The impact of dairy food, vitamin D and calcium consumption on women's health through the life-course

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ABSTRACT OF THE DISSERTATION

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Endometrial and ovarian cancers are hormone related cancers with the most significant risk factors being those related to high estrogen exposure, such as late age at menopause and hormone therapy use. Diet and obesity, two modifiable risk factors, have been implicated in the risk for both cancers. Early life exposures including diet, as well as early onset of puberty, may play a role in the lifetime risk of endometrial and ovarian cancers.

Dairy foods, vitamin D and calcium have been implicated in endometrial and ovarian cancer risk, playing either a direct role or an indirect role through their association with body size. A major source of dietary vitamin D and calcium, dairy foods are also sources of animal derived hormones and growth factors which may have physiologic activity in humans. Obesity is a known risk factor for early sexual development in girls, possibly due to estrogen synthesis in adipose tissue. In this study, data from two population based case-control studies and baseline data from a prospective cohort study were used to examine the association between dairy foods, vitamin D and calcium with risk of endometrial cancer, ovarian cancer, and early breast development

and body size in pre-pubertal girls, respectively. Endometrial cancer risk was significantly inversely associated with dietary calcium intake in our study. Among post-menopausal women, high versus low intake of total calcium was associated with significantly lower risk of endometrial cancer. Less frequent consumption of low-fat cheese was associated with significantly greater risk of endometrial cancer compared to frequent consumption of low-fat type. Effect modification was found by hormone therapy use for total calcium and risk of endometrial cancer, with 'ever' users having greater risk compared to 'never' users. Milk intake was significantly associated with ovarian cancer risk; significant effect modification by hormone therapy use was found for the association between ovarian cancer risk and total vitamin D intake, with 'ever' users having lower risk estimates versus 'never' users. Higher dietary calcium intake was associated with lower mean BMI, fat mass and percent body fat among young girls, while higher total milk and dairy intake were associated with higher adiposity measures. Girls in the highest tertile of total milk were significantly more likely to be overweight and above, as were those in the middle tertile of dietary calcium. In conclusion, our results suggest a possible protective role for calcium with risk of endometrial cancer and reduced adiposity in young girls, and an increased risk for ovarian cancer risk with milk intake. Future research should build on these findings by further elucidating calcium's role in endometrial and ovarian cancer risk and body size in young girls through larger studies focused on high risk populations (i.e., obese and post-menopausal women, young girls) as well as associations with epithelial ovarian cancer tumor sub-types.

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Introduction

Milk and dairy products are among the most important foods in the Western diet, and account for the largest dietary sources of vitamin D and calcium in the US population [1, 2]. While conversion of pro-vitamin D in the skin by UV-B radiation is considered the major source of vitamin D [3, 4], factors such as use of sunscreen, seasonality, latitude, time of day, clothing and skin pigmentation can reduce vitamin D production [5]. Moreover, the World Health Organization and the American Cancer Society advocate avoiding the sun between 10:00 AM – 4:00 PM, and covering the skin with clothing and sunscreen [6, 7] thereby making regular dietary intake of vitamin D a requirement.

Sources of Vitamin D and Calcium and Recommended Intake

Milk is one of the only foods to contain nearly all of the essential nutrients needed for human nutrition, including vitamin D and calcium and as such, suboptimal dairy consumption can be associated with insufficient intake of these nutrients [8, 9]. Several non-dairy foods are rich sources of calcium such as broccoli, spinach and calcium fortified foods, however the presence of constituents such as oxalic acid and fiber found in some plant foods can reduce the bioavailability of calcium [10]. In 2011, an Institute of Medicine Expert Committee increased the dietary reference intake (DRI: average daily requirements) for vitamin D to 600 IU/day for ages 1-70 years [11] Calcium requirements, given as a Recommended Dietary Allowance (RDA), are 1300 mg/day for individuals 9-18 years of age, 1,000 mg/day for women ages 19-50, and 1200 mg/day for adults 50-71+ years [11]. However dietary intakes remain low, particularly among females; data from NHANES 2011-2012 indicate that for vitamin D, girls 6-11 years of

age consumed 216 IU/day, girls 12-19 years consumed 172 IU/day, and women aged 20 years and over consumed 156 IU/day. Calcium intakes were also below recommended amounts, at 1,004 mg/day for girls 6-11 years, 937 mg/day for girls 12-19 years and 867 mg/day for women 20 years and older [12].

Dairy Foods, Vitamin D and Calcium in Carcinogenesis

Milk and milk products are regarded as functional foods, the use of which may have an important direct and indirect effect on health outcomes, and whose consumption has been linked with reduced risk of several cancers [13]. While the classic biological role for vitamin D and calcium is maintenance of bone mineral homeostasis, there is mounting evidence suggesting a protective role in hormone related cancers, including ovarian and endometrial [14]. Several mechanisms have been proposed for a protective effect of vitamin D, including cell growth inhibition and enhanced apoptotic properties of $1,25(OH)_2$ [15], regulation of genes implicated in cancerous transformation and regulation of the cell cycle [16], and suppression of gene expression for aromatase, the enzyme that catalyzes estrogen synthesis from androgenic precursors, and down-regulation the estrogen receptor-alpha (ERα), the nuclear receptor that mediates estrogen actions [17]. For calcium, although a specific mechanism has not been elucidated, possible actions include regulation of cellular proliferation, differentiation and function, and down-regulation of parathyroid hormone (PTH) production which may reduce mitosis and increase apoptosis [18-20].

