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THE ASSOCIATION BETWEEN MEASURES OF DIET QUALITY AND BREAST DENSITY AND BODY COMPOSITION IN PREMENOPAUSAL WOMEN

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Jessica A. Lindgren

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The dissertation of Jessica Lindgren was reviewed and approved* by the following:

Terryl J. Hartman
Adjunct Professor of Nutritional Sciences
Dissertation Advisor
Chair of Committee

Joanne Dorgan
Associate Professor, Fox Chase Cancer Center

Donna Coffman
Research Associate Professor, College of Health and Human Development

Jennifer Savage-Williams
Associate Director, Center for Childhood Obesity Research

Gordon L. Jensen
Professor of Nutritional Sciences and Medicine
Head of the Department of Nutritional Sciences

*Signatures are on file in the Graduate School

ABSTRACT

Breast cancer is the most commonly diagnosed cancer in women in the United States, with 1 in 8 women being diagnosed during their lifetime. There are several well-known risk factors for breast cancer including age, genetic factors, family history, reproductive characteristics, physical attributes (e.g. dense breast tissue) and diet and diet-related factors (e.g. alcohol consumption, weight, and body fatness). While many of these risk factors remain consistent across the pre- and postmenopausal years, it has been established that menopausal status modifies the effect of obesity on breast cancer risk. Body fatness decreases risk in premenopausal women but increases risk in postmenopausal women. However, when assessing body fat distribution, central obesity has been positively associated with breast cancer risk in both pre- and postmenopausal women. Breast density, or the ratio of dense fibroglandular area to total breast area, reflects breast tissue composition and is a strong breast cancer risk factor. Breast density is higher in premenopausal women, postmenopausal women who use hormone replacement therapy (HRT), and those, regardless of menopausal status, who have a lower BMI. In contrast, women who are older, parous, have their first birth at a young age, or are smokers often have lower breast density. These factors can directly (e.g. through altering breast morphology) or indirectly (e.g. through influencing hormones) affect breast density. Breast density may also be a marker for lifestyle exposures (e.g. diet and body size) and their effects on hormone levels.

Because breast density and body fatness have both been shown to be modifiable and susceptible to dietary influences, they are often targeted as potential breast cancer prevention strategies. Many current studies examine single foods or nutrients, which often provide inconsistent results. Our studies focus on measures of overall diet (e.g. dietary energy density and dietary patterns) and its influence on measures of breast density and body fatness. Measures

of overall diet have been shown to more appropriately account for naturally occurring food and nutrient interactions that are often not properly considered in single nutrient studies.

Data for the following cross-sectional studies were collected from young women (25-29y) who were initially enrolled in the Dietary Intervention in Children Study (DISC) and later participated in the DISC06 follow-up study. Informed consent was obtained at all points throughout the study. A total of 663 healthy, pre-pubertal, 8-10 year old children with elevated LDL-C, including 301 girls, were recruited from six clinical centers between 1988-1990. They were randomly assigned to a behavioral dietary intervention or usual care control group. The childhood DISC data collection included anthropometry which was used to calculate the childhood BMI-Z score based on the Center for Disease Control 2000 Growth Charts. Trained study staff measured body composition, anthropometrics, breast density and blood was drawn for DISC06 participants as young adults. Three 24-hour recalls were used to collect dietary information via telephone over the course of two weeks.

The objective of the first study was to evaluate the associations between dietary energy density (ED; kcal/g) and three measures of breast density. Dietary ED is a property of food and can be calculated both with and without beverages. Individual linear mixed effects models were fit by maximum likelihood with robust standard error for each breast density outcome. After multivariate adjustment, a 27.0% (95% CI: 6.3 to 51.7%, p=0.009) change in the average percent dense breast volume (%DBV) and a -18.4% (-32.2 to -1.8%, p=0.03) change in the average absolute non-dense breast volume (ANDBV) was observed in our population for each 1 kcal/g increase of dietary ED from food-alone.

Dietary ED from food and caloric beverages was also significantly inversely associated with the ANDBV with a -13.9% (95% CI: -22.3 to -4.7%, p=0.004) change being observed for each 1 kcal/g unit increase of dietary ED using this method. No associations were observed with the absolute dense breast volume (ADBV). We also stratified the analysis by median childhood

BMI-Z score and observed stronger associations with all three breast density measures and food-only dietary ED in women with a BMI-Z score above 0.2. Overall, this research suggests that dietary ED, particularly from food alone, is associated with measures of breast density.

The objective of the second study was to examine the associations between dietary ED and measures of body fatness among young women. Whole body percent fat as well as android and gynoid fat measurements were obtained via dual-energy X-ray absorptiometry (DXA) and height (nearest 0.5 cm), weight (nearest 0.2 kg), and waist circumference (nearest 0.5 cm), were all obtained by trained study staff. Individual linear mixed effects models were fit by maximum likelihood with robust standard error for each body fatness outcome. After multivariate adjustment, each 1kcal/g unit increase in food-only dietary ED was significantly positively associated with several body fatness measures including BMI [0.59 kg/m2; β (95% CI) = 0.59 (0.04 to 1.14), p=0.03)], whole body percent fat [2.08%; β (95% CI) = 2.08 (0.63 to 3.53), p=0.005], android fat [3.38%; β (95% CI) = 3.38 (2.26 to 4.50), p=<0.0001], gynoid fat [2.00%; β (95% CI) = 2.00 (0.55 to 3.45), p=0.008], and the android:gynoid fat ratio [0.3; β (95% CI) = 0.03 (0.01 to 0.05), p=0.007]. Food-only dietary ED was also positively associated with waist circumference; however, it did not reach statistical significance. This could be due to the larger amount of error associated with waist circumference measurements. There were no associations with dietary ED calculated with either food and caloric beverages or food and all beverages. Overall, this research suggests that dietary ED from food alone is positively associated with several measures of body fatness.

In our third study, the primary objective was to assess the relationship between *a posteriori* dietary patterns derived via finite mixture modeling (FMM) and measures of body fatness and breast density, as described above. Finite mixture modeling, unlike other dietary pattern methodologies, does not restrict an individual to a particular dietary pattern, but rather each subject has a probability of belonging to one of the derived dietary patterns. An individual's

membership to a particular pattern was based on their highest respective posterior class-membership probability. Dietary patterns were derived using complete dietary data from 203 subjects. Five dietary patterns were derived and labeled based on the model-predicted servings of the 15 food groups in each of the intake patterns: "Western" [n=21, (10.3%)], "Dietary Guidelines" [n=40, (19.7%)], "Health Conscious" [n=47, (23.2%), "Alcohol and Vegetables"[n=39, (19.2%)], and "Non-Drinkers" [n=56, 27.6%)]. Briefly, the "Dietary Guidelines" pattern was the most consistent with the current 2010 Dietary Guidelines while the "Health Conscious" pattern had mediocre diet quality (as evidenced by dietary ED), but had a low prevalence of smoking and the highest amounts of physical activity. The "Alcohol and Vegetables" pattern had high intakes of alcohol (primarily beer and wine), healthy fats, and non-starchy vegetables. The "Western" pattern had high intakes of red and processed meats, sweets, and refined grains and finally, the "Non-Drinker" pattern consumed the lowest amount of alcohol. Individual linear mixed effects models were fit by maximum likelihood with robust standard error for each body fatness or breast density outcome. After multivariate adjustment, the "Dietary Guidelines" had a significantly lower dietary ED from food alone and food and all beverages than all of the other patterns. The "Western" pattern had the most unfavorable diet and often the least favorable body fatness outcomes. There were no associations with any of the dietary patterns and breast density measures except for a 34.7% (95% CI: 18.6 to 52.8, p=0.02) and 41.7% (95% CI: 26.7 to 58.5%, p=0.002) change in the ADBV in the "Non-Drinker" and "Alcohol and Vegetables" when compared to the "Dietary Guidelines" pattern.

In conclusion, both dietary ED and dietary patterns were associated with body fatness and breast density, with the least healthful diets (High dietary ED from food-alone and the "Western" dietary pattern) often having the most unfavorable body fatness and breast density outcomes. Our research examines dietary profiles that may better aid in the development of future dietary

recommendations. Future research should include the examination of dietary change across childhood and adolescence and its influence on body fat and breast density in adulthood.

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Chapter 1

Introduction

Overall Background and Significance

Breast cancer is the most commonly diagnosed cancer with 1 in every 8 women being diagnosed by age 80 [1]. It is also the second leading cause of cancer death among women [2]. Several risk factors, both endogenous and exogenous, have been established including alcohol consumption, physical activity, elevated postmenopausal body mass index (BMI) [3], age at menarche and menopause [4], family history and genetic mutations [1].

In addition to the aforementioned breast cancer risk factors, breast density, or the amount of dense fibroglandular tissue present in the breast, has been positively related to breast cancer risk; women who have percent dense areas (PDA) of 75% or more have up to a 4-5-fold increase in risk [5]. Breast density can be measured, assessed, and targeted for potential cancer prevention strategies prior to the clinical onset of cancer [5-8]. Unfortunately, little is known about the mechanism through which BD may affect breast cancer risk [9]; thus, it is unclear which strategies may be most effective in reducing breast density. A recent review (Chapter 2) examined dietary factors and their influence on breast density [10]. Many studies that were included in this review were cross-sectional, included primarily older women with more stable breast tissue, and focused mainly on individual foods and nutrients. From this review, it was determined that vitamin D and calcium were inversely associated and fat and alcohol were positively associated with breast density, particularly in premenopausal women.

Body fatness is also an established breast cancer risk factor; however, it is modified by menopausal status. Weight status is inversely associated with premenopausal breast cancer, but positively associated with postmenopausal breast cancer. Recently, studies have suggested that body fat distribution may be more important to examine than total adiposity because central obesity has been positively associated with both premenopausal and postmenopausal breast cancer. This is further described in chapters 4 and 5.

The study population used in the following research is comprised of young adult women between the ages of 25 and 29 who were originally enrolled in the Dietary Intervention Study in Children (DISC) when they were children (8-10y). The DISC study is a multicenter, randomized, controlled clinical trial sponsored by the National Heart, Lung, and Blood Institute and designed to test a dietary intervention that aimed to lower low-density lipoprotein cholesterol (LDL-C) in children. Complete details regarding trial design and primary aim results are described elsewhere [11-17]. In short, a total of 663 healthy, pre-pubertal, 8-10 year old children with elevated LDL-C, including 301 girls, were recruited from six clinical centers between 1988-1990 and randomly assigned to a behavioral dietary intervention or usual care control group. Informed consent was obtained at all stages of the study. All DISC protocols were approved by Institutional Review Boards at all study locations.

For the DISC06 follow-up study, each female participant attended a single data collection visit at one of the six DISC clinics. Visits were scheduled to take place during the luteal phase of the menstrual cycle with 85% of visits occurring within 14 days of onset of next menses. During the visit, survey questionnaires were completed on several topics such as demographics, medical, reproductive, and menstrual histories, prescription and non-prescription drug use (including extensive information on past and current hormone use), smoking and alcohol use, leisure-time physical activity, and family history of breast cancer. Anthropometrics, total adiposity, body fat distribution and breast density were also assessed by trained study staff. Diet was assessed by three nonconsecutive 24-hour dietary recalls over a two week period with the first being done in person and the subsequent recalls being completed via telephone. All recalls were collected over a two week period by trained interviewers using the Nutrition Data System for Research (NDSR: 2007 University of Minnesota, Minneapolis, MN). All data collection and methodology for all studies is further explained in chapters 3-5.

Data from the DISC are very valuable and allow us to examine a wide array of breast cancer and chronic disease risk factors in premenopausal women, which is a relatively understudied population in regards to breast density. The risks of mammograms do not outweigh the benefits in women under the age of 40, which often means that little breast density data exists in women younger than 40 years of age. However, the DISC trial used 3-D magnetic resonance imaging (MRI), which does not expose women to potentially harmful radiation, to obtain breast density measurements which will be referred to as Percent Dense Breast Volume (%DBV), Absolute Dense Breast Volume (ADBV) and Absolute Non-dense Breast Volume (ANDBV).

Background: Study 1

Breast density is one of the strongest risk factors for breast cancer. Women with high breast densities (greater than 75%) have a 4-fold increase in breast cancer risk [18]. Breast density is modifiable [5-8]; researchers have demonstrated that diet may contribute to breast density modification [19-21]. Most research to date regarding dietary influences on breast density has focused on single foods or nutrients [20, 22, 23]. Few studies have addressed the effects of overall diet on breast density despite the fact that it considers the complex interactions among the foods consumed as components of diets [24-26].

In this study, we examined dietary energy density (ED) and its association with three breast density measures. Dietary ED (kcal/g) assesses diet quality and was calculated three ways: food only, food and caloric beverages, and food and all beverages [27]. Diets high in water and fiber have lower ED due to their large gram weights with less energy contribution to the diet, while diets high in fat have higher ED. Energy-dense diets have been positively associated with body weight in both children and adults [28] and may be contributing to obesity-related chronic disease incidence. Food-only ED estimates have the strongest association with weight status in

both adults and children [29, 30]. To our knowledge, this study is the first to examine dietary ED and breast density measures.

Specific Aim and Hypothesis: Study 1

Study 1: Specific Aim

In the present multi-site, cross-sectional study in young adult women, we explored the association between dietary ED (food-only, food + caloric beverages, and food + all beverages) and breast density (%DBV, ADBV, and ANDBV).

Study 1: Hypothesis

Higher dietary energy densities, particularly from food alone, will be positively associated with breast density.

Background: Study 2

The prevalence of obesity has risen dramatically in the past 20 years in both adults and children [31]. Along with the increase in weight status, an increase in several weight-related diseases has also occurred [31]. Recently, a shift from studying single foods and nutrients to overall diet has increased in popularity. Examining total diet takes food and nutrient interactions into consideration and may aid in the development of sound dietary recommendations. In the present study, we chose to use dietary ED [kcal/ weight of food (g)] as a marker of overall diet

quality [32, 33] and examine its relationship with several measures of body fatness in a cohort of premenopausal women.

It is well established that menopausal status modifies the effect of body fatness on breast cancer risk; higher body fatness decreases risk in premenopausal women and increases risk in postmenopausal women. In contrast, the relationship between body fat distribution and breast cancer does not appear to follow this same pattern [34-36]. Women with higher amounts of central obesity often have lower levels of the sex hormone binding globulin (SHBG) and insulin-like growth factor binding proteins [37-39] which results in higher levels of circulating free estrogen and Insulin-like growth factors. This suggests that central obesity may be contributing to breast cancer risk.

Specific Aim and Hypothesis: Study 2

Study 2: Specific Aim

In the present multi-site, cross-sectional study in young adult women, we explored the association between dietary ED (food-only, food plus caloric beverages, and food plus all beverages) and several body composition and anthropometric measures including BMI, waist circumference, whole body percent fat, android fat, gynoid fat, and the android:gynoid fat ratio.

Study 2: Hypothesis

Higher dietary energy densities, particularly from food alone, will be positively associated with body fatness measures.

Background: Study 3

The prevalence of obesity has risen over the past 20 years as has several weight-related chronic diseases [31]. Dietary pattern analyses have been successfully used to study the effects of total diet on health outcomes**.** In the current cross-sectional study, we examined dietary patterns derived by finite mixture modeling (FMM) and then evaluated associations of dietary patterns with body fatness and breast density outcomes. While being overweight lowers breast cancer risk for premenopausal women, it increases risk for postmenopausal women [3]. In contrast, the relationship between body fat distribution and breast cancer does not appear to follow this same pattern [34-36]. Increased central obesity, measured either by waist circumference or the waist: hip ratio, has been positively associated with both premenopausal [34-36] and postmenopausal breast cancer [34-36] as well as hormonal changes including insulin resistance, hyperinsulinemia and decreases in SHBG and insulin-like growth factor binding proteins (IGFBP) in both pre- and postmenopausal women [37-39]. All of this can result in higher levels of free estrogen and insulin-like growth factors, both of which increase breast cancer risk [40].

Specific Aim and Hypothesis: Study 3

Study 3: Specific Aim

In our multi-site, cross-sectional study, we used FMM to derive dietary patterns and examine the associations between dietary ED (food only, food + caloric beverages, and food + all beverages), BMI, waist circumference, total body fat, android fat, gynoid fat, and the android:gynoid fat ratio. We also explored the association between the dietary patterns and breast density (%DBV, ADBV, and ANDBV).

Study 3: Hypothesis

At least two dietary patterns will be derived. The healthier patterns will be characterized by lower intakes of alcohol, solid fats, and animal proteins and by higher intakes of plant-based foods. This pattern will be inversely associated with all outcome measures. The unhealthy patterns will be the opposite and will be positively associated with outcome measures.

Summary

The research presented in this dissertation uses data from a multi-site clinical trial that includes young adult women between the ages of 25-29. Three main research questions are addressed: The first in Chapter 3 (Study 1), the second in Chapter 4 (Study 2), and the third in Chapter 5 (Study 3). A comprehensive literature review entitled "Diet across the Lifespan and the Association with Breast Density in Adulthood" (Chapter 2) provides background for Study 1 and Study 3 [10]. The second study examines the association between dietary ED and several body fatness measures (Chapter 4). The third study assesses dietary patterns and the association with dietary ED, breast density, and body fatness measures (Chapter 5). Chapter 6 summarizes the findings from chapters 3-5, describes the strengths and limitations of our research, and provides direction for future research.

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Chapter 2

Diet Across the Lifespan and the Association with Breast Density in Adulthood

A reprint of the published version of this can be found in the Appendix.

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Abstract

Studies have shown inconsistent results regarding the association between dietary factors across the lifespan and breast density and breast cancer in women. Breast density is a strong risk factor for breast cancer and the mechanism through which it influences cancer risk remains unclear. Breast density has been shown to be modifiable, potentially through dietary modifications. The goal of this systematic review is to summarize the current studies on diet and diet-related factors across all ages and determine what dietary factors show the strongest association with breast density, the most critical age of exposure, and identify future directions. We identified 28 studies, many of which are cross-sectional, and found that the strongest associations are among vitamin D, calcium, dietary fat, and alcohol in premenopausal women. Longitudinal studies with repeated dietary measures as well as the examination of overall diet over time are needed to confirm these findings.

Introduction

Breast cancer (BC) is the most commonly diagnosed cancer and the second leading cause of cancer death among women [1]. Alcohol consumption, physical activity, elevated postmenopausal body mass index (BMI) [2], age at menarche and menopause [3], family history and genetic mutations [4] are a few of the well-established BC risk factors. In addition, breast density (BD), or the amount of dense fibroglandular tissue present in the breast, has been related to BC risk; women who have breast densities of 75% or more have up to a 4-5-fold increase in BC risk [5]. Consequently, BD is often thought of as an intermediate on the BC development continuum that can be measured, assessed, and targeted for potential cancer prevention strategies [5-8]. Even so, little is known about the mechanism through which BD may affect breast cancer

risk [9]. Breast tissue develops mostly during puberty and continues to undergo changes throughout several life stage events, such as pregnancy [3, 10, 11]. This review will examine research on diet and diet-related factors captured across the lifespan and the association with adult BD.

Methods

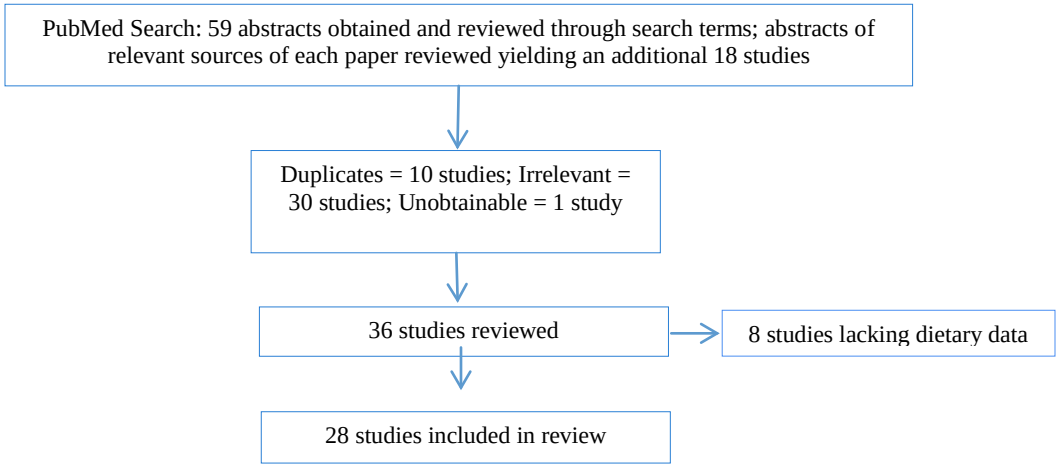

Figure 1-2. Study Selection, "Diet Across the Lifespan and the Association with Breast Density in Adulthood"

A literature search of the PubMed database of the United States National Library of Medicine was conducted to find human studies that evaluated the associations between BD measures and diet in the form of either single nutrients or whole dietary patterns. Both observational and diet intervention studies conducted at any stage of the lifespan were considered. Observational studies were included if they had recorded individual's dietary intake of foods or energy with dietary assessment tools such as a dietary recall (DR), food frequency questionnaire (FFQ), food record (FR), or other relevant assessment tools. Relevant studies were identified using the following search terms in multiple combinations: "adolescent diet and breast density",

"diet and breast density", "childhood and breast density", "diet and parenchymal patterns" and "mammographic breast density and diet." The search was limited to full-text publications written in English. As illustrated in Figure 1, a total of 77 studies were identified. After all exclusions, 28 studies were included in this review.

Measurement of Breast Density

BD can be measured two- (2-D) and three-dimensionally (3-D), with the most common being through 2-D mammography. Mammography measures the area of dense tissue (ADT) and the total area of the breast. Percent dense area (PDA) is often reported and is estimated as the proportion of dense fibroglandular tissue area to total breast area [9]. Area of non-dense tissue (ANDT), which is primarily adipose tissue, also can be estimated. Magnetic resonance imaging (MRI) and ultrasound also are used to measure BD. These 3-D modalities measure volume of dense tissue (VDT) and percent dense volume (PDV). Percent densities measured by mammography and MRI are highly correlated in the general population and among women who have low breast densities ($r = 0.73$) [12], but this correlation is attenuated among women with higher mammographic density greater than 50 percent ($r = 0.26$) [12]. In addition to quantitative measures of PDA and ADT, semi-quantitative and qualitative measures are frequently reported. Either the Wolfe classification, which has been further classified into Tabár, or the Breast Imaging-Reporting and Data System (BI-RADS) classification is used [13-15]. These measures often classify the breast on a four- to five-level scale ranging from low to high levels of fibroglandular tissue. While all methods are able to assess BD, quantitative methods provide more consistent results and a larger gradient of risk. Qualitative measures often have intervals that are too large (fewer categories) and do not capture true risk gradients [16].

Non-Dietary Factors that influence Breast Density

In general, PDA is higher in premenopausal women compared to postmenopausal women as well as in postmenopausal women who use hormone replacement therapy (HRT), and in both pre- and postmenopausal women with a lower BMI; and is lower in women who are parous, experience their first birth at a younger age, or are smokers [9, 17, 18]. Correlates of ADT are less well-studied, but in one study the ADT was inversely associated with age and BMI [18]. The non-dense compartment of the breast is adipose tissue, and higher adiposity, frequently measured by BMI, attenuates the ratio of dense tissue area to total breast area. Other characteristics associated with BD may influence estrogen, insulin-like growth factor-I (IGF-I), or insulin-like growth factor binding proteins (IGBPBs) that affect fibroglandular tissue proliferation [19, 20]. Alternatively, some characteristics, such as parity, could have direct effects on breast morphology that are reflected in PDA.

Dietary Factors

This review will focus primarily on diet and diet-related factors and their potential effects on both PDA and ADT with only limited attention to endogenous risk factors that are well-studied and not modifiable. Observational studies and clinical trials that evaluated dietary intakes during childhood, adolescence and adulthood are described.

Childhood Diet and Adult Breast Density

Much of breast development occurs during puberty; thus, factors such as childhood diet that influence the timing of puberty could potentially affect BD [21, 22]. Three studies have

examined dietary habits during childhood and the effect on BD in adulthood. Mishra and colleagues [23, 24] conducted two studies in a nationally representative longitudinal British sample to examine the association of childhood diet with BD. Childhood diet was assessed at age four years by a single dietary recall completed by mothers and later linked to mammographic BD measures collected at approximately fifty years of age from pre-, peri- and postmenopausal women. After controlling for relevant confounders, the investigators observed no association between PDA and childhood calcium [23], or total energy intake or with three dietary patterns (1. breads and fats, 2. fried potatoes and fish, and 3. milk, fruit and biscuits). A limitation of these studies is that a single dietary recall was used to assess diet, which could have contributed to the null results since multiple recalls are typically required to adequately assess usual diet [25]. Additional time points for dietary data collection, such as during adolescence, may have provided more insight into the effect of early diet on BD.

Haars and colleagues [26] examined the association between short-term transient caloric restriction (i.e. 6-8 mos.) during the Dutch Famine (when women were aged 2-33 years) and adult BD in the Netherlands DOM-Project. While this study does not necessarily fit within our inclusion criteria, it is included in this review because of the limited data available on children. Levels of caloric restriction were retrospectively assessed through three questions regarding hunger, cold, and weight loss and categorized as absent, moderate or severe famine exposure (FE). Degree of famine exposure at 2-9 years of age was significantly inversely associated with ANDT; mean ANDT were 77.8 cm^2, 87.7 cm^2 and 53.1 cm^2 in unexposed, moderately, and severely exposed, respectively ($p_{trend} = 0.03$). Although not significant, the women who were severely energy restricted at this age also had a larger ADT and higher PDA. However, because only 15 subjects were severely restricted, results should be interpreted cautiously.

The three studies that examined childhood diet and its effect on adult BD measures did not find associations with PDA or ADT, although in the study of the Dutch famine, severe caloric

restriction early in life was significantly inversely associated with ANDT later in life [26]. In this cohort, women who were severely calorically restricted had higher levels of both IGF-1 and IGFBP-3 postmenopausally than those who were not restricted [27]. Thus, one mechanism through which caloric restriction at young ages could potentially influence adult BD may be via differential programming of the somatotrophic axis resulting in long-term effects on growth factors such as IGF-1 and IGFBP-3 that are associated with breast density [28]. However, the small sample size and indirect diet assessment limit the inferences that can be drawn from this study. Taken together, the limited data available do not provide strong support for a role of childhood diet in determining breast density, but additional large prospective studies are needed before firm conclusions can be made.

Adolescent Diet and Adult Breast Density

Most of breast development occurs during puberty and diet during this time could have long term effects on BD in adulthood. One of five studies we reviewed found a significant association between diet during adolescence and BD in adulthood [29]. In the study by Tseng et al. [29], higher red meat intakes between the ages of 12-17 years was significantly associated with increased adult PDA in 201 Chinese-American female immigrants. After adjusting for degree of acculturation and other relevant covariates, women with the highest red meat consumption were at 3 times the odds of being in the highest PDA category compared to those with the lowest red meat consumption. When stratified by menopausal status, red meat intake remained significantly positively associated in postmenopausal, but not premenopausal women.

The remaining four studies, including 3 observational studies and one clinical trial, found no associations between dietary components or alcohol consumption during adolescence and BD in adulthood [19, 26, 30, 31]. Two studies used data from the large Minnesota Breast Cancer

Family Study Cohort (MBCFSC) to examine the role of adolescent diet and alcohol consumption on BD in pre- and postmenopausal women. Diet for girls at ages 12-13 years was collected retrospectively 50 years later via a 29-item FFQ focusing on high-fat foods (e.g. meats and other animal fat sources, snacks and desserts). Intakes of fruits, vegetables, fish and chicken were also analyzed. In the first study, Sellers et al. [19] observed no significant associations between any of these food groups and BD in multivariate analyses stratified by menopausal status. In the second analysis, Vachon et al. [30] evaluated alcohol consumption prior to age 18 via a self-reported questionnaire collected when the majority of the women were in their sixties. 'Never drinkers' had lower mean PDA than 'ever drinkers' (22.2 ± 14.3% vs. 26.5% ±15.9%); however, these results were attenuated and not significant after adjustment for age, BMI, HRT use, age at first birth, and parity [30]. In the study by Haars et al. [26] described above, short-term caloric restriction in girls age 10-18 years was not associated with adult BD measures. In a clinical trial, Dorgan et al. [31] examined the long-term effects of a dietary intervention to lower fat and increase fiber intake during childhood and adolescence (the Dietary Intervention Study in Children – DISC) and observed no differences in the VDT or PDV between those participants who received the behavioral intervention and the control group [31]. Thus, similar to childhood diet, the limited data available do not provide much support for a role of adolescent diet in determining adult BD, but additional research is needed.

Adult Diet and Breast Density

The majority of studies that have evaluated associations of diet with BD assessed the effects of adult diet. A total of 26 epidemiological studies and randomized controlled trials that examined dietary intake and BD among adult women are included in this review.

Total Energy

Three studies examined the association of total energy intake in adulthood with BD measures. In a nationally representative British cohort total energy intake around age 36 years was significantly positively associated with PDA and ADT at age 51 years in pre- and postmenopausal women [24]. Sala at al. [32] similarly found that total energy intake was significantly positively associated with PDA. The odds ratio (OR) for being classified in the highest PDA category for women in the highest vs. lowest tertile of energy intake was 1.79 (95%CI: 1.09-2.91). In analysis stratified by menopausal status, energy intake was associated with significantly higher PDA in postmenopausal women only [32]. Finally, in the Dutch famine study described above, caloric restriction in adulthood was not associated with several BD measures suggesting that exposure to short-term caloric restriction may be more important in children.

Dietary Fat

Eight studies [32-39] have examined the association between dietary fat and BD in adulthood. Three studies showed a significant positive association with total fat and BD measures. Nagata and colleagues [37] showed significant associations in a Japanese sample with mean PDA being 15.5% in the highest quartile of total fat intake compared to 9.9% in the lowest quartile ($p_{trend} = 0.04$). In a sample of 31 BC patients, women in the highest quartile of total fat (Mean %Energy (E) = 42.04) compared to the lowest quartile of intake (Mean %E = 34.72) were significantly more likely to be classified as a P2 + DY (high density) pattern compared to the N1 + D1 (low density) pattern ($p<0.01$) [35]. Qureshi et al. [38] showed a positive trend for the

relationship between total fat with increased ADT in a large Norwegian population of postmenopausal women, although it did not reach statistical significance.

Individual fatty acids have also been examined with saturated fatty acids (SFAs) generally being positively associated with increased BD measures. In an analysis based on 645 pre- and postmenopausal women ages of 40-62 years enrolled in the Canadian National Breast Screening Study (CNBSS), SFA intake was significantly positively associated with PDA. Mean PDA was 44.2% in the highest quartile of SFA intake compared to 38.6% in the lowest (p-trend= 0.009) [34]; however, menopausal status was not controlled for or stratified by in this analysis. Similar findings also were reported in pre- and postmenopausal Japanese women; mean PDA was 16.5% in the highest quartile of SFA intake compared to 7.3% in the lowest (p-trend=0.02) [37]. Qureshi and colleagues [38] also showed a positive trend with SFA and PDA in a Norwegian population of postmenopausal women, although statistical significance was not reached. Nordevang and colleagues [35] observed that women who consumed a mean %E of 19.27 from SFA in the highest quartile were more likely to be classified as having a high-risk PDA compared to those who consumed a mean %E of 15.42 from SFA in the lowest quartile (p= <0.05). In contrast, a significant inverse association was observed with SFA in a subset of 283 premenopausal women from the MBCFSC; mean PDA was 37% in those with the highest SFA intake compared to 44% in the lowest consumers after controlling for relevant confounders (p-trend = 0.03) [36]. No associations with dietary fat were observed in postmenopausal women alone in this study.

The essential PUFA, linolenic acid, was inversely associated with PDA in a Mediterranean population of both pre- and postmenopausal women. Women in the highest tertile of intake had 31% lower odds of being classified as high PDA [33]. Elevated PUFA consumption in a sample of BC patients (Mean %E= 5.65 vs. 4.70) and n-6 fatty acids (Mean %E =4.69 vs. 3.81) was also significantly associated with being classified as a P2 or DY (high

density) versus an N1 or P1 (low density) Wolfe parenchymal pattern ($p < 0.05$). Vachon et al. [36] examined a sample of both pre- and postmenopausal women in the MBCFSC and observed women in the highest quartile of PUFA intake had 4% higher PDA compared to those in the lowest quartile ($p = 0.05$). Similar results were observed with the PUFA:SFA ratio in this study.

Finally, Nordevang and colleagues [35] found that women within the highest quartile of MUFA (Mean %E=14.22) were more likely to have a high PDA compared to those in the lowest quartile (Mean %E = 11.98, $p<0.01$). Far fewer associations between dietary factors and BD measures were observed in postmenopausal women, with only increased consumption of MUFAs being significantly associated with high PDA; even though the difference in MUFAs as percent energy between the high and low density groups was small (Mean %E = 12.9 vs. 12.3, $p< 0.05$). A small number of randomized controlled trials (RCTs) have also been conducted to examine dietary fat and BD and have yielded mixed results [40-42]. These studies will be further discussed in the "RCT" section of this review.

