-releasing hormone (GnRH) which is responsible for the onset of puberty (Barraclough & Gorski, 1961; Terasawa, 2006). In humans, early elevated testosterone exposure, as measured by the right hand ratio of the length of the 2^{nd} and 4^{th} digits (2D:4D ratio), suggests that early increased androgen exposure is associated with delayed menarche (Matchock, 2008a). Moreover, it is speculated that *in utero* androgen exposure may offer BCA protection by antagonizing the effects of

estrogens on ductal development in the fetal breast (Troisi et al., 2003c). A recent study of steroid hormone concentration in Chinese and U.S. Caucasian neonates found that after multivariate adjustment, Chinese infants had significantly higher androstenedione (60.5%) and testosterone (185%) compared to U.S. Caucasian infants (Troisi et al., 2008). These data may help explain the lower BCA incidence rates in Asians not explained by adult risk factors. In addition, Asian-Americans and well- nourished Chinese have been reported to have a relatively early menarche compared to U.S. Caucasians, despite lower average fat mass in early adolescence (Elveleth & Tanner, 1990; Lin et al., 1992; Koprowski et al., 1999). Although the number of Asian-American participants in our study is too small (n= 6) to make any conclusions, it is interesting to note that 4/6 had an early age at menarche (< 11.75 y).

We did not detect an independent effect of increased MET score at the visit prior to reaching menarche (a measure of physical activity) on the outcome variable of age at menarche although the analyses were suggestive that girls belonging to the highest quartile of MET score may exhibit a delay in the onset of menarche. It is possible that the time lag between the between onset of menses and the prior visit was too large to detect the effect of recent changes in physical activity.

Several limitations of the present study should be addressed in future studies examining the relationship between birth weight and menarche, such as including other measures of birth size (eg., gestational age and birth length), categorizing childhood growth velocity more precisely, and assessing whether the mother was preeclampsic during the index pregnancy. Preeclampsic prenatal environments may reduce the daughter's risk of developing BCA (Xue & Michels, 2007). Also, elevated maternal

serum androstenedione and testosterone (Troisi et al., 2003c) and lower plasma estradiol (Ranta *et al.*, 1980) have been documented among preeclampsic mothers. Reports investigating exposure to prenatal preeclampsia and age at menarche are limited but fail to detect an independent association (Persson et al., 1999; Ros et al., 2001; Vatten et al., 2003).

Benefits of our study include the availability of prospectively collected measurements beginning at study entry, including detailed information on timing of menarche, measured height and weight prior to and during puberty, and the availability of mother's age at menarche for a subset of study population. Although our sample lacks racial/ethnic diversity, previous research has suggested that perinatal characteristics as surrogates for hormone levels should be limited to a specific ethnic group due to significant variation in effect (Zhang et al., 2007), and hence our racial homogeneity is a strength.

In summary, findings from DISC suggest that decreased birth weight, increased BMI-for-age percentile and younger mother's age at menarche are associated with daughter's earlier age at menarche. Future work should aim to clarify the role of growth velocity (including height, height, and BMI velocity) during specific ages of childhood. Additional work is needed to determine whether birth characteristics (eg. birth weight) are suitable proxy measures for fetal hormones exposure. The mechanisms underlying the association of lower birth weight and earlier puberty are currently unknown, but increased fetal androgen exposure may play a role.

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Chapter 4

BIRTH WEIGHT AND SEX STEROID HORMONE CONCENTRATIONS DURING ADOLESCENCE

Introduction

There is growing interest in the relationship between early life exposures and breast cancer (BCA) risk later in life. The prenatal period and puberty are critical times for maturation of the hypothalamic pituitary ovarian (HPO) axis, which via complex feedback loops maintains ovarian hormone concentrations, including estrogens, progesterone, testosterone and androstenedione in the normal range (Winter et al., 1995). Endogenous exposure per unit of body weight to these hormones is highest *in utero*, surpassing even that experienced in puberty and during pregnancy (Dorgan et al., 2003b; Troisi et al., 2003a). Birth weight, which is associated with maternal estrogen levels during pregnancy (Petridou et al., 1990; Kaijser et al., 2000; Mucci et al., 2003), is a commonly used proxy measure for *in utero* hormone exposure. Furthermore, birth weight has been positively associated with breast cancer (BCA) later in life, thus suggesting a link between fetal hormone exposure and future breast cancer (BCA) risk (Michels & Xue, 2006).

The relationship between measured sex hormone concentrations during childhood and adolescence and later risk of BCA has not been studied although a substantial body of epidemiological, clinical, and experimental research suggests that adult hormone concentrations play a major role in the etiology of female BCA. Higher circulating estrogens and androgens as well as lower SHBG concentrations were associated with increased risk for post-menopausal BCA in a pooled analysis of nine prospective studies (Key *et al.*, 2002). Among pre-menopausal women, the evidence is complicated by the cyclic variation of estrogens and progesterone during the menstrual cycle, but results

from a large, prospective nested case-control study with blood samples timed within the menstrual cycle suggest that elevated circulating estrogens and androgens are important in the etiology of pre-menopausal BCA (Eliassen et al., 2006). These results are supported by studies of breast epithelial tissue that show cell proliferation rates are low during the follicular phase of the menstrual cycle, when estradiol and progesterone levels are low, and higher during the luteal phase when levels are higher (Winter *et al.*, 1995). Since breast tissue is largely undifferentiated prior to age 20 years (Russo & Russo, 1997) and hormone levels are elevated during puberty, it is feasible that adolescence could also be an important period for hormonal influence on future BCA risk.

We hypothesize that birth weight, as a proxy for *in utero* hormone exposure, could potentially influence BCA risk via long-term effects on sexual maturation and pubertal sex hormone levels. To explore the association between birth weight and sex hormones before and during puberty, we evaluated the association of birth weight with serum estrogens, progesterone, androgens and SHBG concentrations among girls participating in the Hormone Ancillary Study (HAS) of the Dietary Intervention Study in Children (DISC). DISC/HAS was a randomized clinical trial designed to assess the efficacy and safety of a low-fat diet intervention on serum cholesterol and serum hormones. We hypothesize that abnormal birth weight (low/high) is associated with increased levels of serum estrogens, progesterone and androgens before and during puberty in girls after controlling for potential confounders such as DISC intervention group and anthropometrics. This hypothesis is consistent with previous reports of an association of lower birth weight with earlier menarche (dos Santos Silva *et al.*, 2002) but

an increased risk of BCA with higher birth weight (Forman *et al.*, 2005; Michels & Xue, 2006).

Subjects and Methods

DISC Study Design

DISC was a two-armed, multi-center, randomized clinical trial sponsored by the National Heart Lung and Blood Institute (NHLBI) and conducted at six clinical centers (Children's' Hospital, New Orleans, LA; Johns Hopkins Hospital, Baltimore, MD; Kaiser Permanente Center for Health Research, Portland, OR; Univ. of Medicine and Dentistry of New Jersey, Newark, NJ; Northwestern Medical School, Chicago, IL; and Univ. of Iowa Hospital, Iowa City, IA). Data management was performed by the coordinating center at Maryland Medical Research Institute, Baltimore, MD. Assent was obtained from DISC participants and written informed consent was obtained from their parents or guardians. Institutional Review Boards at all participating centers approved the DISC protocol, and an NHLBI-appointed independent data and safety monitoring committee provided oversight.