Dairy foods, vitamin D and calcium may also influence cancer risk indirectly by helping to maintain a healthy body weight. As a site for aromatization of androgens, adipose tissue can be a source of endogenous estrogen [21, 22]. Animal studies have

shown that calcium attenuates adipocyte lipid accretion and weight gain, and increases lipolysis via stimulation of lipogenic gene expression resulting in suppressed fat storage and enhanced fat breakdown [23]. Several epidemiologic studies in humans have corroborated this effect showing an inverse association between dairy foods, vitamin D and calcium intakes with body mass index (BMI), body weight and fat mass in children and adults [24-31].

Epidemiology and Risk Factors for Endometrial and Ovarian Cancers

Endometrial cancer is the most common cancer of the female reproductive organs and it is estimated that 61,380 new cases will be diagnosed in 2017, 10,920 (18%) of whom will die from it [32]. As a hormone related cancer, one of the most significant non-modifiable risk factors is late onset of menopause, due to prolonged exposure to high estrogen levels [33]. Modifiable risk factors that are also related to estrogen exposure include hormone therapy use, bearing children, and oral contraceptive use [33]. Obesity, specifically abdominal fatness, is a significant modifiable risk factor for endometrial cancer with 70-90% of estrogen-dependent (Type I) cases being obese; evidence for obesity has been rated 'convincing' as a cause of endometrial cancer by The World Cancer Research Fund and the American Institute of Cancer Research, which has also suggested that food and nutritional factors probably play important roles in the onset of endometrial cancer [33]. Epidemiologic studies that evaluated dairy food consumption in relation to endometrial cancer risk have been largely case-control studies, which have produced conflicting results [34-39] Studies that examined the association between vitamin D and endometrial cancer risk have also been conflicting, showing an increased risk with increased intake of vitamin D [40] and a decreased risk [37]. For calcium the

evidence is variable, showing a null association [41], and a decreased risk with higher intakes [38, 39].

Ovarian cancer, the most lethal of the female reproductive cancers, is also hormone related and accounts for 5% of cancer deaths among women in the US [42]. Like endometrial cancer, a significant non-modifiable risk factor is age at menopause, and also includes early menarche, familial and genetic disposition as an additional risk factors [43]. While few modifiable risk factors are known for ovarian cancer, obesity and hormone therapy may be associated with increased risk while tubal ligation, increasing parity, oral contraceptive use, and hysterectomy may decrease risk [43]. The evidence for dietary factors and ovarian cancer risk has been limited [43]. The role of dairy foods in ovarian cancer risk is also inconclusive, with case-control studies generally showing no association whereas cohort studies have shown a more consistent positive association [44]. Vitamin D was not associated with ovarian cancer risk in a pooled analysis [18], and was inversely associated with risk in a large, population based case-control study [45]. The evidence for calcium's role is inconsistent, showing a null association [46], a positive association [47], and a negative association [45].

Diet and Early Sexual Development in Young Girls

Evidence is growing for the role of early life factors in the etiology of hormone related cancers, including breast and ovarian, suggesting that biological and environmental exposures including diet at critical periods of susceptibility (i.e., peri-puberty) can influence lifetime risk [48-51]. Accelerated growth velocity, final height and early sexual development have been associated with increased lifetime risk of breast and ovarian cancers, and there is evidence that early sexual maturity among girls is on the

rise in the US [49]. Body weight may influence onset of puberty. Girls with greater abdominal fatness are more likely to be obese, and to have higher levels of insulin which can stimulate growth factors and hormones that promote breast tissue growth [52]. Nutrients and food constituents can affect hormonal and metabolic processes as well as energy balance and obesity, thereby influencing growth, body weight, and timing of sexual development in this susceptible population [52, 53].

In young girls, the intake of dairy food and related nutrients may affect key developmental factors. Milk contains a number of components capable of affecting growth and body size, including essential nutrients as well as other growth-enhancing molecules such as fat and insulin like growth factor I (IGF-1), which may alter developmental parameters at critical transition periods [13, 20, 54]. Milk and dairy products are the major source of animal derived estrogens in the human diet, and account for 60-80% of estrogens consumed [55]. Estrone sulfate, the main estrogen in milk, has high bioavailability and once inside the body can be converted to estrone and estradiol; nearly 47% of estrone found in a standard diet is estimated to come from dairy sources [55]. There is evidence that young girls ages 8-11 who consumed milk daily had increased insulin-like growth factor 1(IGF-1) and growth hormone levels [56]. However, the potential anti-estrogenic effects of vitamin D [17], and the anti-obesogenic effects of milk and calcium intake in children [57] make the relationship between dairy food consumption and sexual development complex, in this population.

The role of dairy foods, vitamin D and calcium in sexual development and body size in peri-pubertal girls has received little attention. Age at menarche and dairy food intake has been studied, with mixed results [58, 59], and one study showed a positive

association for dairy foods with height [60]. Obesity may be linked to early sexual development and there is evidence of an inverse association between calcium intake and body fatness in young girls and children [31, 61, 62]. However, other studies have reported no association between calcium and body fatness [63-65]. To date, only one study was identified that examined the association between diary intake and breast development, finding an inverse relationship with yogurt and a positive relationship with sweetened, artificially flavored milk beverages with achieving Tanner Stage 4 [59].