Alcohol

In their 2001 review, Singletary et al. [80] concluded that there was strong evidence for a positive association between alcohol and BD in both pre- and postmenopausal women [43]. Alcohol may influence BD through decreasing the concentration of sex-hormone binding globulin and disturbing estrogen metabolism, increasing serum estrogen metabolites, raising oxidative stress in tissue, and leading to an increase in breast tissue proliferation [44]. The relationship between alcohol and BD may also be related to its positive association with IGF-1 and a negative association with IGFBP-1 that has been shown in post-, but not premenopausal, women [45]. Total alcohol consumption in a multiethnic cohort was associated with a 1-2% higher PDA among pre- and postmenopausal alcohol consumers (median alcohol consumption in

the highest consumers = 12 drinks/wk) when compared to abstainers; however, this association failed to reach statistical significance [46]. In a Mediterranean cohort of both pre- and postmenopausal women, both total wine consumption and total alcohol consumption were significantly positively associated with a 31% and 42% higher odds of having an elevated PDA, respectively [33]. A similar observation was made with total alcohol consumption in premenopausal women with 'Never Drinkers' having a mean PDA of 39% compared 45% for consumers of ≤ 3.9g/d and 42% for consumers of >3.9g/d (p_{trend} = 0.08). When the type of alcohol was examined, comparable results were observed with white wine in postmenopausal women only; however, an inverse association was observed with red wine in postmenopausal women with 'nondrinkers' having a mean PDA of 34% compared to 32% for those consuming ≤1 serving/wk and 28% for those consuming ≥2-4 svg/wk (p_{trend}= 0.02) [36]. The authors suggest that the difference between white and red wine may be due to the polyphenols that are present in red wine, which have been shown to have chemoprotective effects [36]. Tseng et al. [39] and Sala et al. [32] also looked at alcohol intake in pre- and postmenopausal women and found no associations with BD measures.

Soy & Isoflavones

Maskarinec et al. [47] conducted a review of the primarily epidemiological evidence on isoflavones and their association with PDA and concluded that soy products have little to no influence on BD measures regardless of the amount of isoflavones they are consuming in the range 0.1 – 120 mg/d [47]. A meta-analysis of several RCTs that examined the effect of soy and BD measures was also conducted and will be discussed in the "RCT" section of this review.

Calcium and Vitamin D

Vitamin D and calcium have been linked to cellular growth and differentiation in breast tissue [48, 49] and may influence the amount of dense tissue in the breast. Four cross sectional studies found a significant inverse association between vitamin D and calcium intake, alone or in combination, with BD measures [35, 50-52] in premenopausal women. Nordevang et al. [35] found that lower intakes of calcium (1165 vs. 1433 mg/10MJ) were significantly associated with an increased PDA. When examining dietary vitamin D and calcium, Berube et al. [51] observed that premenopausal women in the highest categories of both vitamin D (≥100 IU/d) and calcium (≥750mg/d) intake had 72% lower odds of having high PDA. When intake from both diet and supplements was considered, simultaneous increases of 400 IU of vitamin D/d and 1000 mg of calcium/d were associated with an 8.5% (95% CI: 1.8-15.1%) decrease in PDA in premenopausal women [50]. The association in postmenopausal women was considerably weaker [51] or null [50]. Diorio et al. [52] found comparable results; as dietary vitamin D and calcium increased by 100 IU/d and 250 mg/d respectively, PDA decreased by 1.8% ($p<0.01$). Similar results were found when intake from food and supplements were analyzed together.

Out of the remaining seven studies, two included only postmenopausal women and neither found an association between vitamin D and calcium intake and BD [53, 54]. An additional four studies reported significant associations between vitamin D and calcium overall; however, the results in postmenopausal women were considerably weaker than observed in premenopausal women [23, 39, 55]. Masala et al. [33] observed that Mediterranean women with a higher calcium intake had 33% lower odds of having a high risk mammographic pattern. No association was observed with vitamin D; however, vitamin D intake in this population was very low [33]. In a nationally representative British cohort, an inverse association between calcium intake and PDA, which were both measured among women in their 50's, was observed. Calcium

intakes ≥1180 mg/d compared to 699 mg/d resulted in a 0.53 (95%CI: 0.03-1.02) standard deviation decrease in PDA [23]. No additional associations were observed with the ADT or ADNT in this study. Tseng and colleagues [39] conducted a cross-sectional analysis using a 126-item FFQ to examine several dietary factors including vitamin D and found that after controlling for menopausal status, high-risk women (women with at least one 1st or 2nd degree relative with breast or ovarian cancer) with higher vitamin D intake had 50% lower odds of having high PDA when comparing the highest to the lowest tertile. Finally, serum 25[OH]D and dietary calcium intake obtained from an FFQ in a sample of women from the MBCFSC (73% postmenopausal) were not associated with either PDA or ADT [55]. While the overall trend failed to reach significance, the study did demonstrate that women with the highest mean intake of both calcium (>1,385 mg) and 25(OH)D (>86.2nmol/L) had the lowest PDA and ADT after adjusting for age, BMI, parity, age at first birth, and physical activity. Vachon et al. [36] also reported no associations for calcium and vitamin D from both dietary and supplemental sources with PDA in this cohort.

Overall, this research suggests that vitamin D and calcium are inversely associated with BD in premenopausal women. It is critical to note that as calcium and vitamin D increased from <500 mg/d and <100 IU/d to >1,750 mg/d and >700 IU/d respectively, PDA decreased in a dose-response fashion with clinically relevant decreases in PDA between 8-12% among premenopausal women [50, 52]. This is comparable to the effect of selective estrogen receptor modulators such as tamoxifen [56]. Importantly, Brisson et al. [57] examined serum vitamin D [25(OH)D] levels and found that PDA was lowest in the fall (39%) and highest in the spring (45%) (p=0.003), which was consistent with the rise and fall in serum vitamin D across the seasons. Few studies account for season in which BD was assessed. However, it may be important to consider endogenous vitamin D synthesis in response to sunlight in addition to that contributed by food sources. The biologically active form of vitamin D may decrease BD via its

antiproliferative properties or tissue-specific effects due to breast tissue possessing 1-α-hydroxylase, which converts inactive 25(OH)D to active $1,25(OH)_2D$ [58]. The localized production of $1,25(OH)_2D$ helps to regulate cell growth and promote terminal differentiation which promotes cellular resistance from carcinogenic factors [58]. Premenopausal women have higher levels of estrogen, insulin-like growth factor (IGF), and insulin-like growth factor binding proteins (IGFBPs), which may be associated with increased BD [59, 60]. Vitamin D, calcium, and IGFBP-3 have been proposed to increase each other's beneficial antiproliferative and proapoptoic effects [52]; however, vitamin D alone may help to combat the proliferative effects of estrogen and IGF when these hormones and growth factors are available in abundance, such as in premenopausal women.

Carbohydrates, Protein, and Other

Ten studies have evaluated intakes of carbohydrates, protein, and many other nutrients and their association with BD measures. Eight studies [29, 33, 34, 36-39, 61] used validated FFQs to assess nutrient intake; Sala et al. [32], and Nordevang et al. [35] conducted extensive dietary history interviews. Tseng and colleagues [39] found that in a sample of 90 women with a sporadic family history of BC, total and animal protein intakes above the median intake had 3 to 4 times the odds of an increased PDA; these associations were not observed in women with a strong hereditary pattern (1^{st} or 2^{nd} degree relative) of BC [39]. As mentioned previously, red meat intake during adolescence was significantly positively associated with PDA in adulthood; however, there was no association with red meat intake during adulthood in a sample of 201 Chinese-American immigrants [29].

Although few significant associations are observed among postmenopausal women; both Nagata et al. [37] and Sala et al. [32] found significant associations in both Japanese and

European populations, respectively when evaluating carbohydrates and protein. Sala and colleagues [32] found that protein and carbohydrate were positively associated with PDA in all women. When stratifying by menopausal status, significant positive associations emerged between protein, total meat, and carbohydrates and PDA in postmenopausal women only with those consuming the most having 2.2-2.5 times the odds of having a high-risk PDA. Nagata and colleagues [37] also found that protein was significantly positively associated with PDA with women in the highest quartile of intake having approximately 7% higher PDA than those in the lowest quartile. However, in contrast to the study by Sala, carbohydrates were significantly inversely associated with PDA in 253 postmenopausal Japanese women with those in the highest quartile having 6% lower PDA than the lowest consumers [37]. No associations were observed in premenopausal women [37]. Among pre- and postmenopausal women in the CNBSS, mean PDA was 37.9% in those in the highest quartiles of fiber intake compared to 43.0% in the lowest quartile, and the difference was significant [34]. Comparable results were found in sample of 31 Swedish premenopausal BC patients; lower consumption of carbohydrate and fiber were associated with higher PDA [35].

In a study evaluating dietary factors and mammographic patterns in a Mediterranean population, both pre- and postmenopausal women in the highest tertiles of the following foods and nutrients had 27-34% lower odds of having a high PDA: total vegetables, cheese , β-carotene, vitamin C, and potassium, whereas women in the highest tertile of tomato sauce intake had 34% higher odds of having a high PDA [33]. Similar results with high cheese intake were observed in a sample of 491 premenopausal women in this study [33]. Consistent with these findings, total dairy intake was significantly inversely associated with PDA in premenopausal women in the MBCFSC after controlling for relevant confounders [36]. Among pre- and postmenopausal women in the CNBSS, women in the highest quartiles of carotenoid intake had a 5.4% lower mean PDA when compared to the lowest quartile [34]. Comparable results were found by in

sample of 31 Swedish premenopausal BC patients and found that lower consumption of carotene were associated with increased PDA [35].

Only one study to date has examined multivitamin/multimineral (MVMM) supplement intake and BD outcomes. Berubé and colleagues [61] found that current premenopausal supplement users had a significantly higher adjusted mean PDA of 45% compared to 42.9% of past or 40.2% of never users ($p_{trend} = 0.009$). No association was observed in postmenopausal women. Vachon et al. [36] also found that dietary vitamin E and supplemental vitamin C were significantly positively associated with PDA in premenopausal women with the highest consumers having a 4-5% higher PDA than the lowest consumers. Supplemental vitamin B12, on the other hand, was positively related to PDA in postmenopausal women [36].

In conclusion, the foods or nutrients that were shown to be inversely associated with BD may be, in part, tied to IGF/IGFBP levels and oxidative stress reduction. BD has been associated with increased levels of oxidative stress as evidenced by malonoyldialdhyde (MDA) excretion [62] and IGF/IGFBP, particularly in premenopausal women [28, 59]. Lower intakes of fiber, carotene, and calcium have also been associated with increased breast densities. Carbohydrate intake has been associated with both lower and higher BD measures women. These conflicting results may be attributed to the fact that the types of carbohydrate are often not accounted for and fiber content may influence the way that different carbohydrates affect the IGF/IGFBP pathway and oxidative stress. Finally, higher intakes of total dairy and cheese consumption in premenopausal women are associated with lower BD measures, which may be due to the high amounts of calcium and vitamin D in these products.

Dietary Patterns

Analysis of dietary patterns has recently gained popularity in dietary assessment research, as they capture total diet and are more stable over time than the consumption of single nutrients or foods [63]. Two studies were conducted that examined *a posterori* dietary patterns and their association with BD and one study examined the influence of Mediterranean Diet (measured by Mediterranean Diet Scale - MDS) on BD measures. Dietary patterns were analyzed cross-sectionally in a British cohort and the MBCFSC [24, 64]. After combining data collected from food records collected at ages 36 and 43 years, four patterns emerged in the British cohort (1. low-fat and high fiber; 2. alcohol and fish; 3. high fat and sugar; 4. meat, potatoes, and vegetables). However, none of these patterns was associated with PDA [24]. In the MBCFSC, three dietary patterns emerged from data from a 153-item FFQ (1. fruit, vegetable, and cereal; 2. salad, sauce, and pasta/grain; 3. meat and starch). Only the fruit, vegetable, and cereal pattern was inversely associated with PDA in premenopausal women; however, it did not reach statistical significance [64]. Smoking has been associated with decreased PDA because of its antiestrogenic effects [65]. When all women included in the sample were stratified by smoking status, adherence to the fruit, vegetable, and cereal pattern was significantly inversely associated with PDA in smokers ($p=0.02$) [64]. The salad, sauce, and pasta/grain pattern was also non-significantly inversely associated with PDA in smokers [29]. These patterns are the highest in antioxidant-containing foods, which may benefit women who are under higher oxidative stress, such as smokers.

Tseng et al. [66] cross-sectionally evaluated the MBCFSC using the MDS. The women were scored based on their consumption of vegetables, legumes, fruits and nuts, cereals, fish, and the ratio of monounsaturated fatty acids (MUFA) to saturated fatty acids (SFA) as reported on a 153-item FFQ. For each unit increase in the MDS, PDA was decreased by 1.68% ($p=0.0002$)

among current smokers but not among non-smokers after controlling for relevant confounders including menopausal status [66]. Vegetables, legumes, and cereals were the components of the MDS that had the strongest association with PDA in this population [66].

Overall, it appears that dietary patterns high in antioxidant-containing foods are inversely associated with BD in smokers, who may be experiencing a higher level of oxidative stress than non-smokers. Other research has shown a positive association between BD and MDA, which is a marker for lipid peroxidation and oxidative stress [62].

Randomized Controlled Trials

The epidemiological evidence described above suggests that diet is associated with BD measures and that BD has the potential to be modified. As a result, researchers have conducted clinical trials to examine the association between specific dietary factors with BD outcomes. Boyd et al. [40] first examined a low-fat, high-carbohydrate 2-year dietary intervention in 817 women with PDAs ≥50%. Those who were randomized into the intervention group received intensive instruction to consume 15% of calories from fat, 20% from protein, and 65% from carbohydrate while the control group received general dietary advice and instruction to maintain their current intake of fat. After two years, the average reduction in PDA were 6.1% and 2.1% in the intervention and control groups, respectively ($p=0.01$) [40]. The effect of the intervention remained significant after controlling for age, weight change, and menopausal status [40]. After stratification by menopausal status, significant changes in PDA were only observed in women who were either premenopausal throughout the study or who were premenopausal at baseline but transitioned into menopause by the end of the study, with the greatest change in density occurring in the latter group. Consumption of fat and cholesterol were significantly positively associated

with change in ADT in this subgroup, whereas protein and cholesterol were significantly positively associated with change in PDA [42].

Martin et al. [41] completed a similar larger clinical trial with longer follow-up that included 461 women who were premenopausal at entry and postmenopausal after two years. Several BD measures were assessed (change in breast area, ANDT, ADT, PDA) premenopausally at baseline and later in the postmenopausal phase. Like the previous trial, this trial focused on women with high PDA ≥50% and the intervention group received the same dietary manipulation [40]. This study did not replicate the previous findings from Boyd et al. [40]. After two years, no change was observed in the intervention group and a slightly lower PDA was observed in the control group; the treatment group difference was not significant [41]. The authors suggest these unexpected results were likely due to an increase in the ANDT that occurred with weight gain in the sample.

As previously described in the vitamin D and calcium section, a one year calcium and vitamin D supplementation trial was conducted through the WHI to examine the effects on mammographic PDA in postmenopausal women [53]. Despite the associations observed in observational studies, no change in mammographic PDA was observed with supplementation. The authors suggest that very low PDAs at baseline could have led to a "floor effect" where further supplementation of vitamin D and calcium had no additional benefit. Finally, studies that have examined soy and isoflavone consumption and mammographic PDA have also yielded mixed results. Hooper et al. [67] conducted a meta-analysis of eight RCTs including 1287 total women that compared the administration of supplemental isoflavones versus a placebo for at least six months. Results from the meta-analysis showed a modest non-significant increase in PDA (Mean Difference: 1.83%; 95%CI 0.25-3.40) in premenopausal, but not postmenopausal, women as isoflavone intake increased; however, there was limited evidence of a clear dose-response relationship over the range of isoflavone intake of 40-120 mg/d.

Conclusions

Data from observational studies suggest that the strongest associations between diet and BD measures are among vitamin D, calcium, dietary fat, and alcohol and are found in adult premenopausal women. However, the few clinical trials that have evaluated these associations have failed to demonstrate a significant change in breast density with various dietary interventions. This could be because the foods/nutrients evaluated truly do not influence breast density or could be due to aspects of the study design including duration of the intervention, dose, sample size, or inclusion of predominantly older women in whom breast tissue may be less susceptible to dietary influences.

Limitations

This review has critically examined 28 studies and has identified strengths and weaknesses as well as highlighting several potential directions for new research to advance the field. Many of these studies are cross-sectional in nature and often focus just on PDA. In addition to this, the majority of women who receive mammograms overall and in these studies are >40y; an association between dietary factors and BD measures could be undetected if the critical dietary exposure occurred much earlier in life (and was not measured) before breast tissue is fully differentiated and potentially more vulnerable to exogenous influences.

The majority of studies included in the review assessed BD using 2-D mammography. Even though estimates of BD obtained by mammography and 3-D modalities such as MRI are highly correlated in the general population and in women with less dense breasts [12], correlations are substantially lower in women with more dense breasts in whom density can be more accurately measured using 3-D modalities.

Many studies examined the association of diet with PDA but not the ADT. Fewer associations are observed with the ADT compared to PDA; however, results should be reported when available in order to be more comprehensive, improve comparisons across studies, and enhance interpretability in relation to potential physiological mechanisms. Very few studies controlled for the phase of the menstrual cycle at the time of mammography. Because data on variation of breast density over the menstrual cycle are conflicting [68-71], it seems prudent to consider menstrual cycle day in analyses of breast density when possible. Finally, several methods were used to evaluate BD. Even though many studies used a semi-automated method to reduce variability and error, standardization of assessment would facilitate comparisons across studies.

Future Directions

To date, most studies of the association of diet with BD have been cross-sectional. Longitudinal studies that measure diet and BD over the life course are needed. Studies that evaluate the influence of diet during adolescence, when most breast development occurs, on adult BD could be particularly enlightening. Support for an association of diet with BD from observational studies is stronger for premenopausal women. However, a limited number of short-term clinical trials do not show conclusive evidence that dietary factors influence BD. Clinical trials in younger women could be informative and may provide more definitive results. Lastly, more research on dietary patterns as they relate to BD are needed.

Table 2-2. Studies of Childhood Diet and Breast Density

Author, Year	Study Population, n	Design	Diet/Mammogram Age	Foods/Nutrients of Interest	Dietary Assessment	Outcome	Major Significant Results	Adjustments
Haars, et al. 2009 [Also in Tables 2 & 9] **[26]**	DOM-Project n=144 [The Netherlands]	CS	2-9y/53y	Short-term energy restriction	Retrospective recall of 1944-45 famine ~ 40 yrs later. Exposure to hunger, cold, & weight loss.	BS, DT, NDT, PBD [Mammogram; visual observation]	Severely calorically restricted vs. Unrestricted: NDT: 53.1cm^2 (95%CI: 37.8-72.7) vs. 77 cm^2 (95%CI: 68.8-87.7)	Age at examination, parity, menopausal status, BMI
Mishra, et al. 2010 [Also in Table 9] **[24]**	BBC n =792 [England]	CS	4y/51.5y	Dietary Patterns at age 4: 1) Breads & fats 2) fried potatoes & fish,3) milk, fruit & biscuits	1-24-hr maternal recall of child's diet	PBD, ADT, ANDT [Mammogram; Cumulus]	Null	Mammographic view, age at mammogram, BMI at 53, age at menarche, menopausal status at mammography, HT use, parity, smoking status, PA, social class, the other three dietary patterns, energy
Mishra, et al. 2008 [Also in Table 3] **[23]**	BBC n=979 [England]	PC	4y/51.5y	Dietary Ca & vitamin D	1- 24-hour maternal recall of child's diet	PBD, ADT, ANDT [Mammogram; Cumulus]	Null	Mammographic view, age at mammogram, BMI age 53, energy, age at menarche, parity, smoking status, adult SES.

Table 2-2. Studies of Adolescent Diet and Breast Density

Author, Year	Study Population, n	Design	Diet/Mammogram Age	Foods/Nutrients of Interest	Dietary Assessment	Outcome	Major Significant Results	Adjustments
Haars, et al. 2009 [Also in Tables 1 & 9] [26]	DOM-Project n=356 [The Netherlands]	CS	10-18y/53y	Short-term energy restriction	Described in Table 1	BS, DT, NDT, PBD [Mammogram; visual observation]	Null	Age at exam, parity, menopausal status, BMI
Sellers, et al. 2007 [19]	MBCFSC n=1,552 [United States, NH-White]	CS	12-13 y/60.4y	High-fat meats, dairy, animal fat, high-fat snacks & desserts, high-fat foods, fish & chicken, fruits, vegetables	29-Item FFQ (retrospective recall)	PBD [Mammogram; Cumulus]	Null	Age at mammography, weight at follow-up 1, use of HRT, menopausal status, education, age at menarche, parity, age at 1st birth, OC, alcohol use, smoking
Tseng, et al. 2011 [Also in Table 7] [29]	Chinese-American Immigrants n=201 [US, Asian]	CS	12-17 y/53.1y	Beef, pork, tofu, green veg, fruits	Frequency of consumption: beef, pork, tofu, green veg, fruits (retrospective)	PBD: BIRADS	Red meat intake: All women: (OR_{T3vsT1} = 3.0; 95% CI: 1.5-6.4) Postmenopausal women: (OR_{T3VST1} = 16.9 ; 95% CI 5.4-52.4)	Age, level of acculturation, BMI, # of live births & age at 1st live birth, adult dairy intake
Vachon, Sellers, et al, 2005 [30]	MBCFSC n=1575 [US, NH-White]	CS	<18 y/60.4y	Alcohol	Follow- up questionnaire	PBD [Mammogram; Cumulus]	Null	Age, BMI, HRT, age at 1st birth, # of births, age at menarche, education, adult & adolescent smoking, alcohol, OC, menopausal status
Dorgan et al. 2010 [Also in Table 8]	DISC Premenopausal women n=182 [US, NH-White]	CS [RCT follow-up]	25-29y	Long-term effects of low-fat diet	3-24-hr dietary recalls	PBD & VDT [MRI]	Null	%body fat, age at randomization, age at visit, clinic, BMI-Z score, race, education,

[30]	smoking status, PA at 14-17 years old & separately during the past year, # of full term pregnancies, OC

Table 3-2. Studies of Adult Calcium and Vitamin D Intake and Breast Density

Author, Year	Study Population, n	Design	Diet/Mammogram Age	Foods/Nutrients of Interest	Dietary Assessment	Outcome	Major Significant Results	Adjustments
Bertone-Johnson, et al. 2010 [54]]	MDAS: WHI n=808 Post-menopausal [US,42% NH-White, 39% Black, 20% other races]	CS	50-79 y	Dietary & supplemental vitamin D & Ca	122-item FFQ + supplement inventory	PBD: [Mammogram; computer-assisted method]	Null	Age, race/ethnicity, BMI, age at menarche, parity, OC use & duration, previous HT use/duration, HT trial randomization assignment, family hx of BC, education, alcohol, smoking, total energy, PA, Gail risk, MV use
Bertone-Johnson, et al. 2012 [Also in Table 8] [53]	WHI CaD Trial n=330 Postmenopausal Women [US, 48% NH-White, 36% Black, 15% other]	RCT	50-79y	Daily supplementation of both 1,000 mg of Ca & 400 IU of Vitamin D [1y]	122-item FFQ	PBD: [Mammogram: Computer-assisted method]	Null	Subgroup analyses: Age, race/ethnicity, total vitamin D intake, HT treatment, Gail risk score, BMI, region of residence, category of mammogram density at baseline.
Bérubé, et al. 2005 [51]	Premenopausal women: n=777 Postmenopausal: n=783 [Canada]	CS	Premenopausal: 46.7y Postmenopausal: 61.8y	Dietary & supplemental vitamin D & Ca	161-item FFQ	PBD: [Mammogram; computer-assisted method]	Premenopausal women: Dietary Vitamin D: β= -1.8; Total Vitamin D: β = -1.4; Dietary Calcium: β= -0.7; Total Calcium: β= -0.8. 8.5% ↓mean PBD	Age, BMI, age at menarche, # of full-term pregnancies, age at first full-term pregnancy, duration of OC and/or HRT use, alcohol, daily energy, PA, family

							with simultaneous increases in VD & Ca by 400IU & 1,000mg, respectively. Post-menopausal women.: Null All women: Absolute ↓ in mean $PBD_{Q4Ca\&VitDQ1Ca\&VitD}$ =6.9%,	hx of BC in 1st degree relative, personal history of breast biopsies, smoking status, education (supplement use was also a confounder, determined post-hoc)
Bérubé, et al. 2004 **[50]**	Pre- & Postmenopausal women with extreme densities n=543 [US]	CS	PBD ≤30%: 51.4y PBD≥70%: 46.1y	Vitamin D & Dietary Ca	232-item FFQ	PBD: [Mammogram; visual estimation]	All women: Vitamin D: OR_{Q4vsQ1}=0.24(95 %CI: 0.11-0.53) Calcium: OR_{Q4vsQ1}=0.24(95 %CI: 0.10-0.57) $OR_{EXTvs.FEW\ DENSITIES}$ 0.28(95%CI: 0.15-0.54) (≥100IU Vit D & ≥750mg/d Ca]) Premenopausal women: Vitamin D: OR_{Q4vsQ1} = 0.13 Calcium: OR_{Q4vsQ1} = 0.13 Postmenopausal women: Vitamin D: OR_{Q4vsQ1} =0.30 (p trend = 0.05) Calcium: OR_{Q4vsQ1} = 0.27	Age, mammography, BMI, age at menarche, # of births & age at first birth combined, OCs, menopausal status & use of HRT combined, family hx of BC, education, alcohol, total energy, smoking status

							(p-trend = 0.06) Calcium: OR_{Q4vsQ1} = 0.27 (p-trend = 0.06)	
Diorio, et al. 2006 **[52]**	Premenopausal women n=771 [Canada]	CS	<48y (if a nonsmoker) & <46y (if a smoker)	Dietary & Supplemental Vitamin D & Ca	FFQ	PBD: [Mammogram: computer-assisted method]	FOOD ONLY: Vitamin D: β for 100IU/d = -1.8; Calcium: β for 250mg/d = -1.8 FOOD & SUPP: Vitamin D: β for 100IU/d = -1.4 Calcium: β for 250mg/d = -1.9	Alcohol, total energy, age, BMI, age at menarche, age at first full-term pregnancy, # of full-term pregnancies, # of breast biopsies, duration of past use of OC & of HRT, family history of BC in 1st degree relative, PA, education, smoking status
Masala, et al. 2006 [Also in Tables 4, 5, & 7] **[33]**	Mediterranean Population – Florence, Italy section of EPIC n=1,668	CS	Pre-, post- & peri-menopausal women	Vitamin D & Ca	160-item validated FFQ	Wolfe Classification [P2+DY vs. N1+P1] & semi-quantitative	All Women: P2+DY vs. N1+P1: Calcium $OR_{T3vs.T1}$= 0.67 (95%CI: 0.47-0.94)	Age, education, BMI, menopausal status, total energy (log), each food separately (tertiles)
Mishra, et al. 2008 [Also in Table 1] **[23]**	BBC [n's ranged from 674-979 women] Cross-sectional n: Total: n=287 [England]	C	36,43,53y/51.5y	Dietary Ca & vitamin D [age 53 follow-up: included supplement data]	5-day food records	PBD, ADT, ANDT [Mammogram;Cumulus]	Null Cross-sectional findings: Postmenopausal women: ≥1180 mg/d^{-1} vs. ≤699 mg/d^{-1}, 0.53 s.d. lower PBD (95% CI: 0.03-1.02)	Mammographic view, age at mammogram, BMI at 53, energy, age at menarche, parity, smoking status, adult SES
Nordevang, et al. 1993 [Also in	BC Patients [stage I-II n=238	CS	57.5y	Ca	Dietary hx interview within 4 months of BC	Wolfe Classification [N1+ P1 vs.	Premenopausal women: P2+Dy vs. N1+P1:	BMI, age, ER status

Tables 5 & 7] [35]	[Sweden]				diagnosis	P2+Dy]	Calcium (1165 vs. 1433 mg/10 MJ)	
Knight, et al. 2006 [55]	MBCFCS n=487 [US, NH-White]	CS	56.4y	Vitamin D [25(OH)D] & dietary Ca	FFQ	PBD, TDA [Mammogram: Cumulus]	Null	Full model: age, BMI, parity, age at first birth, PA
Tseng, et al. 2007 [Also in Tables 4, 5, & 7] [39]	Women with at least 1 1st or 2nd degree relative with BC or OV cancer n=157 [US, NH-White]	CS	50y	Vitamin D & Ca	126-item FFQ	PBD: BIRADS	OR: Vitamin D intake $_{T3vsT1}$, 0.5 (95% CI: 0.2-1.1)	Age, BMI, caloric intake, age at menarche, menopausal status, history of HRT, family history
Vachon, et al, 2000 [Also in Tables 4, 5, & 7] [36]	MBCFCS n = 1508 [US, NH-White]	CS	61.4y	Vitamin D & Ca	153-item validated FFQ	PBD [Mammogram: visual estimation]	Null	Energy, age, BMI, WHR, PA, age at menarche, age at first birth & # of births (combined), alcohol, smoking, family hx of BC, HRT (all & postmenopausal women) & OC use (premenopausal women)

Table 4-2. Studies of Alcohol Intake in Adulthood and Breast Density

Author, Year	Study Population, n	Design	Age	Foods/Nutrients of Interest	Dietary Assessment	Outcome	Major Significant Results	Adjustments
Maskarinec, et al. 2005 [46]	BEAN (n=217 premenopausal women) & MEC (n=582 cases & n=658 controls) [Multiethnic cohort]	BEAN = CS MEC = CC	BEAN = 43y MEC (cases & controls) = 57y	Alcohol	Validated FFQ	Mammogram: Computer-assisted method]	Null	Age, BMI, ethnicity, HRT use, age at first live birth, parity, age at menarche, menopausal status, group status, family hx of BC when appropriate.
Masala, et al. 2006 [Also in Tables 3, 5, & 7] [33]	Mediterranean Population – Florence section of EPIC n=1,668 [Italy]	CS	Pre-, post- & peri-menopausal women	Alcohol	160-item validated FFQ	Wolfe Classification [P2+DY vs. N1+P1] & semi-quantitative method ["entirely fat"; <25%, "25-75%, >75% high density area]	All Women: P2+DY vs. N1+P1: Overall alcohol: $OR_{T3vs.T1}$=1.31 (95%CI: 1.01-1.72) Premenopausal women at enrollment [n=491]: Wine: OR_{Q4vsQ1}: 1.84 (95%CI: 1.07-3.16); High alcohol consumption OR_{Q4vsQ}1:1.86 (95%CI:1.03-3.38)	Age, education, BMI, menopausal status, total energy (log), each food separately (tertiles)
Sala, et al. 2000[Also in Tables 5 & 7] [32]	EPIC-Norfolk Cases: P2/DY Controls: N1/P1 [n=203 cases & n=203 controls] [UK]	NCC	Cases & Controls: 59y	Alcohol	7-day food record	Wolfe Patterns: [High Risk: P2 & DY; Low Risk: N1 & P1]	Null	Menopausal status, parity, HRT, BMI
Tseng, et al, 2007 [Also in Tables 3, 5, & 7] [39]	Women with 1st degree or 2nd degree relative with BC or OV cancer n=157 [US, NH-White]	CS	50y	Alcohol	126 item validated FFQ	PBD: BIRADS	Null	Age, BMI, energy, age at menarche, menopausal status, hx of HRT, family hx.

Vachon, et al. 2000 [Also in Tables 3, 5, & 7] **[36]**	MBCFCS n = 1508 [US, NH-White]	CS	61.4y	Alcohol	153-item validated FFQ	PBD [Mammogram: visual estimation]	Postmenopausal women: White wine: Nondrinkers vs. ≥2-4 svg/wk = 29%(95%CI: 26-32%) vs. 34%(95%CI: 30-37%), Red wine: Nondrinkers vs. ≥2-4 svg/wk: 34%(95%CI:31-36%) vs. 28%(95%CI:24-33%)	Energy intake, age, BMI, WHR, PA, age at menarche, age at first birth & # of births (combined), alcohol, smoking, family hx of BC, HRT (all & postmenopausal women), OC (premenopausal women)

Table 5-2. Studies of Dietary Fat Intake in Adulthood and Breast Density

Author, Year	Study Population, n	Design	Age	Foods/Nutrients of Interest	Dietary Assessment	Outcome	Major Significant Results	Adjustments
Brisson, et al. 1989 [Also in Table 7] **[34]**	CNBSS – Newly Diagnosed BC patients Cases: n=290 Controls:n= 645 Total n=935 [Canada]	CC	40-62 y	Dietary fats	114-item FFQ + questions on vitamin A	Wolfe Classification [High Risk: P2+DY; Low Risk: N1+ P1] [Mammogram: visual estimation]	Controls (Total Densities): Saturated fat_{Q4vsQ1}: 44.2% vs. 38.6%, β=0.370 (SE=0.141)	Age, body weight, parity, education, energy
Masala, et al. 2006 [Also in Tables 3, 5, & 7] **[33]**	Mediterranean Population – Florence section of EPIC n=1,668 [Italy]	CCS	Pre-, post- & peri-menopausal women	Dietary fats	160-item FFQ	Wolfe Classification [P2+DY vs. N1+P1] & semi-quantitative method	All Women: P2+DY vs. N1+P1: Olive Oil $OR_{T3vs.T1}$ 0.73 (95%CI: 0.55-0.98) Linolenic acid $OR_{T3vs.T1}$ =0.69 (95%CI: 0.47-0.99, p-trend = 0.05)	Age, education, BMI, menopausal status, total energy (log), each food separately (tertiles)
Nagata, et al. 2005 [Also in Table 7] **[37]**	Japanese Women n=601 [Japan]	CS	Premenopausal women: 42.6y Postmenopausal women: 57.8y	Dietary fats	169-item FFQ	PBD [Mammogram: fully-automated method]	Postmenopausal women: Total Fat: Q4vsQ1 = 15.5 (95%CI: 10.8-21.2) vs. 9.9% (95%CI: 6.8-13.7; Saturated fat: Q4vsQ1=16.5% (95CI:11.3-22.6%) vs. 7.3%(95%CI:4.7-10.4%	Age, BMI, smoking status, # of births, hx of breast feeding for premenopausal women & for age, BMI, education, age at menopause for postmenopausal women. Nutrient intakes were adjusted for total energy.