A total of 663 participants (362 boys and 301 girls), 8-10 years old with elevated LDL-cholesterol levels were randomly assigned to either dietary intervention (n = 334) or usual care (n= 329) between 1988 and 1990. Overall trial results have been published (Van Horn et al., 2003). The initial DISC protocol was designed for three years of intervention and was subsequently extended with planned intervention and follow-up until participants reached 18 years of age. However, DISC was terminated in 1997, when the mean age of participants was 16.7 years, because the participants in the two treatment

groups did not have statistically significantly different serum LDL-cholesterol levels (Obarzanek et al., 2001). In total, five visits for the female participants are included in the present analysis: baseline, Year-1, Year-3 Year-5 and last visit (median duration 7.0 years, range = 6.4-9.1 y).

Diet Intervention

The DISC dietary intervention featured a food pattern designed to reduce intake of total fat to ≤28% calories and saturated fat to ≤8% calories, increase polyunsaturated fat intake to 9% calories and monounsaturated fat to 11% calories, reduce cholesterol intake to 75 mg per 1000 calories (to a maximum of 150 mg per day), maintain protein intake at 14% calories (2/3 animal protein, 1/3 vegetable protein), and maintain carbohydrate intake at 58% calories. At the year-3 and last visit, the intervention girls achieved 28.7% and 27.4% calories dietary fat, 10.3% and 9.6% calories from saturated fat, 95 mg and 99 mg dietary cholesterol / 1000 calories, respectively (Dorgan et al., 2003b).

Female Participants in the DISC

Subjects were recruited through schools, health maintenance organizations and pediatric practices. Female eligibility requirements for the overall DISC trial included baseline age of 8 to 10 years, a serum LDL cholesterol level in the 80-90th percentiles (Department of Health and Human Services, 1980), no major illnesses, and no medications that could affect blood lipid levels or growth. In addition, participants had to be at or above the 5th percentile for height and in the 5-90th percentiles for weight-for-

height according to growth data from the Bogalusa Heart Study (Dorgan et al., 2003b), be Tanner Stage 1 for breast and public hair development (Tanner, 1962), and have normal cognitive and psycho-social development as assessed by use of the Achenbach Child Behavior Checklist (Achenbach & Edelbrook, 1983). Girls whose families or themselves were already following a low-fat diet, with a parent with early heart disease, with a planned family move in the next 3 years or with known behavioral problems were excluded. Subjects remained under the care of their primary care physicians during the study and the LDL-cholesterol criteria were set so that children with severe hypercholesterolemia in need of possible pharmaceutical intervention were excluded.

Of the 301 girls randomly assigned to one of two study arms in DISC, 286 participated in the HAS at one or more visits and 203 had a documented birth weight. The HAS was initiated after the randomization in DISC. Therefore, baseline blood samples (which were collected prior to randomization) were not available for 218 of the 286 girls and year-1 samples were unavailable for 97 of the 286 girls. Girls were ineligible to participate in HAS if they were pregnant or were using oral contraceptives during the three months prior to blood collection, if they were post-menarcheal but were missing data on the date of their next menses, or if their next menses started more than 33 days after blood collection.

Data Collection

Data were collected prior to randomization and annually thereafter by trained staff who were blinded to treatment assignment. Demographic characteristics, medical history, and use of medications were obtained via self-report with parental assistance

where necessary. Birth weight was included in the mothers'/guardians' questionnaire and reported at the year-3 visit to the closest ounce or gram. Maternal recalled birth weight is generally very close to the true birth weight, with correlations as high as 0.94 (Tomeo et al., 1999; O'Sullivan et al., 2000; Walton et al., 2000; Buka et al., 2004). Height was measured with stadiometers centrally constructed by the Medical Instruments Unit of the University of Iowa. Weight was measured using a beam balance or digital scales that were calibrated weekly against a range of standard weights ranging between 20 -100 kg. BMI was calculated with measured height and weight (BMI: weight in kg/ height in m^2). Diet information was obtained through a series of three 24-hour recalls; one in person and two by telephone by means of the Nutrition Data System (version 2.2-2.4, Nutrition Coordinating Center, University of Minnesota). Physical activity was assessed with a questionnaire designed to determine time spent in five intensity levels of physical activity, from which an estimated-metabolic-equivalent (MET) score was calculated by multiplying the number of hours spent on each level of activity intensity by a MET (multiple of resting energy expenditure).

A single blood sample was collected by venipuncture in the morning after an overnight fast at baseline and at the year-1, year-3, year-5, and last visits. Serum was separated out then aliquoted and stored in glass vials at -80°C until it was analyzed for hormone, lipid and micronutrient levels (DISC Collaborative Research Group, 1995; Obarzanek *et al.*, 1997; Obarzanek *et al.*, 2001). Post-menarche girls completed menstrual cycle calendars for six weeks before and six weeks after their blood collection. Blood collections were not timed to the menstrual cycle instead the day of the menstrual

cycle that corresponded to the date of the blood collection was determined from the menstrual cycle calendars and taken account for in the analyses.

Hormone Assays

Hormone assays were performed by Esoterix Endocrinology, Inc. (Calabasas Hills, CA). Steroid hormones were measured by radioimunnoassay, and SHBG was measured by an immunoradiometric assay (Dorgan et al., 2001; Dorgan et al., 2002). The concentration of non-SHBG bound estradiol was calculated as the product of the total estradiol concentration and the percent non-SHBG bound estradiol, which was measured by ammonium sulfate precipitation (Dorgan et al., 2001). To monitor the quality of hormone assays, masked quality control samples were included with participant samples in each batch. These samples were aliquots from three serum quality control pools that were created by serially diluting serum from adults with charcoal stripped serum to cover the range of steroid concentrations expected in participant samples. Within-visit coefficients of variation (CV) estimated from quality control samples were 8%-29% for estradiol, 12%-31% for estrone, 12%-17% for estrone sulfate, 4%-10% for progesterone, and 15% for SHBG. Low concentrations of hormones in some quality control samples may have contributed to the higher CVs observed for some hormones. For example, the mean concentrations of estradiol in samples from the three quality control pools were 0.9 ng/dl, 2.8 ng/dl, and 11.3 ng/dl and their corresponding within-visit CVs were 29%, 11%, and 8% (Dorgan et al., 2003b).

Statistical Analysis

Statistical analyses were performed using SAS software (SAS System for Windows, version 9.1; SAS Institute, Cary, NC). Birth weight was categorized into one of three categories: < 1 standard deviation (SD) below the sample mean ('low' birth weight), within +/- 1 SD of the sample mean ('average' birth weight), or > 1 SD above the sample mean ('high' birth weight). BMI-for-age percentile, weight-for-age percentile, and height-for-age percentile were calculated using the Centers for Disease Control SAS Program for Growth Charts (U.S. Centers for Disease Control, 2007). One-way ANOVA analyses were used to compare differences in mean baseline BMI-for-age percentile, weight-for-age percentile, height-for-age percentile, total daily energy intake, and age at menarche between low, average, and high birth weight groups. Chi-square tests were used to compare differences in treatment groups.