The Present Dissertation

With the recently updated dietary guidelines continuing to reinforce daily consumption of dairy products [66], it is important to understand the potential role that dairy foods and related nutrients play in endometrial and ovarian cancers, as well as early sexual development in young girls. Clarification of the public health impact of dairy food consumption, given the potentially conflicting effects of growth enhancing components present in milk and dairy foods, with the beneficial anti-proliferative, anti-obesity and anti-estrogenic effects of calcium and vitamin D, is needed for optimal dietary intervention.

In this dissertation, the association between dairy foods, vitamin D and calcium intake in relation to the risk of endometrial and ovarian cancers and risk of early sexual development in young girls was examined. Data from two population based case-control studies and the baseline assessment of a cohort study were used in the current analysis: The Estrogen, Diet, Genetics, and Endometrial Cancer (EDGE) Study, The New Jersey Ovarian Cancer and The Jersey Girl Study, respectively.

To our knowledge, this is the first study to assess the relationship between dairy food, vitamin D and calcium with risk factors for early sexual development among young girls in the US. Findings from these analyses will contribute to the growing body of evidence regarding the role of these dietary factors in the risk of ovarian and endometrial cancers.

Chapter 1: Dairy Foods, Related Nutrients and Endometrial Cancer Risk, The EDGE Study

Introduction

Despite advances in the early detection and treatment of endometrial cancer, death rates have been increasing [67]. In the US, an estimated 61,380 cases of endometrial cancer will be diagnosed in 2017, with an estimated 10,920 deaths [32] Many modifiable and non-modifiable risk factors for endometrial cancer pertain to a woman's hormonal status and include hormone therapy use, not bearing children, and late natural menopause which increase women's exposure to unopposed estrogens [33]. Obesity/body fatness is a convincing cause of endometrial cancer according to the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR), possibly due to increased levels of hormones that may promote growth of cancer cells or increased aromatization of androstenedione to estrone in adipose tissue [33]. Between 2013-2014, the prevalence of obesity among women in the US was 40%; 9.9% of obese women had a BMI $\geq$40 (Class 3) [68]. With rates of obesity expected to increase [69], endometrial cancer will become a more significant public health problem in the future making the identification of effective preventive measures critical. Diet, either through direct action of food components or as a determinant of obesity, is a modifiable factor that may have a profound impact on the risk of this disease.

Dairy foods, vitamin D and calcium have been implicated in the risk of endometrial cancer, but the evidence is inconsistent [36, 41]. A systematic literature review conducted by the WCRF/AICR has rated the evidence on milk and dairy foods and endometrial cancer as limited and inconclusive [70]. Given the scant and conflicting evidence on the relationship between these dietary factors and endometrial cancer risk

and the increasing public health issue this disease represents, this study examined the association between dairy foods, vitamin D and calcium intake with endometrial cancer risk using data from a population based case-control study conducted in NJ, The Estrogen, Diet, Genetics and Endometrial Cancer (EDGE) Study.

Subjects and Methods

Study Population

The EDGE Study is a population-based case-control study described in detail elsewhere [71]. In brief, participants were women living in one of six NJ counties (Bergen, Essex, Hudson, Middlesex, Morris, Union). To be eligible, women had to be aged 21 years or older and speak English or Spanish. Informed consent was obtained from all cases and controls following passive consent from their physicians. Cases were women with newly diagnosed and histologically confirmed diagnoses of epithelial endometrial cancer, who were identified using rapid case ascertainment by the NJ State Cancer Registry, between July 1, 2001 and June 30, 2005; data were supplemented with review of state cancer registry data to identify cases diagnosed out of the area. Out of 1,559 eligible cases, 1,104 could be contacted within one year of diagnosis; of those, 469 (42%) completed the study interview.

Controls met the same eligibility criteria of the cases, but could not have had a history of endometrial cancer or hysterectomy. Random digit dialing (RDD) was used to locate women <65 years of age. One hundred seventy-five (175) of the 355 eligible women (49%) completed the interview. Women ≥65 years were initially identified by random selection using lists purchased from the Centers for Medicare and Medicaid

Services (CMS). Three hundred sixteen (316) were identified, of which 68 (22%) completed the interview. Area sampling for controls identified an additional 524 eligible women, of whom 224 (43%) completed the interview. A total of 467 controls completed the study interview.

Of the 469 cases and 467 controls that completed the study interview, 424 (90%) cases and 398 controls (85%) provided dietary data, which were used for this analysis. Fourteen (14) cases and 10 controls were excluded due to implausibly low or high caloric intake (<500 or >3,500 kcal/day) [45], and an additional 14 cases and 7 controls were excluded for missing values on other key covariates including smoking status, race, menopause, time spent outdoors, age at menarche, and hormone therapy use resulting in a final sample size of 396 cases and 381 controls for analysis.

Data Collection

Trained interviewers conducted in-depth interviews via telephone with participants to collect information on demographic characteristics and major risk factors including personal and family history of cancer, reproductive history, hormone use, medical history and lifestyle factors.

Dietary data were collected using a self-administered Block 98.2 Food Frequency Questionnaire (FFQ), a validated instrument that is used to collect usual intake of approximately 110 food items and dietary supplements [72]. Participants were asked to estimate their usual consumption of each food item over 6 months prior to diagnosis for cases, and interview date for controls. Pictures depicting reference portions were provided to facilitate recall and quantification. Nutrient intake levels were derived from

the FFQ through the Block Dietary Data Systems which is based on the US Department of Agriculture National Nutrient Database for Standard Reference [73].