Nordevang, et al. 1993 [Also in Tables 3 & 7] **[35]**	BC Patients [stage I-II n=238 [Sweden]	CS	57.5y	Dietary fats	Dietary history interview within 4 months of BC diagnosis	Wolfe Classification [N1+ P1 vs. P2+Dy]	Premenopausal women: P2+Dy vs. N1+P1: Total fat (42.04 vs. 34.72%E); Saturated fat (19.27 vs. 15.42%E), MUFA (14.22 vs. 11.98%E); PUFA (5.65 vs. 4.70), n-6 FA (4.69 vs. 3.81%E) Postmenopausal women: P2+Dy vs. N1+P1: MUFA[12.88 vs. 12.32%E)	BMI, age, ER status
Sala, et al. 2000 [Also in Tables 4 & 7] **[32]**	EPIC-Norfolk Cases: P2/DY Controls: N1/P1 [n=203 cases & n=203 controls] [UK]	NCC	Cases & Controls: 59y	Dietary fats	7-day food record	Wolfe Patterns: [High Risk: P2 & DY; Low Risk: N1 & P1]	Null	Menopausal status, parity, HRT, BMI
Tseng, et al. 2007 [Also in Table 3, 4, & 7] **[39]**	1st degree or 2nd degree relative with BC or ovarian cancer n=157 [US, NH-White]	CS	50y	Dietary fats	126 item validated FFQ	PBD: BIRADS	Null	Age, BMI, caloric intake, age at menarche, menopausal status, history of HRT, family history category.
Qureshi, et al. 2012 [Also in Table 7] **[38]**	NBCSP n=2,252 Postmenopausal women [Norway]	CS	58y	Dietary fats	180-item validated FFQ	PBD & AD [Mammogram: Computer-assisted method]	PBD: Saturated fat $_{Q4vsQ1}$: 19.7 (95%CI: 18.7-20.7%) vs 17.0 (95%CI: 15.6-18.3, p-trend = 0.06)	Age at mammography, education (y), age at menarche, # of pregnancies, age at 1st FT pregnancy for

								parous women, HRT, BMI, total energy
Vachon, et al. 2000 [Also in Tables 3, 4, & 7] **[36]**	MBCFCS n = 1508 [US, NH-White]	CS	61.4y	Dietary fats	153-item FFQ	PBD [Mammogram: visual estimation]	Premenopausal women: PUFAs: Q4vsQ1: 42%(95%CI:35-49%) vs.38%(95%CI:37-51%) PUFA:SFA: 43%(95%CI: 36-50%) vs.38%(33-44%,); SFA: Q4vsQ1: 37% (95%CI:32-43%)vs.44%(95% CI: 37-51%)	Energy, age, BMI, WHR, PA, age at menarche, age at first birth and # of births (combined), self-reported alcohol intake, smoking, family hx of BC, HRT (all & postmenopausal women), OC (premenopausal women)

Table 6-2. Studies of Dietary Patterns in Adulthood and Breast Density

Author, Year	Study Population, n	Design	Age	Foods/Nutrients of Interest	Dietary Assessment	Outcome	Major Significant Results	Adjustments
Mishra, et al. 2010 [Also in Table 1] **[24]**	BBC n =700 [England]	C	36,43y/51y ["habitual adult" dietary patterns]	Dietary Patterns: 1) low fat, fiber 2) alcohol & fish 3) high fat & sugar 4) meat, potatoes & vegetables	5-day food records	PBD, ADT, ANDT [Mammogram;Cumulus]	Null	Mammographic view, age at mammogram, BMI at 53, age at menarche, menopausal status at the time of mammography, HT use, parity, smoking status, PA, social class, other three dietary patterns, energy
Tseng, et al. 2008 **[66]**	MBCFSC n=1,286 [US, NH-White]	CS	57y	MDS	153-item validated FFQ	PBD [Mammogram: semi-automated threshold method]	Current smokers (n=176) & the MDS (continuous): β= -1.68(SE=0.55) MDS Category: $\beta_{CAT3vsCAT1}$ = -7.17 (SE = 2.77)	Age, total energy, menopausal status, education, HRT, BMI, WHR, age at menarche, parity & age at first live birth (combined variable), alcohol, relation to proband.
Tseng, et al. 2008 **[64]**	MBCFSC n=1,286 [US, NH-White]	CS	57y	Dietary patterns: 1) fruit-vegetable-cereal pattern 2) salad-sauce-pasta/grain pattern 3)meat-starch pattern	153-item validated FFQ	PBD [Mammogram: Semi-automated method]	Smokers: Fruit-vegetable-cereal pattern: β= -0.30 (SE = 0.13) Salad-sauce-pasta/grain pattern: (β= -0.27(SE=0.15, p=0.06)	Age, total energy, menopausal status, education, PA, HRT, BMI, WHR, age at menarche, parity & age at first birth, alcohol, relation to proband

Table 7-2 Studies of Selected Nutrients in Adulthood and Breast Density

Author, Year	Study Population, (n)	Design	Age	Foods/Nutrients of Interest	Dietary Assessment	Outcome	Major Significant Results	Adjustments
Bérubé, et al. 2008 **[61]**	Premenopausal women: n=777 Postmenopausal women: n=783 [Canada]	CS	Premenopausal: 47y Postmenopausal: 60y	MVMM supplements	161-item FFQ	PBD: [Mammogram: computer-assisted method]	Premenopausal women: Current users (45%, SE: 1.64%), past (42.9%, SE: 1.28%), never users (40.2% SE: 1.05%)	Age, education, BMI, age at menarche, # of full-term pregnancies, age at first full-term pregnancy, duration of OC & HRT, smoking status, PA, family hx of BC in first degree relative, personal hx of breast biopsy, chronic illness, mean energy, alcohol, vitamin & mineral supplements, following special diet, dietary vitamin D & calcium intake, season of mammography
Brisson, et al. 1989 [Also in Table 5] **[34]**	CNBSS – Newly Diagnosed BC patients Cases: n=290 Controls:n= 645 Total n=935 [Canada]	CC	40-62 y	Several dietary factors, especially vitamin A	114-item FFQ + additional questions on vitamin A	Wolfe Classification [High Risk: P2+DY; Low Risk: N1+ P1] [Mammogram: visual estimation]	Controls (Total Densities): Carotenoids $_{Q4vsQ1}$: 38.2% vs. 43.6%, β= -392 (SE=171) ; Fiber$_{Q4vsQ1}$:37.9% vs. 43.0%, β=-1.02 (SE=0.41)	Age, bodyweight, parity, education, energy
Masala, et al. 2006 [Also in Tables 3, 4, & 5]	Mediterranean Population – Florence section of EPIC n=1,668 [Italy]	CS	Pre-, post-& peri-menopausal women	Several dietary factors	160-item validated FFQ	Wolfe Classification [P2+DY vs. N1+P1] & semi-quantitative	All Women: P2+DY vs. N1+P1: Vegetables: $OR_{T3vs.T1}$= 0.66 (95%CI:0.50-0.88); Cheese: $OR_{T3vs.T1}$: 0.73 (95%CI: 0.55-0.99); β-	Age, education, BMI, menopausal status, total energy(log), each food separately (tertiles)

[33]						method	carotene $OR_{T3vs.T1}$= 0.71 (95%CI: 0.53-0.94), Vitamin C $OR_{T3vs.T1}$= 0.75 (95%CI: 0.56-0.99); Potassium $OR_{T3vs.T1}$= 0.69 (95%CI: 0.48-1.00, p-trend = 0.05), Tomato sauce: $OR_{T3vs.T1}$ =1.34 (95%CI: 1.01-1.77) Premenopausal women at enrollment [n=491]: High consumption of cheese: OR_{Q4vsQ1} 0.44 (95%CI: 0.23-0.84)	
Nagata, et al. 2005 [Also in Table 5] **[37]**	Japanese Women n=601 [Japan, Asian]	CS	Premenopausal women: 42.6y Postmenopausal women: 57.8y	Protein, dietary fiber, & soy isoflavones	169-item validated FFQ	PBD [Mammogram: fully-automated method]	Postmenopausal women: Protein: Q4vsQ1 = 13.9%(95%CI:10.4-18.0%) vs.6.7%(95%CI: 3.6-10.7%; CHO: Q4vsQ1 = 9.6%(95%CI: 6.5-13.2) vs. 15.6%(95%CI: 11.1-20.9%)	Age, BMI, smoking status, # of births, & hx of breast feeding for premenopausal women and for age, BMI, # of births, education, age at menopause; nutrient intakes were adjusted for total energy.
Nordevang, et al. 1993 [Also in Tables 3 & 5] **[33]**	BC Patients [stage I-II n=238 [Sweden]	CS	57.5y	Various nutrients	Dietary history interview within 4 months of BC diagnosis	Wolfe Classification [N1+ P1 vs. P2+Dy]	Premenopausal women: P2+Dy vs. N1+P1: CHO: (40.41 vs. 47.37%E); Fiber (19.05 vs. 26.09mg/10MJ), Carotene (3.80 vs. 5.62 mg/MJ)	BMI, age, ER status
Sala, et al. 2000 [Also in Tables 4, 5 & 9] **[32]**	EPIC-Norfolk Cases: P2/DY Controls: N1/P1 [n=203 cases & n=203 controls] [UK]	CC	Cases & Controls: 59y	Vitamin A, Vitamin C, Vitamin E, protein, carbohydrate, fiber, vegetables, cereals & breads, fruits, red meat, white meat,	7-day food record	Wolfe Patterns: [High Risk: P2 & DY; Low Risk: N1 & P1]	All Women: Protein: $OR_{ORT3vs.T1}$ =2.00 (95%CI:1.06-3.77)**; Total CHO: $OR_{ORT3vs.T1}$ =1.93, 95%CI: 1.03-3.59)**	*Unadjusted **Menopausal status, parity, HRT, BMI

				total meat, milk, dairy products, fish.			Postmenopausal Women: Protein: (OR $_{ORT3vs.T1}$ -=2.20, 1.04-4.63, p=0.03)**, Total CHO: (OR $_{ORT3vs.T1}$ =2.22,1.02-4.79)**, Total meat intake: (OR $_{ORT3vs.T1}$ =2.50, 1.09=5.69)**	
Tseng, et al. 2007 [Also in Table 3, 4, & 5] **[39]**	At 1st degree or 2nd degree relative with BC or ovarian cancer n=157 [US, NH-White]	CS	50y	Calories, cholesterol, protein, animal protein, carbs, dietary fiber, carotene, folate, vitamin E , meats, fruits, vegetables, tofu.	126 item FFQ	PBD: BIRADS	Women who do not have hereditary cancer patterns: Protein [OR: 3.0 (95% CI: 1.3-6.9)] & animal protein [OR: 4.3 (95%CI: 1.8-10.3)	Age, BMI, energy, age at menarche, menopausal status, hx of HRT, family hx category.
Tseng, et al. 2011 [Also in Table 2] **[29]**	Chinese-American Immigrant Women n=201 [US, Asian]	CS	53.1y	Red meat	88-item FFQ	PBD:BIRADS	Null	Age, level of acculturation, BMI, combined variable representing # of live births & age at first live birth, adult weekly frequency of dairy food intake
Qureshi, et al. 2012 [Also in Table 5] **[38]**	NBCSP n=2,252 Postmenopausal women [Norway]	CS	58y	Various nutrients & vitamins	180-item FFQ	PBD & AD [Mammogram: Computer-assisted method]	PBD: Saturated fat $_{Q4vsQ1}$: 19.7 (95%CI: 18.7-20.7%) vs 17.0 (95%CI: 15.6-18.3, p-trend = 0.06)	Age at mammography, y of education, age at menarche, # of pregnancies, age at first full-term pregnancy for parous women, HRT, BMI, total energy

Vachon, et al. 2000 [Also in Tables 3, 4, & 5] **[36]**	MBCFCS n = 1508 [US, NH-White]	CS	61.4y	Vitamin A, retinol, carotene, crude & dietary fiber, total carbohydrates, cholesterol, B12, folate, vitamins C, E, total protein, total energy	153-item FFQ	PBD [Mammogram: visual estimation]	Premenopausal women: Vit E: Q4vsQ1: 42%(95%CI: 36-47%) vs. 38%(95%CI: 33-46%, p-trend = 0.05); Total Dairy Intake:T3vs.T1=38% (95%CI=32-44%) vs. 44%(95%CI: 37-51%) Postmenopausal women: Vit B12 [sup only]: Q4vQ1: 34%(95%CI: 31-36%) vs. 32%(95%CI:30-34%, p-trend = 0.05]	Energy intake, age, BMI, WHR, PA, age at menarche, age at first birth and # of births (combined), alcohol smoking, family hx of BC, HRT (all and postmenopausal women) and OC use (premenopausal women)

Table 8-2. Randomized Controlled Trials in Adulthood of Diet and Breast Density

Author, Year	Study Population, n	Design	Age	Foods/Nutrients of Interest	Dietary Assessment	Outcome	Major Significant Results	Adjustments
Bertone-Johnson, et al. 2012 [Also in Table 3] **[53]**	WHI Ca+D Trial n=330 Postmenopausal women with low BD [8.4% ± 10.2%] [US]	RCT	I & C, respectively: 61.8y, 62.0y	Daily supplementation of 1,000 mg of Ca & 400 IU of Vitamin D [1 y]	122-item FFQ	PBD: [Mammogram: Computer-assisted method]	Null	Subgroup analyses: age, race/ethnicity, total vitamin D, HT treatment, Gail risk score, BMI, region of residence, category of mammogram density at baseline.
Boyd, et al. 1997 **[40]**	≥50% PBD n=817 [Canada]	RCT	I & C, respectively: 46.5y, 45.9y	Low-fat, high-CHO diet [2 y]	3-day food records	AD, PBD at baseline & 2 years [Mammogram: Automated]	Intervention Group: BA ↓ by an average 2.4%. The average ↓ in PBD was 6.1%. Control Group: BA was ↑ by 0.3% & PBD was ↓ by 2.1%.	Group assignment, age, weight, menopausal status.
Martin, et al. 2009 **[41]**	≥50% PBD [premenopausal at entry, postmenopausal during follow-up] n=461 [Canada]	RCT	I & C, respecively: 48.7y, 48.6y.	Low-fat, high CHO intervention vs. control [2 y]	Food records	TB, DA, NDA, PBD [Mammogram: Computer-assisted method]	Null	Family hx of BC, OC use, HRT, menopausal status, dietary fat
Knight, et al. 1999 **[42]**	Premenopausal at entry & postmenopausal at follow-up Total: n=78 [Canada]	RCT	I & C, respectively; 49.5y, 49.2y.	Low-fat, high CHO intervention vs. control [2 y]	3 food records	ADT, PBD at baseline & 2 years [Mammogram: Automated]	Total fat (median change: 57-31 g/d) was associated with an average 5.61cm^2 ↓ in the ADT. Sat. fat (median change: 21-11g/d) & was associated with an average 5.54cm^2 ↓ in the ADT & a 3.93% ↓ in PBD. Dietary cholesterol (median change: 229-	Total energy, weight change (included in all models); age, family hx, smoking status, parity, ever breastfeeding, OC use, age at menarche, age at first birth, PA

							150mg/d) was associated in an average 3.27cm^2 ↓ in the ADT & a 3.52% ↓ in PBD.	
Dorgan et al. 2010 [Also in Table 2] **[30]**	DISC Premenopausal women n=182 [US, NH-White]	CS [RCT follow-up]	25-29y	Long-term effects of low-fat diet	3-24-hr dietary recalls	PBD & VDT [MRI]	Null	%body fat, age at randomization, age at visit, clinic, BMI-Z score, race, education, smoking status, PA at 14-17 years old & separately during the past year, # of full term pregnancies, hormonal contraceptives

Table 9-2. Studies of Total Energy and Adult Breast Density

Author, Year	Study Population, n	Design	Age	Foods/Nutrients of Interest	Dietary Assessment	Outcome	Major Significant Results	Adjustments
Haars, et al. 2009 [Also in Table 1 & 2] [26]	DOM-Project, The Netherlands n=535 [The Netherlands]	CS	>18y/53y	Short-term energy restriction	Described in Table 1	BS, DT, NDT, PBD: [Mammogram ; visual observation]	Null	Age at examination, parity, menopausal status, BMI
Sala, et al. 2000 [Also in Tables 4 & 5] [32]	EPIC-Norfolk Cases: P2/DY Controls: N1/P1 [n=203 cases & n=203 controls] [UK]	CC	Cases & Controls: 59y	Total energy	7-day food record	Wolfe Patterns: [High Risk: P2 & DY; Low Risk: N1 & P1]	All Women: Total energy**:** $OR_{T3vs.T1}$ = 1.79, 95%CI :1.09-2.91) Postmenopausal Women: Total Energy: ($OR_{T3vs.T1}$ =2.27, 1.20-4.26)	Unadjusted
Mishra, et al. 2010 [Also in Table 1] [24]	BBC n =700 [England]	C	36,43y/51y ["habitual adult" dietary patterns]	Total energy	5-day food records	PBD, ADT, ANDT [Mammogram ;Cumulus]	All women: Energy: PBD: Per SD 0.12 (95%CI: 0.01, 0.23) ADT: Per SD: 0.12 (95%CI: 0.00-0.25).	Mammographic view, age at mammogram, BMI at 53, age at menarche, menopausal status at mammography, HRT, parity, smoking status, PA, SES, other 3 dietary patterns, energy intake.

Legend: PC: Prospective Cohort; CS: Cross-Sectional; CC: Case-Control; NCC: Nested Case-Control; RCT: Randomized Controlled Trial; I: Intervention; C: Control; BS: Breast Size; PBD: Percent Breast Density; VDT: Volume of Dense Tissue; ADT: Area of Dense Tissue; ANDT: Area of Non-Dense Tissue; DT: Dense Tissue; NDT: Non-Dense Tissue; TDA: Total Dense Area; BMI: Body Mass Index; HRT: Hormone Replacement Therapy; MBCFSC: Minnesota Breast Cancer Family Study Cohort; BBC: British Birth Cohort; MDAS-WHI: Mammogram Density Ancillary Study-Women's Health Initiative; WHI CaD: Women's Health Initiative Calcium and Vitamin D Trial; DOM-Project: Diagnostisch Onderzoek Mammacarcinoom-Project; EPIC: European Investigation into Cancer and Nutrition; NBCSP: Norwegian Breast Cancer Screening Program; CNBSS:Canadian National Breast Screening Study; DISC: Dietary Intervention Study in Children; BEAN: The Breast, Estrogens, and Nutrition Study; MEC: The Multiethnic Cohort; CHO: Carbohydrate; MUFA: Monounsaturated Fatty Acids; PUFA; Polyunsaturated Fatty Acids; n-6-FA: Omega 6 Fatty Acids; BC: Breast Cancer; MVMM: Multivitamin/Multimineral Supplement; NH-White: Non-Hispanic White; OR: Odds Ratio; FFQ: Food Frequency Questionnaire; BI-RADS: Breast Imaging-Reporting and Data System; MDS: Mediterranean Diet Score; WHR: Waist-to-Hip Ratio; PA: Physical Activity; ER Status: Estrogen Receptor Status; OC: Oral Contraceptive; Hx: History; MV: Multivitamin

Table 10-2. Summary of Nutrient Relationships with Breast Density and their Proposed Mechanisms

Premenopausal Women: ↑ intakes of:	***Mechanism of Action (i.e. IGF/IGFBP/E2/ROS)***	***Postmenopausal Women: ↑ intakes of:***	***Mechanism of Action (i.e. IGF/IGFBP/E2/ROS)***
Total fat	↑↓IGF, ↓IGFBP, May ↑Estrogen	Protein	Veg Pro =↑IGFBP Total Pro =↑IGF
SFA [?]	↓IGFBP, ↑IGF, May ↑Estrogen	Total fat	↓IGFBP, ↑↓IGF, May ↑Estrogen
MUFAs	↓IGFBP, ↑IGF, May ↑Estrogen	Saturated fat	↓IGFBP, ↑IGF, May ↑Estrogen
n-6 FA	↓IGFBP, ↑IGF, May ↑Estrogen	Vitamin B12 [supplemental]	?
PUFA	↑IGF, ↓IGFBP, May ↑Estrogen	White wine	↑Estrogen metabolites, ↑ Estrogen responsiveness, ↓SHBG, ↑IGF, ↓IGFBP ↑Oxidative stress
PUFA:SFA	↑IGF, ↓IGFBP, May ↑Estrogen [?]	Meat	↑Oxidative Stress
Vitamin C [supplemental]	?	Carbohydrates [?]	↑IGF [Need to distinguish between whole v. refined, many studies do not do this]
Wine	↑Estrogen metabolites, ↑ Estrogen responsiveness, ↓SHBG, ↑IGF, ↓IGFBP ↑Oxidative stress	Total energy	↑ Estrogen, ↑IGF availablility, ↑DNA replication rate & ↓apoptosis
Overall alcohol consumption	↑Estrogen metabolites, ↑ Estrogen responsiveness, ↓SHBG, ↑IGF, ↓IGFBP ↑Oxidative stress		
MVMM supplements	MAY ↑IGF, ↑IGFBP		
Total energy [excess consumed in midlife may affect densities in later life or restriction early in life]	↑ Estrogen, ↑IGF availability, ↑DNA replication rate & ↓apoptosis		
↓ intakes of:			
Carbohydrates	↑IGF [Need to distinguish between whole v. refined, many studies do not do this]		
Fiber	↓Oxidative stress [?], may ↑SHBG, ↑IGFBP		
Carotene	↓Oxidative stress [?], ↑IGFBP		
Calcium	Ameliorates IGF action & enhances IGFBP action [see paper in review], ↑IGF [?]		

Table 11-2. Summary of Nutrient Relationships with Breast Density and their Proposed Mechanisms

Nutrients that are associated with a ↓ in Breast Density (absolute density or % breast density)

Premenopausal Women: ↑intakes of:	**Mechanism of Action (i.e. IGF/IGFBP/E2/ROS)**	**Postmenopausal Women: ↑intakes of:**	**Mechanism of Action (i.e. IGF/IGFBP/E2/ROS)**
Calcium	May ameliorate IGF action & enhances IGFBP action, ↑IGF [?]	Carbohydrate [?]	↑IGF [Need to distinguish between whole v. refined, many studies do not do this]
Vitamin D	May ameliorate IGF action & enhances IGFBP action, breast tissue may be able to locally synthesis 25(OH)D→1,25(OH)2D	Red Wine	↓Oxidative stress [?]
SFA [?]	[?]	MUFA	↓Oxidative stress [?]
Total dairy	↑IGF, ↑IGFBP, vitamin D & calcium may negate these effects [VD & Ca have stronger effects when IGF/IGFBP are high]	Carotenoids	↓Oxidative stress [?],↑IGFBP
Cheese consumption	↑IGF, ↑IGFBP, vitamin D & calcium may negate these effects [VD & Ca have stronger effects when IGF/IGFBP are high]	Fiber	↓Oxidative stress [?], may ↑SHBG, ↑IGFBP

Legend: SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; n-6 FA: Omega-6 Fatty Acids; PUFA: Polyunsaturated Fatty Acids; MVMM: Multivitamin/Multimineral Supplements; IGF: Insulin Growth Factor; IGFBP: Insulin Growth Factor Binding Proteins; SHBG: Sex Hormone Binding Globulin; VD: Vitamin D; Ca: Calcium.

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Chapter 3

Dietary Energy Density is Positively Associated with Breast Density among Young Women

Abstract

Breast density is associated with breast cancer risk; however, little is known about dietary influences on breast density. The objective of this study was to determine if dietary energy density (ED) is associated with percentage dense breast volume (%DBV), absolute dense breast volume (ADBV), and absolute non-dense breast volume (ANDBV) in young women.

A cross-sectional analysis was conducted with 172 females ages 25-29 from the Dietary Intervention Study in Children Follow-Up Study (DISC06). Percent DBV and ADBV were measured by magnetic resonance imaging (MRI) and diet was assessed by three 24-hour recalls. Percent DBV was calculated from the total dense breast volume (TDBV) and the ADBV. The ANDBV was calculated using the %DBV and ADBV. Dietary ED (kcal/g) was calculated using three methods: (1) food-only, (2) food plus caloric beverages, and (3) food plus all beverages. Analysis was conducted using linear mixed effects models.

The median dietary ED in this population from food alone was 1.67 kcal/g (range = 0.91-3.42 kcal/g). Food-only dietary ED was positively associated with %DBV and inversely associated with ANDBV. There were no statistically significant associations for the ED variables with ADBV. After adjusting for race, smoking status, education, duration of hormone use, % body fat, childhood BMI-Z score, and parity, each 1 kcal/g unit increase in ED was associated with a 27.0% (95% CI: 6.3 to 51.7%) increase in %DBV (p=0.009). There were no significant associations for ED with %DBV or ADBV when ED was calculated as food plus caloric beverages or food plus all beverages. After stratifying by median childhood BMI-Z score, the associations between ED and all breast density measures became stronger. A significant positive association was observed for food-only ED with %DBV and a significant inverse association with ANDBV in women who had higher childhood BMI-Z scores. The ADBV was also positively associated with food-only ED in women who had a higher childhood BMI-Z score; however, it failed to reach statistical significance.

This is the first report to suggest a potential role for dietary ED in breast density. The effects of long-term exposure to high ED diets on breast cancer risk remain unknown.

Introduction

Breast density, quantified as the amount of fibroglandular tissue relative to total breast area, is one of the strongest risk factors for breast cancer. Women with high breast densities (greater than 75%) have a 4-fold increase in breast cancer risk [1]. Consequently, breast density is considered by some to be an intermediate phenotype along the breast cancer continuum [2]. Breast density is modifiable [2-5]; researchers have demonstrated that diet may be a contributing factor. [6-8]. Most research to date regarding dietary influences on breast density has focused on single foods or nutrients [7, 9, 10]. Few studies have addressed the effects of overall diet on breast density despite the fact that it may more aptly account for the complex interactions among the foods consumed as components of diets [11-13].

Dietary energy density (ED) is a relatively novel measure of diet quality that estimates the amount of energy per unit of food (kcal/g) consumed. Dietary ED can be calculated for food only, food and caloric beverages, and food and all beverages [14]. Diets high in water and fiber have lower ED due to their large gram weights with less energy contribution to the diet. In contrast, diets high in fat have higher ED. Energy-dense diets have been positively associated with body weight in both children and adults [15] and may be contributing to obesity-related chronic disease incidence. While there is no consensus for measuring dietary ED, a recent comprehensive review suggested that ED from food alone provides the most consistent results and that including the energy from caloric beverages as a covariate aids in accounting for caloric beverages without attenuating the ED value due to a large gram weight [16]. Food-only ED estimates have the strongest association with weight status in both adults and children [17, 18]. For the present analysis, dietary ED and its relationship with percent dense breast volume

(%DBV), absolute dense breast volume (ADBV), and absolute non-dense breast volume (ANDBV) was evaluated in young women.

Materials and Methods

Design

The Dietary Intervention Study in Children (DISC) was a multicenter, randomized, controlled clinical trial sponsored by the National Heart, Lung, and Blood Institute and designed to test a dietary intervention intended to lower low-density lipoprotein cholesterol (LDL-C) in children. Complete details regarding trial design and primary aim results have been described previously [19-25]. Briefly, a total of 663 healthy, pre-pubertal, 8-10 year old children with elevated LDL-C, including 301 girls, were recruited from six clinical centers between 1988-1990. They were randomly assigned to a behavioral dietary intervention or usual care control group. Informed consent was initially obtained from the parents or guardians prior to randomization and again when the female DISC participants enrolled in the DISC06 follow-up study as adults. All DISC protocols were approved by Institutional Review Boards at the involved centers.

Participants

All 301 female DISC participants were invited to DISC06 follow-up visits between 2006-2008 when they were between the ages of 25-29; a total of 260 participated. Women who were pregnant or breastfeeding during or within 12 weeks before the follow-up visit (n=30), as well as those who had breast implants or reduction surgery (n=16), were not eligible for the current analysis, leaving a total of 214 women. Of these, 38 had technically unacceptable or missing

breast density or total body fat measures and four additional women were missing dietary assessments, resulting in a final analytical sample of 172 women.

Data Collection

For the DISC06 follow-up study, each female participant attended a one day data collection visit at one of the six DISC clinics. Visits were scheduled to take place during the luteal phase of the menstrual cycle whenever possible with 85% of visits occurring within 14 days of onset of next menses. During the visit, participants provided a fasting blood sample and completed survey questions regarding demographics, medical, reproductive, and menstrual histories, prescription and non-prescription drug use (including extensive information on past and current hormone use), smoking and alcohol use, leisure-time physical activity, and family history of breast cancer. Height, weight and waist circumference were measured by trained study staff and body composition was assessed by dual-energy x-ray absorptiometry (DXA) [20]. Total adiposity was characterized by percent whole body fat mass calculated as the ratio of whole body fat mass: whole body total mass. Percent DBV was calculated by using the total dense breast volume (TDBV) and the ADBV that were measured by magnetic resonance imaging (MRI) and processed by a single investigator using customized imaging software [20]. Total volumes of fibroglandular and fatty tissue were computed separately for each breast. The volumes of dense fibroglandular tissue and non-dense breast tissue alone were also examined. Childhood BMI-Z scores were calculated from the measured heights and weights at DISC study entry and based on the Center for Disease Control 2000 Growth Charts [26].

Diet was assessed using three nonconsecutive 24-hour dietary recalls collected over the course of two weeks. Assessment was conducted by trained interviewers using the Nutrition Data System for Research (NDS-R: 2007 University of Minnesota, Minneapolis, MN). Dietary ED was calculated using three methods: (a) using food only, (b) using all food plus caloric beverages,

and (c) using all foods and all beverages. First, food and beverage codes were assigned to each food item in the food file generated by NDS-R. Second, the daily average of total energy intake (kcal) was calculated for each participant. Additional daily average total energy intakes were calculated for food-only intake, and food and caloric beverage intakes. Likewise, the average amount of food (g), food and caloric beverages (g) and food and all beverages (g) were estimated. Each of the 3 measures of ED were then calculated as energy (kcal)/amount (g).

Statistical Analysis

Percent DBV, ADBV, and ANDBV were transformed to natural logarithms to improve normality. To evaluate associations with dietary ED, linear mixed effects models (SAS PROC MIXED) were fit by maximum likelihood with robust standard errors separately for %DBV, ADBV, and ANDBV. Dietary EDs estimated from food only, food and caloric beverages, and food and all beverages were included as independent continuous variables in separate models. Clinic was included in models as a random effect and all other variables were included as fixed effects. Associations between each breast density measure and dietary ED method were first examined individually in an unadjusted model. Subsequent models were further adjusted for risk factors and confounders such as smoking status (current vs. never), BMI-Z score at baseline, race (white vs. non-white), education (no college vs. attended college), duration of use of hormonal contraceptives and other sex hormones (years), whole-body percent fat, and number of full-term pregnancies. Whole body percent fat was an important confounder and accounted for over 50% of the variance in the model. DISC treatment group and energy from caloric beverages (in the food-only ED model only) also were evaluated as potential covariates, but were not included in the final model because they were not significant confounders and did not contribute appreciably to the model. Percentage differences in %DBV, ADBV, and ANDBV associated with a 1 kcal/g unit increase of ED were calculated as $\%\Delta = (\exp(\beta)-1)x100$. Effect modification by baseline

BMI-Z score was assessed by testing the significance of the cross-product terms of BMI-Z score with the continuous dietary ED variables in separate multivariate models and by stratified analyses. For stratified analyses, BMI-Z score was dichotomized based on the median and resulting subgroups were analyzed controlling for important covariates in the models. All statistical testing was conducted using two-sided tests, with significance determined at $p< 0.05$. All data were analyzed using SAS 9.3 (SAS Institute, Cary, NC).

Results

The majority of participants were non-Hispanic White (89.5%) with a median age of 27.1 years old (IQR = 26.5-27.8). Among all women the median BMI was 23.9 kg/m^2 (IQR = 21.1 to 28.2 kg/m^2) with 25% of the sample categorized as overweight and 16% as obese. The median dietary EDs were 1.67 kcal/g (interquartile range (IQR) = 1.46–2.06 kcal/g) for food only, 1.21 kcal/g (IQR = 1.05-1.44 kcal/g) for foods + caloric beverages, and 0.66 kcal/g (IQR = 0.48-0.81 kcal/g) when using foods + all beverages. The median %DBV, ADBV, and ANDBV were 24.9% (IQR = 11.1-42.5%), 92.0 cc (IQR = 47.0-140.0 cc), and 286.1 cc (IQR = 154.1–473.1 cc) respectively. Additional demographic characteristics are described in **Table 1.**

Food-only dietary ED was not associated with %DBV in unadjusted analyses [%Δ, (95% CI) = 9.4% (-7.6 to 30.0%), p=0.29]; however, it was significantly positively associated with %DBV after controlling for relevant covariates [%Δ, (95% CI) = 27.0% (6.3 to 51.7%), p = 0.009] (**Table 2**). Food-only dietary ED was also not associated with the ANDBV in unadjusted analyses [%Δ, (95% CI) = 5.1% (-8.8 to 22.1%), p=0.47]; however, after controlling for covariates, a significant inverse association was observed [%Δ, (95% CI) = -18.4% (-32.2 to -1.8%), p=0.03]. In contrast, food-only ED was not associated with ADBV in either unadjusted or adjusted analysis. ED calculated using food + caloric beverages or all beverages was not associated with %DBV or ADBV in either unadjusted or adjusted analyses. However, food +

caloric beverages, but not food + all beverages ED, was significantly inversely associated with ANDBV [%Δ, (95% CI) = -13.9% (-22.3 to -4.7%), p=0.004] in the adjusted analyses.