Mixed linear regression models (SAS Proc Mixed) were used to calculate the mean hormone concentrations during follow-up according to category of birth weight after controlling for potential covariates. Differences between the mean values of each category were computed using the least squares means of fixed effects. Where indicated, serum hormone data were transformed before analysis to improve normality and the means were reverse-transformed to compute the reported geometric means and 95% confidence intervals (95% CI). Separate models were used to fit pre- and post-menarcheal samples, with additional separate models for the follicular and luteal phases for estradiol, non-SHBG bound estradiol, estrone, estrone sulfate, and progesterone. Progesterone was only measured for the post-menarcheal girls. Post-menarche serum samples collected <15 days before the next menses were classified as luteal, and samples

collected 15 through 33 days before or on the day of onset of the next menses were classified as follicular. Post-menarcheal girls whose next menses started more than 33 days after blood collection were not included in the analyses. Androgens and SHBG, unlike estrogens and progesterone, do not vary substantially over the course of the menstrual cycle although the level of SHBG produced by the liver declines at puberty. These anticipated variations were reflected in the decision to use five separate regression models (pre-menarcheal samples for all hormones and SHBG, pre- and post-menarcheal samples combined for androgens, post-menarcheal samples for androgens and SHBG, and separate luteal and follicular phase samples for the estrogens and progesterone) with two sets of covariates. The first set of covariates adjusted for visit number, treatment group, age in years, height-for-age percentile, weight-for-age percentile, mean daily energy / body weight, and a categorical variable for day of menstrual cycle the blood was collected (post-menarche girls, only). The second set adjusted for the aforementioned variables plus years until menarche for the pre-menarche samples and years since menarche for the post-menarche samples. Our previous analyses found an association of birth weight with age at menarche (Chapter 3). Therefore, if birth weight is associated with hormones without a menarche timing variable in the model but is not associated once this variable is included, it would suggest that birth weight is associated with hormones via effect on age at menarche. These covariates were chosen for their ability to explain the most variance after examining over 25 potential variables.

Results

Baseline Characteristics

A total of 203 (71%) of the 286 girls who participated in the HAS had a documented birth weight. The mean birth weight was 3399 g (SD= 508, range 1899-5103). Nine girls (4%) were of clinical low birth weight (≤ 2500g at birth) and twenty girls (10%) weighed ≥ 4000g. Baseline characteristics of the participants according to category of birth weight are presented in Table 4.1. There were no significant differences in unadjusted BMI-for-age percentile, height-for-age percentile, weight-for-age percentile, MET score at baseline, or total daily energy across categories of birth weight. Mean age at menarche was significantly higher for girls in the high birth weight category compared to girls with the low birth weight category. This finding was explored in greater depth in Chapter 3.

Table 4.1**: Characteristics of girls who participated in the Dietary Intervention Study/ Hormone Ancillary Study according to birth weight**

	Birth Weight Category (range in grams)			
	Low Birth Weight (1899-2863) *n* = 28	**Average Birth Weight** (2892-3884) *n* =151	**High Birth Weight** (3941-5103) *n* = 24	***p*-value**
Birth Weight, mean (SD)	2571 (257.99)	3412 (262.78)	4288 (282.29)	--
Treatment Group Intervention (*n*)	13	75	14	0.67‡
Usual Care (*n*)	15	76	14	
BMI-for-age percentile at study baseline [1], mean (SD)	50.23 (29.75)	56.87 (27.32)	57.17 (27.52)	0.25[a] 0.96[b] 0.37[c]
Height-for-age percentile at Study baseline, mean (SD)	46.51 (28.76)	49.07 (27.64)	44.23 (27.93)	0.66[a] 0.43[b] 0.77[c]
Weight-for-age percentile at Study baseline mean (SD)	47.88 (30.28)	53.18 (28.55)	51.10 (24.76)	0.37[a] 0.74[b] 0.68[c]
MET score at baseline, mean (SD)	333.28 (50.57)	330.01 (44.13)	316.66 (39.87)	0.72[a] 0.18[b] 0.18[c]
Total daily energy intake at baseline, mean (SD)	1635.46 (320.14)	1631.26 (425.47)	1653.79 (308.77)	0.96[a] 0.80[b] 0.87[c]
Age at Menarche, y, Mean, mean (SD)	12.61 (1.05)	12.75 (1.03)	13.31 (1.12)	0.53[a] 0.06[b] 0.05[c]
Race† (*n*) **White**	23	134	21	
Black	3	8	3	
Asian	0	6	0	

SD= standard deviation
[1] mean age at baseline= 9.14 y, range 8.02 y – 10.35 y
[+] p-value from ANOVA unless noted otherwise
[a] comparing low to average
[b] comparing average to high
[c] comparing low to high
‡ *p*-value from χ^2 test
† Race not reported for *n*= 5 individuals

Hormone Concentrations in Pre-menarcheal Girls

The median duration until menarche for girls at the time of the pre-menarche blood collections was 2.50 y (range 0.003 – 7.05 y). The model without years until menarche detected a significant association between birth weight and DHEAS concentration (p= 0.05) and androstenedione concentration (p= 0.05) (Table 4.2). The association of birth weight and concentration of non-SHBG bound estradiol and testosterone concentration approached statistical significance using the same set of covariates (p= 0.11 and 0.09, respectively). No significant effect of birth weight with any of the hormones or SHBG was observed in the models that adjusted for years until menarche.

Mean concentrations of estradiol, estrone, estrone sulfate and SHBG were generally similar across categories of birth weight. The mean non-SHBG bound estradiol, androstenedione, testosterone and DHEAS concentrations were significantly higher among girls with low birth weight compared to girls with normal birth weight in the model that did not adjust for years until menarche ($p\leq$ 0.05). These results indicate that prior to the onset of menses, the association of birth weight with DHEAS and androstenedione concentrations likely occurs via an effect of birth weight on age at menarche. Furthermore, the effect is being driven by higher hormone concentrations among the girls in the low birth weight category. The evidence is suggestive that a similar phenomenon may occur with non-SHBG bound estradiol and testosterone concentrations prior to menarche although these relationships did not achieve statistical significance.

Table 4.2 **Mean pre-menarche serum hormone and SHBG concentrations in DISC girls according to birth weight**

	n samples *n* individuals (max samples per individual)	**Low Birth Weight** (< 2891 g)	**Average Birth Weight** (2892-3907 g)	**High Birth Weight** (>3908 g)	***p*-value**
		Mean (95% CI)			
Estradiol, ng / dL†	316 192 (4)	2.51 (1.99, 3.09)	2.06 (1.82, 2.32)	2.48 (1.98, 3.04)	0.11
Estradiol, ng / dL‡	346 202 (5)	1.98 (1.28, 2.82)	1.53 (1.09, 2.06)	1.80 (1.15, 2.59)	0.22
Non-SHBG-bound estradiol, ng/dL†	265 192 (3)	1.34 (1.05, 1.66)	1.10 (0.95, 1.27)	1.27 (0.99, 1.58)	0.15
Non-SHBG-bound estradiol, ng/dL‡	290 202(4)	0.97 [a] (0.57, 1.49)	0.67 [a] (0.41, 1.00)	0.69 (0.36, 1.13)	0.11
Estrone, ng / dL†	316 192 (4)	2.06 (1.76, 2.39)	1.91 (1.77, 2.07)	2.17 (1.87, 2.49)	0.22
Estrone, ng / dL‡	346 202 (5)	1.63 (1.25, 2.07)	1.42 (1.15, 1.71)	1.52 (1.16, 1.94)	0.32
Estrone Sulfate, ng /dL†	245 192 (3)	47.29 (39.24, 57.01)	42.44 (38.20, 47.03)	48.05 (39.85, 57.95)	0.36
Estrone Sulfate, ng /dL‡	267 202 (4)	42.79 (32.26, 56.76)	35.72 (28.85, 44.23)	36.81 (27.77, 48.78)	0.21
Androstenedione, ng / dL†	312 192 (3)	83.20 (70.59, 95.81)	72.93 (66.92, 78.93)	78.78 (66.29, 91.28)	0.24
Androstenedione, ng / dL‡	340 202 (5)	76.88 [a] (60.49, 93.27)	61.03 [a] (49.63, 72.42)	61.45 (45.36, 77.54)	**0.05**
Testosterone, ng / dL†	260 192 (3)	15.24 (11.97, 19.40)	12.58 (10.97, 14.43)	14.07 (11.09, 17.84)	0.20
Testosterone, ng / dL‡	284 202 (4)	14.28 [a] (9.88, 20.64)	10.80 [a] (8.13, 14.335)	11.21 (7.80, 16.11)	0.09
DHEAS, µg/dL†	317 192 (4)	54.33 (42.15, 70.04)	42.30 (37.76, 47.36)	46.56 (36.21, 59.87)	0.16
DHEAS, µg/dL‡	347 202 (5)	57.55 [a] (42.57, 77.80)	41.72 [a] (34.39, 50.62)	43.84 (32.56, 59.03)	**0.05**
SHBG, nmol†	317 192 (4)	107.00 (91.35, 122.65)	97.02 (89.94, 104.09)	99.82 (84.33, 115.31)	0.47
SHBG, nmol‡	347 202 (5)	100.39 (83.50, 117.27)	100.60 (89.33, 111.88)	109.60 (92.98, 126.22)	0.44