Dietary Assessment

For the present analysis, dairy food and nutrient intakes were assessed from major dairy sources and included milk as a beverage and used on cereal; yogurt, including frozen yogurt; cheese, including sliced cheese or cheese spread; and ice cream, including ice milk and ice cream bars. Soy milk and rice milk were included in the analysis since they are often fortified with vitamin D and calcium. Frequency of consumption was assessed as 'never', 'a few times per year', 'once per month', '2-3 times per month', 'once per week', '2 times per week', '3-4 times per week, '5-6 times per week', and 'everyday'. Portion size was assessed as glasses of milk, cups of yogurt, cups of ice cream, and slices of cheese. One glass of milk was assumed to represent 1 cup, or 8 fluid ounces, based on reference amounts customarily consumed [74]. Milk subtype usually consumed was assessed as 'whole', 'reduced fat 2%', low-fat 1%', 'non-fat', or 'I don't drink milk or soy milk'. Cheese and ice cream subtypes consumed were assessed as 'usually low-fat', 'sometimes low-fat', or 'hardly ever low-fat'. Average daily vitamin D and calcium supplemental intake was assessed by querying frequency of multiple and single vitamin/nutrient supplement use (i.e., 'don't take', 'a few days per month', '1-3 days/week', '4-6 days/week', 'every day') among participants who indicated regular use of vitamins/minerals.

For analysis, weekly consumption of dairy foods was derived using raw dietary data by converting frequency into times per week, and multiplying by portion size. Next, weekly intake totals of all dairy foods were converted to 'milk equivalents', which allows

for consistency when comparing the nutrient content across different dairy foods, based on the following reference categories (Table A) [75]:

Table A: One Cup Milk Equivalents, MyPyramid Food Guidance System

Milk Products	**Amount that counts as 1 cup equivalent of milk**	**Common portions and respective cup equivalents of milk**
Milk	1 cup 1 half-pint container ½ cup evaporated milk	1 cup
Yogurt	1 regular container (8 fl ounces) 1 cup	1 small container (6 ounces) = ¾ cup 1 snack size container (4 ounces) = ½ cup
Cheese	1 ½ ounces hard cheese (cheddar, mozzarella, Swiss, Parmesan) 1/3 cup shredded cheese 2 ounces American cheese ½ cup ricotta cheese 2 cups cottage cheese	1 slice of hard cheese is equivalent to ½ cup milk 1 slice processed cheese is equivalent to 1/3 cup milk ½ cup cottage cheese is equivalent to ¼ cup milk
Milk-based desserts	1 cup pudding made with milk 1 cup frozen yogurt 1 ½ cups ice cream	1 scoop ice cream is equivalent to 1/3 cup milk

Because recommended dietary allowances for nutrients are based on daily intake, daily intakes of vitamin D (IU) and calcium (mg) from dietary, supplemental and total (daily + supplemental) sources were used for analysis.

Data Analysis

Quartiles of intake for total dairy (servings/week), total milk (milk equivalents/week), total cheese (milk equivalents/week), yogurt (milk equivalents/week), total ice cream (milk equivalents/week), total, dietary and supplemental vitamin D (IU/day), and total, dietary and supplemental calcium (mg/day) were created based on the

distribution among controls, using the lowest quartile (Q1) as the reference [76]. User groups were created for supplemental vitamin D and supplemental calcium based on the median average daily intake among user controls; individuals with intakes below the median were categorized as 'low', and those above the median as 'high', with 'non-users' as the reference category. Analysis of milk subtype was conducted among users, with non-fat type as the reference and excluded participants whose subtype was unknown. Cheese and ice cream subtype analyses were conducted among users of the food items, with 'usually low fat' as the reference category.

Descriptive statistics (frequencies and proportions for categorical variables, means and standard deviations for continuous variables) for selected demographic and risk factors were calculated to describe the study population. Distributions were compared between cases and controls using chi-square tests for categorical variables and student's t-test for continuous variables. Analysis of covariance (ANCOVA) was used to compare age adjusted means of total dairy, dairy foods and nutrients between cases and controls. Multivariable logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the main effects of the primary exposures of interest, total dairy and dairy foods, vitamin D and calcium on the risk of endometrial cancer. For each exposure, two models were developed: Model 1 adjusted for reference age (continuous), defined as 'age at diagnosis' for cases and 'age at baseline interview' for controls, and total energy intake (continuous). Model 2 further adjusted for a priori confounders or risk factors for endometrial cancer including education (high school or less, college, graduate degree); race (white, black, other); oral contraceptive use (ever, never); hormone therapy (HT) use (ever, never); age at menarche (<12, 12-13,>13);

menopause (pre/postmenopausal); parity (0, 1-2, >3); BMI (<25 kg/m^2, 25-<30 kg/m^2, ≥30 kg/m^2; based on self-reported weight and height 1 year prior), and ever diagnosed with diabetes (yes/no). Smoking (never, past, current), time spent outdoors (hours/week; continuous), coffee consumption (yes/no), and consumption of total added sugars (continuous) were evaluated as potential confounders by assessing the magnitude of change in risk estimates in the fully adjusted models using backward elimination. Because the magnitude of change in effect estimates was less than 10% none of the four covariates was retained in the final main effects models. Tests of linear trend were derived by assigning the median intake value to each quantile and including this as a continuous variable in the fully adjusted logistic regression models.