We observed a significant interaction between food-only ED and childhood BMI-Z score for %DBV and ADBV models (both p=<0.0001), but not for ANDBV. As a result, we stratified by the median BMI-Z score (0.2) (**Table 3**). In stratified results, food-only ED was significantly positively associated with %DBV [82.3% (35.8 to 144.8%), p <0.0001] only among participants with higher BMI-Z scores. Food-only ED was also positively associated with the ADBV in this group [44.3% (3.4 to 115.2%), p=0.07]; however, it failed to reach statistical significance. Food-only ED was significantly inversely associated with ANDBV [-26.3% (-43.6 to -3.8%), p=0.03] in women with higher BMI-Z scores. A significant interaction was also observed for BMI-Z score with Food + All Beverages ED. There were no statistically significant associations with the breast density outcomes within BMI-Z strata with the exception of a marginally significant inverse association with %DBV [-19.6% (-36.1 to 1.2%), p=0.06] among participants with a low BMI-Z score.

Discussion

In this analysis of young premenopausal women, a significant positive association between food-only dietary ED and %DBV and a significant inverse association between food-only dietary ED and ANDBV was observed. Each 1 kcal/g unit increase of food-only ED corresponded with a 27.0% increase in %DBV and an 18.4% decrease in the ANDBV. A significant inverse association was also observed between the ANDBV when ED was calculated using the food + caloric beverages dietary ED method with each unit increase in food + caloric beverage ED corresponding to a 13.9% decrease in ANDBV.

While our findings of an inverse association between food-only dietary ED, food + caloric beverages ED and ANDBV, or the fatty portion of the breast, appear counterintuitive, a

2011 study by Pettersson and colleagues [27], detected a significant inverse association between the non-dense portion of the breast and breast cancer risk, which suggests that higher amounts of fatty breast tissue may be protective against breast cancer. Additionally, another study [28] reported that non-dense breast tissue was positively correlated with lobular involution, which is inversely associated with breast cancer risk.

Though the changes in the breast density measures observed with a one unit change in ED are substantial, it is important to note that a change in one unit of ED is not inconsequential. For example, a woman consuming 1800kcal/day (by eating 1200g food) would have to consume an additional 1200kcal (and still eat 1200g food) to increase dietary ED by 1 unit. Nevertheless, a wide range of dietary EDs existed in the study population (0.91-3.42 kcals/g).

When the data was stratified by the median childhood BMI-Z score, we observed that higher food-only ED was positively associated with %DBV and ADBV and inversely associated with ANDBV primarily in women who were heavier as children. It is possible that heavier children had a poorer quality diet which continued throughout the critical period of breast development. No associations were observed in women with lower BMI-Z scores ≤ 0.2 and little was explained by further investigating additional ED methods and breast density measures within BMI-Z strata (**Table 3**). Our results were consistent with those of other investigators who noted that the strongest association with dietary ED are observed with the food only ED method [15].

Although the effect of adult weight status on breast cancer risk differs between pre- and postmenopause, higher childhood adiposity has been shown to be protective against breast cancer in both pre- and postmenopausal women [29]. While this phenomenon is not well understood, some have suggested that it could be attributed to the inverse correlation between elevated childhood body fatness and insulin-like growth factor-1 (IGF-1) levels, which would slow growth velocity (a possible risk factor for breast cancer) and decrease breast cancer risk [30] [31]. Our results of significant associations with dietary ED and all three breast density measures only in women who were heavier as children may seem counterintuitive; however, one study from the

Nurses' Health Study and Nurses' Health Study II populations [29] found that the inverse association with adolescent adiposity and breast cancer risk only existed in girls who weighed less than 8.5 pounds at birth. Birth weight has been positively associated with breast cancer risk in premenopausal women and as a result, weight gain after birth may not be protective in these women. In our sample, birth weight was higher, although not statistically significant, in girls with a BMI-Z score above the median [Median (IQR) = 7.49 lbs (1.13) vs. 7.40 lbs (1.10) p=0.62]. Our sample size (n=172) is considerably smaller than the large NHS and NHS II, which may have contributed to the lack of significance between the two strata.

This study reinforces the importance of examining associations between health outcomes and dietary ED in several ways. A 2012 review by Pérez-Escamilla et al. [15] emphasizes that beverages have a different effect on satiety than food; therefore, associations between outcomes measures and dietary ED methods that contain beverages are often weakened and not accurate. Several factors influence ED, of the macronutrients, fat has the most influence on ED since it contributes a large amount of energy for a relatively small weight. Water has the opposite effect, contributing a large amount of weight without adding energy; therefore, it is not surprising that the inclusion of beverages attenuated the associations between ED and %DBV and ANDBV, much like it results observed for weight status [16-18]. Our study shows a linear decrease in ED values across methods as beverages, both caloric and non-caloric, are added into the calculation.

To our knowledge, this is the first study to examine and report the relationship between dietary ED and breast density outcomes. Dietary ED provides information about the complete diet, and can be thought of as a novel measure of dietary quality in a manner similar to dietary patterns and score-based assessments such as the Healthy Eating Index-2005 [32]. While much of the research to date surrounding the contribution of dietary factors to breast density remains mixed, it appears that diet may be more influential in premenopausal versus postmenopausal women [6, 33, 34]. Breast tissue among premenopausal women is very dynamic because of changes that occur throughout the menstrual cycle and during pregnancy [35-37] and is thought to

be more susceptible to dietary factors such as vitamin D, calcium, and alcohol [6, 34, 38]. At least four epidemiologic studies have been conducted that included premenopausal women in their sample and examined relationships of dietary factors, including fat intake, to breast density measures. All four studies showed a positive association with dietary fat intake and %DBV; however only two reached statistical significance [7, 39]; while the other two did not [8, 40].

Few studies have examined as many breast density measures as we did [11, 41, 42]. Many studies only reported associations with percent dense breast area (%DBA), which suggests that further investigation into all measures of breast density is warranted. This may provide more insight into potential mechanisms through which breast composition can influence breast cancer risk. With that being said, %DBV alone remains one of the strongest risk factors for breast cancer, which is consistent with our findings [1].

Many studies focus on the association between single nutrients and BD with only a small number evaluating the relationship between dietary patterns and BD in both pre- and postmenopausal women [11, 13]. One study demonstrated that BD is inversely associated with diets higher in fruits, vegetables, and cereals, all of which may lower the overall ED of a diet, in pre- and postmenopausal smokers [13]. This association was also observed in the subset of premenopausal women; however, it failed to reach statistical significance in this group [13]. Fat and sugar both contribute to an increased overall dietary ED, and diets higher in fat and sugar were associated with increased %DBV in a sample of both pre- and postmenopausal women [11].

Our study had several strengths. Participants were 25-29 years old, which is an under-represented age group in studies of breast density. All data were collected by trained and certified individuals. Breast density was measured using MRI, which is more accurate than mammography particularly for dense breasts typical of young women [43]. Diet was assessed by three 24-hour diet recalls and adiposity was assessed by whole body DXA scans. Dietary ED was calculated in several ways to capture the influence of all types of food and beverages on breast density measures. Examining whole diets can provide a better understanding of the effect of diet

on disease or disease risk factors by taking into account interactions among foods, which cannot be done when examining single nutrients alone. Our study also had some weaknesses. In particular, participants were mostly non-Hispanic white women who had elevated LDL-C as children, which may limit generalizability of results to a larger population.

In summary, results of our study suggest that dietary ED is positively associated with %DBV and inversely associated with ANDBV, the fatty portion of the breast. When the data is stratified by BMI-Z score, associations with food-only ED and %DBV and ANDBV remain similar; however, a markedly stronger, although not statistically significant, association with ADBV emerges in women who had a higher childhood BMI. These results suggest that additional measures beyond %DBV need to be assessed in order to make sound conclusions regarding the effect of diet on breast density and subsequent cancer risk. Future directions should include longitudinal analyses of several ED methods in childhood and adolescence and their relationship with a wide variety of adult breast density measures in the DISC cohort.

Tables

Table 1-3. Selected Characteristics of Participants (n=172)

Characteristics	**Median (IQR)[1]**
Age at visit (y)	27.1 (26.5 – 27.8)
BMI (kg/m^2)	23.9 (21.1-28.2)
Body Fat (DXA)	34.8 (28.8 to 42.5)
Waist Circumference (cm)	79.0 (73.6 – 90.8)
BMI Z-score at baseline (8-10y)	0.19 (-0.47 – 0.97)
Duration of hormone use (y)	5.1 (1.9 – 8.0)
Percent dense breast tissue (%)	24.9 (11.1 - 42.5)
Fibroglandular breast volume (cc)	92.0 (47.0 to 140.0)
Non-dense breast volume (cc)	286.1 (154.1 to 473.1)
Characteristics	**%**
Non-Hispanic White	89.5
Current Smokers	23.8
>High School Education	90.1
% Full Term Pregnancy	26.2
Dietary Energy Density Method	**Median (IQR)[1]**
Food-Only	1.67 (1.46 - 2.06)
Food + Caloric Beverages Only	1.21 (1.05 – 1.44)
Food + All Beverages	0.66 (0.48 - 0.81)

[1]IQR: Interquartile Range

Table 2-3. Geometric Means and Percent Change (%Δ, (95% CI) in Breast Density, Fibroglandular Volume, and the Area of Non-Dense Tissue with Increasing Dietary Energy Density (n=172)

Variable	***%Δ (95% CI)* (n=172)***	***P-value***	***%Δ (95% CI)** (n=172)***	***P-value***
% Dense Breast Volume (%DBV)				
Food-Only ED	9.4% (-7.6 to 30.0%)	0.29	27.0% (6.3 to 51.7%)	0.009
Food + Caloric Beverages ED	23.4% (-18.3 to 86.7%)	0.31	21.3 % (-11.9 to 67.0%)	0.23
Food + All Beverages ED	-23.7% (-54.3 to 27.3%)	0.30	9.2% (-29.0 to 67.8%)	0.69
Fibroglandular Volume (ADBV)				
Food-Only ED	13.9% (-9.0 to 41.6%)	0.26	5.4% (-18.1 to 36.3%)	0.67
Food + Caloric Beverages ED	22.1% (-18.5 to 82.9%)	0.33	8.1% (-26.5 to 59.0%)	0.69
Food + All Beverages ED	4.1% (-36.1 to 70.8%)	0.86	-0.1% (-36.2 to 57.1%)	0.99
Non-Dense Breast Volume (ANDBV)				
Food-Only ED	5.1 (-8.8 to 22.1%)	0.47	-18.4% (-32.2 to -1.8%)	0.03
Food + Caloric Beverages ED	-6.8 (-29.6 to 24.6 %)	0.65	-13.9% (-22.3 to -4.7%)	0.004
Food + All Beverages ED	43.3 (-3.2 to 112.5 %)	0.07	-14.9%(-46.9 to 36.7%)	0.55

*Estimates from linear mixed-effects models including clinic as a random effect and dietary energy density as fixed effects.
** Estimates from linear mixed-effects models using clinic as a random effect and dietary energy density, race, smoking status, education, duration of hormone use, percent body fat, childhood BMI-Z score, and parity as fixed effects.

Table 3-3. Geometric Means (95% CI) and Percent Change (%Δ, (95% CI) in Breast Density, Fibroglandular Volume, and the Area of Non-Dense Tissue with Increasing Dietary Energy Density by strata of BMI-Z Score

	BMI-Z Score ≤ 0.2 (low) (n=86) ***β Coefficient (SE)***	***P-Value***	***BMI-Z Score > 0.2 (high) (n=86)*** ***β Coefficient (SE)***	***P-Value***	***P-for interaction***
% Dense Breast Volume (%DBV)					
Food-Only ED	1.3 %(-22.9 to 33.2%)	0.92	82.3% (35.8 to 144.8%)	<0.0001	<0.0001
Food + Caloric Beverages	4.4% (-26.2 to 47.9%)	0.81	42.4% (-14.4 to 137.1%)	0.17	0.47
Food + All Beverages	-19.6% (-36.1 to 1.2%)	0.06	65.8% (14.8 to 222.7%)	0.13	0.01
Absolute Dense Breast Volume (ADBV)					
Food-Only ED	-4.1% (-26.0 to 24.3%)	0.75	44.3% (3.4 to 115.2%)	0.07	<0.0001
Food + Caloric Beverages	-13.5% (-40.8 to 26.3 %)	0.45	59.2% (2.1 % to 148.3%)	0.04	0.21
Food + All Beverages	-20.0% (-53.5 to -27.3%)	0.41	50.0% (-28.3 to 214.1%)	0.28	0.13
Absolute Non-Dense Breast Volume (ANDBV)					
Food-Only ED	-10.9% (-28.9 to 11.7%)	0.31	-26.3% (-43.6 to -3.8%)	0.03	0.12
Food + Caloric Beverages	-23.8% (-37.4 to -7.1%)	0.008	-3.9% (-16.8% to 30.2%)	0.73	0.94
Food + All Beverages	-7.1% (-45.6 to 58.6%)	0.78	-18.5% (-48.6 to 29.1%)	0.38	0.10

*Estimates from linear mixed-effects models using clinic as a random effect and dietary energy density, percent body fat, race, smoking status, hormone use, and parity as fixed effects.

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Chapter 4

Dietary Energy Density is Associated With Body Composition in Young, Premenopausal Women: Results from The Dietary Intervention Study In Children Follow-Up Study (DISC06)

Abstract

Total adiposity has been shown to be inversely associated with premenopausal breast cancer risk; however, the effect of body fat distribution on risk remains inconclusive. We examined the association between dietary energy density (ED) (or the ratio of total energy / gram weight) and several measures of body fatness. Dietary ED has been successfully used to study the effects of total diet on weight status. A cross-sectional analysis was conducted on 203 adult women (25-29y) originally enrolled into the Dietary Intervention Study in Children (DISC). Anthropometry and body composition measures (DXA) were assessed by trained individuals. Dietary ED was calculated from 24-hour recall data in three ways: 1) Food-only; 2) Food plus caloric beverages; and 3) Food plus all beverages. The median dietary ED (inter-quartile range – IQR) from food alone was 1.7 (IQR: 1.5-2.1). Food-only dietary ED was significantly positively associated with body mass index (BMI), total body, android, and gynoid fat, and the android:gynoid fat ratio in multivariate adjusted linear mixed effects models. These results suggest that dietary ED from food alone is associated with body fatness and body fat distribution in young women.

Introduction

The prevalence of obesity has risen dramatically in the past 20 years in both adults and children [1]. Along with the increase in weight status, an increase in several weight-related diseases including type 2 diabetes and some cancers, including colon and breast, also has occurred [1]. It is well established that menopausal status modifies the effect of body fatness on breast cancer risk; higher body fatness decreases risk in premenopausal women and increases risk in postmenopausal women. In contrast, the relationship between body fat distribution and breast

cancer does not appear to follow this same pattern [2-4]. Women with higher amounts of central obesity, as measured by waist circumference and the waist:hip ratio, often have lower levels of the sex hormone binding globulin (SHBG) and insulin-like growth factor binding proteins (IGFBP) [5-7] which results in higher levels of circulating free estrogen and Insulin-like growth factor. Central obesity [8], over the past few decades may be partially contributing to the increase in premenopausal breast cancer risk.

Diet is often targeted when studying chronic disease risk because of its ability to be easily modified. Many studies focus on single nutrients; however, there has recently been a growing interest in assessing total diet because it more aptly captures food and nutrient interactions that are not considered when examining single nutrients or foods alone. The evaluation of total diet provides a more comprehensive and realistic understanding of the effect of diverse diets on chronic disease risk.

Energy density is a property of food and can be calculated using the ratio of kilocalories / gram of a single food, meal, or overall diet and is most often influenced by the amount of fat and water in the diet. Fat provides the most energy per gram (9 kcal/g) and high intakes of fat and added sugars characterize a high ED diet. In contrast, water provides no energy per gram (0 kcal/g) and high intakes of water, often in the form of fruits and vegetables, define low ED diets. Low ED diets are often associated with a healthier weight status, which may reduce the risk of several obesity-related chronic diseases [9]. Low ED diets also tend to be higher quality and provide more micronutrients, antioxidants, and bioactive components per kilocalorie that may help to reduce the risk of chronic diseases independent of healthy weight maintenance [10, 11]. Additionally, the American Institute for Cancer Research (AICR) and World Cancer Research Fund (WCRF) recommend consuming low energy-dense foods (<2.25 kcal/g) for cancer prevention [12].

The strongest and most consistent associations between ED and weight status have been observed with food-only dietary ED, while more inconsistent results have been observed with the inclusion of beverages [9, 13]. Beverages, regardless of their energy content, contain a large amount of water and often attenuate the association with weight status [9]. In our study, we examined the associations between anthropometric and body fatness measures and dietary ED calculated using three different methods: food-only, food plus caloric beverages, and food plus all beverages.

Materials and Methods

Design

The Dietary Intervention Study in Children (DISC) was a multicenter, randomized, controlled clinical trial sponsored by the National Heart, Lung, and Blood Institute and designed to test a dietary intervention to reduce low-density lipoprotein cholesterol (LDL-C) levels in children. Complete details regarding trial design and primary aim results are described elsewhere [14-20]. Briefly, a total of 663 healthy, pre-pubertal, 8-10 year old children with elevated LDL-C, including 301 girls, were recruited from six clinical centers between 1988-1990 and randomly assigned to a behavioral dietary intervention or usual care control group. Informed consent was initially obtained from the parents or guardians prior to randomization and again when the female DISC participants enrolled in the DISC06 follow-up study as adults. All DISC protocols, both past and present, were approved by Institutional Review Boards at all involved centers.

Participants

All 301 female DISC participants were invited to DISC06 follow-up visits from 2006-2008 when they were between the ages of 25-29; a total of 260 participated. Women who were pregnant or breastfeeding during or within 12 weeks before the follow-up visit (n=30) were not eligible for the current analysis, leaving 230 women. Of these, 27 had technically unacceptable or missing body composition measures, resulting in a final analytical sample of 203 women.

Data Collection

For the DISC06 follow-up study, each female participant attended a data collection visit at one of the six DISC clinics. Participants provided a fasting blood sample and completed survey questions regarding demographics, medical, reproductive, and menstrual histories, prescription and non-prescription drug use (including extensive information on past and current hormone use), smoking and alcohol use, leisure-time physical activity, and family history of breast cancer. All anthropometric measurements were done by trained study staff. Height was measured by a stadiometer, weight was measured by a balance beam or electronic scale and waist circumference was measured at the level of the uppermost lateral border of the right iliac crest using an anthropometric measuring tape. All measures were taken twice and averaged if difference in the two measures were within allowable tolerances (0.5 cm for height and waist circumference and 0.2 kg for weight). If differences were larger, measurements were taken a third time and the two closest were averaged. Body composition was assessed by dual-energy x-ray absorptiometry (DXA) [15]. Total adiposity was characterized by percent whole body fat mass, calculated as the ratio of whole body fat mass: whole body total mass. The percentage of android, or trunk and upper body fat, and gynoid, or fat around the hips, as well as the

android:gynoid fat ratio were also evaluated. Childhood BMI-Z scores were calculated from the measured heights and weights taken at DISC study entry and based on the Center for Disease Control 2000 Growth Charts [21].

Diet was assessed from three unannounced, nonconsecutive 24-hour dietary recalls. The first recall was conducted at the clinic site while the subsequent recalls were collected via telephone over the following two weeks. Assessment was conducted by trained interviewers via telephone using the Nutrition Data System for Research (NDSR: 2007 University of Minnesota, Minneapolis, MN). Several food files were generated from the recalls that included total energy, macro and micronutrients, and individual foods and beverages.

Dietary ED was calculated using three methods: (a) using food only, (b) using all food plus caloric beverages, and (c) using all foods and all beverages. To achieve this, food and beverage codes were first assigned to each food item in the food file generated by NDSR. Secondly, the daily average of total energy intake (kcal) was calculated for each participant with additional daily average total energy intakes calculated for food-only intake, and food and caloric beverage intakes. Similarly, the average amount of food (g), food and caloric beverages (g) and food and all beverages (g) were estimated. Each of the 3 measures of ED were then calculated as energy (kcal)/amount (g).

Statistical Analysis

Linear mixed effects models (SAS PROC MIXED) were fit by maximum likelihood with robust standard errors separately for all body composition measures. Dietary EDs estimated from food only, food and caloric beverages, and food and all beverages were included as independent continuous variables in separate models. Clinic was included in models as a random effect and all others variables were included as fixed effects. Associations between each body composition

measure and dietary ED method were first examined individually in unadjusted models. Subsequent models were further adjusted for risk factors and confounders including BMI-Z score at baseline (continuous), race (white vs. non-white), education (no college vs. attended college), and physical activity (continuous met-minutes). DISC treatment group and smoking status (current [2 cigarettes/day] vs. never) were evaluated as potential covariates, but were not included in the final models because they did not contribute appreciably to the model. Effect modification by education and physical activity were assessed by testing the significance of the cross-product terms of the individual education and physical activity variables with the continuous dietary ED variables in separate multivariate models and by stratified analyses. For stratified analyses, physical activity was dichotomized based on the median and education was dichotomized based on high school education or less versus college or higher. Resulting subgroups were analyzed controlling for important covariates in the models. Statistical testing was conducted using two-sided tests, with significance determined at $p< 0.05$. All data were analyzed using SAS 9.3 (SAS Institute, Cary, NC).

Results

The majority of participants were non-Hispanic White (89.7%), with a median age of 27.2 years old (IQR=26.6-27.9 y). The majority of the sample had a healthy weight (59%); however, 24.6% were overweight and 16.3% were obese. In addition, 87.2% of the participants were meeting the AICR/WCRF energy density recommendation of a dietary ED <2.25 kcal/g for cancer prevention [12]. Additional demographic characteristics are described in **Table 1.**

Overall, results for unadjusted and fully adjusted models were similar; therefore we only present the multivariate adjusted results. Food-only dietary ED was significantly positively associated with several measures of body fatness. For each 1 kcal/g unit of food-only dietary ED,

total percent body fat increased by 2.08% [β (95% CI) = 2.08 (0.63 to 3.53), p=0.005], android fat increased by 3.38% [β (95% CI) = 3.38 (2.26 to 4.50), p=<0.0001], gynoid fat increased by 2.00% [β (95% CI) = 2.00 (0.55 to 3.45), p=0.008], and the A:G fat ratio increased by 0.3 [β (95% CI) = 0.03 (0.01 to 0.05), p=0.007] **(Table 2).** Food-only dietary ED was also significantly positively associated with BMI; for each 1 kcal/g unit increase in food-only dietary ED, BMI increased by 0.59 kg/m^2. [β (95% CI) = 0.59 (0.04 to 1.14), p=0.03)] **(Table 2)**. No association was observed with waist circumference in any of the dietary ED methods. Additionally, no associations were observed with any of the other dietary ED methods **(Table 2)**. While the changes in body fatness measures observed with a one unit change in ED are large, it is important to note that a wide range of dietary EDs existed in the study population (0.91-3.42 kcals/g) and a change in one unit of ED is possible. The data was also stratified by education and median physical activity (298 met-min/week). There were no significant interactions with food-only dietary ED, but some significant interactions with food plus caloric beverages and food plus all beverages were observed. These results may suggest that dietary ED is a stronger predictor of body fatness outcomes in women with less education and physical activity (See **Tables 3 and 4**).

Discussion

In our population, food-only dietary ED was significantly positively associated with whole body percent fat, android fat, gynoid fat, the android:gynoid fat ratio, and BMI. No significant associations were observed between the other two dietary ED methods and the aforementioned outcome variables. No significant associations were observed between any of the dietary ED methods and waist circumference. The lack of association with waist circumference may be because of the larger error margin compared to other measures [22]. Finally, the addition of the energy from beverages, as suggested by a 2009 review [13] that examined the validity of

the inclusion of beverages in the ED calculation, as a covariate in the food-only dietary ED models was not significant and did not further explain the overall results.

Our findings are consistent with other research that have found significant associations between food-only ED methods [9]; however, examining the other two methods of dietary ED provides a more comprehensive picture. Several hypotheses have been made as to why the food-only ED method is often the only method associated with body composition and weight status. For example, the ED of a food, meal, or overall diet is heavily dependent on the water and fat content. When beverages are included in the ED calculation, they will attenuate the ED because of their large gram weight, even if they are caloric, and weaken the strength of the ED variable. Another hypothesis is that beverages have a different effect on satiety than foods and therefore should be examined separately from foods [23, 24].

While premenopausal breast cancer is not an endpoint in this study, we attempted to identify women who may be at a higher risk for developing this and other chronic diseases through examining dietary behaviors and body fatness measures. Diet is a low cost modifiable risk factor, hence why it is so frequently targeted in cancer and chronic disease research. Incorporating low ED foods into existing diets is a fairly simple way to reduce the risk of obesity and increase the amount of essential nutrients in the diet that can aid in disease prevention.

This study also reinforces the importance of studying overall diet as compared to single nutrients. Knowing the effects that types of diets have on health outcomes has the potential to aid in the development of sound dietary recommendations that are focused on achieving and maintaining a healthy weight and preventing chronic diseases such as cancer, cardiovascular disease, and diabetes. High ED and Low ED diets are repeatedly characterized by vastly different, yet easily identifiable foods that can be translated into recommendations for obesity and chronic disease prevention.

Our study has several strengths. All data including anthropometric, body composition, and dietary, were collected by highly trained staff. Total adiposity was measured by DXA, a very reliable method of assessment [25]. Dietary data was collected via 24-h recall by trained staff via standardized procedures with accuracy enhanced by the use the NDSR. We also examined three different methods of dietary ED and six body composition measures. In other studies that examine dietary ED and weight status, the relationships between BMI and waist circumference are often shown [26-28]. Our study not only examined BMI and waist circumference, but also total adiposity, android and gynoid fat, and the android:gynoid fat ratio. It is well known that BMI has several limitations including the lack of differentiation between adiposity and lean muscle mass [29] and that assessing body fat via DXA is a more precise measure of total adiposity.

The study also has some limitations. Because this was a cross-sectional study, causality cannot be inferred. The women in DISC were initially enrolled as children due to abnormal blood lipid concentrations. It is possible that DISC participants, particularly those randomized to the intervention group, adopted long-term dietary practices which influenced adult dietary ED. The original results of the DISC trial showed that the adjusted differences of percent of energy from total dietary fat and saturated fat were significantly lower in the intervention group throughout the entire seven-year intervention period, although the differences began to taper around the fifth year [19]. Energy intake was also lower in the intervention group throughout the first three years of the intervention; however, these differences were not sustained through the subsequent visits [19]. Diets were examined during the DISC06 follow-up study as well; the intervention group had significantly less saturated fat intake [9.8g (7.9-12.0) vs. 11.3g (9.5-13.3), p=<0.001] and significantly higher dietary fiber intake [8.9g/1000 kcals (6.3-11.2) vs. 7.3g/1000 kcals (5.8-10.5), p= 0.01] [15]; however treatment group had no effect on our outcomes of

interest. This suggests that the participants may have implemented dietary practices that impacted adult dietary ED.

In conclusion, food-only dietary ED was significantly positively associated with several measures of body fatness in our sample of premenopausal women. Future research should include longitudinal studies to evaluate the associations between dietary ED and chronic disease including risk factors for chronic disease such as inflammatory markers, glucose, and insulin over time. It is not known whether consuming a higher ED diet in childhood through adolescence is associated with body composition and other health outcomes in adulthood.

Tables

Table 1-4. Selected Demographic Characteristics (n=203)

Characteristics	**Median (IQR)[1]**
Age at visit (y)	27.2 (26.6 - 27.9)
BMI (kg/m^2)	24.0 (21.3 - 27.9)
Body Fat (%, DXA)	34.8 (29.0 - 42.2)
Waist Circumference (cm)	80.4 (73.8 - 91.3)
Android Fat (%)	40.2 (30.5 - 49.7)
Gynoid Fat (%)	46.5 (41.0 -51.4)
Android:Gynoid Fat Ratio	0.86 (0.71 - 0.99)
Dietary Energy Density Method	**Median (IQR)[1]**
Food-Only	1.7(1.5 - 2.1)
Food + Caloric Beverages Only	1.2(1.0 - 1.4)
Food + All Beverages	0.6 (0.5 - 0.8)
Characteristics	**%**
Non-Hispanic White	89.7%
Current Smokers	25.6%
>High School Education	89.7%

[1]IQR: Interquartile Range

Table 2-4. The Association between Dietary Energy Density and Selected Body Composition Measures in DISC Participants

Variable		*β Coefficient (95% CI)**	*P-Value**	*β Coefficient (95% CI)***	*P-Value***
Waist Circumference (cm)					
Food-Only ED	203	1.47 (-0.45 to 3.39)	0.13	1.13 (-0.71 to 2.97)	0.23
Food + Caloric Beverages	203	1.21 (-2.81 to 5.23)	0.55	0.71 (-1.94 to 3.36)	0.60
Food + All Beverages	203	2.79 (-3.34 to 5.92)	0.37	-1.05 (-8.69 to 6.59)	0.79
Total Body Fat (%)					
Food-Only ED	203	2.21 (0.94 to 3.48)	0.0008	2.08 (0.63 to 3.53)	0.005
Food + Caloric Beverages	203	0.42 (-0.4 to 1.39)	0.67	-0.68 (-2.01 to 0.65)	0.32
Food + All Beverages	203	4.70 (-1.36 to 10.76)	0.13	2.32 (-2.83 to 7.47)	0.38
BMI (kg/m^2)					
Food-Only ED	203	0.49 (0.14 to 0.84)	0.005	0.59 (0.04 to 1.14)	0.03
Food + Caloric Beverages	203	0.33 (-1.63 to 2.29)	0.74	0.19 (-0.83 to 1.21)	0.71
Food + All Beverages	203	1.98 (-0.29 to 4.25)	0.09	0.89 (-1.23 to 3.01)	0.41
Android Fat (%)					
Food-Only ED	203	3.45 (1.71 to 5.19)	0.0001	3.38 (2.26 to 4.50)	<0.0001
Food + Caloric Beverages	203	0.54 (-3.81 to 4.89)	0.81	-0.64 (-3.52 to 2.24)	0.67
Food + All Beverages	203	5.76 (-1.04 to 12.56)	0.10	2.80 (-3.08 to 8.68)	0.35
Gynoid Fat (%)					
Food-Only ED	203	2.24 (1.24 to 3.24)	<0.0001	2.00 (0.55 to 3.45)	0.008
Food + Caloric Beverages	203	1.02(-0.98 to 3.02)	0.32	0.15 (-1.57 to 1.87)	0.87
Food + All Beverages	203	3.89 (-3.73 to 11.51)	0.19	2.28 (-3.74 to 8.30)	0.46
Android:Gynoid Fat Ratio					
Food-Only ED	203	0.04 (0.00 to 0.08)	0.04	0.03 (0.01 to 0.05)	0.007
Food + Caloric Beverages	203	0.001 (-0.08 to 0.08)	0.98	-0.004 (-0.06 to 0.06)	0.86
Food + All Beverages	203	0.06 (-0.06 to 0.18)	0.15	0.03 (-0.05 to 0.11)	0.44

*Estimates from linear mixed-effects models using clinic as random effect and dietary energy density as a fixed effect.

*Estimates from linear mixed-effects models using clinic as a random effect and dietary energy density, race, education, childhood BMI-Z score, as fixed effects.

Table 3-4. The Association between Dietary Energy Density and Selected Body Composition Measures in DISC Participants Stratified by Education

	High School or Lower (n=21) *β Coefficient (SE)*	*P-Value*	*College or Higher (n= 182)* *β Coefficient (SE)*	*P-Value*	*P-for interaction*
Waist Circumference (cm)					
Food-Only ED	5.80 (2.92)	0.07	1.21 (1.18)	0.31	0.88
Food + Caloric Beverages	13.15 (0.78)	<0.0001	-1.62 (1.61)	0.32	0.0003
Food + All Beverages	11.16 (5.86)	0.08	-1.95 (4.01)	0.63	0.21
Total Body Fat (%)					
Food-Only ED	2.53 (1.97)	0.001	1.97 (0.72)	0.007	0.43
Food + Caloric Beverages	7.76 (3.11)	0.03	-2.49 (0.93)	0.008	0.0005
Food + All Beverages	7.51 (5.65)	0.21	1.51 (2.01)	0.45	0.26
BMI (kg/m^2)					
Food-Only ED	2.05 (1.30)	0.14	0.60 (0.45)	0.18	0.90
Food + Caloric Beverages	5.70 (1.64)	0.005	-0.68 (0.93)	0.46	0.02
Food + All Beverages	5.55 (3.02)	0.09	-0.06 (1.15)	0.10	0.006
Android Fat (%)					
Food-Only ED	4.06 (3.80)	0.31	3.48 (0.67)	<0.0001	0.99
Food + Caloric Beverages	9.95 (4.07)	0.03	-2.75 (2.29)	0.23	0.02
Food + All Beverages	8.71 (7.61)	0.28	1.73 (2.51)	0.49	0.16
Gynoid Fat (%)					
Food-Only ED	1.67 (2.09)	0.44	1.66 (0.67)	0.01	0.85
Food + Caloric Beverages	3.41(2.80)	0.25	-0.62 (1.09)	0.57	0.21
Food + All Beverages	7.70 (5.67)	0.20	1.34 (2.26)	0.55	0.49
Android:Gynoid Fat Ratio					
Food-Only ED	0.07 (0.06)	0.28	0.04 (0.02)	0.04	0.78
Food + Caloric Beverages	0.15 (0.04)	0.002	-0.04 (0.04)	0.24	0.001
Food + All Beverages	0.01 (0.09)	0.93	0.03 (0.04)	0.46	0.26

Estimates from linear mixed-effects models using clinic as a random effect and dietary energy density, race, physical activity, and childhood BMI-Z score as fixed effects.