SD= standard deviation CI= Confidence Interval, SHBG = sex hormone binding globulin,
DHEAS = dehydroepiandrosterone sulfate

† Geometric means and 95% CI adjusted for visit number, treatment, age, height-for-age percentile, weight-for-age percentile, average kcals/ body weight, years until menarche

‡ Geometric means and 95% CI adjusted for visit number, treatment, age, height-for-age percentile, weight-for-age percentile, average kcals/ body weight

[a] items in same row marked with same letter differ at $p \leq 0.05$

Androstenedione, Testosterone, DHEAS and SHBG in Pre- and Post-Menarche Girls

Birth weight was associated with DHEAS concentration in both the model including years until or years since menarche (p= 0.05) as well as in the model not adjusted for menarcheal timing (p= 0.02) for the combined pre- and post-menarche samples (Table 4.3). None of the other hormones were significantly associated with birth weight using either set of covariates. Concentrations of androstenedione were generally similar across categories of birth weight. The mean DHEAS concentration was significantly higher among individuals with low birth weight compared to individuals with normal birth weight ($p\leq$ 0.05) in both sets of models (with and without years since menarche). The mean testosterone concentration for low birth weight girls was significantly higher compared to average birth weight girls ($p\leq$ 0.05) in the model not adjusted for years until/ since menarche.

In sum, the findings are indicative of a relationship between birth weight and DHEAS that is independent of the effect of birth weight on age at menarche. Moreover, the effect is occurring largely though the higher DHEAS concentrations among girls with lower birth weight.

Table 4.3 **Mean serum hormone concentrations in DISC girls (pre- and post-menarche combined), according to birth weight**

	n samples *n* individuals (max samples per individual)	**Low Birth Weight** (< 2891 g)	**Average Birth Weight** (2892-3907 g)	**High Birth Weight** (>3908 g)	***p*-value**
		Mean (95% CI)			
Androstenedione, ng / dL†	531 191 (4)	116.79 (102.85, 130.74)	106.04 (100.37, 111.72)	105.13 (90.91, 119.35)	0.35
Androstenedione, ng / dL‡	622 203 (5)	110.05 (96.77, 123.33)	98.84 (92.95, 104.74)	100.39 (87.11, 113.66)	0.30
Testosterone, ng / dL†	527 191 (4)	19.94 (13.33, 29.82)	16.82 (11.48, 24.64)	16.71 (11.00, 25.37)	0.22
Testosterone, ng / dL‡	552 201 (4)	20.86 [a] (17.43, 24.95)	17.33 [a] (16.12, 18.63)	17.72 (14.81, 21.21)	0.16
DHEAS, µg/dL†	536 191 (4)	102.85 [a] (75.61, 139.91)	79.38 [a] (61.34, 102.70)	85.76 (62.41, 117.82)	**0.05**
DHEAS, µg/dL‡	562 201 (4)	99.48 [a] (81.64, 121.23)	73.20 [a] (67.51, 79.38)	78.43 (64.12, 95.92)	**0.02**

SD= standard deviation CI= Confidence Interval, SHBG = sex hormone binding globulin,
DHEAS = dehydroepiandrosterone sulfate
† Geometric means and 95% CI adjusted for visit number, treatment, age, height-for-age percentile, weight-for-age percentile, average kcals/ body weight, menarche status, years until or since menarche
‡ Geometric means and 95% CI adjusted for visit number, treatment, age, height-for-age percentile, weight-for-age percentile, average kcals/ body weight, menarche status
[a] items in same row marked with same letter differ at $p \leq 0.05$

Androstenedione, Testosterone, DHEAS and SHBG in Post-Menarche Girls

The median duration since menarche at the time of blood collection was 2.12 y (range 0.01 – 6.69 y) for the post-menarche samples assessing androstenedione, testosterone, DHEAS, and SHBG. Birth weight was not associated with any of the hormone or SHBG concentrations although the association neared statistical significance for DHEAS and SHBG (Table 4.4). For DHEAS, the significance of the birth weight effect was the same in the model including and the model excluding years since menarche (p= 0.09), and for SHBG the effect was slightly stronger in the model including years since menarche compared with the model excluding this variable (p= 0.06 and p= 0.08, respectively). This association between birth weight and SHBG concentration was further strengthened when the models were calculated using birth weight as a continuous variable (p= 0.03 and p= 0.05 for the model including and excluding years since menarche, respectively).

Concentrations of androstenedione and testosterone were generally similar across categories of birth weight. The mean DHEAS concentration was significantly higher among individuals with low birth weight compared to individuals with normal birth weight ($p\leq$ 0.05) in both sets of models (with and without years since menarche). This suggests that the nearly statistically significant effect of DHEAS is being driven by higher concentrations in the low birth weight girls. The mean SHBG concentration for low birth weight girls was significantly lower compared to high birth weight girls ($p\leq$ 0.05) for both models.

Table 4.4 **Mean post-menarche serum hormone and SHBG concentrations in Dietary Intervention Study in Children (DISC) girls, according to birth weight**

	n samples *n* individuals (max samples per individual)	**Low Birth Weight** (< 2891 g)	**Average Birth Weight** (2892-3907 g)	**High Birth Weight** (>3908 g)	***p*-value**
		Mean (95% CI)			
Androstenedione, ng / dL†	270 161 (3)	149.52 (130.49, 168.55)	140.10 (131.18, 149.03)	148.00 (128.97, 167.02)	0.52
Androstenedione, ng / dL‡	271 162 (3)	148.72 (129.25, 168.19)	138.47 (129.41, 147.54)	140.86 (122.25, 159.46)	0.61
Testosterone, ng / dL†	268 161 (3)	30.75 (25.53, 37.04)	29.50 (27.13, 32.07)	32.07 (26.81, 38.36)	0.64
Testosterone, ng / dL‡	269 162 (3)	30.54 (25.30, 36.87)	29.12 (26.77, 31.67)	30.53 (25.68, 36.30)	0.80
DHEAS, µg/dL†	271 161 (3)	129.53 [a] (106.43, 157.64)	104.56 [a] (95.84, 114.09)	116.54 (96.19, 141.19)	0.09
DHEAS, µg/dL‡	272 162 (3)	128.88 [a] (105.82, 156.98)	103.66 [a] (95.03, 113.06)	115.57 (96.17, 138.89)	0.09
SHBG, nmol†	272 161 (3)	61.82 [a] (48.97, 74.67)	72.07 (66.48, 77.67)	83.09 [a] (70.63, 95.55)	0.06[1] **0.03[2]**
SHBG, nmol‡	273 162 (3)	61.37 [a] (48.34, 74.40)	71.26 (65.63, 76.89)	81.56 [a] (69.52, 93.59)	0.08[1] **0.05[2]**