A sensitivity analysis for main effects was conducted for total dairy, total milk, total vitamin D, dietary vitamin D, supplemental vitamin D, total calcium, dietary calcium and supplemental calcium as continuous variables to determine if their relationship with the outcome changed as a result of creating categorical variables from the continuous measures. Models adjusted for total fat (continuous) instead of total energy were also tested. Additional analyses were done to test for effect modification by BMI, hormone therapy use and menopause on the association between endometrial cancer risk with total vitamin D and total calcium intakes, using the Likelihood Ratio Test. All analyses were performed using SAS software, version 9.4 (Cary, NC).

Results

As presented in Table 1, cases were significantly younger than controls with a mean age 62.33 vs. 64.86, respectively ($p<0.001$) and a lower proportion were White

(p=0.02). The analysis found the distributions of endometrial cancer risk factors to be in the expected directions. Compared to controls, fewer cases had a graduate degree (p=0.04), and fewer ever used hormone therapy (p=0.03). More cases than controls reached menarche before age 12 (p=0.01), had fewer children (p=0.001), were obese (p<.0001), had greater waist-to-hip ratio (p=0.002), and were diabetic (p=0.007). Oral contraceptive use, menopause status, smoking status and time spent outdoors did not differ significantly between cases and controls.

Age adjusted means for dairy foods, vitamin D and calcium for cases and controls are presented in Table 2. Mean intake of total calcium was significantly lower among cases compared to controls (p=0.02). A suggestion of lower intake among cases for total dairy (p=0.08), yogurt (p=0.08) and supplemental vitamin D (IU/day) (p=0.06) was observed.

Fully adjusted multivariable analysis showed suggestion of decreased risk for total dairy intake (Table 3). Neither total milk nor milk subtype was significantly associated with the risk of endometrial cancer. A suggestion of increased risk with yogurt intake was observed, although 95% confidence intervals included the null. Odds ratios for total cheese consumption suggested an increased risk for each quartile of intake compared to the lowest quartile, although there was no evidence of a dose-response association. Cheese subtype was significantly associated with endometrial cancer risk, among users; 'sometimes' and 'hardly ever low-fat' users were associated with a statistically significant increased risk compared to 'usually low fat' users, ('sometimes low-fat' OR: 2.29; 95% CI: 1.36, 3.84; 'hardly ever low-fat' OR: 1.70; 95% CI: 1.04, 2.74). Higher intakes of total ice cream suggested increased risk compared to the lowest

quartile, and ORs for ice cream subtype among users suggested increased risk for less frequent low-fat consumption.

Multivariable analyses showed no association between total, dietary, and supplemental vitamin D with endometrial cancer risk (Table 4). For calcium, age and energy adjusted analysis found a statistically significant reduced risk for total calcium at the highest (Q4) versus the lowest (Q1) intake (OR: 0.58; 95% CI: 0.37, 0.90), however this association was attenuated in the fully adjusted multivariable model. Dietary calcium was associated with a statistically significant reduced risk at the highest level (Q4) compared to the lowest (Q1) in age and energy adjusted models (OR: 0.52; 95% CI: 0.32, 0.84), which retained its statistical significance in the fully adjusted model (OR: 0.63; 95% CI: 0.41, 0.97; p trend =0.05). Supplemental calcium intake did not contribute to the risk of endometrial cancer in our analyses.

Similar magnitudes of association were observed between continuously scaled total milk, total dairy, total vitamin D, dietary vitamin D, supplemental vitamin D, total calcium, dietary calcium and supplemental calcium in sensitivity testing (data not shown). Results did not change in models adjusting for total fat, excluding total energy (data not shown). We did not find statistically significant interaction by BMI or HT use on the association between total vitamin D and endometrial cancer risk (Table 5). However, significant interaction was found by hormone therapy use with total calcium and endometrial cancer risk, with never users having ORs <1.0 and ever users having ORs >1.0, although none of the ORs were statistically significant (Table 5). The small number of pre-menopausal women in our study prevented the assessment of statistical interaction by menopause status with total vitamin D and total calcium intake. In

stratified analysis among post-menopausal women, however, a statistically significant inverse association was observed for the highest versus lowest quartile of total calcium (OR: 0.54; 95% CI: 0.29, 0.98).

Discussion

The present analysis from the EDGE population based case-control study found evidence to support a reduced risk of endometrial cancer with calcium intake. A significant inverse relationship was found between endometrial cancer risk at the highest versus the lowest quartile of dietary calcium intake, a finding that contradicts the null association found with dietary calcium in another case-control study [41]. Although total calcium intake was not clearly associated with endometrial cancer risk in our study, a finding consistent with another case-control study [40] but contrary to others [37, 39, 41], we did find stastically significant interaction by HT use with lower risk for never users and greater risk for ever users, although none of the risk estimates were statistically significant. No other studies were identified that examined effect modification by hormone therapy use on the association between endometrial cancer risk and calcium intake, to support or contradict our finding.

In this analysis, post-menopausal women at the highest quartile of total calcium intake were at significantly reduced risk for endometrial cancer. While we did not find an association with supplemental calcium intake, which is consistent with findings from a large, randomized trial of calcium + vitamin D supplementation [77], Terry and colleagues observed a statistically significant inverse association among post-menopausal supplemental calcium users, but only among those with low dairy consumption [38].