Table 4-4. The Association between Dietary Energy Density and Selected Body Composition Measures in DISC Participants Stratified by Physical Activity

	Physical Activity (< 298 met-min/wk) (n=100)		*Physical Activity (≥ 298 met-min/wk) (n= 103)*		
	β Coefficient (SE)	***P-Value***	***β Coefficient (SE)***	***P-Value***	***P-for interaction***
Waist Circumference (cm)					
Food-Only ED	2.44 (1.25)	0.05	1.20 (2.85)	0.68	0.39
Food + Caloric Beverages	1.57 (3.03)	0.61	2.54 (2.78)	0.36	0.52
Food + All Beverages	0.92 (4.14)	0.82	0.70 (5.03)	0.89	0.90
Total Body Fat (%)					
Food-Only ED	2.58 (1.09)	0.02	2.73 (2.09)	0.19	0.52
Food + Caloric Beverages	1.19 (1.25)	0.35	-2.06 (2.20)	0.35	0.09
Food + All Beverages	0.38 (1.14)	0.74	7.01 (4.02)	0.08	0.30
BMI (kg/m^2)					
Food-Only ED	1.32 (0.23)	<0.0001	0.86 (0.85)	0.32	0.38
Food + Caloric Beverages	0.91 (0.54)	0.10	1.01 (1.26)	0.43	0.26
Food + All Beverages	1.96 (0.84)	0.02	2.04 (2.25)	0.37	0.30
Android Fat (%)					
Food-Only ED	4.25 (1.75)	0.02	4.24 (1.60)	0.01	0.39
Food + Caloric Beverages	1.93 (2.64)	0.47	-2.21 (2.26)	0.33	0.02
Food + All Beverages	0.65 (2.48)	0.79	8.52 (4.94)	0.09	0.43
Gynoid Fat (%)					
Food-Only ED	1.48 (1.17)	0.21	3.43 (2.33)	0.14	0.88
Food + Caloric Beverages	1.00 (1.14)	0.38	-0.51 (2.69)	0.85	0.20
Food + All Beverages	-1.17 (1.11)	0.29	6.89 (5.24)	0.19	0.21
Android:Gynoid Fat Ratio					
Food-Only ED	0.06 (0.02)	0.01	0.01 (0.03)	0.69	0.15
Food + Caloric Beverages	0.02 (0.05)	0.64	-0.04 (0.03)	0.13	0.04
Food + All Beverages	0.04 (0.05)	0.43	0.06 (0.06)	0.36	0.39

Estimates from linear mixed-effects models using clinic as a random effect and dietary energy density, race, education, and childhood BMI-Z score as fixed effects.

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Chapter 5

Dietary Patterns are Associated With Measures of Body Fatness and Breast Density in Young, Premenopausal Women: Results from The Dietary Intervention Study In Children Follow-Up Study (DISC06)

Abstract

The goal of the present cross-sectional study was to identify dietary patterns (DPs) among 203 young adult women (25-29y) and evaluate associations of dietary patterns with measures of body fatness and breast density. Trained study staff measured anthropometrics (height, weight, waist circumference) and body composition was assessed by dual-energy x-ray absorptiometry (DXA). Percent dense breast volume (%DBV) and absolute dense breast volume (ADBV) were measured by magnetic resonance imaging (MRI). The absolute non-dense breast volume (ANDBV) was calculated using %DBV and ADBV. Dietary intake was assessed by trained interviewers using three nonconsecutive 24-hour dietary recalls collected over a two week period. Dietary energy density (ED) was calculated as energy (kcal)/amount (g) three ways: food-only, food + caloric beverages, and food + all beverages. Using finite mixture modeling, 15 food group variables were used to derive five DPs which we designated as "Western", "Dietary Guidelines", "Health Conscious", "Alcohol and Vegetables", and "Non-drinking" patterns. A number of characteristics were significantly different across DPs including body mass index (BMI $p=0.001$), overall body fatness ($p= <0.0001$), waist circumference ($p=0.02$), percent dense breast volume ($p = 0.01$), absolute non-dense breast volume ($p=0.007$), total energy intake ($p<0.0001$), and dietary energy density from food alone (ED; $p<0.0001$). Women in the "Western" pattern, characterized by higher intakes of red meat, refined grains, and starchy vegetables, had the highest mean body fatness ($41.8 \pm 8.6\%$), waist circumference (91.0 ± 13.8 cm), and food-only ED (2.01 ± 0.27kcals/g). Women in the "Dietary Guidelines" pattern, characterized by higher intakes of fruit, whole grains, and lean protein, had a significantly lower mean ED (1.39 ± 0.32 kcals/g) than all of the other DPs (all $p<0.05$).

Introduction

Over the past 20 years, obesity has risen dramatically and remained elevated in the United States [1]. Currently, two-thirds of Americans are classified as either overweight or obese [1]. With this increase in overweight and obesity also comes a rise in several weight-related chronic diseases including cardiovascular disease, type 2 diabetes and certain cancers such as colon and breast [1]. A recent study examined dietary patterns over a 10 year period and concluded that individuals are most likely to stay in their originally designated pattern. If transition did occur, they most often moved to a "healthier", as deemed by a higher Dietary Guidelines Adherence Index (DGAI) score, pattern. The most persistent patterns were "alcohol and snacks" [DGAI (sd): 8.31 (2.24)], "healthier" [DGAI (sd): 11.95 (1.94)], and "meat and soda" [DGAI (sd): 7.29 (2.11)] with the least healthful patterns predicting greater BMI and increased risk of obesity over time [2].

It is well known that menopausal status modifies the effect of total body fatness on breast cancer risk. Being overweight lowers risk for premenopausal women but increases risk for postmenopausal women. In contrast, the relationship between body fat distribution and breast cancer does not appear to follow this same pattern. Increased central obesity, measured either by waist circumference or the waist: hip ratio, has been positively associated with both premenopausal [3-5] and postmenopausal breast cancer [3-5]. Central obesity has also been associated with hormonal changes including insulin resistance, hyperinsulinemia and decreases in concentrations of sex-hormone binding globulin (SHBG) and insulin-like growth factor binding proteins (IGFBP) in both pre- and postmenopausal women [6-8]. The result, higher levels of free estrogen and insulin-like growth factors, are known to increase breast cancer risk [9].

Breast density, or the amount of dense fibroglandular tissue in the breast, has also been identified as a strong breast cancer risk factor. Women with mammographic densities >75% have a 4-5 fold increase in breast cancer risk [10]. Breast density can also be modified, potentially

through diet, and is often a target for cancer prevention strategies. Breast tissue is very dynamic throughout adolescence and childbearing ages and changes with several life events such as pregnancy [11, 12], suggesting that premenopausal breast tissue may be more susceptible to modifications that will impact breast cancer risk in the future.

We identified *a posteriori,* or data-driven, dietary patterns derived via finite mixture modeling (FMM) and examined their associations with several chronic disease risk factors including breast density, anthropometric, and overall body composition measures in a cohort of premenopausal women between the ages of 25-29. Unlike single nutrient analyses, examining whole dietary patterns provides a more comprehensive assessment of diet because it considers food and nutrient interactions. Dietary patterns have been associated with several chronic diseases including cardiovascular disease, certain cancers, as well as measures of adiposity [13-15]. Examining dietary patterns may also provide a better foundation for future dietary recommendations and interventions by determining how overall food profiles, rather than individual foods, influence health and disease.

Materials and Methods

Design

The Dietary Intervention Study in Children (DISC) was a multicenter, randomized, controlled clinical trial sponsored by the National Heart, Lung, and Blood Institute and designed to test a dietary intervention that aimed to lower low-density lipoprotein cholesterol (LDL-C) in children. Complete details regarding trial design and primary aim results were described previously [16-22]. Briefly, a total of 663 healthy, pre-pubertal, 8-10 year old children (301 female) with elevated LDL-C were recruited from six clinical centers between 1988-1990 and randomly assigned to a behavioral dietary intervention or usual care control group. Informed

consent was initially obtained from the parents or guardians prior to randomization and again when the adult female DISC participants enrolled in the DISC06 follow-up study. All DISC protocols were approved by Institutional Review Boards at all involved centers.

Participants

The 301 female DISC participants were invited to DISC06 follow-up visits at ages 25-29 between 2006-2008. A total of 260 women participated. For our analyses, women who were pregnant or breastfeeding during or within 12 weeks before the follow-up visit (n=30) were not eligible. Of these 230 women, 27 had at least one technically unacceptable or missing measure, resulting in a final analytical sample of 203 for body composition analyses.

Not all women with body composition measures also had complete or acceptable breast density data. An additional 16 women were not eligible for the analysis due to having breast implants or reduction surgery and another 16 had technically unacceptable or missing breast density measures, resulting in a final analytical sample of 171 women for the analysis of breast density and dietary patterns. All women included in both analyses had complete dietary data.

Data Collection

For the DISC06 follow-up study, each female participant attended a one day data collection visit at one of the DISC clinics. Visits were scheduled to take place during the luteal phase of the menstrual cycle whenever possible with 85% of visits occurring within 14 days of onset of next menses. During the visit, participants provided a fasting blood sample and completed survey questions regarding demographics, medical, reproductive, and menstrual histories, prescription and non-prescription drug use (including extensive information on past and current hormone use), smoking and alcohol use, leisure-time physical activity, and family history

of breast cancer, cardiovascular disease, and type 2 diabetes. All anthropometric measurements were done by trained study staff. Height was measured by a stadiometer, weight was measured by a balance beam or electronic scale and waist circumference was measured at the level of the uppermost lateral border of the right iliac crest using an anthropometric measuring tape. All measures were taken twice and averaged if difference in the two measures were within allowable tolerances (0.5 cm for height and waist circumference and 0.2 kg for weight). If differences were larger, measurements were taken a third time and the two closest were averaged. Body composition was assessed by dual-energy x-ray absorptiometry (DXA) [17]. Total adiposity was characterized by whole body percent fat mass, calculated as the ratio of whole body fat mass: whole body total mass. The whole-body DXA scans also allowed the examination of the body fat distribution by reporting the android and gynoid regions of the body in addition to whole body measures. The android:gynoid (A:G) relative fat distribution was calculated and examined in analyses. Diet was assessed using three nonconsecutive 24-hour dietary recalls collected over the course of two weeks. Assessment was conducted by trained interviewers using the Nutrition Data System for Research (NDSR: 2007 University of Minnesota, Minneapolis, MN). Several food files were generated from the recalls that included total energy, macro and micronutrients, and individual foods and beverages.

All breast density measures were assessed by magnetic resonance imaging (MRI) and processed by a single investigator using customized imaging software [17]. Percent dense breast volume (DBV) was calculated from the total dense breast volume (TDBV) and the absolute dense breast volume (ADBV). Total volumes of fibroglandular and fatty tissue were computed separately for each breast. The volumes of dense fibroglandular tissue (ADBV) and non-dense breast tissue (ANDBV) alone were also examined. The area of non-dense breast volume (ANDBV) was calculated using the %DBV and ADBV. Childhood BMI-Z scores were calculated from the measured heights and weights taken at DISC study entry based on the Center for Disease Control 2000 Growth Charts [23].

We calculated dietary ED as a measure of overall diet quality. Dietary ED was calculated from the 24-dietary recalls using three methods: (a) using food only, (b) using all food plus caloric beverages, and (c) using all foods and all beverages [24]. To achieve this, food and beverage codes were first assigned to each food item generated by NDSR. Second, the daily average of total energy intake (kcal) was calculated for each participant; additional daily average intakes were calculated for food-only intake, and food + caloric beverage intake and food + all beverage intake. Similarly, the average amount of food (g), food and caloric beverages (g) and food and all beverages (g) were estimated. Each of the 3 measures of ED was then calculated as energy (kcal)/amount (g).

Statistical Analysis

Dietary pattern analyses were conducted using FMM (LatentGold, version 4.5, Statistical Innovations Inc., Belmont, MA) using an exploratory *a posteriori*, or data-driven, method. FMM detects groups in a population that are otherwise unable to be observed. In the present study, each latent class is considered a dietary pattern. All raw dietary data was initially collapsed into a smaller set of 22 food groups that contained similar types of foods. Food groups were then further collapsed into a final set of 15 food groups that were used as indicators in the analysis. These food groups are further described in **Table 1.** All 15 food groups were analyzed as indicators; the best model was selected using the Bayesian Information Criterion (BIC) and the Akaike Information Criterion (AIC) as well as through basic model interpretability (**Figure 1-5**). FMM, unlike other dietary pattern methodologies such as cluster analysis, does not confine an individual to a particular dietary pattern, but rather each subject has a probability of belonging to each of the identified dietary patterns. An individual's membership to a particular pattern was based on their highest respective posterior class-membership probability. Once dietary patterns were derived, one-way analysis of variance (ANOVA), Chi-Square and Fisher's Exact test were

done using SPSS and SAS (Version 9.3, Cary Institute, NC) to examine differences between variables of interest and the dietary patterns.

To further evaluate associations with body composition, anthropometric, breast density, and dietary outcome variables, mixed models (SAS PROC MIXED) were also were fit by maximum likelihood with robust standard errors separately for all outcomes. All breast density measures were log-transformed prior to analysis to improve normality. Body composition, dietary, breast density, and anthropometric variables were included as independent continuous variables in separate models. Clinic was included in models as a random effect and all other variables were included as fixed effects. Associations between each outcome measure and dietary patterns were first examined individually in an unadjusted model (data not shown). Subsequent body composition, food group, and nutrient models were further adjusted for risk factors and confounders such as smoking status (current vs. never), childhood BMI-Z score, race (white vs. non-white), education (no college vs. attended college), and physical activity (met-minutes). Two final models are presented: one is energy adjusted (**Table 5**) while the other is not (**Table 4**).

The breast density models are adjusted for the same variables as the body composition models with the exception of physical activity and the addition of the duration of use of hormonal contraceptives and other sex hormones (years), whole-body percent fat, and number of full-term pregnancies. DISC treatment group also was evaluated as a potential covariate, but was not included in the final model because it was not a significant confounder and did not contribute appreciably to the model. Dietary patterns were modeled on all 203 participants with complete dietary and covariate data; however, only 171 women had complete breast density data. Percentage differences in %DBV, ADBV, and ANDBV associated with a unit increase of ED were calculated as $\%\Delta = (\exp(\beta)-1)x100$. All statistical testing was conducted using two-sided tests, with significance determined at $p< 0.05$. All data were analyzed using SAS 9.3 (SAS Institute, Cary, NC).

Results

Sample Characteristics

Characteristics of the study sample are presented in **Table 2.** The sample was predominantly Non-Hispanic White (90%) and had at least a high school education (90%). The majority of the sample were nonsmokers (74%). The median (IQR) age (years) and total energy intake (kcal) of the 203 participants was 27.2 (26.6 – 27.9) and 1631 (1369 to 2012), respectively.

Description of Dietary Patterns

Dietary patterns were derived using complete dietary data from 203 subjects. Five dietary patterns were derived and labeled based on the model-predicted servings of the 15 food groups in each of the intake patterns (**Table 3**) "Western" [n=21, (10.3%)], "Dietary Guidelines" [n=40, (19.7%)], "Health Conscious" [n=47, (23.2%), "Alcohol and Vegetables"[n=39, (19.2%)], and "Non-Drinkers" [n=56, 27.6%)].

The "Western" dietary pattern (n=21) was characterized by the highest intakes of red and processed meat, sweets, other fats, refined grains, starchy and fried vegetables, and miscellaneous foods that included sauces, condiments, and pickled foods. This pattern had significantly higher energy intake and dietary food-only ED than all other patterns [Mean Energy (SD) = 2144 kcals (634) and Mean Food-Only Dietary ED (SD) = 2.01 kcal/g (0.27)]. Although the association was not as strong, the "Western" pattern also had the highest dietary ED using the Food + Caloric Beverages and the Food + All Beverages methods (**Table 4**) and consumed more than the USDA Dietary Guidelines recommendation for calories of total fat (37.4%) and saturated fat (12.7%).

The "Dietary Guidelines" pattern (n=40) consumed high amount of fruits, whole grains, lean protein, fiber, and unsweetened beverages such as coffee and tea. This group also consumed

a high amount of non-starchy vegetables (3.02 servings). Additionally, the pattern was characterized by the lowest intakes of other fats, sauces and condiments, refined grains, and whole milk dairy products. The "Dietary Guidelines" pattern had the lowest dietary ED (from all 3 methods) out of all of the DPs [Mean (SD) Food-Only Dietary ED = 1.39 kcal/g (0.32); Mean (SD) Food + Caloric Beverages ED = 1.15 (0.23); Mean (SD) Food + All Beverages ED = 0.46 (0.15)] and the lowest total energy [Mean (SD) Total Energy = 1582 (413)]. Most likely this low energy consumption is a result of their high consumption of fruit and non-starchy vegetables and low intake of fat (26.7% of kcals from fat and 8.6% of kcals from saturated fat). After controlling for all relevant covariates, all dietary patterns had a significantly higher Food-only ED and Food + All beverages ED than the "Dietary Guidelines" pattern (all $p < 0.0001$) **(Table 5)**. A similar pattern labeled "Health Conscious" (n=47) consumed the highest amounts of low-fat and fat-free dairy products and the lowest amounts of red/processed meat, sweets, fruit and starchy/fried vegetables.

Women in the "Alcohol and Vegetables" pattern (n=39) consumed approximately 1.2 daily servings (16.4 g, 6.0% total kcals) of alcohol, primarily in the form of beer and wine, which was the highest of all five DPs. This pattern also consumed high amounts of healthy fats, lean protein, non-starchy vegetables, and whole milk and the second highest amount of starchy vegetables. In contrast, the Non-Drinker pattern (n=56) consumed the lowest amount of alcohol (0.003 servings, 0.05 g, 0.35% of total kcals) as well as healthy fats, whole grains, non-starchy vegetables, and unsweetened beverages across all DPs. This pattern also consumed the second highest amount of sweets, second only to the "Western" pattern.

Demographics

The "Western" DP had the lowest amount of post-high school education (81.0%) and the highest percentage of current smokers (47.7%) across all patterns. The "Dietary

Guidelines" and "Health Conscious" patterns had the lowest percentage of current smokers; 15.0% and 14.9%, respectively. The "Health Conscious" pattern also had the highest amount of physical activity (321.4 ± 57.2 met-min/wk). Much like the "Western" pattern, the "Alcohol and Vegetables" pattern also had a high percentage of current smokers (43.6%) (See **Table 4**).

Breast Density Measures

When examining breast density measures, there were no significant differences observed between %DBV and ANDBV among any of the dietary patterns after controlling for parity, hormone use, childhood BMI-Z score, whole-body percent fat, race, education, and smoking status. When examining the ADBV, both the "Alcohol and Vegetables" and "Non-Drinker" pattern had significantly higher amounts of dense breast tissue compared to the "Dietary Guidelines" pattern **(Table 7)**.

Dietary Patterns and Body Fatness

The "Western" DP was the only pattern to exceed the 'at-risk' waist circumference (>88 cm) [25] [Mean (SD) Waist Circumference = 91.0 cm (13.8)] and BMI threshold (>25) [26] [Mean (SD) BMI = 29.0 kg/m^2 (5.2)]. The "Western" pattern also has the largest A:G fat ratio, which indicates they have higher levels of central obesity, which can increase the risk for certain chronic diseases including premenopausal breast cancer [3-5]. The "Dietary Guidelines" pattern had the lowest A:G fat ratio [Mean (SD) 0.80 (0.19)].

After controlling for relevant covariates, all dietary patterns, with the exception of the "Alcohol and Vegetables" pattern, also had significantly higher total body fat and android fat (all $p < 0.05$) than the "Dietary Guidelines" pattern. The only pattern to have significantly higher gynoid fat was the "Non-Drinker" pattern; however, most patterns had a significantly higher A:G

Fat ratio compared to the "Dietary Guidelines" pattern (all $p < 0.05$) with the exception of the "Western" pattern that failed to reach statistical significance. All patterns had a higher mean BMI than the "Dietary Guidelines" pattern; however, only the "Western" and the "Non-Drinker" pattern were statistically significant. Finally, the "Alcohol and Vegetables" and "Western" patterns had a significantly higher mean waist circumference than the "Dietary Guidelines" pattern (all $p < 0.05$). The "Health Conscious" pattern also had a higher mean waist circumference, although it only reached modest significance ($p=0.05$). All of these results are described in **Table 5** and **Table 6.**

Discussion

Our study utilized FMM to identify five dietary patterns using both model fit statistics and basic interpretability, with the two extremes being the "Western" and the "Dietary Guidelines" patterns. The "Western" DP is the most energy-dense pattern making it the most unfavorable of all five patterns. Dietary ED is the amount of energy per gram of food in the diet [kcal/ amount (g)] and can be calculated with or without beverages [27]. We analyzed three forms of dietary ED: food-only, food + caloric beverages, and food + all beverages. While the ED variable is often less accurate with the inclusion of beverages [27], regardless of the beverage energy content, the "Western" pattern consistently had the highest dietary ED across all three methods. This pattern was characterized by the highest intakes of red/processed meat, sweets, other fats, refined grains, and starchy/fried vegetables, all of which commonly define a high ED diet, and was significantly associated with several measures of body fatness.

The "Dietary Guidelines" pattern had the lowest dietary energy density (ED) across all three methods. Low ED is generally associated with higher dietary quality which is consistent with dietary recommendations [28, 29]. The "Dietary Guidelines" pattern was also high in fruits, whole grains, lean protein, and the second highest in non-starchy vegetables. It was the lowest in

other fats, sauces and condiments, and refined grains. High consumption of water and fiber, often in the form of fruits and vegetables, are hallmarks of a low ED diet. This pattern also had the most favorable A:G fat ratio of all the patterns. Diets low in ED have been shown to be of higher diet quality [28, 29] and are associated with a lower BMI in both adults and children [27]. Low ED diets are also advocated by the 2010 Dietary Guidelines committee for their role in weight management [30] and by the American Institute for Cancer research for cancer prevention [31]. In a recent 2013 review, it was concluded that dietary patterns that were higher in fruits, vegetables, and lean protein and lower in fat and alcohol may also be beneficial in regards to overall breast cancer risk [32]. Taylor and colleagues [33] conducted a meta-analysis of case-control and cohort studies that examined the association between red meat intake and breast cancer risk in premenopausal women. This meta-analysis suggested that high red meat consumption may increase breast cancer risk in young women [Summary Relative Risk (95% CI) = 1.24 (1.08-1.42)]. Additionally, Ronco and colleagues [34] conducted a hospital-based, case-control study and observed similar effects on premenopausal breast cancer with high intakes of red meat [Odds Ratio (OR, 95% CI) = 2.20 (1.35 – 3.60)]. In addition to red meat, this study also reported that fried foods may also increase risk [Odds Ratio (OR, 95% CI) = 1.79 (1.12 – 2.84)] while plant-based foods may be protective [Odds Ratio (OR, 95% CI) = 0.41 (0.26 – 0.65)], particularly in premenopausal women.

In addition to the "Dietary Guidelines" and "Western" patterns, we also identified "Alcohol and Vegetables", "Non-Drinker", and "Health Conscious" patterns. The "Alcohol and Vegetables" pattern consumed 1.2 servings (16.42g) of alcohol while the "Non-Drinker" pattern consumed <0.05 servings (0.05 g) of alcohol daily. We felt that identifying these patterns was important since alcohol is considered a risk factor for breast cancer in both pre- and postmenopausal women [35]. Studies have consistently shown that for each 10g of ethanol consumed per day, risk increases by 10% [35]. This suggests that women in the "Alcohol and

Vegetables" pattern may be at an elevated risk and the "Non-Drinker" pattern may be at a decreased risk, for developing breast cancer, regardless of their body composition measures.

As mentioned previously, menopausal status modifies the effect of total adiposity on breast cancer risk. Being overweight lowers risk for premenopausal women but increases risk for postmenopausal women. In contrast, the relationship between body fat distribution and breast cancer does not appear to be the same. Unlike postmenopausal breast cancer, total body fatness has been shown to be protective against breast cancer in premenopausal women [35] because of the increased number of anovular menstrual cycles and decreased levels of progesterone and estrogen that are associated with weight gain in premenopausal women [3]. However, body fat distribution may be more important when assessing this relationship with studies observing that central obesity may increase breast cancer risk in premenopausal women [3, 5]. In an attempt to identify women who may be at a higher risk for premenopausal breast cancer, our study observed that the "Western" dietary pattern had the highest waist circumference, BMI, total percent body fat, percent android fat, percent gynoid fat, and android:gynoid fat ratio. This shows that the "Western" dietary pattern is associated with several body composition measures in our population that may increase their risk for several chronic diseases, including premenopausal breast cancer.

Recently, a study by Pachucki and colleagues [2] examined dietary patterns, BMI, and long-term risk of obesity. Over ten years of follow-up, it was observed that many people tend to stay in a particular dietary pattern and often do not transition into other patterns. It was also observed that less healthy dietary patterns over time led to an increased BMI and risk of obesity [2]. This suggests that women in unhealthier dietary patterns at young ages may be at risk for becoming overweight or obese postmenopausally [35]. This study [2] also showed that if a transition did occur, it was almost always to a healthier pattern. This may imply that dietary interventions in women in the unhealthy dietary patterns may be beneficial and help to reduce future risk of obesity and chronic disease.

In addition to the dietary patterns examining total diet profiles, overall lifestyle patterns also emerged. As mentioned previously, the "Western" and "Alcohol and Vegetables" pattern, which are deemed to be the highest risk patterns in terms of dietary factors, also have the highest percentages of current smokers. The "Western" pattern also has the lowest percentage of college-educated women and the lowest amount physical activity. In contrast, the "Dietary Guidelines", the "Health Conscious" and the "Non-drinker" patterns, which are considered to be "lower risk", have the lowest percentages of current smokers. The "Dietary Guidelines", "Health Conscious", and "Alcohol and Vegetables" patterns have the highest amount of physical activity.

It is interesting to note that while the "Dietary Guidelines" and "Heath Conscious" patterns appear similar and both have BMI and waist circumference measures within the healthy range, the "Dietary Guidelines" pattern appears to place more emphasis on diet and less emphasis on other lifestyle factors, such as physical activity, in terms of maintaining adequate health. The "Health Conscious" group is highly educated and appears to be knowledgeable about healthy lifestyle factors; however, this pattern places a higher emphasis on non-dietary lifestyle factors rather than total diet quality, as evidenced by their higher dietary ED. Regarding diet, they do consume most things, such as alcohol (0.98 servings), sweets (1.6 servings), and starchy/fried vegetables (0.40 servings), in moderation; however, they have the lowest intake of fruit and a low intake of non-starchy vegetables. They also have the highest level of physical activity and education and the lowest prevalence of current smokers, which suggests that they place more emphasis on non-dietary lifestyle factors to maintain health and reduce their risk of disease. A recent study by Kossman et al. [36] showed that higher levels of physical activity are able to reduce estrogen and progesterone levels in a group of high-risk premenopausal women, which suggests that overall lifestyle patterns may be beneficial in reducing the risk of premenopausal breast cancer and other chronic diseases.

In addition to examining several anthropometric and body composition measures, we also examined associations with three breast density measures and the dietary patterns. To date, few

studies have been conducted that examine the association between dietary patterns and breast density [37-39]. This has been reviewed and discussed in detail elsewhere [40]. Briefly, a study by Mishra and colleagues [37] examined dietary patterns in childhood and breast density in adulthood and observed no relationship. Other studies were conducted in older adult women from the Minnesota Breast Cancer Family Study and only observed significant inverse associations between percent breast density and a dietary pattern high in fruits, vegetables, and cereal [39] and an increasing Mediterranean Diet Score in smokers [38].

The "Alcohol and Vegetables" and the "Non-drinker" patterns had a significantly higher amount of dense breast tissue than the "Dietary Guidelines" pattern. While it is interesting to note that the pattern defined by the highest amount of alcohol consumption had a significantly higher amount of dense breast tissue than the "Dietary Guidelines" pattern, the "Non-drinker" pattern also had significantly higher amounts of dense breast tissue than the "Dietary Guidelines" pattern group.

Finally, our study focuses on dietary assessment and body composition measures prior to age 30. A recent review has shown that BMI prior to middle age has a positive association with coronary heart disease (CHD) later in life [41]. This suggests that dietary interventions to lower body composition measures and subsequently reduce chronic disease risk, may be particularly important at this age.

Strengths and Limitations

Our study has several strengths. First, the dietary ED values were consistent with the dietary patterns observed; highest in the "Western" pattern and lowest in the "Dietary Guidelines" pattern. This confirms our assumptions about these patterns. Second, several reliable body composition measures were assessed, including total body fat mass as well as measures of body fat distribution. This is particularly important because BMI and total adiposity do not consider

the location of fat on the body and central obesity has been shown to be more metabolically active and more influential in the development of certain diseases, including premenopausal breast cancer [3, 4]. Our study also had high quality data collected by trained study staff including repeated 24-hour dietary recalls, DXA, and MRI for assessing diet, body composition, and breast density, respectively. All data was collected by trained study staff that followed standardized procedures.

A few limitations also exist in this research. When analyzing dietary patterns, a high level of subjectivity exists. In our case, we needed to reduce the number of food groups down into 15 analyzable categories because of the FMM methodology. This could be problematic because in doing this, we run the risk of missing an important or influential food group if it has been combined with others. Finally, our study was cross-sectional, which means that causation cannot be determined.

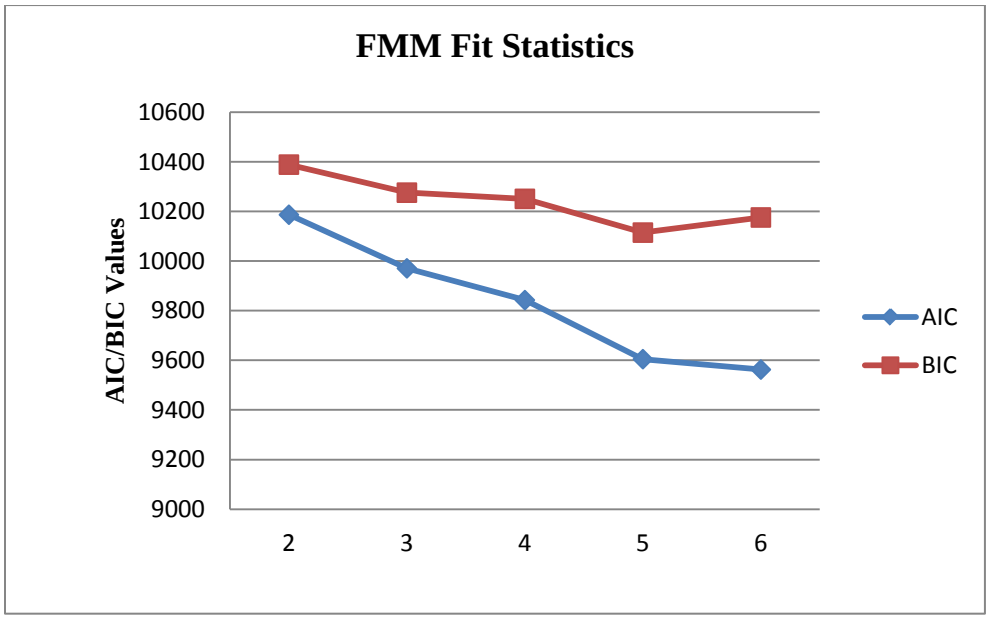
In conclusion, the “”Western” pattern was comprised primarily of red and processed meat, refined grains, and starchy vegetables and had the highest of all body composition measures. This pattern was also the only pattern where several of the body composition measures (BMI and WC) crossed into the unhealthy or “at-risk” range. The “Dietary Guidelines” pattern had the highest diet quality as evidenced by their low dietary ED and high intakes of fruits, whole grains, coffee, tea, and lean protein. The “Alcohol and Vegetables”, “Non-Drinker”, and “Health Conscious” patterns were moderate in diet quality. Future directions should include longitudinal research of dietary patterns established at an early age and examined over time to assess their association with chronic disease onset in adulthood as well as the development of consistent dietary pattern methodology that will allow for increased accuracy when comparing dietary patterns across studies.