SD= standard deviation CI= Confidence Interval, SHBG = sex hormone binding globulin,
DHEAS = dehydroepiandrosterone sulfate

† Geometric means and 95% CI adjusted for visit number, treatment, age, height-for-age percentile, weight-for-age percentile, average kcals/ body weight, years since menarche

‡ Geometric means and 95% CI adjusted for visit number, treatment, age, height-for-age percentile, weight-for-age percentile, average kcals/ body weight

[a] items in same row marked with same letter differ at $p \leq 0.05$

[1] *p*-value for categorical birth weight [2] *p*-value for continuous birth weight

Estradiol, Non-SHBG bound Estradiol, Estrone, Estrone Sulfate and Progesterone in Luteal Phase Samples

The median duration since menarche at the time of blood collection was 1.98 y (range 0.04 – 6.61 y) for the luteal phase samples of estradiol, non-SHBG bound estradiol, estrone, estrone sulfate and progesterone. No independent effect of categorical birth weight was detected for any of the luteal phase hormone samples. Furthermore, there were no significant differences in the mean hormone concentrations across categories of birth weight, although some comparisons were close to being statistically significant. In the model adjusted for years since menarche, the high birth weight group had higher mean estradiol and estrone concentrations compared to the normal birth weight group (p=0.08 and p= 0.06, respectively). No differences in hormone concentrations were detected between birth weight groups in the models which excluded years until menarche (Table 4.5).

We decided to examine the relationship with birth weight as a continuous variable since the relatively small number of individuals in the high birth weight category with luteal phase samples may have hindered our ability to detect a categorical effect. These analyses yielded positive, significant effects of estradiol (p= 0.04 including years until menarche, p= 0.08 excluding years until menarche), non-SHBG bound estradiol (p= 0.03 including years until menarche, p= 0.06 excluding years until menarche), and estrone (p= 0.05 including years until menarche, p= 0.18 excluding years until menarche). These results suggest that birth weight is positively associated with estradiol, non-SHBG bound estradiol, and estrone concentration during the luteal phase of the menstrual cycle.

Table 4.5 **Mean serum hormone and SHBG concentrations in Dietary Intervention Study in Children (DISC) girls, luteal phase of menstrual cycle, according to birth weight**

	n samples *n* individuals (max samples per individual)	**Low Birth Weight** (< 2891 g)	**Average Birth Weight** (2892-3907 g)	**High Birth Weight** (>3908 g)	***p*-value**
		Mean (95% CI)			
Estradiol, ng / dL †	129 101 (3)	9.46 (6.63, 13.50)	10.01 (8.54, 11.73)	13.46 (9.80, 18.49)	0.19[1] **0.04[2]**
Estradiol, ng / dL ‡	129 101 (3)	9.44 (6.58, 13.55)	9.87 (8.40, 11.60)	12.69 (9.24, 17.43)	0.31[1] 0.08[2]
Non-SHBG-bound Estradiol, ng/dL †	129 101 (3)	5.11 (3.37, 7.21)	5.62 (4.77, 6.55)	6.89 (5.05, 9.01)	0.36[1] **0.03[2]**
Non-SHBG-bound estradiol, ng/dL ‡	129 101 (3)	5.12 (3.36, 7.23)	5.58 (4.72, 6.51)	6.62 (4.83, 8.68)	0.48[1] 0.06[2]
Estrone, ng / dL†	129 101 (3)	5.40 (4.09, 7.13)	5.23 (4.61, 5.93)	6.75 (5.26, 8.67)	0.17[1] **0.05[2]**
Estrone, ng / dL‡	129 101 (3)	5.20 (3.90, 6.94)	5.12 (4.49, 5.84)	6.23 (4.83, 8.05)	0.36[1] 0.18[2]
Estrone Sulfate, ng /dL†	130 101 (3)	135.75 (91.12, 202.21)	138.06 (115.10, 165.62)	167.10 (117.88, 236.87)	0.58[1] 0.21[2]
Estrone Sulfate, ng /dL‡	130 101 (3)	132.50 (88.89, 197.53)	135.86 (113.11, 163.15)	158.25 (112.15, 223.30)	0.30[1] 0.38[2]
Progesterone, ng / dL†	128 101 (3)	105.60 (52.78, 211.26)	110.71 (81.06, 151.20)	101.59 (55.11, 187.26)	0.96[1] 0.93[2]
Progesterone, ng / dL‡	128 101 (3)	103.78 (51.48, 209.20)	108.98 (79.60, 149.23)	90.17 (49.15, 165.44)	0.84[1] 0.71[2]

SD= standard deviation CI= Confidence Interval, SHBG = sex hormone binding globulin,
† Geometric means and 95% CI adjusted for visit number, treatment, age, height-for-age percentile, weight-for-age percentile, average kcals/ body weight, menstrual cycle day, years since menarche
‡ Geometric means and 95% CI adjusted for visit number, treatment, age, height-for-age percentile, weight-for-age percentile, average kcals/ body weight, menstrual cycle day
[1] *p*-value for categorical birth weight [2] *p*-value for continuous birth weight

Estradiol, Non-SHBG bound estradiol, Estrone, Estrone Sulfate and Progesterone in Follicular Phase Samples

The median duration since menarche for the post-menarche, follicular phase estrogens and progesterone samples was 2.59 y (range 0.01 – 6.69 y). No independent effect of birth weight was detected for any of the follicular phase hormone samples (estradiol, non-SHBG bound estradiol, estrone, estrone sulfate and progesterone) (Table 4.6). In addition, there were no significant differences in the mean hormone concentrations across categories of birth weight.

Table 4.6 **Mean serum hormone concentrations in Dietary Intervention Study in Children (DISC) girls, follicular phase of menstrual cycle, according to birth weight**

	n samples *n* individuals (max samples per individual)	**Low Birth Weight** (< 2891 g)	**Average Birth Weight** (2892-3907 g)	**High Birth Weight** (>3908 g)	***p*-value**
		Mean (95% CI)			
Estradiol, ng / dL †	140 112 (3)	4.98 (3.50, 6.71)	4.50 (3.76, 5.32)	5.46 (3.59, 7.73)	0.57
Estradiol, ng / dL ‡	141 113 (3)	4.96 (1.87, 6.70)	4.45 (1.92, 5.27)	5.56 (1.94, 7.72)	0.46
Non-SHBG-bound estradiol, ng/dL †	140 112 (3)	4.98 (3.50, 6.71)	4.50 (3.76, 5.32)	5.46 (3.59, 7.73)	0.57
Non-SHBG-bound estradiol, ng/dL ‡	141 113 (3)	3.26 (2.40, 4.24)	2.44 (2.05, 2.87)	2.57 (1.70, 3.61)	0.19
Estrone, ng / dL†	140 112 (3)	4.03 (3.32, 4.90)	3.55 (3.22, 3.93)	3.87 (3.05, 4.92)	0.39
Estrone, ng / dL‡	141 113 (3)	4.03 (3.29, 4.92)	3.51 (3.16, 3.89)	3.98 (3.15, 5.02)	0.29
Estrone Sulfate, ng /dL†	140 112 (3)	95.14 (70.90, 127.68)	83.56 (71.57, 97.56)	82.84 (57.81, 118.71)	0.69
Estrone Sulfate, ng /dL‡	141 113 (3)	94.73 (70.51, 127.26)	82.71 (70.80, 96.61)	85.94 (61.20, 120.67)	0.66
Progesterone, ng / dL†	134 112 (3)	38.78 (24.41, 61.60)	31.36 (24.64, 39.91)	27.83 (15.50, 49.99)	034
Progesterone, ng / dL‡	135 113 (3)	38.18 (23.46, 62.14)	30.39 (23.56, 39.21)	35.03 (19.65, 62.44)	0.61