Our study did not find a significant association between dairy food intake and endometrial cancer risk. Dairy food consumption has been variably associated with endometrial cancer risk with studies showing null associations [35, 36, 38, 39], increased risk [78, 79] and an inverse association [37, 80]. These inconsistent findings could be due in part to different methodologies of quantifying dairy intake, which has been reported as weekly servings [36, 38], monthly intake [35], and cumulative averge intake [79] making direct comparisons to our results challenging. Our study is unique in that we quantified all dairy food consumption in milk equivalents to facilitate comparisons across various dairy foods characterized by large differences in nutrient profiles. No other studies reported an analytic approach to allow for these differences between dairy foods.

Few data exist on the association between specific dairy foods and endometrial cancer risk. In our study, there was suggestion of increased risk for intakes of total yogurt, total cheese and total ice cream, with stronger risk for less frequent consumption of low fat varieties. We found that among cheese users, less frequent use of low-fat cheese was associated with a statistically significant increased endometrial cancer risk compared to frequent low-fat cheese use. Although our study did not specifically quantify cheese intake by fat subtype, hard cheeses are generally higher in fat content and consumption of hard cheese has been associated with a significantly greater risk of endometrial cancer in a large cohort study [79]. Our null association for milk and endometrial cancer risk is consistent with evidence from another case-control study [81], however it contradicts findings from another which found a significantly lower intake of milk among cases compared with controls [34]. Milk type was not associated with endometrial cancer risk in our study.

We examined total (diet + supplements), dietary, and supplemental vitamin D intake. Multivariable analysis did not support an association between total vitamin D intake and endometrial cancer risk, a result that is consistent with a population based case-control study conducted in Canada [41] and evidence reported by the World Cancer Research Fund and the American Institute for Cancer Research [33]. Our findings contradict those from a case-control study conducted in Mexico that found a statistically significant reduced risk for endometrial cancer for women in the highest vs. lowest quartile of total vitamin D [37], and another population based, case-control study that reported a stastically significant increased risk among those in the highest quartile of total vitamin D [40].

For supplemental vitamin D there was little indication of an association in our study. There are few studies examining vitamin D supplementation and endometrial cancer risk to draw upon for comparing our results. One case-control study conducted in the UK found no association between vitamin D supplement use (+/- calcium) and endometrial cancer risk in postmenopausal women [82]. Similarly, results from the Women's Health Initiative randomized trial of calcium plus vitamin D supplementation in post-menopausal women found no significant difference in endometrial cancer risk between the group receiving supplementation and placebo [77].

In summary, greater consumption of dairy products and total vitamin D levels were not associated with endometrial cancer in our population based, case-control study of mostly postmenopausal women. However, our results support existing evidence for an inverse association between endometrial cancer risk and dietary calcium intake, and total calcium intake among post-menopausal women. Our findings of reduced risk among

post-menopausal women, together with the significant interaction by hormone therapy with ORs <1.0 for 'never' users provides evidence that the effects of calcium on endometrial cancer risk may be modified by estrogen exposure. From a biological perspective the chemopreventive role of calcium is not fully understood, although it has been shown to regulate a wide variety of cellular functions involved in carcinogenesis [14]. There is evidence that calcium helps reduce body weight and loss of fat mass, and its potentially protective effect on endometrial cancer may operate indirectly through this mechanism since endometrial cancer is closely related to obesity [26, 27, 29, 83]. In our study, however, we did not find statistically significant effect modification by BMI.

Our study is characterized by a number of strengths including its population based design and histological confirmation of disease among cases, reducing the potential for misclassification error. The assessment of a wide range of potentially confounding lifestyle factors allowed for control of known confounders, in particular those related to estrogen exposure which is a well known risk factor for endometrial cancer [83].

We recognize several limitations to our study which include the potential for dietary recall bias inherent to case-control studies, as cases may recall and report their dietary intake differently than controls, although we attempted to reduce this bias by asking cases to report usual intake 6 months prior to diagnosis. The highly correlated nature of vitamin D and calcium both biologically and in foods, may have complicated the assessment of their individual mechanistic effects. A small sample size may have lacked power for some statistical analyses. We were unable to stratify our analyses by type of hormone therapy used due to the limited number of 'ever' users in our sample. It should be noted that potential differences in associations may exist between Type 1 and

Type II endometrial tumor types, which we were unable to address in this study. Our analyses included multiple tests at the α=0.05 significance level, allowing for the possiblity that some findings may be due to chance. Finally, we cannot exclude the possibility that components of dairy foods other than vitamin D and calcium might explain the observed associations with endometrial cancer risk. However, vitamin D and calcium were selected *a priori* based on their potential role in endometrial carcinogenesis.

In conclusion, our study adds to existing evidence for the role of calcium in reducing the risk of endometrial cancer. Larger, prospective studies are needed to confirm calcium's protective role in endometrial carcinogenesis, and to identify the subgroups in which the associations would be greatest.