Tables:

Table 1-5. Description of Food Groupings used to Derive Dietary Patterns in The Dietary Intervention Study in Children (DISC06) Follow-up Study

Food Group Name	***Examples of Food Items***
Fruit	Whole fruit and juice (citrus and non-citrus),
Non-Starchy Vegetables	Dark leafy greens, tomatoes, celery, cucumbers
Starchy & Fried Vegetables	White potatoes, French fries, potato chips
Red & Processed Meat	Beef, cold-cuts, sausage
Lean Protein	Poultry, fish, eggs
Whole Grains	Breads, rolls, flours, snack chips, crackers, pastas all made with 100% whole grain, popcorn
Some-whole and Refined Grains	Breads, rolls, flours, crackers, pastas all made with some-whole grain or refined grains
Healthy Fats	Oils, nuts and seeds, nut butters
Other Fats	Regular salad dressing, butter & animal fats, margarine, non-dairy cream
Whole Milk Products	Milk, cheese, and yogurts made with whole milk
Low-Fat & Fat-Free Milk Products	Milk, cheese, and yogurts made with low-fat or fat-free milk
Unsweetened Beverages	Low calorie/calorie-free beverages, water, coffee, and tea
Sweets & Sweetened Beverages	Cookies, cakes, pastries, frozen dairy desserts, and sweetened beverages
Alcohol	Beer, wine, liquor
Miscellaneous	Reduced fat sauces and condiments, pickled foods

Figure 1-5. Finite Mixture Modeling Fit Statistics



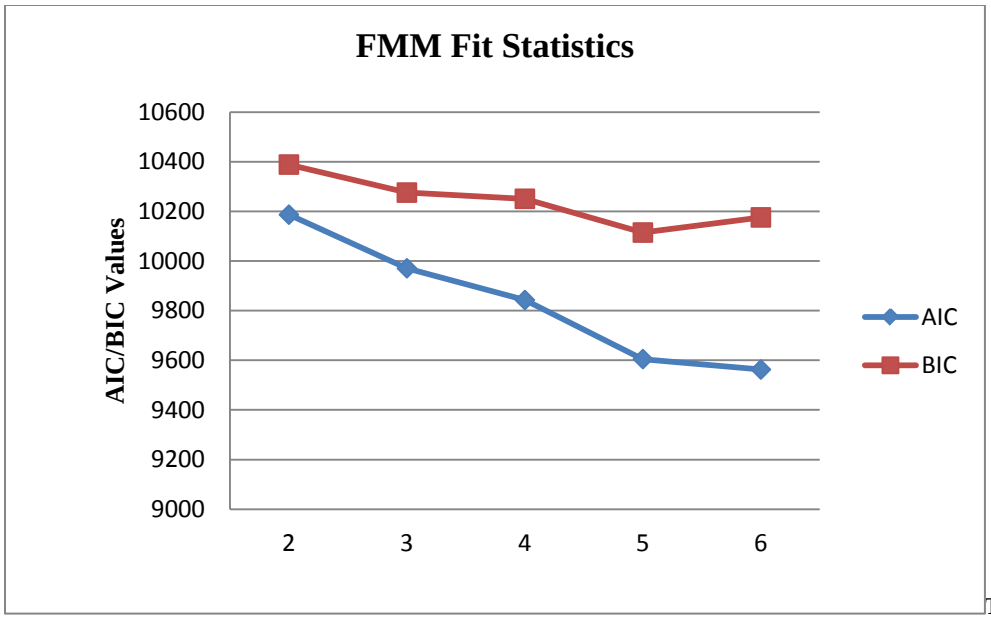


Table 2-5. Selected Demographic Characteristics (n=203)

Descriptive Characteristics	
Non-Hispanic White	89.7%
Current Smokers	25.6%
>High School Education	89.7%
Age at visit [Median (IQR)]	27.2y (26.6 - 27.9)
Physical Activity [Median (IQR)]	298.0 (271.5 - 337.5)
Dietary Energy Density	**Median (IQR)[1]**
Food-Only	1.7(1.5 - 2.1)
Food + Caloric Beverages Only	1.2(1.0 - 1.4)
Food + All Beverages	0.6 (0.5 - 0.8)
Body Composition Characteristics	**Median (IQR)[1]**
BMI (kg/m^2)	24.0 (21.3 - 27.9)
Body Fat (%, DXA)	34.8 (29.0 - 42.2)
Waist Circumference (cm)	80.4 (73.8 - 91.3)
Android Fat (%)	40.2 (30.5 - 49.7)
Gynoid Fat (%)	46.5 (41.0 -51.4)
Android:Gynoid Fat Ratio	0.86 (0.71 - 0.99)
Breast Density Characteristics (n=171)	**Median (IQR)[1]**
Percent Dense Volume (%)	24.9 (10.5-42.5)
Absolute Dense Volume (cc)	91.2 (46.6 – 139.4)
Absolute Non-Dense Volume (cc)	287.0 (156.0 – 474.0)

[1]IQR: Interquartile Range

Table 3-5. Model-predicted Servings of the 15 Food Groups in Each of the 5 Dietary Patterns (n=203)

	Non-Drinkers	***Health Conscious***	***Alcohol & Vegetables***	***Dietary Guidelines***	***Western***

Cluster Size	27.6%	23.2%	19.2%	19.7%	10.3%
Indicators (mean svgs)					
Fruit	1.03	0.62	1.71	1.90	0.77
Red/Processed Meat	2.03	1.18	1.61	1.69	2.56
Lean Protein	2.01	2.54	3.03	3.02	2.81
Non-Starchy Vegetables	1.39	1.55	3.26	3.06	1.67
Sweets & Sweetened Beverages	3.05	1.68	1.78	2.19	4.52
Alcohol	0.003	0.99	1.19	0.47	0.06
Healthy Fats	0.98	1.12	3.27	1.10	1.60
Other Fats	1.68	2.28	1.80	1.53	4.07
Whole Grains	0.75	1.31	0.87	2.32	1.10
Some Whole/Refined Grains	4.98	4.43	5.54	2.87	5.86
Starchy/Fried Vegetables	0.64	0.41	0.49	0.68	1.41
Whole Fat Dairy	0.49	0.35	0.85	0.29	0.55
Low-Fat/Fat-Free Dairy	0.78	1.17	0.51	0.80	0.86
Unsweetened Beverages	3.19	4.42	5.53	8.95	4.64
Miscellaneous	0.87	1.47	1.22	0.81	4.07

Table 4-5. Selected Characteristics across the 5 Dietary Patterns among DISC Participants (n=203)

Characteristic	***Dietary Guidelines (n=40)***	***Western (n=21)***	***Alcohol and Vegetables (n=39)***	***Non-Drinkers (n=56)***	***Health Conscious (n=47)***	***P-Value***
DEMOGRAPHICS						
% NH-White	92.5	85.7	92.3	85.7	91.5	0.74
% Some college education or higher	90.0	81.0	92.3	83.9	97.9	0.08
% Current Smokers	15.0	47.7	43.6	21.4	14.9	0.001
Physical Activity (met-min/wk)	320.9 (58.7)	297.3 (46.7)	320.1 (56.9)	293.5 (50.5)	321.4 (57.2)	0.03
DIETARY CHARACTERISTICS						
Total Energy (kcals)	1582 (413)	2144 (634)	1922 (447)	1647 (467)	1600 (360)	<0.0001
Energy from Beverages (kcals)	289 (244)	388 (248)	410 (303)	334 (247)	436 (398)	0.15
Energy Density (Food-Only, kcal/g)	1.39 (0.32)	2.01 (0.27)	1.78 (0.51)	1.86 (0.41)	1.80 (0.36)	<0.0001
Energy Density (Food + Caloric Beverages, kcal/g)	1.15 (0.23)	1.36 (0.31)	1.27 (0.38)	1.27 (0.39)	1.23 (0.31)	0.15
Energy Density (Food + All Beverages, kcal/g)	0.46 (0.15)	0.82 (0.26)	0.68 (0.23)	0.73 (0.20)	0.65 (0.02)	<0.0001
Average Non-Starchy Vegetable (Svg)	3.06 (1.97)	1.67 (1.00)	3.26 (2.47)	1.39 (0.75)	1.55 (0.94)	<0.0001
Average Fruit (Svg)	1.90 (1.57)	0.77 (0.64)	1.71 (1.39)	1.03 (0.96)	0.62 (0.47)	<0.0001
Total Alcohol (g) / [%kcal]	6.63 (6.93) / [2.9]	0.88 (1.80) / [0.3]	16.43 (14.17) / [6.0]	0.05 (0.09) / [<0.01]	13.14 (13.99) / [5.8]	<0.0001

Total Fiber (g)	19.14 (7.48)	14.62 (5.56)	16.58 (7.43)	12.30 (5.30)	12.83 (5.06)	<0.0001
Total Sodium (mg)	2764.17 (1074.00)	3512.34 (1039.53)	3240.75 (1076.50)	2722.69 (869.33)	2670.20 (566.92)	0.0006
Total Cholesterol (mg)	191.24 (93.91)	302.18 (161.55)	248.02 (113.25)	210.83 (102.42)	181.33 (97.13)	0.0002
Total Protein (g) / [%kcal]	71.9 (18.9) / [18.2]	81.5 (24.3) / [15.2]	75.6 (18.4) / [15.7]	81.1 (118.1) / [19.7]	65.2 (17.3) / [16.3]	0.8
Total Carb (g) / [%kcal]	215.0 (72.9) / [54.4]	258.9 (84.3) / [48.3]	217.3 (71.0) / [62.9]	218.6 (70.5) / [78.8]	193.3 (49.8) / [65.2]	0.01
Total Fat (g) / [%kcal]	46.98 (17.71) / [26.7]	89.14 (30.31) / [37.4]	73.21 (26.26) / [34.3]	59.98 (22.57) / [41.3]	53.87 (16.79) / [30.3]	<0.0001
Total MUFA (g) / [%kcal]	17.64 (7.38) / [10.0]	32.25 (12.07) / [13.5]	27.48 (11.21) / [12.9]	22.08 (9.00) / [12.1]	19.96 (6.69) / [11.2]	<0.0001
Total PUFA (g) / [%kcal]	10.04 (4.16) / [5.7]	19.53 (7.49) / [8.2]	15.90 (6.85) / [7.4]	11.55 (4.22) / [6.3]	11.73 (3.68) / [6.6]	<0.0001
Total SFA (g) / [%kcal]	15.13 (6.40) / [8.6]	30.25 (11.46) / [12.7]	23.97 (9.48) / [11.2]	21.37 (9.08) / [17.2]	17.89 (7.15) / [10.0]	<0.0001
BODY FATNESS/ ANTHROPOMETRICS						
Waist Circumference (cm)	81.4 (11.8)	91.0 (13.8)	80.1 (10.7)	84.7 (12.7)	82.9 (13.8)	0.02
Total Body Fat (%)	33.7 (6.5)	41.8 (8.6)	31.8 (8.1)	37.2 (9.8)	35.21 (8.6)	<0.0001
Gynoid Fat (%)	45.6 (6.6)	51.2 (6.9)	43.6 (7.2)	46.9 (7.8)	45.6 (7.8)	0.005
Android Fat (%)	36.8 (10.2)	48.0 (12.8)	35.9 (11.8)	42.2 (13.6)	39.4 (11.3)	0.001
Android:Gynoid Fat Ratio	0.80 (0.19)	0.93 (0.21)	0.81 (0.18)	0.88 (0.21)	0.85 (0.16)	0.06
BMI (kg/m^2)	24.6 (4.2)	29.0 (5.2)	23.6 (4.5)	25.4 (5.4)	24.5 (4.6)	0.001

Table 5-5. The Associations between Dietary Patterns and Selected Dietary and Body Fatness Measures: Results from Multivariate-Adjusted Models [Not Adjusted for Energy, n=203][1]

	Alcohol & Vegetables			*Health Conscious*			*Non-Drinker*			*Western*		
	Estimate[2]	**S.E.**	**P-Value**	**Estimate[2]**	**S.E.**	**P-Value**	**Estimate[2]**	**S.E.**	**P-Value**	**Estimate[2]**	**S.E.**	**P-Value**
DIETARY CHARACTERISTICS												
Food-Only Dietary ED (kcal/g)	0.36	.06	<0.0001	0.41	.06	<0.0001	0.38	.1	<0.0001	.57	.06	0.0001
Food + Caloric Beverages Dietary ED (kcal/g)	1.5	.13	0.06	0.13	.07	0.18	0.09	.1	0.05	0.2	.08	0.01
Food + All Beverages Dietary ED (kcal/g)	0.23	.03	<0.0001	0.2	.05	<0.0001	0.23	0	<0.0001	0.34	0.05	<0.0001
Total Energy (kcal)	414.92	196.8	<0.0001	47.88	92.5	0.71	12.32	127	0.74	540.21	37.6	0.0003
Caloric Beverage Energy (kcal)	128.06	41.43	0.002	157.37	69.4	0.02	46.23	23	0.04	83.46	39	0.03
Non-Starchy Vegetables (svg)	0.4	0.42	0.34	-1.51	0.33	<0.0001	-1.56	0.3	<0.0001	-1.15	0.34	0.001
Fruit (svg)	-0.04	0.2	0.84	-1.24	0.14	<0.0001	-0.8	0.2	<0.0001	-1.07	0.11	<0.0001
Alcohol (g)	9.88	.54	0.0001	6.59	.62	0.01	-6.05	.3	<0.0001	-5.3	.38	0.0002
Fiber (g)	-1.27	.24	0.31	-6.24	.52	<0.0001	-6.53	.1	<0.0001	-3.92	.4	0.006
Cholesterol (mg)	70.99	8.27	0.0001	-7.24	6.9	0.79	14.95	3	0.24	117.88	2.6	<0.0001
Total Fat (g)	28.79	.51	<0.0001	9.16	.54	0.05	9.91	.5	<0.0001	41.14	.97	<0.0001
Saturated Fat (g)	9.89	.97	<0.0001	3.65	.83	0.05	5.02	.7	<0.0001	14.55	.94	<0.0001
Monounsaturated Fat (g)	10.95	.9	<0.0001	3.31	.69	0.05	3.19	.5	<0.0001	14.05	.15	<0.0001
Polyunsaturated Fat (g)	6.14	.97	<0.0001	1.87	.18	0.11	1.12	.5	0.02	9.74	.06	<0.0001
Sodium (mg)	557.87	93.3	0.06	-103.2	82	0.57	115.89	16	0.32	699.83	294	0.02
BODY FATNESS, ANTHROPOMETRICS, & PHYSICAL ACTIVITY												
Total Body Fat (%)	0.03	2.11	.99	2.98	.12	0.008	3.81	.7	<0.0001	6.1	.64	0.02
BMI (kg/m2)	.4	0.55	.47	0.87	.87	0.32	1.39	.6	0.02	3.25	.73	<0.001

Waist Circumference (cm)	.13	0.86	.01	2.27	.16	0.05	3.01	.3	0.19	7.03	.67	0.009
Andriod Fat (%)	.62	2.53	.52	4.58	.61	0.005	6.04	.8	<0.0001	8.56	.39	0.01
Gynoid Fat (%)	0.86	1.92	.65	1.71	.06	0.11	1.65	.8	0.04	3.93	.18	0.07
Android:Gynoid Fat Ratio	.05	0.02	.02	0.07	.02	0.002	0.09	0	<0.0001	0.1	.05	0.07
Physical Activity (met-min)**	.71	6.54	.91	2.23	.21	0.76	-26.86	10	0.01	-25.9	.59	0.0001

[1]All dietary patterns were compared to the reference "Dietary Guidelines" pattern. [2]Estimates (β-coefficients) represent the change in the outcome of interest for each dietary pattern after multivariate adjustment when compared to the "Dietary Guidelines" pattern.

*Adjusted for: Smoking status (current vs. never), Education (Some college vs. No college), Race (White vs. Non-White), Childhood BMI-Z score, Physical Activity (met-minutes)

**Adjusted for: Smoking status (current vs. never), Education (Some college vs. No college), Race (White vs. Non-White), Childhood BMI-Z score [Clinic was included as a random effect for all models].

Table 6-5. The Associations between Dietary Patterns and Selected Dietary and Body Fatness Characteristics: Results from Multivariate Adjusted Models [Energy Adjusted, n=203][1]

	Alcohol & Vegetables			*Health Conscious*			*Non-Drinker*			*Western*		
	Estimate[2]	**S.E.**	**P-Value**	**Estimate[2]**	**S.E.**	**P-Value**	**Estimate[2]**	**S.E.**	**P-Value**	**Estimate[2]**	**S.E.**	**P-Value**
DIETARY CHARACTERISTICS												
Non-Starchy Vegetables (svg)	0.18	.45	0.7	-1.53	.32	<0.0001	-1.57	.3	<0.0001	-1.47	.4	<0.0001
Fruit (svg)	-0.2	.15	0.16	-1.25	.11	<0.0001	-0.86	0.2	<0.0001	-1.31	.2	<0.0001
Alcohol (g)	7.98	.41	0.001	6.34	.51	0.01	-6.07	.3	<0.0001	-7.52	.4	<0.0001
Fiber (g)	-3.72	.17	0.002	-6.34	.05	<0.0001	-6.57	1	<0.0001	-7.37	.5	<0.0001
Cholesterol (mg)	30.53	8.3	0.1	-10.84	1.5	0.61	12.49	2	0.28	59.23	4	<0.0001
Total Fat (g)	12.92	.59	0.006	6.73	.41	<0.0001	9.15	.9	<0.0001	19.83	.5	<0.0001
Saturated Fat (g)	2.64	.98	<0.0001	-1.42	.94	0.008	4.69	.9	<0.0001	6.79	.1	<0.0001
Monounsaturated Fat (g)	4.9	.92	<0.0001	2.21	.23	0.07	2.82	.8	0.0006	5.84	.8	<0.0001
Polyunsaturated Fat (g)	3.36	.51	<0.0001	1.46	.68	0.03	0.94	.4	0.008	5.65	.7	0.001
Sodium (mg)	-58.78	93	0.76	-135.36	19	0.27	-112.27	26	0.37	-76.78	77	0.66
BODY FATNESS, ANTHROPOMETRICS, & PHYSICAL ACTIVITY												
Total Body Fat (%)	0.22	.01	0.91	3	1.09	0.007	3.83	.7	<0.0001	6.47	2.5	0.009
BMI (kg/m2)	0.25	.41	0.54	0.86	0.86	0.32	1.38	.6	0.02	3.02	0.6	<0.0001
Waist Circumference (cm)	2.31	.93	0.01	2.29	1.13	0.04	3.02	.3	0.2	7.25	3.1	0.02
Andriod Fat (%)	1.78	.36	0.45	4.6	1.6	0.005	6.05	.8	<0.0001	8.79	2.9	0.003
Gynoid Fat (%)	-0.77	.82	0.68	1.72	1.05	0.1	1.65	.8	0.04	4.06	1.9	0.04
Android:Gynoid Fat Ratio	0.05	.02	0.05	0.07	0.02	0.003	0.09	0	<0.0001	0.1	0.1	0.07
Physical Activity (met-min)**	6.31	.22	0.31	2.78	7.34	0.7	-26.23	10	0.01	-17.11	4.6	0.0003

[1]All dietary patterns were compared to the reference "Dietary Guidelines" pattern.
[2]Estimates (β-coefficients) represent the change in the outcome of interest for each dietary pattern after multivariate adjustment when compared to the "Dietary Guidelines" pattern.
*Adjusted for: Smoking status (current vs. never), Education (Some college vs. No college), Race (White vs. Non-White), Childhood BMI-Z score, Physical Activity (met-minutes), Total energy (kcal) **Adjusted for: Smoking status (current vs. never), Education (Some college vs. No college), Race (White vs. Non-White), Childhood BMI-Z score, Total Energy (kcal) [Clinic was included as a random effect in all models].

Table 7-5. Geometric Means and Percent Change (%Δ, (95% CI) in Breast Density, Fibroglandular Volume, and the Area of Non-Dense Tissue across 5 Dietary Patterns (n=171)[1]

Variable[1]	***%Δ***	***95% CI***	***P-Value***
Percent Breast Density			
Alcohol and Vegetables	10.0%	(-5.2 to 27.6%)	0.52
Health Conscious	19.6%	(7.0 to 33.7%)	0.11
Non-Drinkers	22.0%	(5.8 to 40.8)	0.17
Western	4.7%	(-35.5 to 40.9)	0.90
Area of Dense Breast Volume (ADBV)			
Alcohol and Vegetables	34.7%	(18.6 to 52.8%)	0.02
Health Conscious	23.3%	(1.1 to 50.2%)	0.29
Non-Drinkers	41.7%	(26.7 to 58.5)	0.002
Western	3.2%	(27.4 to 46.7%)	0.93
Area of Non- Dense Breast Volume (ANDBV)			
Alcohol and Vegetables	13.7%	(3.1 to 25.4%)	0.19
Health Conscious	-12.1%	(-21.1 to -2.1)	0.24
Non-Drinkers	-5.4%	(-15.8 to 6.4%)	0.64
Western	-7.0%	(-16.5 to 3.7%)	0.51

Adjusted for: Race (White vs. Non-White), Smoking Status (Current vs. Never), Education (Some college vs. No College), Duration of Hormone use, % body fat, Childhood BMI-Z score, and Parity. Clinic was included as a random effect.

[1]All dietary patterns were compared to the reference "Dietary Guidelines" pattern

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Chapter 6

Conclusions

Summary of research findings and implications

Research presented in this dissertation is cross-sectional in design and focuses on how overall diet and diet quality are associated with body composition and breast density in young premenopausal women. Diet is relatively easy to modify and small changes early in life may prevent the development of chronic disease in the future [1] . In order to investigate our objectives, we examined dietary ED (food-only, food + caloric beverages, and food + all beverages) and derived *a posteriori* dietary patterns to assess overall diet quality and evaluate the associations with several measures of both breast density and body fatness. Our overall findings suggest that diets high in ED are associated with less favorable body fatness and breast density outcomes.

In Study 1 (Chapter 3), dietary ED and three measures of breast density were explored. Food-only dietary ED was positively associated with the percent dense breast volume and inversely associated with the area of non-dense breast volume. There were no statistically significant associations for any of the ED variables with the volume of dense breast tissue. There were also statistically significant interactions between childhood BMI Z-score and food-only ED, percent dense breast volume, and the area of dense breast volume. A significant positive association was observed for food-only ED with percent dense breast volume and a significant inverse association with the area of non-dense breast volume in women who had higher childhood BMI-Z scores. The volume of dense breast tissue was also positively associated with food-only ED in women who had a higher childhood BMI-Z score; however, it failed to reach statistical significance. While many studies of diet and breast density have focused on single nutrients, our study was the first to report an association between dietary ED and breast density.

In our second study (Chapter 4), we examined the associations between dietary ED and several measures of body fatness in premenopausal women. We found that food-only ED was

significantly positively associated with body mass index (BMI), total body, android, and gynoid fat, and the android:gynoid fat ratio. Food-only ED was not associated with waist circumference. The dietary ED calculations that contained caloric beverages and all beverages were not associated with any of the measures of body fatness.

In our third and final study (Chapter 5), we derived five dietary patterns using finite mixture modeling (FMM): 1) Western; 2) Dietary Guidelines; 3) Alcohol and Vegetables; 4) Non-Drinkers; and 5) Health Conscious. The two extreme patterns were the "Western" and the "Dietary Guidelines". The "Western" pattern was the most unfavorable and characterized by the highest intakes of unhealthy foods including red and processed meats, sweets, starchy and fried vegetables, and refined grains, among others. The "Western" pattern also had the highest energy and dietary ED across all three methods (food-only, food + caloric beverages, food + all beverages). Finally, the "Western" pattern had the highest percentage of current smokers, the lowest percentage of individuals with post-high school education, and was the only pattern to have high-risk BMI (<25) and waist circumference (< 88cm). In contrast, the "Dietary Guidelines" pattern was the most optimal and was characterized by the highest intakes of healthy foods including fruits, whole grains, and lean protein. The "Dietary Guidelines" pattern had a very low prevalence of current smokers (15%) and had the lowest energy intake both overall and from beverages alone. This pattern also had the most favorable Android:Gynoid fat ratio of all the patterns.

There were no associations with any of the dietary patterns with the percent dense breast volume or the volume of non-dense tissue. When exploring the volume of dense tissue, both the "Alcohol and Vegetables" and "Non-Drinker" patterns were significantly positively associated.

Strengths

The research presented has several strengths. To begin, all 301 girls who participated in the original Dietary Intervention in Children Study (DISC) were then invited to participate in the Dietary Intervention in Children Follow-Up Study (DISC06) when they were between the ages of 25-29. A total of 260 girls participated. A large amount of high quality data were collected that allowed for the examination of diet (24-h recall) and several breast cancer risk factors including body composition (DXA) and breast density (MRI). In addition, this age group of women is highly understudied in the diet and breast density literature because breast density is often assessed via mammography which does not begin at least until age 40.

Limitations

The research presented in this dissertation was not without limitations. One shortcoming of this research is that all of the studies were cross-sectional in nature which does not allow for causal inferences to be made. Another consideration of this research was that the women included in this study were primarily non-Hispanic white and initially enrolled into the DISC trial because of elevated LDL-C and half underwent a dietary intervention regarding dietary fat intake. Even though adherence to the dietary intervention diminished after approximately the third year of follow-up, the adjusted differences between the usual care and the intervention group remained significantly different throughout the duration of the seven-year intervention [2]. This still may limit the generalizability of this research to the general population because of the potential adoption of lasting dietary habits. Additionally, breast tissue is very dynamic throughout the premenopausal years and breast density has been shown to differ even across the menstrual cycle [3, 4] which makes it difficult to measure accurately. Our study attempted to account for these

changes by measuring breast density during the same phase (luteal) of all participants. As with any study that uses dietary data, underreporting of unhealthy foods or overreporting of healthy foods may also exist. Finally, we tried to control for many potential confounders through multivariate-adjusted models; however, the possibility of an unknown confounder that was unable to be controlled for still remains and must be taken into account when interpreting all results.

Future directions

We examined the cross-sectional association between measures of overall diet quality and body fatness and breast density in a cohort of young women. These studies were important to do because they expand the literature on an understudied population and examine overall diet which translates easier into recommendations for the general population.

Also, we know that individuals tend to stay on diet and weight trajectories [5] so intervening at this age may alter that trajectory before chronic disease of any sort has the opportunity to develop. Continued research should include, but not be limited to, the longitudinal analysis of diet in childhood, how it changes throughout the critical years of development, and the effect it has on body fatness and breast density measures into adulthood using the DISC cohort. Dietary data from the original DISC trial would be available to use for this unique longitudinal analysis; however, it would require additional processing before any analyses could occur. Additionally, intervention studies in young women that focus on the dietary profiles such as the low ED "Dietary Guidelines" pattern that are favorably associated with body fatness outcomes may also be beneficial. Breast tissue develops during adolescence and continues to differentiate and change throughout young adulthood. This suggests that the strongest impact of many environmental factors, including diet, on breast cancer risk may occur at this time.

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Appendix

“Diet Across the Lifespan and the Association With Breast Density in Adulthood”

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Review Article

Diet across the Lifespan and the Association with Breast Density in Adulthood

Jessica Lindgren,[1] Joanne Dorgan,[2] Jennifer Savage-Williams,[3] Donna Coffman,[4] and Terryl Hartman[1]

[1] *Department of Nutritional Sciences, The Pennsylvania State University, 110 Chandlee Laboratory, University Park, PA 16802, USA*
[2] *Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111, USA*
[3] *Department of Nutritional Sciences, Center for Childhood Obesity Research, The Pennsylvania State University, 129 Noll Laboratory, University Park, PA 16802, USA*
[4] *The Methodology Center, The Pennsylvania State University, 400 Calder Square II, State College, PA 16801, USA*

Correspondence should be addressed to Jessica Lindgren; jal5150@psu.edu

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Studies have shown inconsistent results regarding the association between dietary factors across the lifespan and breast density and breast cancer in women. Breast density is a strong risk factor for breast cancer, and the mechanism through which it influences cancer risk remains unclear. Breast density has been shown to be modifiable, potentially through dietary modifications. The goal of this paper is to summarize the current studies on diet and diet-related factors across all ages, determine which dietary factors show the strongest association with breast density, the most critical age of exposure, and identify future directions. We identified 28 studies, many of which are cross-sectional, and found that the strongest associations are among vitamin D, calcium, dietary fat, and alcohol in premenopausal women. Longitudinal studies with repeated dietary measures as well as the examination of overall diet over time are needed to confirm these findings.

1. Introduction

Breast cancer (BC) is the most commonly diagnosed cancer and the second leading cause of cancer death among women [1]. Alcohol consumption, physical activity, elevated after menopausal body mass index (BMI) [2], age at menarche and menopause [3], and family history and genetic mutations [4] are a few of the well-established BC risk factors. In addition, breast density (BD), or the amount of dense fibroglandular tissue present in the breast, has been related to BC risk; women who have breast densities of 75% or more have up to a 4-5-fold increase in BC risk [5]. Consequently, BD is often thought of as an intermediate on the BC development continuum that can be measured, assessed, and targeted for potential cancer prevention strategies [5–8]. Even so, little is known about the mechanism through which BD may affect breast cancer risk [9]. Breast tissue develops mostly during puberty and continues to undergo changes throughout several life stage events, such as pregnancy [3, 10, 11]. This paper will examine research on diet and diet-related factors captured across the lifespan and the association with adult BD.

2. Methods

A literature search of the PubMed database of the United States National Library of Medicine was conducted to find human studies that evaluated the associations between BD measures and diet isn the form of either single nutrients or whole dietary patterns. Both observational and diet intervention studies conducted at any stage of the lifespan were considered. Observational studies were included if they had recorded individual's dietary intake of foods or energy with dietary assessment tools such as a dietary recall (DR), food frequency questionnaire (FFQ), food record (FR), or other

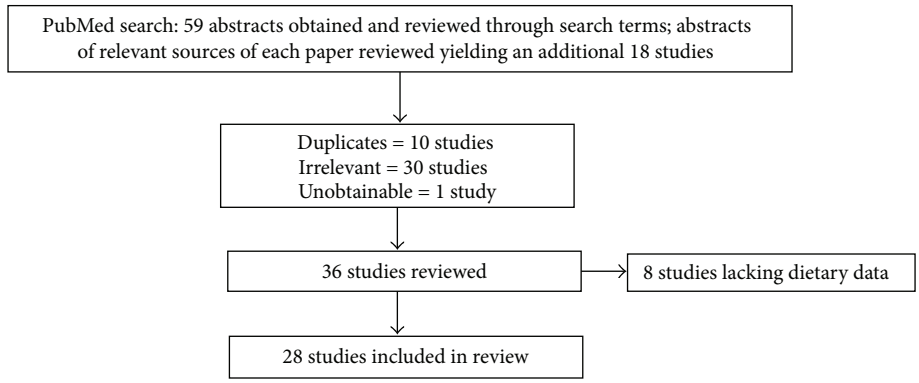

Figure 1: Lindgren, Dorgan, Savage-Williams, Coffman, and Hartman "Diet across the Lifespan and the Association with Breast Density in Adulthood."

relevant assessment tools. Relevant studies were identified using the following search terms in multiple combinations: "adolescent diet and breast density," "diet and breast density," "childhood and breast density," "diet and parenchymal patterns" and "mammographic breast density and diet." The search was limited to full-text publications written in English. As illustrated in Figure 1, a total of 77 studies were identified. After all exclusions, 28 studies were included in this paper.

2.1. Measurement of Breast Density. BD can be measured two (2D) and three dimensionally (3D), with the most common being through 2D mammography. Mammography measures the area of dense tissue (ADT) and the total area of the breast. Percent dense area (PDA) is often reported and is estimated as the proportion of dense fibroglandular tissue area to total breast area [9]. Area of nondense tissue (ANDT), which is primarily adipose tissue, also can be estimated. Magnetic resonance imaging (MRI) and ultrasound also are used to measure BD. These 3D modalities measure volume of dense tissue (VDT) and percent dense volume (PDV). Percent densities measured by mammography and MRI are highly correlated in the general population and among women who have low breast densities ($r = 0.73$) [38], but this correlation is attenuated among women with higher mammographic density greater than 50 percent ($r = 0.26$) [38]. In addition to quantitative measures of PDA and ADT, semiquantitative and qualitative measures are frequently reported. Either the Wolfe classification, which has been further classified into Tabár, or the breast imaging-reporting and data system (BI-RADS) classification is used [39–41]. These measures often classify the breast on a four- to five-level scale ranging from low-to-high levels of fibroglandular tissue. While all methods are able to assess BD, quantitative methods provide more consistent results and a larger gradient of risk. Qualitative measures often have intervals that are too large (fewer categories) and do not capture true risk gradients [42].

2.2. Nondietary Factors That Influence Breast Density. In general, PDA is higher in premenopausal women compared to postmenopausal women as well as in postmenopausal women who use hormone replacement therapy (HRT), and in both pre- and postmenopausal women with a lower BMI; and is lower in women who are parous, experience their first birth at a younger age, or are smokers [9, 43, 44]. Correlates of ADT are less well-studied, but in one study the ADT was inversely associated with age and BMI [44]. The nondense compartment of the breast is adipose tissue, and higher adiposity, frequently measured by BMI, attenuates the ratio of dense tissue area to total breast area. Other characteristics associated with BD may influence estrogen, insulin-like growth factor-I (IGF-I), or insulin-like growth factor binding proteins (IGBPBs) that affect fibroglandular tissue proliferation [15, 45]. Alternatively, some characteristics, such as parity, could have direct effects on breast morphology which are reflected in PDA.

2.3. Dietary Factors. This review will focus primarily on diet and diet-related factors and their potential effects on both PDA and ADT with only limited attention to endogenous risk factors that are well-studied and not modifiable. A summary of these findings can be found in Tables 10 and 11. Observational studies and clinical trials that evaluated dietary intakes during childhood, adolescence and adulthood are described.

3. Childhood Diet and Adult Breast Density

Much of breast development occurs during puberty; thus, factors such as childhood diet that influence the timing of puberty could potentially affect BD [46, 47]. Three studies have examined dietary habits during childhood and the effect on BD in adulthood. Mishra and colleagues [13, 14] conducted two studies in a nationally representative longitudinal British sample to examine the association of childhood diet with BD. Childhood diet was assessed at age four years by a single dietary recall completed by mothers and later linked to mammographic BD measures collected at approximately fifty years of age from pre-, peri- and postmenopausal women. After controlling for relevant confounders, the investigators observed no association between PDA and childhood calcium [14], or total energy intake or with three dietary patterns ((1) breads and fats, (2) fried potatoes and fish, and (3) milk,

fruit and biscuits). A limitation of these studies is that a single dietary recall was used to assess diet, which could have contributed to the null results since multiple recalls are typically required to adequately assess usual diet [48]. Additional time points for dietary data collection, such as during adolescence, may have provided more insight into the effect of early diet on BD.