SD= standard deviation CI= Confidence Interval, SHBG = sex hormone binding globulin,
DHEAS = dehydroepiandrosterone sulfate

† Geometric means and 95% CI adjusted for visit number, treatment, age, height-for-age percentile, weight-for-age percentile, average kcals/ body weight, menstrual cycle day, years since menarche

‡ Geometric means and 95% CI adjusted for visit number, treatment, age, height-for-age percentile, weight-for-age percentile, average kcals/ body weight, menstrual cycle day

Discussion

In this study, birth weight was associated with DHEAS concentration across the seven years of follow-up, androstenedione concentration prior to menarche, and SHBG concentration during post-menarche visits. Concentrations of DHEAS and pre-menarche androstenedione among girls belonging to the low birth weight category were higher than girls in the average birth weight category, but no differences between the average and high birth weight categories were observed. Post-menarche concentrations of SHBG, a glycoprotein that binds to sex hormones and inhibits bioavailability, were found to be higher among the girls belonging to the high birth weight category compared to girls in the low birth weight category. Some luteal phase estrogens were positively associated with birth weight modeled as a continuous variable. Thus, our initial hypothesis that both the low and high birth weight categories would have higher hormone concentrations compared to the average category is partially supported.

Analyses were run with and without a covariate for years until / years since menarche based on our previous work indicating a significant association between birth weight and age at menarche. The effect of birth weight on pre-menarche DHEAS and androstenedione concentrations appears to occur in part via birth weight's effect on age at menarche. Among the post-menarche DHEAS samples, the effect of birth weight was marginally significant (p= 0.09) in both models, suggesting that the effect of birth weight on post-menarche DHEAS concentration is independent of its effect on age at menarche. Post-menarche SHBG appeared to be positively associated with birth weight, with the significance of the association greater when the analyses were adjusted for years since age at menarche. This association was further strengthened when the models were

calculated using birth weight as a continuous variable (p= 0.03 and p= 0.05 for the model including and excluding years since menarche, respectively). Among the luteal phase hormones, there was a positive relationship between birth weight and concentration of estradiol, non-SHBG bound estradiol and estrone although this relationship was only significant when birth weight was modeled as a continuous variable and included adjustment for years since menarche. No associations were found between birth weight and any of the follicular phase hormones. Our blood collections were not timed with the menstrual cycle and categorization of menstrual cycle phase was dependent on the accuracy of the girls' daily menstrual calendars. Since estradiol has greater variation in the follicular phase compared to the luteal phase, it is possible that inaccurate reporting on the menstrual calendars may have hindered our ability to detect an association between birth weight on the follicular phase hormone concentrations.

There are only a few published studies investigating the relationship of birth size with serum sex hormones during childhood or adolescence. A handful of cross-sectional studies have documented higher concentrations of DHEAS during childhood and adolescence among individuals born small-for-gestational age (SGA) compared to individuals born average-for-gestational (AGA) (Francois & de Zegher, 1997; Ibanez et al., 1998b; Tenhola et al., 2002). A cross-sectional study of pre-pubertal females reported a negative correlation between birth weight and estradiol and no association of birth weight with DHEAS (Tam et al., 2006). A study of adult women (aged 24-36 years) detected a positive association of ponderal index at birth (a measure of body size, calculated as birth weight/birth length^{3}x100) with salivary estradiol over the course of one menstrual cycle (Jasienska et al., 2006). Although it is impossible to make a direct

comparison between the latter two studies investigating estradiol, these findings illuminate the possibility that birth size may have different associations with the same hormones at different stages of life. Our study found no relationship between birth weight and estradiol prior to menarche, but detected a positive association of birth weight and estradiol among post-menarche luteal phase samples; DHEAS concentrations were associated with birth weight throughout the seven years of follow-up.

High birth weight and increased risk of BCA has been documented in both case-control and cohort studies across various geographic regions. Birth weight is likely a marker for an unknown mechanism influencing mammary carcinogenesis (Michels & Xue, 2006). Our study sought to investigate whether birth weight is associated with sex hormone concentrations during adolescence. Breast tissue is largely undifferentiated during adolescence and has increased susceptibility to mutations (Russo & Russo, 1997). If such a relationship between birth weight and adolescent sex hormone concentrations exists, it offers one possible pathway by which birth weight is associated with BCA risk.

Birth weight is a commonly used proxy measure for fetal hormone exposure. In a study of umbilical cord hormone concentrations and neonatal factors, the lowest unadjusted concentration of DHEA, DHEAS, estrone and testosterone were found among cord blood samples from infants in the lowest category of birth weight. Moreover, the same study found that birth weight was positively associated with umbilical cord DHEAS in an adjusted regression analysis (Troisi et al., 2003a). At term, approximately 90% of estriol (the major fetal estrogen) is produced by the placenta from fetal plasma 16α-hydroxy-DHEAS and 50% of the estradiol is produced from placental DHEAS (Casey & MacDonald, 1992). As such, the production of DHEAS by the fetal adrenal glands has a

major impact on fetal estrogen concentrations. In addition, positive associations between maternal circulating estrogens during pregnancy and offspring's birth weight have been documented (Petridou et al., 1990; Kaijser et al., 2000; Mucci et al., 2003) although concern has been raised over whether mother's circulating hormones are representative of fetal exposure (Troisi et al., 2003b).

The hypothesized role of endogenous estrogen exposure during early life and adolescence on future BCA risk is complex. Estrogens promote growth and induce breast cell proliferation by binding to estrogen receptors and regulating gene transcription (Tsai & O'Malley, 1994; Russo & Russo, 1997). Increased cell proliferation and mutagenic properties of estrogens give rise to the potential for carcinogenesis (Liehr, 2000), but estrogens can also promote cell differentiation which should offer some protection against mutations in the mammary gland in early life (Hilakivi-Clarke et al., 2002). It is hypothesized that *in utero* androgen exposure may offer BCA protection by antagonizing the effects of estrogens on ductal development in the fetal breast (Troisi et al., 2003c), possibly through a reduction in breast stem cell population (Baik et al., 2005; Michels & Xue, 2006; Troisi et al., 2008). In our study, low birth weight girls had higher levels of serum DHEAS and androstenedione and lower luteal phase estradiol, non-SHBG bound estradiol and estrone. We speculate that lower birth weight may be associated with higher androgen concentrations in childhood and adolescence which protect the developing breast tissue in a way not fully understood. In addition, our research suggests that circulating levels of some estrogens may be lower during the luteal phase of the menstrual cycle among lower birth weight girls. This finding is intriguing since breast cell proliferation rates are greater in the luteal phase compared to the follicular phase of

the menstrual cycle (Winter et al., 1995). Additional work is needed to understand the mechanisms underlying these complex relationships.