Table 1. Selected characteristics of cases and controls, The EDGE Study

	Cases (*N* =396) *n* (%)	Controls (N =381) *n* (%)	P[1]
Age (y) Mean (SD)	62.33 (9.45)	64.86 (11.03)	<0.001
Education			
High school or less	145 (36)	129 (34)	
College	175 (44)	150 (39)	0.04
Graduate degree	76 (19)	102 (27)	
Race			
White	353 (89)	350 (92)	
Black	33 (8)	16 (4)	0.02
Other	10 (3)	15 (4)	
Oral contraceptive use			
Ever	187 (47)	186 (49)	0.66
Never	209 (53)	195 (51)	
Hormone therapy use			
Ever	79 (20)	101 (27)	0.03
Never	317 (80)	280 (73)	
Age at menarche (y)			
<12	106 (27)	91 (24)	0.01
12-13	222 (56)	191 (50)	
>13	68 (17)	99 (26)	
Menopause status			
Premenopausal	57 (14)	48 (13)	0.46
Postmenopausal	339 (86)	333 (87)	
Parity			
0	82 (21)	48 (13)	0.001
1- 2	190 (48)	174 (46)	
>3	124 (31)	159 (42)	
Body mass index 1y before ref (kg/m^2)[3]			
<25	101 (26)	185 (49)	<.0001
25 – <30	115 (29)	114 (30)	
≥30	180 (45)	82 (21)	
Waist to Hip Ratio (WHR) (cm)			
<0.85	167 (43)	203 (54)	0.002
≥0.85	221 (57)	172 (46)	
Smoking			
Never	221 (56)	204 (53)	
Past	150 (38)	144 (38)	0.45
Current	25 (6)	33 (9)	
Diabetes[3]			
Yes	58 (15)	32 (8)	0.007
No	338 (85)	349 (92)	
Time spent outdoors (hours/wk, mean (SD))	16.13 (12.98)	17.88 (15.22)	0.08

[1]Chi Square Test test for categorical variables, Student's t-test for continuous variables
[3]Before diagnosis for cases, interview date for controls

Table 2. Age adjusted means (± SE) for dietary factors among cases and controls, the EDGE Study

Food/Nutrient Intake	Cases (N=396)			Controls (N=381)			P[1]
	Mean	(SE)	Median	Mean	(SE)	Median	
Total energy intake (kcal/day)	1592.65	(28.28)	1503.80	1575.71	(29.39)	1486.40	0.68
Total dairy (weekly number of servings)	6.77	(0.29)	5.60	7.53	(0.30)	6.30	0.08
Total Milk (weekly milk equivalents)[2,3]	3.53	(0.24)	1.38	4.04	(0.25)	1.75	0.14
Yogurt (weekly milk equivalents)	0.76	(0.75)	0.15	0.95	(0.76)	0.16	0.08
Cheese (weekly milk equivalents)	1.38	(0.08)	0.66	1.26	(0.08)	0.66	0.34
Ice cream (weekly milk equivalents)	1.06	(0.09)	0.47	0.97	(0.09)	0.23	0.51
Total vitamin D (IU/day)	336.87	(11.44)	357.17	352.39	(11.44)	428.06	0.33
Dietary vitamin D (IU/day)	132.87	(5.05)	113.27	138.76	(5.15)	110.11	0.42
Supplemental vitamin D (IU/day) *(Users: Cases=230; Controls=225)*	348.80	(5.82)	400.00	364.23	(5.89)	400.00	0.06
Total calcium (mg/day)	1113.89	(31.93)	970.75	1219.26	(32.56)	1230.10	0.02
Dietary calcium (mg/day)	634.52	(16.79)	574.00	671.27	(17.12)	604.90	0.13
Supplemental calcium (mg/day) (*Users: Cases=272; Controls=281)*	691.65	(27.14)	1000.00	749.08	(26.70)	1000.00	0.13

[1] ANCOVA
[2] Milk equivalent= 1 cup milk; 1 cup yogurt; 1 ½ oz. natural cheese, 2 oz. processed cheese
[3] Includes milk as a beverage and on cereal; whole, reduced fat 2%, low fat 1%, non-fat, soy and rice types

Table 3. Association between dairy food intake and endometrial cancer risk, the EDGE Study