Haars and colleagues [12] examined the association between short-term transient caloric restriction (i.e., 6–8 mos.) during the Dutch Famine (when women were aged 2–33 years) and adult BD in The Netherlands DOM-project. While this study does not necessarily fit within our inclusion criteria, it is included in this paper because of the limited data available on children. Levels of caloric restriction were retrospectively assessed through three questions regarding hunger, cold, and weight loss and categorized as absent, moderate, or severe famine exposure (FE). Degree of famine exposure at 2–9 years of age was significantly inversely associated with ANDT; mean ANDT were 77.8 cm^2, 87.7 cm^2 and 53.1 cm^2 in unexposed, moderately, and severely exposed, respectively (P_{trend} = 0.03). Although not significant, the women who were severely energy restricted at this age also had a larger ADT and higher PDA. However, because only 15 subjects were severely restricted, results should be interpreted cautiously.

The three studies that examined childhood diet and its effect on adult BD measures did not find associations with PDA or ADT although, in the study of the Dutch famine, severe caloric restriction early in life was significantly inversely associated with ANDT later in life [12]. In this cohort, women who were severely calorically restricted had higher levels of both IGF-1 and IGFBP-3 postmenopausally than those who were not restricted [49]. Thus, one mechanism through which caloric restriction at young ages could potentially influence adult BD may be via differential programming of the somatotrophic axis resulting in long-term effects on growth factors such as IGF-1 and IGFBP-3 that are associated with breast density [50]. However, the small sample size and indirect diet assessment limit the inferences that can be drawn from this study. Taken together, the limited data available do not provide strong support for a role of childhood diet in determining breast density, but additional large prospective studies are needed before firm conclusions can be made.

4. Adolescent Diet and Adult Breast Density

Most of breast development occurs during puberty, and diet during this time could have long-term effects on BD in adulthood. One of five studies we found a significant association between diet during adolescence and BD in adulthood [16]. In the study by Tseng et al. [16], higher red meat intakes between the ages of 12–17 years were significantly associated with increased adult PDA in 201 Chinese-American female immigrants. After adjusting for degree of acculturation and other relevant covariates, women with the highest red meat consumption were at 3 times the odds of being in the highest PDA category compared to those with the lowest red meat consumption. When stratified by menopausal status, red meat intake remained significantly positively associated in postmenopausal, but not premenopausal women.

The remaining four studies, including 3 observational studies and one clinical trial, found no associations between dietary components or alcohol consumption during adolescence and BD in adulthood [12, 15, 17, 18]. Two studies used data from the large Minnesota Breast Cancer Family Study Cohort (MBCFSC) to examine the role of adolescent diet and alcohol consumption on BD in pre- and postmenopausal women. Diet for girls at ages 12-13 years was collected retrospectively 50 years later via a 29-item FFQ focusing on high-fat foods (e.g., meats and other animal fat sources, snacks, and desserts). Intakes of fruits, vegetables, fish, and chicken were also analyzed. In the first study, Sellers et al. [15] observed no significant associations between any of these food groups and BD in multivariate analyses stratified by menopausal status. In the second analysis, Vachon et al. [17] evaluated alcohol consumption prior to age 18 via a self-reported questionnaire collected when the majority of the women were in their sixties. "Never drinkers" had lower mean PDA than "ever drinkers" (22.2 ± 14.3% versus 26.5% ± 15.9%); however, these results were attenuated and not significant after adjustment for age, BMI, HRT use, age at first birth, and parity [17]. In the study by Haars et al. [12] described above, short-term caloric restriction in girls age 10–18 years was not associated with adult BD measures. In a clinical trial, Dorgan et al. [18] examined the long-term effects of a dietary intervention to lower fat and increase fiber intake during childhood and adolescence (the Dietary Intervention Study in Children-DISC) and observed no differences in the VDT or PDV between those participants who received the behavioral intervention and the control group [18]. Thus, similar to childhood diet, the limited data available do not provide much support for a role of adolescent diet in determining adult BD, but additional research is needed.

5. Adult Diet and Adult Breast Density

The majority of studies that have evaluated associations of diet with BD assessed the effects of adult diet. A total of 26 epidemiological studies and randomized controlled trials that examined dietary intake and BD among adult women are included in this paper.

5.1. Total Energy. Three studies examined the association of total energy intake in adulthood with BD measures (Table 9). In a nationally representative British cohort total energy intake around age 36 years was significantly positively associated with PDA and ADT at age of 51 years in pre- and postmenopausal women [13]. Sala et al. [29] similarly found that total energy intake was significantly positively associated with PDA. The odds ratio (OR) for being classified in the highest PDA category for women in the highest versus lowest tertile of energy intake was 1.79 (95% CI: 1.09–2.91). In analysis stratified by menopausal status, energy intake was associated with significantly higher PDA in postmenopausal women only [29]. Finally, in the Dutch famine study described above, caloric restriction in adulthood was not associated

with several BD measures suggesting that exposure to short-term caloric restriction may be more important in children.

5.2. Dietary Fat. Eight studies [24, 25, 27, 29–32, 51] have examined the association between dietary fat and BD in adulthood. Three studies showed a significant positive association with total fat and BD measures. Nagata and colleagues [31] showed significant associations in a Japanese sample with mean PDA being 15.5% in the highest quartile of total fat intake compared to 9.9% in the lowest quartile (P_{trend} = 0.04). In a sample of 31 BC patients, women in the highest quartile of total fat (mean % energy (E) = 42.04) compared to the lowest quartile of intake (mean %E = 34.72) were significantly more likely to be classified as a P2 + DY (high density) pattern compared to the N1 + D1 (low density) pattern ($P < 0.01$) [25]. Qureshi et al. [32] showed a positive trend for the relationship between total fat with increased ADT in a large Norwegian population of postmenopausal women although it did not reach statistical significance.

Individual fatty acids have also been examined with saturated fatty acids (SFAs) generally being positively associated with increased BD measures. In an analysis based on 645 pre- and postmenopausal women ages of 40–62 years enrolled in the Canadian National Breast Screening Study (CNBSS), SFA intake was significantly positively associated with PDA. Mean PDA was 44.2% in the highest quartile of SFA intake compared to 38.6% in the lowest (P trend = 0.009) [30]; however, menopausal status was not controlled for or stratified by in this analysis. Similar findings also were reported in pre- and postmenopausal Japanese women; mean PDA was 16.5% in the highest quartile of SFA intake compared to 7.3% in the lowest (P trend = 0.02) [31]. Qureshi and colleagues [32] also showed a positive trend with SFA and PDA in a Norwegian population of postmenopausal women although statistical significance was not reached. Nordevang and colleagues [25] observed that women who consumed a mean %E of 19.27 from SFA in the highest quartile were more likely to be classified as having a high-risk PDA compared to those who consumed a mean %E of 15.42 from SFA in the lowest quartile ($P \leq 0.05$). In contrast, a significant inverse association was observed with SFA in a subset of 283 premenopausal women from the MBCFSC; mean PDA was 37% in those with the highest SFA intake compared to 44% in the lowest consumers after controlling for relevant confounders (P trend = 0.03) [51]. No associations with dietary fat were observed in postmenopausal women alone in this study.

The essential PUFA, linolenic acid, was inversely associated with PDA in a mediterranean population of both pre- and postmenopausal women. Women in the highest tertile of intake had 31% lower odds of being classified as high PDA [24]. Elevated PUFA consumption in a sample of BC patients (mean %E = 5.65 versus 4.70) and n-6 fatty acids (Mean %E = 4.69 versus 3.81) was also significantly associated with being classified as a P2 or DY (high density) versus an N1 or P1 (low density) Wolfe parenchymal pattern ($P < 0.05$). Vachon et al. [51] examined a sample of both pre- and postmenopausal women in the MBCFSC and observed women in the highest quartile of PUFA intake had 4% higher PDA compared to those in the lowest quartile ($P = 0.05$). Similar results were observed with the PUFA : SFA ratio in this study.

Finally, Nordevang and colleagues [25] found that women within the highest quartile of MUFA (mean %E = 14.22) were more likely to have a high PDA compared to those in the lowest quartile (mean %E = 11.98, $P < 0.01$). Far fewer associations between dietary factors and BD measures were observed in postmenopausal women, with only increased consumption of MUFAs being significantly associated with high PDA even though the difference in MUFAs as percent energy between the high and low density groups was small (mean %E = 12.9 versus 12.3, $P < 0.05$). A small number of randomized controlled trials (RCTs) have also been conducted to examine dietary fat and BD and have yielded mixed results [35–37]. These studies will be further discussed in the "RCT" section of this paper.

5.3. Alcohol. In their 2001 review, Singletary and Gapstur [52] concluded that there was strong evidence for a positive association between alcohol and BD in both pre- and postmenopausal women [52]. Alcohol may influence BD through decreasing the concentration of sex-hormone binding globulin and disturbing estrogen metabolism, increasing serum estrogen metabolites, raising oxidative stress in tissue, and leading to an increase in breast tissue proliferation [53]. The relationship between alcohol and BD may also be related to its positive association with IGF-1 and a negative association with IGFBP-1 that has been shown in post-, but not premenopausal, women [54]. Total alcohol consumption in a multiethnic cohort was associated with a 1-2% higher PDA among pre- and postmenopausal alcohol consumers (median alcohol consumption in the highest consumers = 12 drinks/wk) when compared to abstainers; however, this association failed to reach statistical significance [28]. In a mediterranean cohort of both pre- and postmenopausal women, both total wine consumption and total alcohol consumption were significantly positively associated with a 31% and 42% higher odds of having an elevated PDA, respectively [24]. A similar observation was made with total alcohol consumption in premenopausal women with "Never Drinkers" having a mean PDA of 39% compared 45% for consumers of ≤3.9 g/d and 42% for consumers of >3.9 g/d ($P_{\text{trend}} = 0.08$). When the type of alcohol was examined, comparable results were observed with white wine in postmenopausal women only; however, an inverse association was observed with red wine in postmenopausal women with "nondrinkers" having a mean PDA of 34% compared to 32% for those consuming ≤1 serving/wk and 28% for those consuming ≥2–4 svg/wk ($P_{\text{trend}} = 0.02$) [51]. The authors suggest that the difference between white and red wine may be due to the polyphenols that are present in red wine, which have been shown to have chemoprotective effects [51]. Tseng et al. [27] and Sala et al. [29] also looked at alcohol intake in pre- and postmenopausal women and found no associations with BD measures.

5.4. Soy and Isoflavones. Maskarinec et al. [55] conducted a review of the primarily epidemiological evidence on

isoflavones and their association with PDA and concluded that soy products have a little-to-no influence on BD measures regardless of the amount of isoflavones they are consuming in the range 0.1–120 mg/d [55]. A meta-analysis of several RCTs that examined that the effect of soy and BD measures was also conducted and will be discussed in the "RCT" section of this paper.

5.5. Calcium and Vitamin D. Vitamin D and calcium have been linked to cellular growth and differentiation in breast tissue [56, 57] and may influence the amount of dense tissue in the breast. Four cross-sectional studies found a significant inverse association between vitamin D and calcium intake, alone or in combination, with BD measures [21–23, 25] in premenopausal women. Nordevang et al. [25] found that lower intakes of calcium (1165 versus 1433 mg/10MJ) were significantly associated with an increased PDA. When examining dietary vitamin D and calcium, Bérubé et al. [22] observed that premenopausal women in the highest categories of both vitamin D (≥100 IU/d) and calcium (≥750 mg/d) intake had 72% lower odds of having high PDA. When intake from both diet and supplements was considered, simultaneous increases of 400 IU of vitamin D/d and 1000 mg of calcium/d were associated with an 8.5% (95% CI: 1.8–15.1%) decrease in PDA in premenopausal women [21]. The association in postmenopausal women was considerably weaker [22] or null [21]. Diorio et al. [23] found comparable results; as dietary vitamin D and calcium increased by 100 IU/d and 250 mg/d, respectively, PDA decreased by 1.8% ($P < 0.01$). Similar results were found when intake from food and supplements were analyzed together.

Out of the remaining seven studies, two included only postmenopausal women and neither found an association between vitamin D and calcium intake and BD [19, 20]. An additional four studies reported significant associations between vitamin D and calcium overall; however, the results in postmenopausal women were considerably weaker than observed in premenopausal women [14, 26, 27]. Masala et al. [24] observed that Mediterranean women with a higher calcium intake had 33% lower odds of having a high-risk mammographic pattern. No association was observed with vitamin D; however, vitamin D intake in this population was very low [24]. In a nationally representative British cohort, an inverse association between calcium intake and PDA, which were both measured among women in their 50's, was observed. Calcium intakes ≥1180 mg/d compared to 699 mg/d resulted in a 0.53 (95% CI: 0.03–1.02) standard deviation decrease in PDA [14]. No additional associations were observed with the ADT or ADNT in this study. Tseng and colleagues [27] conducted a cross-sectional analysis using a 126-item FFQ to examine several dietary factors including vitamin D and found that, after controlling for menopausal status, high-risk women (women with at least one 1st or 2nd degree relative with breast or ovarian cancer) with higher vitamin D intake had 50% lower odds of having high PDA when comparing the highest to the lowest tertile. Finally, serum 25[OH]D and dietary calcium intake obtained from an FFQ in a sample of women from the MBCFSC (73% postmenopausal) were not associated with either PDA or ADT [26]. While the overall trend failed to reach significance, the study did demonstrate that women with the highest mean intake of both calcium (>1,385 mg) and 25(OH)D (>86.2 nmol/L) had the lowest PDA and ADT after adjusting for age, BMI, parity, age at first birth, and physical activity. Vachon et al. [51] also reported no associations for calcium and vitamin D from both dietary and supplemental sources with PDA in this cohort.

Overall, this research suggests that vitamin D and calcium are inversely associated with BD in premenopausal women. It is critical to note that as calcium and vitamin D increased from <500 mg/d and <100 IU/d to >1,750 mg/d and >700 IU/d, respectively, PDA decreased in a dose-response fashion with clinically relevant decreases in PDA between 8 and 12% among premenopausal women [21, 23]. This is comparable to the effect of selective estrogen receptor modulators such as tamoxifen [58]. Importantly, Brisson et al. [59] examined serum vitamin D [25(OH)D] levels and found that PDA was lowest in the fall (39%) and highest in the spring (45%) ($P = 0.003$), which was consistent with the rise and fall in serum vitamin D across the seasons. Few studies account for season in which BD was assessed. However, it may be important to consider endogenous vitamin D synthesis in response to sunlight in addition to that contributed by food sources. The biologically active form of vitamin D may decrease BD via its antiproliferative properties or tissue-specific effects due to breast tissue possessing 1-α-hydroxylase, which converts inactive 25(OH)D to active $1,25(OH)_2D$ [60]. The localized production of $1,25(OH)_2D$ helps to regulate cell growth and promote terminal differentiation which promotes cellular resistance from carcinogenic factors [60]. Premenopausal women have higher levels of estrogen, insulin-like growth factor (IGF), and insulin-like growth factor binding proteins (IGFBPs), which may be associated with increased BD [61, 62]. Vitamin D, calcium, and IGFBP-3 have been proposed to increase each other's beneficial antiproliferative and proapoptotic effects [23]; however, vitamin D alone may help to combat the proliferative effects of estrogen and IGF when these hormones and growth factors are available in abundance, such as in premenopausal women.

5.6. Carbohydrates, Protein, and Other. Ten studies have evaluated intakes of carbohydrates, protein, and many other nutrients and their association with BD measures. Eight studies [16, 24, 27, 30–32, 34, 51] used validated FFQs to assess nutrient intake; Sala et al. [29] and Nordevang et al. [25] conducted extensive dietary history interviews. Tseng and colleagues [27] found that, in a sample of 90 women with a sporadic family history of BC, total and animal protein intakes above the median intake had from 3 to 4 times the odds of an increased PDA; these associations were not observed in women with a strong hereditary pattern (1st or 2nd degree relative) of BC [27]. As mentioned previously, red meat intake during adolescence was significantly positively associated with PDA in adulthood; however, there was no association with red meat intake during adulthood in a sample of 201 Chinese-American immigrants [16].

Although few significant associations are observed among postmenopausal women; both Nagata et al. [31] and Sala et al. [29] found significant associations in both Japanese and European populations, respectively, when evaluating carbohydrates and protein. Sala and colleagues [29] found that protein and carbohydrate were positively associated with PDA in all women. When, stratifying by menopausal status, significant positive associations emerged between protein, total meat, and carbohydrates and PDA in postmenopausal women only with those consuming the most having 2.2–2.5 times the odds of having a high-risk PDA. Nagata and colleagues [31] also found that protein was significantly positively associated with PDA with women in the highest quartile of intake having approximately 7% higher PDA than those in the lowest quartile. However, in contrast to the study by Sala, carbohydrates were significantly inversely associated with PDA in 253 postmenopausal Japanese women with those in the highest quartile having 6% lower PDA than the lowest consumers [31]. No associations were observed in premenopausal women [31]. Among pre- and postmenopausal women in the CNBSS, mean PDA was 37.9% in those in the highest quartiles of fiber intake compared to 43.0% in the lowest quartile, and the difference was significant [30]. Comparable results were found in a sample of 31 Swedish premenopausal BC patients; lower consumption of carbohydrate and fiber was associated with higher PDA [25].

In a study evaluating dietary factors and mammographic patterns in a Mediterranean population, both pre- and postmenopausal women in the highest tertiles of the following foods and nutrients had 27–34% lower odds of having a high PDA: total vegetables, cheese, β-carotene, vitamin C, and potassium, whereas women in the highest tertile of tomato sauce intake had 34% higher odds of having a high PDA [24]. Similar results with high cheese intake were observed in a sample of 491 premenopausal women in this study [24]. Consistent with these findings, total dairy intake was significantly inversely associated with PDA in premenopausal women in the MBCFSC after controlling for relevant confounders [51]. Among pre- and postmenopausal women in the CNBSS, women in the highest quartiles of carotenoid intake had a 5.4% lower mean PDA when compared to the lowest quartile [30]. Comparable results were found by in sample of 31 Swedish premenopausal BC patients and found that lower consumption of carotene was associated with increased PDA [25].

Only one study to date has examined multivitamin/multimineral (MVMM) supplement intake and BD outcomes. Bérubé and colleagues [34] found that current premenopausal supplement users had a significantly higher adjusted mean PDA of 45% compared to 42.9% of past or 40.2% of never users ($P_{\text{trend}} = 0.009$). No association was observed in postmenopausal women. Vachon et al. [51] also found that dietary vitamin E and supplemental vitamin C were significantly positively associated with PDA in premenopausal women with the highest consumers having a 4-5% higher PDA than the lowest consumers. Supplemental vitamin B12, on the other hand, was positively related to PDA in postmenopausal women [51].

In conclusion, the foods or nutrients that were shown to be inversely associated with BD may be, in part, tied to IGF/IGFBP levels and oxidative stress reduction. BD has been associated with increased levels of oxidative stress as evidenced by malonoyldialdhyde (MDA) excretion [63] and IGF/IGFBP, particularly in premenopausal women [50, 61]. Lower intakes of fiber, carotene, and calcium have also been associated with increased breast densities. Carbohydrate intake has been associated with both lower and higher BD measures women. These conflicting results may be attributed to the fact that the types of carbohydrate are often not accounted for and fiber content may influence the way that different carbohydrates affect the IGF/IGFBP pathway and oxidative stress. Finally, higher intakes of total dairy and cheese consumption in premenopausal women are associated with lower BD measures, which may be due to the high amounts of calcium and vitamin D in these products.

5.7. Dietary Patterns. Analysis of dietary patterns has recently gained popularity in dietary assessment research, as they capture total diet and are more stable over time than the consumption of single nutrients or foods [64]. Two studies were conducted that examined *a posteriori* dietary patterns and their association with BD and one study examined the influence of Mediterranean Diet (measured by Mediterranean diet scale (MDS)) on BD measures. Dietary patterns were analyzed cross-sectionally in a British cohort and the MBCFSC [13, 33]. After combining data collected from food records collected at ages 36 and 43 years, four patterns emerged in the British cohort ((1) low-fat and high fiber; (2) alcohol and fish; (3) high fat and sugar; (4) meat, potatoes, and vegetables). However, none of these patterns was associated with PDA [13]. In the MBCFSC, three dietary patterns emerged from data from a 153-item FFQ ((1) fruit, vegetable, and cereal; (2) salad, sauce, and pasta/grain; (3) meat and starch). Only the fruit, vegetable, and cereal pattern was inversely associated with PDA in premenopausal women; however, it did not reach statistical significance [33]. Smoking has been associated with decreased PDA because of its antiestrogenic effects [65]. When all women included in the sample were stratified by smoking status, adherence to the fruit, vegetable, and cereal pattern was significantly inversely associated with PDA in smokers ($P = 0.02$) [33]. The salad, sauce, and pasta/grain pattern was also nonsignificantly inversely associated with PDA in smokers [16]. These patterns are the highest in antioxidant-containing foods, which may benefit women who are under higher oxidative stress, such as smokers.

Tseng et al. [66] cross-sectionally evaluated the MBCFSC using the MDS. The women were scored based on their consumption of vegetables, legumes, fruits and nuts, cereals, fish, and the ratio of monounsaturated fatty acids (MUFA) to saturated fatty acids (SFA) as reported on a 153-item FFQ. For each unit increase in the MDS, PDA was decreased by 1.68% ($P = 0.0002$) among current smokers but not among nonsmokers after controlling for relevant confounders including menopausal status [66]. Vegetables, legumes, and cereals were the components of the MDS that had the strongest association with PDA in this population [66].

Table 1: Studies of childhood diet and breast density.

Author, year	Study population, n	Design	Diet/mammogram Age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Haars, et al. 2010 [12] (also in Tables 2 and 9)	DOM-Project $n = 144$ (The Netherlands)	CS	2–9 y/53 y	Short-term energy restriction	Retrospective recall of 1944-45 famine ~40 yrs later. Exposure to hunger, cold, and weight loss.	BS, DT, NDT, PBD (mammogram; visual observation)	Severely calorically restricted versus unrestricted: NDT: 53.1 cm^2 (95% CI: 37.8–72.7) versus 77 cm^2 (95% CI: 68.8–87.7)	Age at examination, parity, menopausal status, BMI
Mishra, et al. 2011 [13] (also in Tables 9 and 6)	BBC $n = 792$ (England)	PC	4 y/51.5 y	Dietary patterns at age 4: (1) breads and fats (2) fried potatoes and fish, (3) milk, fruit, and biscuits	1–24-hr maternal recall of child's diet	PBD, ADT, ANDT (mammogram; Cumulus)	Null	Mammographic view, age at mammogram, BMI at 53, age at menarche, menopausal status at mammography, HT use, parity, smoking status, PA, social class, the other three dietary patterns, energy
Mishra, et al. 2008 [14] (also in Table 3)	BBC $n = 979$ (England)	PC	4 y/51.5 y	Dietary Ca and vitamin D	1–24-hour maternal recall of child's diet	PBD, ADT, ANDT (mammogram; Cumulus)	Null	Mammographic view, age at mammogram, BMI age 53, energy, age at menarche, parity, smoking status, adult SES.

Table 2: Studies of adolescent diet, and breast density.

Author, year	Study population, n	Design	Diet/mammogram age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Haars et al. 2010 [12] (also in Tables 1 and 9)	DOM-project $n = 356$ (The Netherlands)	CS	10–18 y/53 y	Short-term energy restriction	Described in Table 1	BS, DT, NDT, PBD (mammogram; visual observation)	Null	Age at examination, parity, menopausal status, BMI
Sellers et al. 2007 [15]	MBCFSC $n = 1{,}552$ (United States, NH-White)	CS	12-13 y/60.4 y	High-fat meats, dairy, animal fat, high-fat snacks and desserts, high-fat foods, fish and chicken, fruits, vegetables	29-item FFQ (retrospective recall)	PBD (mammogram; Cumulus)	Null	Age at mammography, weight at follow-up 1, use of HRT, menopausal status, education, age at menarche, parity, age at first birth, OC use, alcohol use, smoking hx
Tseng et al. 2011 [16] (also in Table 7)	Chinese-American immigrants $n = 201$ (US, Asian)	CS	12–17 y/53.1 y	Beef, pork, tofu, green veg, fruits	Frequency of consumption: beef, pork, tofu, green veg, fruits (retrospective)	PBD: BIRADS	Red meat intake: all women: ($OR_{T3\ \text{versus}\ T1} = 3.0$; 95% CI: 1.5–6.4) postmenopausal women: ($OR_{T3\ \text{versus}\ T1} = 16.9$; 95% CI 5.4–52.4)	Age, level of acculturation, BMI, number of live births and age at first live birth, adult dairy intake
Vachon et al. 2005 [17]	MBCFSC $n = 1575$ (US, NH-White)	CS	<18 y/60.4 y	Alcohol	Follow-up questionnaire	PBD (mammogram; Cumulus)	Null	Age, BMI, HRT, age at first birth, number of births, age at menarche, education, adult and adolescent smoking status, alcohol, OC use, menopausal status
Dorgan et al. 2010 [18] (also in Table 8)	DISC premenopausal women $n = 182$ (US, NH-White)	CS (RCT followup)	25–29 y	Long-term effects of low-fat diet	3–24-hr dietary recalls	PBD and VDT (MRI)	Null	% body fat, age at randomization, age at visit, clinic, BMI-Z score, race, education, smoking status, PA at 14–17 years old and separately during the past year, number of full-term pregnancies, hormonal contraceptives

Table 3: Studies of adult calcium and vitamin D intake and breast density.

Author, year	Study population, n	Design	Age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Bertone-Johnson, et al. 2010 [19]	MDAS: WHI n = 808 Postmenopausal (US, 42% NH-White, 39% Black, 20% other races)	CS	50–79 y	Dietary and supplemental vitamin D and Ca	122-item FFQ + supplement inventory	PBD: (mammogram; computer-assisted method)	Null	Age, race/ethnicity, BMI, age at menarche, parity, OC use and duration, previous HT use/duration, HT trial randomization assignment, family hx of BC, education, alcohol, smoking, total energy, PA, Gail risk, MV use
Bertone-Johnson et al. 2012 [20] (also in Table 8)	WHI CaD trial n = 330 postmenopausal women (US, 48% NH-White, 36% Black, 15% other)	RCT	50–79 y	Daily supplementation of both 1,000 mg of Ca and 400 IU of vitamin D (1y)	122-item FFQ	PBD: (Mammogram: computer-assisted method)	Null	Subgroup analyses: age, race/ethnicity, total vitamin D intake, HT treatment, Gail risk score, BMI, region of residence, category of mammogram density at baseline.
Bérubé et al. 2005 [21]	Premenopausal women: n = 777 Postmenopausal: n = 783 (Canada)	CS	Premenopausal: 46.7 y Post-menopausal: 61.8 y	Dietary and supplemental vitamin D and Ca	161-item FFQ	PBD: (mammogram; computer-assisted method)	Premenopausal women: dietary vitamin D: $\beta = -1.8$; total vitamin D: $\beta = -1.4$; dietary calcium: $\beta = -0.7$; total calcium: $\beta = -0.8$. 8.5% ↓mean PBD with simultaneous increases in VD and Ca by 400 IU and 1,000 mg, respectively. Postmenopausal women: null All women: absolute ↓ in mean $PBD_{Q4Ca \text{ and } VitDQ1Ca \text{ and } VitD} =$ 6.9%,	Age, BMI, age at menarche, number of full-term pregnancies, age at first full-term pregnancy, duration of OC and/or HRT use, alcohol, daily energy, PA, family hx of BC in 1st degree relative, personal history of breast biopsies, smoking status, education (supplement use was also a confounder, determined post hoc)

Table 3: Continued.

Author, year	Study population, n	Design	Age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Bérubé et al. 2004 [22]	Pre- and postmenopausal women with extreme densities $n = 543$ (US)	CS	PBD ≤ 30%: 51.4 y PBD ≥ 70%: 46.1 y	Vitamin D and dietary Ca	232-item FFQ	PBD: (mammogram; visual estimation)	All women: vitamin D: $OR_{Q4\ versus\ Q1} = 0.24$ (95% CI: 0.11–0.53); calcium: $OR_{Q4\ versus\ Q1} = 0.24$ (95% CI: 0.10–0.57) $OR_{EXT\ versus\ FEW\ DENSITIES}$ 0.28 (95% CI: 0.15–0.54) (≥100 IU Vit D and ≥750 mg/d Ca)=) premenopausal women: vitamin D:$OR_{Q4\ versus\ Q1} = 0.13$; calcium: $OR_{Q4\ versus\ Q1} = 0.13$ postmenopausal women: vitamin D: $OR_{Q4\ versus\ Q1} = 0.30$ (P trend = 0.05) calcium: $OR_{Q4\ versus\ Q1} = 0.27$ (P-trend = 0.06)	Age, mammography, BMI, age at menarche, number of births and age at first birth combined, OCs, menopausal status and use of HRT combined, family hx of BC, education, alcohol, total energy, smoking status
Diorio et al. 2006 [23]	Premenopausal women $n = 771$ (Canada)	CS	<48 y (if a nonsmoker) and <46 y (if a smoker)	Dietary and supplemental vitamin D and Ca	FFQ	PBD: (mammogram: computer-assisted method)	Food only: vitamin D: β for 100 IU/d = −1.8; calcium: β for 250 mg/d = −1.8 Food and supp: vitamin D: β for 100 IU/d = −1.4; calcium: β for 250 mg/d = −1.9	Alcohol, total energy, age, BMI, age at menarche, age at first full-term pregnancy, number of full-term pregnancies, number of breast biopsies, duration of past use of OC and of HRT, family history of BC in 1st degree relative, PA, education, smoking status
Masala et al. 2006 [24] (also in Tables 4, 5, and 7)	Mediterranean population—florence section of EPIC $n = 1, 668$ (Italy)	CS	Pre-, post-, and perimenopausal women	Vitamin D and Ca	160-item validated FFQ	Wolfe classification (P2 + DY versus N1 + P1) and semiquantitative method	All women: P2 + DY versus N1 + P1: calcium $OR_{T3\ versus\ T1} = 0.67$ (95% CI: 0.47–0.94)	Age, education, BMI, menopausal status, total energy (log), each food separately (tertiles)

Table 3: Continued.

Author, year	Study population, *n*	Design	Age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Mishra et al. 2008 [14] (also in Table 1)	BBC (*n*'s ranged from 674 to 979 women) *Cross-sectional n*:total: $n = 287$ (England)	PC	36, 43, 53 y/51.5 y	Dietary Ca and vitamin D (age 53 follow-up: included supplement data)	5-day food records	PBD, ADT, ANDT (mammogram; cumulus)	Null *cross-sectional findings:* postmenopausal women: ≥1180 mg/d^{-1} versus ≤699 mg/d^{-1}, 0.53 s.d. lower PBD (95% CI: 0.03–1.02)	Mammographic view, age at mammogram, BMI at 53, energy, age at menarche, parity, smoking status, adult SES
Nordevang et al. 1993 [25] (also in Tables 5 and 7)	BC patients (stage I-II) $n = 238$ (Sweden)	CS	57.5 y	Ca	Dietary hx interview within 4 months of BC diagnosis	Wolfe classification (N1 + P1 versus P2 + Dy)	Premenopausal women: P2 + Dy versus N1 + P1: calcium (1165 versus 1433 mg/10 MJ)	BMI, age, ER status
Knight et al., 2006 [26]	MBCFCS $n = 487$ (US, NH-white)	CS	56.4 y	Vitamin D (25(OH)D) and dietary Ca	FFQ	PBD, TDA (mammogram: Cumulus)	Null	Full model: age, BMI, parity, age at first birth, PA
Tseng et al. 2007 [27] (also in Tables 4, 5, and 7)	Women with at least one 1st degree or 2nd degree relative with BC or ovarian cancer $n = 157$ (US, NH-White)	CS	50 y	Vitamin D and Ca	126-item FFQ	PBD: BIRADS	OR: vitamin D intake$_{T3 \text{ versus } T1}$, 0.5 (95% CI: 0.2–1.1)	Age, BMI, caloric intake, age at menarche, menopausal status, history of HRT, family history category.
Vachon et al., 2000 [9] (also in Tables 4, 5, and 7)	MBCFCS $n = 1508$ (US, NH-White)	CS	61.4 y	Vitamin D and Ca	153-item validated FFQ	PBD (Mammogram: visual estimation)	null	Energy, age, BMI, WHR, PA, age at menarche, age at first birth and number of births (combined), alcohol, smoking, family hx of BC, HRT (all and postmenopausal women) and OC use (premenopausal women)

Table 4: Studies of alcohol intake in adulthood and breast density.