This research has several imitations. Data for insulin and insulin-like-growth factor (IGF) were not available for the present analyses but should be a consideration in future work. Insulin and IGF stimulate production of androgens by the ovaries as well as stimulate adrenal production of androstenedione and DHEAS (Nestler & Strauss, 1991; Stoll et al., 1994; Stoll, 1998a, b; Guercio et al., 2003). Furthermore, insulin may inhibit hepatocyte production of SHBG (Ibanez et al., 1997; Ibanez et al., 1998a). Another consideration of our investigation is that the study population included girls with elevated serum LDL-cholesterol levels and who were between the 5th and 95th weight for height percentiles. As such, our results may not be generalizable to the greater population. Also, given that all of the measured hormones plus SHBG are biochemically related it is difficult to pinpoint the individual relationship of any one sex hormone with birth weight. As suggested previously, timing of the blood collection with the menstrual cycle as well as assessing ovulation and analyzing girls with anovulatory cycles separately could also improve the accuracy of our estimations. Availability of additional measure of infant birth size such as including gestational age and birth length would also improve our study. A study by Ibanez et al. (Ibanez et al., 2002) found that during a 3-month study period normal weight adolescent girls born small-for-gestational age were more likely to be anovulatory than average-for-gestational age girls (40% vs. 4%, p= 0.002). If lower birth weight girls also experience a greater frequency of anovulatory cycles, our results would likely be affected.

The present investigation has numerous strengths. It is the first study to investigate the association of birth weight with longitudinal hormone measurements during adolescence and was conducted as part of a randomized, controlled clinical trial of children who were pre-pubertal at baseline. Serum hormone assays were performed using highly specific radioimmunoassays, and estradiol, estrone, estrone sulfate and testosterone radioimmunoassay were preceded by a chromatographic purification step. Lastly, the anthropometric data was high quality, we controlled for phase of the menstrual cycle, and data were collected by trained personnel.

In conclusion, birth weight is associated with DHEAS, androstenedione, certain luteal phase estrogens and SHBG concentrations during adolescence. For pre-menarche DHEAS and androstenedione concentrations, the effect likely occurs via the influence of birth weight on age at menarche. Conversely, birth weight is positively associated with luteal phase estradiol, non-SHBG bound estradiol, estrone and post-menarche SHBG independent of the effect of birth weight on age at menarche. It remains to be determined how these associations may affect long term BCA risk. A better understanding of early life risk factors may help individuals with a family history of BCA to modify behaviors to decrease risk. Furthermore, given the etiologic complexity of BCA, understanding early life risk factor plays an important role in unraveling disease development.

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Chapter 5

CONCLUSIONS

The results of the present studies provide additional insight on the association between birth weight and BCA risk factors. Early menarche, a known risk factor for BCA, was found to be associated with lower birth weight after adjusting for BMI-for-age percentile, physical activity, mother's age at menarche, and race. Girls weighing less than 2891 g at birth (< 1 SD below sample mean, categorically defined as 'low birth weight'), were nearly twice as likely to experience an earlier menarche compared to girls with birth weight within +/- 1 SD of sample mean (HR= 1.92, 95% CI = 1.07, 3.43, p= 0.03). The direct relationship between birth weight and age at menarche also was upheld when birth weight was modeled as a continuous variable (p= 0.05) (Chapter 3). Elevated levels of hormones in adulthood are associated with increased BCA risk (Key et al., 2002; Eliassen et al., 2006), but the association between adolescent hormone concentrations and future BCA risk is unknown. In Chapter 4, birth weight was associated with DHEAS concentrations throughout the 7 years of follow-up, pre-menarche androstenedione concentrations, post-menarche SHBG and certain luteal phase estrogen concentrations. For pre-menarche DHEAS and androstenedione concentrations, the association with birth weight likely occurred via birth weight's effect on age at menarche. Conversely, the relationship between post-menarche DHEAS, post-menarche SHBG, and luteal phase estradiol, non-SHBG estradiol and estrone appeared to be independent of the effect of birth weight on age at menarche. Together, these results offer support to the fetal origins for BCA hypothesis first proposed by Trichopoulos more than 15 years ago (Trichopoulos, 1990). Trichopolos' hypothesis focused on *in utero* estrogen exposure. More recent work, including our own, lends support to the hypothesis

that *in utero* and early life androgen exposure may also have an important role in determining future BCA risk. There are a number of possible explanations why birth weight is associated with age at menarche, serum hormone concentrations and ultimately BCA risk in adulthood. Likewise, there are a number of questions yet to be answered. We will explore these concurrently in the following sections.

Is birth weight a marker for fetal hormone exposure?

Birth weight is a commonly assumed proxy for *in utero* hormone exposure, but in reality, limited research on the relationship between measured fetal hormones and birth weight exists. What evidence does exist suggests a positive relationship between some hormones and birth weight. Troisi and colleagues (Troisi *et al.*, 2003a) found that cord DHEA, DHEAS, estrone and testosterone were lowest in the lightest infants (< 2500 g), and results of a regression analysis indicated that birth weight remained positively associated with cord DHEAS (p= 0.01) after adjustment for covariates. Birth weight was also positively associated with serum DHEAS at birth in an early study of ill infants (Turnipseed *et al.*, 1976). These studies, unlike some work suggesting a link between birth weight and *in utero* estrogen exposure using maternal concentrations of circulating hormones during pregnancy (Petridou et al., 1990; Kaijser et al., 2000; Mucci et al., 2003) are based on actual measurements from the fetal compartment. Some studies (Simmons *et al.*, 1994; Shibata *et al.*, 2002; Troisi *et al.*, 2003a), but not all (Nagata *et al.*, 2006), suggest that concentrations in the maternal serum and fetal cord blood are not highly correlated and furthermore, that umbilical cord estrogen concentrations are not associated with birth weight. This suggests that maternal circulating hormones are not

representative of fetal exposure. As such, direct measurements from the fetal circulation may be more appropriate for assessment of *in utero* exposure. However, methods in addition to measuring hormonal concentration in the cord blood should be pursued. The stress of labor and delivery may give rise to hormone concentrations in the umbilical cord atypical from those during normal gestational growth and development. Also, hormone levels change over the course of the pregnancy, and cord blood measurements provide only one snapshot of *in utero* exposure.

Assuming birth weight is a marker for *in utero* hormone exposure, how does fetal hormone exposure influence age at menarche and sex steroid hormone concentrations in adolescence?

Both fetal androgen exposure (Matchock, 2008b) and higher birth weight are associated with later age at menarche (dos Santos Silva *et al.*, 2002). On a related note, females with conditions characterized by elevated androgens, including polycystic ovary syndrome (PCOS) and congenital adrenal hyperplasia have older age at menarche (Sadrzadeh *et al.*, 2003; New, 2004). Also, development of PCOS is associated with smaller size at birth (Ibanez *et al.*, 1998b). Infants born small-for-gestational age (SGA) or low birth weight have increased prevalence of insulin resistance in childhood, adolescence, and adulthood (Hofman *et al.*, 1997; Ibanez *et al.*, 1998b; Gluckman & Hanson, 2004). Some evidence suggests that insulin resistance and its impact on hormone production may be the mechanism underlying the link between lower birth weight with earlier menarche and adolescent hormone concentrations. In mice, low birth weight is associated with chronic hyperactivity of the HPO axis, resulting in increased plasma corticosterone, hepatic gluconeogenesis and insulin resistance (Buhl *et al.*, 2007).

Although insulin data were not available for the present analyses, we suspect that the lower birth weight girls may have higher levels of circulating insulin. In turn, the insulin may stimulate ovarian androgen production and adrenal production of androstenedione and DHEAS (Nestler & Strauss, 1991; Stoll *et al.*, 1994; Stoll, 1998a, b; Guercio *et al.*, 2003). Furthermore, insulin is associated with reduced hepatic production of SHBG, thereby increasing the amount of available, unbound estradiol which ultimately may act upon estrogen receptive tissues including the breast (Ibanez *et al.*, 1997; Ibanez *et al.*, 1998a). In addition to the observed earlier menarche among lower birth weight girls, our research detected higher levels of DHEAS and androstenedione and lower concentrations of SHBG and some luteal phase estrogens among lower birth weight girls. These results are in keeping with the proposed influence of insulin on hormone production in lower birth weight girls. More work is required to elucidate the actual effect of insulin on adolescent hormone concentrations and investigate other possible mechanisms.