Author, year	Study population, n	Design	Age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Maskarinec et al. 2006 [28]	BEAN (n = 217 premenopausal women) and MEC (n = 582 cases and n = 658 controls) (multiethnic cohort)	BEAN = CS MEC = CC	BEAN = 43 y MEC (cases and controls) = 57 y	Alcohol	Validated FFQ	Mammogram: (computer-assisted method)	Null	Age, BMI, ethnicity, HRT use, age at first live birth, parity, age at menarche, menopausal status, group status, family hx of BC when appropriate.
Masala et al. 2006 [24] (also in Tables 3, 5, and 7)	Mediterranean Population—florence section of EPIC n = 1,668 (Italy)	CS	Pre-, post-, and peri-menopausal women	Alcohol	160-item validated FFQ	Wolfe classification (P2 + DY versus N1 + P1) and semiquantitative method ("entirely fat"; <25%, 25–75%, >75% high density area)	All Women: P2 + DY versus N1 + P1: Overall alcohol: $OR_{T3\ versus\ T1}$ = 1.31 (95% CI: 1.01–1.72) Premenopausal women at enrollment (n = 491): Wine: $OR_{Q4\ versus\ Q1}$: 1.84 (95% CI: 1.07–3.16); high alcohol consumption $OR_{Q4\ versus\ Q1}$: 1.86 (95% CI: 1.03–3.38)	Age, education, BMI, menopausal status, total energy (log), each food separately (tertiles)
Sala et al. 2000 [29] (also in Tables 5 and 7)	EPIC-Norfolk Cases: P2/DY Controls: N1/P1 (n = 203 cases and n = 203 controls) (UK)	NCC	Cases and controls: 59 y	Alcohol	7-day food record	Wolfe Patterns: (high risk: P2 and DY; low risk: N1 and P1)	Null	Menopausal status, parity, HRT, BMI
Tseng et al., 2007 [27] (also in Tables 3, 5, and 7)	Women with at least one 1st degree or 2nd degree relative with BC or ovarian cancer n = 157 (US, NH-White)	CS	50 y	Alcohol	126 item validated FFQ	PBD: BIRADS	Null	Age, BMI, energy, age at menarche, menopausal status, hx of HRT, family hx category.
Vachon et al. 2000 [9] (also in Tables 3, 5, and 7)	MBCFCS n = 1508 (US, NH-White)	CS	61.4 y	Alcohol	153-item validated FFQ	PBD (Mammogram: visual estimation)	Postmenopausal women: white wine: nondrinkers versus ≥2–4 svg/wk = 29% (95% CI: 26–32%) versus 34% (95% CI: 30–37%), Red wine: nondrinkers versus ≥2–4 svg/wk: 34% (95% CI: 31–36%) versus 28% (95% CI: 24–33%)	Energy intake, age, BMI, WHR, PA, age at menarche, age at first birth and number of births (combined), alcohol, smoking, family hx of BC, HRT (all and postmenopausal women), OC (premenopausal women)

Table 5: Studies of dietary fat intake in adulthood and breast density.

Author, year	Study population, n	Design	Age	Foods/Nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Brisson et al. 1989 [30] (Also in Table 7)	CNBSS—newly Diagnosed BC patients cases: $n = 290$ controls: $n = 645$ total $n = 935$ (Canada)	CC	40–62 y	Dietary fats	114-item FFQ + questions on vitamin A	Wolfe classification (high risk: P2 + DY; low risk: N1 + P1) (mammogram: visual estimation)	Controls (total densities): saturated $fat_{Q4\ versus\ Q1}$: 44.2% versus 38.6%, $\beta = 0.370$ (SE = 0.141)	Age, body weight, parity, education, energy
Masala et al. 2006 [24] (also in Tables 3, 5, and 7)	Mediterranean population—florence section of EPIC $n = 1{,}668$ (Italy)	CS	Pre-, post-, and perimenopausal women	Dietary fats	160-item FFQ	Wolfe classification (P2 + DY versus N1 + P1) & semiquantitative method	All women: P2 + DY versus N1 + P1: Olive Oil $OR_{T3\ versus\ T1}$ 0.73 (95% CI: 0.55–0.98) linolenic acid $OR_{T3\ versus\ T1} = 0.69$ (95% CI: 0.47–0.99, P trend = 0.05)	Age, education, BMI, menopausal status, total energy (log), each food separately (tertiles)
Nagata et al. 2005 [31] (also in Table 7)	Japanese women $n = 601$ (Japan)	CS	Premenopausal women: 42.6 y Postmenopausal women: 57.8 y	Dietary fats	169-item FFQ	PBD (Mammogram: fully-automated method)	Postmenopausal women: Total Fat: $Q4$ versus $Q1$ = 15.5 (95% CI: 10.8–21.2) versus 9.9% (95% CI: 6.8–13.7; Saturated fat: $Q4$ versus $Q1$ = 16.5% (95 CI:11.3–22.6%) versus 7.3% (95% CI: 4.7–10.4%)	Age, BMI, smoking status, number of births, hx of breast feeding for premenopausal women and for age, BMI, education, age at menopause for postmenopausal women. Nutrient intakes were adjusted for total energy.
Nordevang et al. 1993 [25] (also in Tables 3 and 7)	BC Patients (stage I-II) $n = 238$ (Sweden)	CS	57.5 y	Dietary fats	Dietary history interview within 4 months of BC diagnosis	Wolfe classification (N1 + P1 versus P2 + Dy)	Premenopausal women: P2 + Dy versus N1 + P1: total fat (42.04 versus 34.72% E); saturated fat (19.27 versus 15.42% E), MUFA (14.22 versus 11.98% E); PUFA (5.65 versus 4.70), n-6 FA (4.69 versus 3.81% E) postmenopausal women: P2 + Dy versus N1 + P1: MUFA (12.88 versus 12.32% E)	BMI, age, ER status
Sala et al. 2000 [29] (also in Tables 4 and 7)	EPIC-Norfolk Cases: P2/DY Controls: N1/P1 ($n = 203$ cases and $n = 203$ controls) (UK)	NCC	Cases and controls: 59 y	Dietary fats	7-day food record	Wolfe patterns: (high risk: P2 & DY; low risk: N1 & P1)	Null	Menopausal status, parity, HRT, BMI

Table 5: Continued.

Author, year	Study population, n	Design	Age	Foods/Nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Tseng et al. 2007 [27] (also in Table 3, 4, and 7)	1st degree or 2nd degree relative with BC or ovarian cancer $n = 157$ (US, NH-White)	CS	50 y	Dietary fats	126 item validated FFQ	PBD: BIRADS	Null	Age, BMI, caloric intake, age at menarche, menopausal status, history of HRT, family history category.
Qureshi et al. 2011 [32] (also in Table 7)	NBCSP $n = 2,252$ Postmenopausal women (Norway)	CS	58 y	Dietary fats	180-item validated FFQ	PBD & AD (mammogram: computer-assisted method)	PBD: Saturated $\text{fat}_{Q4 \text{ versus } Q1}$: 19.7 (95% CI: 18.7–20.7%) versus 17.0 (95% CI: 15.6–18.3, P-trend = 0.06)	Age at mammography, y of education, age at menarche, number of pregnancies, age at first full-term pregnancy for parous women, HRT, BMI, total energy
Vachon et al. 2000 [9] (also in Tables 3, 4, and 7)	MBCFCS $n = 1508$ (US, NH-White)	CS	61.4 y	Dietary fats	153-item FFQ	PBD (mammogram: visual estimation)	Premenopausal women: PUFAs: $Q4$ versus $Q1$: 42% (95% CI: 35–49%) versus 38% (95% CI: 37–51%) PUFA : SFA: 43% (95% CI: 36–50%) versus 38% (33–44%); SFA: $Q4$ versus $Q1$: 37% (95% CI: 32–43%) versus 44% (95% CI: 37–51%)	Energy, age, BMI, WHR, PA, age at menarche, age at first birth and number of births (combined), self-reported alcohol intake, smoking, family hx of BC, HRT (all and postmenopausal women), OC (premenopausal women)

Table 6: Studies of dietary patterns in adulthood and breast density.

Author, year	Study population, n	Design	Age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Mishra et al. 2011 [13] (also in Table 1)	BBC $n = 700$ (England)	PC	36, 43 y/51 y ("habitual adult" dietary patterns)	Dietary patterns: (1) low fat, fiber (2) alcohol and fish (3) high fat and sugar (4) meat, potatoes, and vegetables	5-day food records	PBD, ADT, ANDT (Mammogram; Cumulus)	Null	Mammographic view, age at mammogram, BMI at 53, age at menarche, menopausal status at the time of mammography, HT use, parity, smoking status, PA, social class, other three dietary patterns, energy
Tseng et al. 2008 [33]	MBCFSC n=1,286 (US, NH-White)	CS	57 y	MDS	153-item validated FFQ	PBD (Mammogram: semiautomated threshold method)	CCurrent smokers ($n = 176$) and the MDS (continuous): $\beta = -1.68$ (SE = 0.55) MDS category: $\beta_{\text{CAT3 versus CAT1}} = -7.17$ (SE = 2.77)	Age, total energy, menopausal status, education, HRT, BMI, WHR, age at menarche, parity and age at first live birth (combined variable), alcohol, relation to proband
Tseng et al. 2008 [33]	MBCFSC $n = 1,286$ (US, NH-White)	CS	57 y	Dietary patterns: (1) fruit-vegetable-cereal pattern (2) salad-sauce-pasta/grain pattern (3) meat-starch pattern	153-item validated FFQ	PBD (Mammogram: semiautomated method)	Smokers: fruit-vegetable-cereal pattern: $\beta = -0.30$ (SE = 0.13) Salad-sauce-pasta/grain pattern: ($\beta = -0.27$) (SE = 0.15, $P = 0.06$)	Age, total energy, menopausal status, education, PA, HRT, BMI, WHR, age at menarche, parity and age at first birth, alcohol, relation to proband

Table 7: Studies of selected nutrients in adulthood and breast density.

Author, year	Study population, (n)	Design	Age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Bérubé et al. 2008 [34]	Premenopausal women: $n = 777$ Postmenopausal women: $n = 783$ (Canada)	CS	Premenopausal: 47 y Postmenopausal: 60 y	MVMM supplements	161-item FFQ	PBD: (Mammogram: computer-assisted method)	Premenopausal women: current users (45%, SE: 1.64%), past (42.9%, SE: 1.28%), never users (40.2% SE: 1.05%)	Age, education, BMI, age at menarche, number of full-term pregnancies, age at first full-term pregnancy, duration of OC and HRT, smoking status, PA, family hx of BC in first degree relative, personal hx of breast biopsy, chronic illness, mean energy, alcohol, vitamin and mineral supplements, following special diet, dietary vitamin D and calcium intake, season of mammography
Brisson et al. 1989 [30] (also in Table 5)	CNBSS—newly diagnosed BC patients Cases: $n = 290$ Controls: $n =$ 645 Total $n = 935$ (Canada)	CC	40–62 y	Several dietary factors, especially vitamin A	114-item FFQ + additional questions on vitamin A	Wolfe classification (high risk: P2 + DY; low risk: N1 + P1) (Mammogram: visual estimation)	Controls (Total Densities): $\text{Carotenoids}_{Q4\ \text{versus}\ Q1}$: 38.2% versus 43.6%, $\beta = -392$ (SE = 171); $\text{Fiber}_{Q4\ \text{versus}\ Q1}$: 37.9% versus 43.0%, $\beta = -1.02$ (SE = 0.41)	Age, bodyweight, parity, education, energy
Masala et al. 2006 [24] (also in Tables 3, 4, and 5)	Mediterranean Population—Florence section of EPIC $n = 1{,}668$ (Italy)	CS	Pre-, post-, and peri-menopausal women	Several dietary factors	160-item validated FFQ	Wolfe classification (P2 + DY versus N1 + P1) and semi-quantitative method	All Women: P2 + DY versus N1 + P1: Vegetables: $\text{OR}_{T3\ \text{versus}\ T1} = 0.66$ (95% CI:0.50–0.88); Cheese: $\text{OR}_{T3\ \text{versus}\ T1}$: 0.73 (95% CI: 0.55–0.99); β-carotene $\text{OR}_{T3\ \text{versus}\ T1} = 0.71$ (95% CI: 0.53–0.94), Vitamin C $\text{OR}_{T3\ \text{versus}\ T1} = 0.75$ (95% CI: 0.56–0.99); Potassium $\text{OR}_{T3\ \text{versus}\ T1} = 0.69$ (95% CI: 0.48–1.00, P-trend = 0.05), Tomato sauce: $\text{OR}_{T3\ \text{versus}\ T1} =$ 1.34 (95% CI: 1.01–1.77) Premenopausal women at enrollment ($n = 491$): High consumption of cheese: $\text{OR}_{T3\ \text{versus}\ T1}$ 0.44 (95% CI: 0.23–0.84)	Age, education, BMI, menopausal status, total energy(log), each food separately (tertiles)

Table 7: Continued.

Author, year	Study population, (n)	Design	Age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Nagata et al. 2005 [31] (also in Table 5)	Japanese women $n = 601$ (Japan, Asian)	CS	Premenopausal women: 42.6 y Postmenopausal women: 57.8 y	Protein, dietary fiber, and soy isoflavones	169-item validated FFQ	PBD (mammogram: fully automated method)	Postmenopausal women: protein: Q4 versus Q1 = 13.9% (95% CI: 10.4–18.0%) versus 6.7% (95% CI: 3.6–10.7%; CHO: Q4 versus Q1 = 9.6% (95% CI: 6.5–13.2) versus 15.6% (95% CI: 11.1–20.9%)	Age, BMI, smoking status, number of births, and hx of breast feeding for premenopausal women and for age, BMI, number of births, education, age at menopause; nutrient intakes were adjusted for total energy.
Nordevang et al. 1993 [25] (also in Tables 3 and 5)	BC patients (stage I-II) $n = 238$ (Sweden)	CS	57.5 y	Various nutrients	Dietary history interview within 4 months of BC diagnosis	Wolfe classification (N1 + P1 versus P2 + Dy)	Premenopausal women: P2 + Dy versus N1 + P1: CHO: (40.41 versus 47.37% E); Fiber (19.05 versus 26.09 mg/10 MJ), Carotene (3.80 versus 5.62 mg/MJ)	BMI, age, ER status
Sala et al. 2000 [29] (also in Tables 4, 5 and 9)	EPIC-Norfolk cases: P2/DY controls: N1/P1 ($n = 203$ cases and $n = 203$ controls) (UK)	NCC	Cases and controls: 59 y	Vitamin A, vitamin C, vitamin E, protein, carbohydrate, fiber, vegetables, cereals and breads, fruits, red meat, white meat, total meat, milk, dairy products, fish.	7-day food record	Wolfe patterns: (high risk: P2 and DY; low risk: N1 and P1)	All women: protein: $OR_{OR\ T3\ versus\ T1} = 2.00$ (95%CI:1.06–3.77)**; total CHO: $OR_{OR\ T3\ versus\ T1} = 1.93$, 95% CI: 1.03–3.59)** Postmenopausal women: Protein: ($OR_{OR\ T3\ versus\ T1} = 2.20$, 1.04–4.63, $P = 0.03$)**, Total CHO: ($OR_{OR\ T3\ versus\ T1} = 2.22$, 1.02–4.79)**, Total meat intake: ($OR_{OR\ T3\ versus\ T1} = 2.50$, 1.09 = 5.69)**	*Unadjusted **Menopausal status, parity, HRT, BMI
Tseng et al. 2007 [27] (also in Table 3, 4, and 5)	At 1st degree or 2nd degree relative with BC or ovarian cancer $n = 157$ (US, NH-White)	CS	50 y	Calories, cholesterol, protein, animal protein, carbs, dietary fiber, carotene, folate, vitamin E, meats, fruits, vegetables, tofu.	126 item FFQ	PBD: BIRADS	Women who do not have hereditary cancer patterns: protein (OR: 3.0 (95% CI: 1.3–6.9)) and animal protein (OR: 4.3 (95% CI: 1.8–10.3)	Age, BMI, energy, age at menarche, menopausal status, hx of HRT, family hx category.

Table 7: Continued.

Author, year	Study population, (n)	Design	Age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Tseng et al. 2011 [16] (also in Table 2)	Chinese-American immigrant women $n = 201$ (US, Asian)	CS	53.1 y	Red meat	88-item FFQ	PBD: BIRADS	Null	Age, level of acculturation, BMI, combined variable representing # of live births and age at first live birth, adult weekly frequency of dairy food intake
Qureshi et al. 2011 [32] (also in Table 5)	NBCSP $n = 2,252$ Postmenopausal women (Norway)	CS	58 y	Various nutrients and vitamins	180-item FFQ	PBD and AD (mammogram: computer-assisted method)	PBD: Saturated $\text{fat}_{\text{Q4 versus Q1}}$: 19.7 (95% CI: 18.7–20.7%) versus 17.0 (95% CI: 15.6–18.3, P-trend = 0.06)	Age at mammography, y of education, age at menarche, number of pregnancies, age at first full-term pregnancy for parous women, HRT, BMI, total energy
Vachon et al. 2000 [9] (also in Tables 3, 4, and 5)	MBCFCS $n = 1508$ (US, NH-White)	CS	61.4 y	Vitamin A, retinol, carotene, crude and dietary fiber, total carbohydrates, cholesterol, B12, folate, vitamins C, E, total protein, total energy	153-item FFQ	PBD (mammogram: visual estimation)	Premenopausal women: vit E: $Q4$ versus $Q1$: 42% (95% CI: 36–47%) versus 38% (95% CI: 33–46%, P trend = 0.05); total dairy intake: $T3$ versus $T1$ = 38% (95% CI = 32–44%) versus 44% (95% CI: 37–51%) Postmenopausal women: Vit B12 (sup only): $Q4$ versus $Q1$: 34% (95% CI: 31–36%) versus 32% (95% CI: 30–34%, P trend = 0.05)	Energy intake, age, BMI, WHR, PA, age at menarche, age at first birth and number of births (combined), alcohol smoking, family hx of BC, HRT (all and postmenopausal women) and OC use (premenopausal women)

Table 8: Randomized controlled trials in adulthood of diet and breast density.

Author, year	Study population, (n)	Design	Age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Bertone-Johnson et al. 2012 [20] (also in Table 3)	WHI Ca + D trial n = 330 postmenopausal women with low BD (8.4%± 10.2%) (US)	RCT	I and C, respectively: 61.8 y, 62.0 y	Daily supplementation of 1,000 mg of Ca and 400 IU of vitamin D (1 y)	122-item FFQ	PBD: (mammogram: computer-assisted method)	Null	Subgroup analyses: age, race/ethnicity, total vitamin D, HT treatment, Gail risk score, BMI, region of residence, category of mammogram density at baseline.
Boyd et al. 1997 [35]	≥50% PBD n = 817 (Canada)	RCT	I and C, respectively: 46.5 y, 45.9 y	Low-fat, high-CHO diet (2 y)	3-day food records	AD, PBD at baseline and 2 years (mammogram: automated)	Intervention group: BA ↓ by an average 2.4%. The average ↓ in PBD was 6.1%. Control group: BA was ↑ by 0.3% and PBD was ↓ by 2.1%.	Group assignment, age, weight, menopausal status.
Martin et al. 2009 [36]	≥50% PBD (premenopausal at entry, postmenopausal during followup) n = 461 (Canada)	RCT	I and C, respectively: 48.7 y, 48.6 y.	Low-fat, high CHO intervention versus control (2 y)	Food records	TB, DA, NDA, PBD (mammogram: computer-assisted method)	Null	Family hx of BC, OC use, HRT, menopausal status, dietary fat
Knight et al. 1999 [37]	Premenopausal at entry and postmenopausal at followup Total: n = 78 (Canada)	RCT	I and C, respectively: 49.5 y, 49.2 y.	Low-fat, high CHO intervention versus control (2 y)	3 food records	ADT, PBD at baseline and 2 years (mammogram: automated)	Total fat (median change: 57–31 g/d) was associated with an average 5.61 cm^2 ↓ in the ADT. Saturated fat (median change: 21–11 g/d) and was associated with an average 5.54 cm^2 ↓ in the ADT and a 3.93 % ↓ in PBD. Dietary cholesterol (median change: 229–150 mg/d) was associated in an average 3.27 cm^2 ↓ in the ADT and a 3.52 % ↓ in PBD.	Total energy, weight change (included in all models); age, family hx, smoking status, parity, ever breast feeding, OC use, age at menarche, age at first birth, PA

Table 8: Continued.

Author, year	Study population, (n)	Design	Age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Dorgan et al. 2010 [18] (also in Table 2)	DISC Premenopausal women $n = 182$ (US, NH-White)	CS (RCT followup)	25–29 y	Long-term effects of low-fat diet	3–24-hr dietary recalls	PBD and VDT (MRI)	Null	% body fat, age at randomization, age at visit, clinic, BMI-Z score, race, education, smoking status, PA at 14–17 years old and separately during the past year, number of full term pregnancies, hormonal contraceptives

Table 9: Studies of total energy and adult breast density.

Author, year	Study population, (*n*)	Design	Age	Foods/nutrients of interest	Dietary assessment	Outcome	Major significant results	Adjustments
Haars et al. 2010 [12] (also in Table 1 and 2)	DOM-project, The Netherlands $n = 535$ (The Netherlands)	CS	>18 y/53 y	Short-term energy restriction	Described in Table 1	BS, DT, NDT, PBD: (mammogram; visual observation)	Null	Age at examination, parity, menopausal status, BMI
Sala et al. 2000 [29] (also in Tables 4 and 5)	EPIC-Norfolk Cases: P2/DY Controls: N1/P1 ($n = 203$ cases & $n = 203$ controls) (UK)	NCC	Cases & Controls: 59 y	Total energy	7-day food record	Wolfe Patterns: (High Risk: P2 & DY; Low Risk: N1 & P1)	All women: total energy: $OR_{T3\ versus\ T1} = 1.79$, 95% CI: 1.09–2.91) Postmenopausal women: total energy: ($OR_{T3\ versus\ T1} = 2.27$, 1.20–4.26)	Unadjusted
Mishra et al. 2011 [13] (also in Tables 1 and 6)	BBC $n = 700$ (England)	PC	36, 43 y/51 y ("habitual adult" dietary patterns)	Total energy	5-day food records	PBD, ADT, ANDT (mammogram; Cumulus)	All women: energy: PBD: Per SD 0.12 (95% CI: 0.01, 0.23) ADT: Per SD: 0.12 (95% CI: 0.00–0.25).	Mammographic view, age at mammogram, BMI at 53, age at menarche, menopausal status at the time of mammography, HT use, parity, smoking status, PA, social class, other three dietary patterns, energy intake.

PC: prospective cohort; CS: cross-sectional; CC: case control; NCC: nested case-control; RCT: randomized controlled trial; I: intervention; C: control; BS: breast size; PBD: percent breast density; VDT: volume of dense tissue; ADT: area of dense tissue; ANDT: area of non-dense tissue; DT: dense tissue; NDT: non-dense tissue; TDA: total dense area; BMI: body mass index; HRT: hormone replacement therapy; MBCFSC: minnesota breast cancer family study cohort; BBC: british birth cohort; MDAS-WHI: mammogram density ancillary study-women's health initiative; WHI CaD: women's health initiative calcium and vitamin d trial; DOM-Project: diagnostisch onderzoek mammacarcinoom-project; EPIC: european investigation into cancer and nutrition; NBCSP: norwegian breast cancer screening program; CNBSS: canadian national breast screening study; DISC: dietary intervention study in children; BEAN: the breast, estrogens, and nutrition study; MEC: the multiethnic cohort; CHO: carbohydrate; MUFA: monounsaturated fatty acids; PUFA; polyunsaturated fatty acids; n-6-FA: omega 6 fatty acids; BC: Breast Cancer; MVMM: multivitamin/multimineral supplement; NH-White: non-hispanic white; OR: odds ratio; FFQ: food frequency questionnaire; BI-RADS: breast imaging-reporting and data system; MDS: mediterranean diet score; WHR: waist-to-hip ratio; PA: physical activity; ER Status: estrogen receptor status; OC: oral contraceptive; Hx: history; MV: multivitamin.

Table 10: Summary of nutrient relationships with breast density and their proposed mechanisms.

Nutrients that are associated with an ↑ in breast density (absolute density or % breast density)	
Premenopausal women:	*mechanism of action (i.e., IGF/IGFBP/E2/ROS)*
Higher intakes of	
Total fat	↑↓IGF, ↓IGFBP, May ↑Estrogen
SFA (?)	↓IGFBP, ↑IGF, May ↑Estrogen
MUFAs	↓IGFBP, ↑IGF, May ↑Estrogen
n-6 FA	↓IGFBP, ↑IGF, May ↑Estrogen
PUFA	↑IGF, ↓IGFBP, May ↑Estrogen
PUFA : SFA	↑IGF, ↓IGFBP, May ↑Estrogen (?)
Vitamin C (supplemental)	?
Wine	↑Estrogen metabolites, ↑Estrogen responsiveness, ↓SHBG, ↑IGF, ↓IGFBP ↑Oxidative stress
Overall alcohol consumption	↑Estrogen metabolites, ↑Estrogen responsiveness, ↓SHBG, ↑IGF, ↓IGFBP ↑Oxidative stress
MVMM supplements	MAY ↑IGF, ↑IGFBP
Total energy (excess consumed in midlife may affect densities in later life or restriction early in life)	↑Estrogen, ↑IGF availability, ↑DNA replication rate & ↓apoptosis
Lower intakes of	
Carbohydrates	↑IGF (Need to distinguish between whole v. refined, many studies do not do this)
Fiber	↓Oxidative stress (?), may ↑SHBG, ↑IGFBP
Carotene	↓Oxidative stress (?), ↑IGFBP
Calcium	Ameliorates IGF action & enhances IGFBP action (see paper in review), ↑IGF (?)
Protein	Veg Pro = ↑IGFBP Total Pro = ↑IGF
Total fat	↓IGFBP, ↑↓IGF, May ↑Estrogen
Saturated fat	↓IGFBP, ↑IGF, May ↑Estrogen
Vitamin B12 (supplemental)	?
White wine	↑Estrogen metabolites, ↑Estrogen responsiveness, ↓SHBG, ↑IGF, ↓IGFBP ↑Oxidative stress
Meat	↑Oxidative stress
Carbohydrates (?)	↑IGF (Need to distinguish between whole versus refined, many studies do not do this)
Total energy	↑Estrogen, ↑IGF availability, ↑DNA replication rate & ↓apoptosis

Overall, it appears that dietary patterns high in antioxidant-containing foods are inversely associated with BD in smokers, who may be experiencing a higher level of oxidative stress than nonsmokers. Other research has shown a positive association between BD and MDA, which is a marker for lipid peroxidation and oxidative stress [63].

5.8. Randomized Controlled Trials. The epidemiological evidence described above suggests that diet is associated with BD measures and that BD has the potential to be modified. As a result, researchers have conducted clinical trials to examine the association between specific dietary factors with BD outcomes (Table 8). Boyd et al. [35] first examined a low-fat, high-carbohydrate 2-year dietary intervention in 817 women with PDAs ≥50%. Those who were randomized into the intervention group received intensive instruction to consume 15% of calories from fat, 20% from protein, and 65% from carbohydrate while the control group received general dietary advice and instruction to maintain their current intake of fat. After two years, the average reduction in PDA was 6.1% and 2.1% in the intervention and control groups, respectively, ($P = 0.01$) [35]. The effect of the intervention remained significant after controlling for age, weight change, and menopausal status [35]. After stratification by menopausal status, significant changes in PDA were only observed in women who were either premenopausal throughout the study or who were premenopausal at baseline but transitioned into menopause by the end of the study, with the greatest change in density occurring in the latter group. Consumption of fat and cholesterol was significantly positively associated with change in ADT in this subgroup, whereas protein and cholesterol were significantly positively associated with change in PDA [37].

Table 11: Summary of nutrient relationships with breast density and their proposed mechanisms.

Nutrients that are associated with a ↓ in breast density (absolute density or % breast density)	
Premenopausal women: ↑intakes of:	*Mechanism of action (*i.e., *IGF/IGFBP/E2/ROS)*
Calcium	May ameliorate IGF action and enhances IGFBP action, ↑IGF (?)
Vitamin D	May ameliorate IGF action and enhances IGFBP action, breast tissue may be able to locally synthesis 25(OH)D → 1,25(OH)2D
SFA (?)	(?)
Total dairy	↑IGF, ↑IGFBP, vitamin D and calcium may negate these effects (VD and Ca have stronger effects when IGF/IGFBP are high)
Cheese consumption	↑IGF, ↑IGFBP, vitamin D and calcium may negate these effects (VD and Ca have stronger effects when IGF/IGFBP are high)
Carbohydrate (?)	↑IGF (need to distinguish between whole v. refined, many studies do not do this)
Red Wine	↓Oxidative stress (?)
MUFA	↓Oxidative stress (?)
Carotenoids	↓Oxidative stress (?), ↑IGFBP
Fiber	↓Oxidative stress (?), may ↑SHBG, ↑IGFBP

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; n-6 FA: omega-6 fatty acids; PUFA: polyunsaturated fatty acids; MVMM: multivitamin/multimineral supplements; IGF: insulin growth factor; IGFBP: insulin growth factor binding proteins; SHBG: sex hormone binding globulin; VD: vitamin d; Ca: calcium.

Martin et al. [36] completed a similar larger clinical trial with longer followup that included 461 women who were premenopausal at entry and postmenopausal after two years. Several BD measures were assessed (change in breast area, ANDT, ADT, PDA) premenopausally at baseline and later in the postmenopausal phase. Like the previous trial, this trial focused on women with high PDA ≥50% and the intervention group received the same dietary manipulation [35]. This study did not replicate the previous findings from Boyd et al. [35]. After two years, no change was observed in the intervention group and a slightly lower PDA was observed in the control group; the treatment group difference was not significant [36]. The authors suggest that these unexpected results were likely due to an increase in the ANDT that occurred with weight gain in the sample.

As previously described in the vitamin D and calcium section, a one-year calcium and vitamin D supplementation trial was conducted through the WHI to examine the effects on mammographic PDA in postmenopausal women [20]. Despite the associations observed in observational studies, no change in mammographic PDA was observed with supplementation. The authors suggest that very low PDAs at baseline could have led to a "floor effect" where further supplementation of vitamin D and calcium had no additional benefit. Finally, studies that have examined soy and isoflavone consumption and mammographic PDA have also yielded mixed results. Hooper et al. [67] conducted a meta-analysis of eight RCTs including 1287 total women that compared the administration of supplemental isoflavones versus a placebo for at least six months. Results from the meta-analysis showed a modest nonsignificant increase in PDA (mean difference: 1.83%; 95% CI 0.25–3.40) in premenopausal, but not postmenopausal, women as isoflavone intake increased; however, there was limited evidence of a clear dose-response relationship over the range of isoflavone intake of 40–120 mg/d.

6. Conclusions

Data from observational studies suggest that the strongest associations between diet and BD measures are among vitamin D, calcium, dietary fat, and alcohol and are found in adult premenopausal women. However, the few clinical trials that have evaluated these associations have failed to demonstrate a significant change in breast density with various dietary interventions. This could be because the foods/nutrients evaluated truly do not influence breast density or could be due to aspects of the study design including duration of the intervention, dose, sample size, or inclusion of predominantly older women in whom breast tissue may be less susceptible to dietary influences.

6.1. Limitations. This paper has critically examined 28 studies and has identified strengths and weaknesses as well as highlighting several potential directions for new research to advance the field. Many of these studies are cross-sectional in nature and often focus just on PDA. In addition to this, the majority of women who receive mammograms overall and in these studies are >40 y; an association between dietary factors and BD measures could be undetected if the critical dietary exposure occurred much earlier in life (and was not measured) before breast tissue is fully differentiated and potentially more vulnerable to exogenous influences.

The majority of studies included in the paper assessed BD using 2D mammography. Even though estimates of BD obtained by mammography and 3D modalities such as MRI are highly correlated in the general population and in women with less dense breasts [38], correlations are substantially lower in women with more dense breasts in whom density can be more accurately measured using 3D modalities.

Many studies examined the association of diet with PDA but not the ADT. Fewer associations are observed with the ADT compared to PDA; however, results should be reported

when available in order to be more comprehensive, improve comparisons across studies, and enhance interpretability in relation to potential physiological mechanisms. Very few studies controlled for the phase of the menstrual cycle at the time of mammography. Because data on variation of breast density over the menstrual cycle are conflicting [68–71], it seems prudent to consider menstrual cycle day in analyses of breast density when possible. Finally, several methods were used to evaluate BD. Even though many studies used a semiautomated method to reduce variability and error, standardization of assessment would facilitate comparisons across studies.

6.2. Future Directions. To date, most studies of the association of diet with BD have been cross-sectional. Longitudinal studies that measure diet and BD over the life course are needed. Studies that evaluate the influence of diet during adolescence, when most breast development occurs, on adult BD could be particularly enlightening. Support for an association of diet with BD from observational studies is stronger for premenopausal women. However, a limited number of short-term clinical trials do not show conclusive evidence that dietary factors influence BD. Clinical trials in younger women could be informative and may provide more definitive results. Lastly, more research on dietary patterns as they relate to BD are needed.

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VITA

Jessica A. Lindgren

EDUCATION

2013 Ph.D., Nutritional Sciences, The Pennsylvania State University
2010 B.S, Nutritional Sciences, Applied Option, The Pennsylvania State University

SELECTED PUBLICATIONS

1) **Lindgren J**, Dorgan J, Savage-Williams J, Coffman D, Hartman T. "Diet across the Lifespan and the Association with Breast Density in Adulthood," *Int J Breast Cancer*, Vol. 2013. doi:10.1155/2013/808317.

2) **Lindgren JA**, Vernarelli JA, Savage-Williams, J, Hartman TJ. 2013. "Is Usual Dietary Pattern Related to the Risk of Developing Breast Cancer?" *Curr Nutr Rep* 2013. doi: 10.1007/s13668-013-0039-1.

HONORS AND AWARDS

2012 Travel Award, AICR Annual Research Conference
2011 Travel Award, NIH Office of Dietary Supplements Summer Practicum