How does birth weight's effect on age at menarche and sex steroid hormone concentrations during adolescence affect future risk of breast cancer?

Early age at menarche is a known BCA risk factor (American Cancer Society, 2007) and is associated with an increased number of lifetime ovulatory menstrual cycles (Kvale & Heuch, 1988; Ursin *et al.*, 2000). Endogenous estradiol and progesterone concentrations peak with ovulation-- thus the more ovulatory menstrual cycles a woman experiences the greater her lifetime exposure to these hormones. Accordingly, this is one suspected causal pathway linking early menarche to increased BCA risk. As discussed previously, we suspect that differences in insulin concentrations may be a mechanism

underlying the observation of earlier menarche among the lower birth weight girls. Yet, numerous studies suggest that lower birth weight girls are at an overall decreased risk of BCA (Michels & Xue, 2006), which creates an incongruous risk profile over the lifecourse. Although we did not detect a significant effect of growth velocity, our data lacked measurements between birth and study entry. It is during this age span that other research groups have found childhood growth velocity to mediate the relationship between birth weight and menarche (Adair, 2001; dos Santos Silva *et al.*, 2004). If true, this could be one explanation for increased risk of early menarche but decreased risk of BCA among lower birth weight girls. Another explanation is the possibility of increased frequency of anovulatory menstrual cycles among the lower birth weight girls. Ibanez and colleagues found girls born SGA were ten times more likely to be anovulatory over a 3-month period compared to peers of a similar body size who were born average-for-gestational age (AGA) (Ibanez *et al.*, 2002). If the frequency of anovulation persists into adulthood, lower birth weight girls would experience lower lifetime exposure to endogenous estradiol and progesterone and a likely decreased BCA risk despite an earlier menarche.

The influence of endogenous sex steroid hormone concentrations during adolescence on later risk of BCA is unknown. Prospective studies that begin prior to puberty and follow girls into adulthood are needed to accurately describe the relationship. There are a number of hypothesized mechanisms by which future risk of BCA could be affected. Higher concentrations of estrogens during adolescence could increase cell proliferation and the potential for a carcinogenic DNA mutation (Laidlaw *et al.*, 1995) and some evidence suggests that estrogen itself is a mutagen (Liehr, 2000). However,

estrogens can also promote cell differentiation, which could offer some protection against mutations in the mammary gland in early life (Sutherland et al., 1998b; Hilakivi-Clarke et al., 2002). Although biologic data are not available to support the hypothesis, it is speculated that *in utero* androgen exposure may offer BCA protection by antagonizing the effects of estrogens on ductal development in the fetal breast (Troisi et al., 2003c), and possibly reduce the breast stem cell population (Michels & Xue, 2006; Troisi *et al.*, 2008). Whether higher concentrations of circulating androgens during adolescence could antagonize the effects of estrogens on breast terminal end buds and ducts during adolescence is unknown. In our study, lower birth weight girls had higher levels of serum DHEAS and androstenedione, lower luteal phase estradiol, non-SHBG bound estradiol and estrone, and lower post-menarche SHBG. We speculate that the higher levels of DHEAS and androstenedione in our sample of lower birth weight girls may protect the developing breast tissue through an unknown mechanism. Given that breast cell proliferation rates are greater in the luteal phase compared to the follicular phase of the menstrual cycle (Winter *et al.*, 1995), the observation of higher estrogens in the luteal phase among the higher birth weight girls offers another piece of evidence supporting the direct relationship between birth weight and BCA risk. Although we did not have hormone measurements from the umbilical cord or in infancy, it is important to recognize the possible long term impact of estrogen exposure on BCA risk. Schmidt and colleagues (Schmidt *et al.*, 2002) found serum estradiol levels were positively associated with breast tissue size in 3-month old girls, thus suggesting that breast tissue is sensitive to sex steroid hormones in very early life.

Additional Considerations

There are a number of directions in which the present body of research can be expanded to improve our knowledge of the *in utero* influence on BCA risk factors. Continued attention must be given to the variables confounding the relationship between birth weight, BCA risk factors, and BCA. These include other birth characteristics that influence birth weight such as birth length and gestational age. Also, other attributes of childhood development such as growth velocity could be important mediators in the relationship. Greater attention is needed to the potential modifying effects of environmental influences including maternal diet during pregnancy, exposure to suspected endocrine disruptors *in utero* and during childhood, and diet during infancy. Some research suggests that mother's consumption of polyunsaturated fat during pregnancy may be positively associated with umbilical cord estriol (Nagata *et al.*, 2007). Such data were not available in the present analyses, but would be a worthwhile addition to future studies.

A recent review article concluded that children are extremely sensitive to estradiol even at serum levels below detection limits, which the raises the possibility of adverse effects stemming from exposure to endocrine disruptors, chiefly through environmental pollutants, chemicals in food packaging, consumption of growth promoters used in cattle production (Aksglaede et al., 2006). The National Toxicology Program Draft Brief on Bisphenol A (BPA), used the production of polycarbonate plastics and epoxy resins) concluded that there is some concern for fetal, infant, and childhood exposure to BPA at current human exposures on the prostate gland, mammary gland, and earlier age for puberty in females (National Toxicology Program, 2008). The release of the report was

followed by a proposed ban of the use of BPA in the manufacture of baby bottles by the Canadian government (Health Canada, 2008). It is likely that research in this area will grow as further regulatory and policy actions are taken. Other early life characteristics such as infant feeding method (ie., breast versus formula and the use of soy-based formulas) are deserving of research attention.

Final Conclusions

Based on the findings of the present studies and the existing relevant literature, birth weight is associated with certain risk factors for BCA. Although lower birth weight is associated with an overall decreased BCA risk, it is also associated with an earlier age at menarche. We suspect that differences in anovulatory cycles or growth velocity may be important mediators in these relationships. Lower birth weight was also associated with higher levels of DHEAS, pre-menarche androstenedione and lower concentrations of some luteal phase estrogens and post-menarche SHBG. These results suggest that characteristics of the fetal environment can have long term effects on sexual maturation and sex hormone concentrations during adolescence that ultimately may affect future risk of BCA. As the mechanisms underlying these associations are elucidated there is great potential for the development of BCA prevention strategies during childhood.

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VITA

Elizabeth Hill Ruder

Education

August 2008	Ph.D., Nutritional Sciences, The Pennsylvania State University
July 2001	Dietetic Registration, American Dietetic Association, The Cleveland Clinic Foundation
May 2000	B.S. with Honors in Nutritional Science, Nutritional Sciences, Cornell University

Awards and Fellowships

2007	World Cancer Research Fund/American Institute of Cancer Research Conference Scholarship Award
2007	NIH National Graduate Student Research Festival Scholarship
2004-2005	University Fellow, Pennsylvania State University

Publications

1) **Ruder EH**, Dorgan JF, Kranz S, Kris-Etherton PM, Hartman TJ (2008). Examining Breast Cancer Lifestyle Risk Factors: Early life and Childhood. Clinical Breast Cancer. Accepted May, 28 2008.
2) **Ruder EH**, Hartman TJ, Blumberg JB, Goldman MJ (2008). Oxidative Stress and Female Reproductive Failure. Human Reproduction Update. Accepted April 15, 2008.
3) **Ruder EH**, Patterson J. Reducing Risk of Childhood Overweight Among Immigrant Families from Asia: A Tool for Educators. Journal of Nutrition Education and Behavior 2008 Jan;40(10): 57-58.
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