Dairy Food Intake (weekly milk equivalents)	Cases N=396 *n(%)*	Controls N=381 *n(%)*	OR[1] (95% CI)	OR[2] (95% CI)	P for Trend
Total dairy (servings/week)					
Q1 (<2.8)	102 (26)	85 (23)	REF	REF	0.18
Q2 (2.8-<6.3)	110 (28)	97 (25)	0.94 (0.63-1.41)	0.97 (0.63-1.48)	
Q3 (6.3-<10.5)	96 (24)	94 (24)	0.81 (0.54-1.23)	0.88 (0.57-1.36)	
Q4 (≥10.5)	88 (22)	105 (28)	0.68 (0.45-1.04)	0.76 (0.48-1.19)	
Total milk[3]					
Q1 (<0.19)	91 (23)	96 (25)	REF	REF	0.29
Q2 (0.19-<1.75)	118 (30)	92 (24)	1.38 (0.93-2.07)	1.40 (0.92-2.14)	
Q3 (1.75-<7.0)	98 (25)	87 (23)	1.19 (0.79-1.81)	1.29 (0.83-1.99)	
Q4 (≥ 7.0)	89 (22)	106 (28)	0.93 (0.31-1.41)	0.96 (0.62-1.49)	
Milk Type Usually Consumed					
(Users: cases=284; controls=284)					NA
Non-fat	97 (34)	105 (38)	REF	REF	
Low-fat 1%	70 (25)	66 (23)	1.27 (0.73-1.75)	1.04 (0.65-1.67)	
Reduced fat 2%	63 (22)	61 (21)	1.07 (0.68-1.68)	0.83 (0.50-1.38)	
Whole	41 (14)	34 (12)	1.30 (0.76-2.24)	1.15 (0.63-2.11)	
Other (soy & rice)	13 (5)	18 (5)	0.71 (0.33-1.53)	0.60 (0.26-1.39)	
Yogurt					
Q1 (<0.03)	81 (20)	95 (25)	REF	REF	
Q2 (0.03-<0.16)	104 (26)	88 (23)	1.30 (0.86-1.97)	1.34 (0.87-2.08)	0.68
Q3 (0.16-<1.0)	110 (28)	86 (23)	1.39 (0.92-2.10)	1.47 (0.95-2.29)	
Q4 (≥1.0)	101 (26)	112 (29)	0.97 (0.65-1.46)	1.14 (0.74-1.76)	
Total Cheese					
Q1 (<0.21)	73 (19)	82 (22)	REF	REF	
Q2 (0.21-<0.66)	88 (22)	76 (20)	1.27 (0.81-1.97)	1.26 (0.79-2.02)	0.73
Q3 (0.66-<1.82)	127 (32)	119 (31)	1.16 (0.77-1.75)	1.14 (0.74-1.77)	
Q4 (≥1.82)	108 (27)	104 (27)	1.13 (0.73-1.74)	1.19 (0.75-1.88)	
Cheese Type Usually Consumed					
(*Users: cases=346 ;controls=315)*					
Usually low-fat	41 (10)	64 (17)	REF	REF	
Sometimes low-fat	122 (31)	87 (23)	2.14 (1.32-3.47)	2.29 (1.36-3.84)	NA
Hardly ever low-fat	183 (46)	164 (43)	1.69 (1.08-2.64)	1.70 (1.04-2.74)	
Total Ice Cream					
Q1 (<0.09)	61 (15)	69 (18)	REF	REF	
Q2 (0.09-<0.23)	103 (26)	101 (27)	0.93 (0.59-1.47)	1.16 (0.72-1.89)	0.57
Q3 (0.23-<0.94)	116 (29)	114 (29)	1.19 (0.77-1.84)	1.27 (0.80-2.01)	
Q4 (≥0.94)	97 (25)	97 (25)	1.31 (0.83-2.07)	1.27 (0.78-2.07)	
Ice Cream Type					
(Users: cases=358 ;controls=318)					
Usually low-fat	65 (16)	69 (18)	REF	REF	
Sometimes low-fat	127 (32)	88 (23)	1.52 (0.98-2.35)	1.53 (0.96-2.43)	NA
Hardly ever low-fat	166 (42)	161 (42)	1.09 (0.73-1.63)	1.11 (0.72-1.73)	

[1] Model 1 adjusted for age (continuous), total energy intake (continuous)

[2] Model 2 adjusted for age (continuous), total energy intake (continuous), parity, BMI, hormone therapy use, oral contraceptive use, menopause status, age at menarche, diabetes, education, race

[3] Includes milk as beverage and on cereal; whole, reduced fat 2%, low fat 1%, non-fat, soy and rice types

Table 4. Association between vitamin D and calcium intakes with endometrial cancer risk, the EDGE Study

Daily Nutrient Intake	Cases N=396 *n(%)*	Controls N=381 *n(%)*	OR[1] (95% CI)	OR[2] (95% CI)	P for Trend
Total Vitamin D (IU)					
Q1 (<122.18)	100 (25)	95 (25)	REF	REF	0.75
Q2 (122.18-<428.06)	115 (29)	95 (25)	1.14 (0.76-1.69)	1.12 (0.74-1.69)	
Q3 (428.06-<522.56)	87 (22)	97 (25)	0.89 (0.59-1.35)	1.03 (0.67-1.59)	
Q4 (≥522.56)	94 (25)	94 (25)	0.99 (0.66-1.49)	1.09 (0.71-1.69)	
Dietary Vitamin D (IU)					
Q1 (<61.51)	94 (24)	95 (25)	REF	REF	0.70
Q2 (61.51-<110.11)	99 (25)	95 (25)	1.02 (0.68-1.54)	1.02 (0.67-1.56)	
Q3 (110.11-<185.55)	121 (31)	95 (25)	1.22 (0.81-1.84)	1.18 (0.78-1.79)	
Q4 (≥185.55)	82 (21)	96 (25)	0.84 (0.54-1.32)	0.88 (0.57-1.36)	
Supplemental Vitamin D (IU)[3]					
Non-users	166 (42)	156 (41)	REF	REF	0.56
Low (>0-<400.0)	63 (16)	43 (11)	1.32 (0.84-2.07)	1.32 (0.83-2.12)	
High (≥ 400.0)	167 (42)	182 (48)	0.91 (0.67-1.24)	1.07 (0.77-1.49)	
Total Calcium (mg)					
Q1 (<664.45)	122 (31)	95 (25)	REF	REF	
Q2 (664.45-<1230.10)	104 (26)	95 (25)	0.89 (0.53-1.19)	0.86 (0.57-1.30)	0.05
Q3 (1230.10-<1715.60)	95 (24)	95 (25)	0.78 (0.53-1.17)	1.06 (0.69-1.63)	
Q4 (≥1715.60)	75 (19)	96 (25)	0.58 (0.37-0.90)	0.81 (0.52-1.25)	
Dietary Calcium (mg)					
Q1 (<428.70)	122 (31)	95 (25)	REF	REF	
Q2 (428.70-<604.90)	89 (22)	95 (25)	0.66 (0.44-0.99)	0.77 (0.51-1.17)	0.05
Q3 (604.90-<875.80)	109 (28)	96 (25)	0.77 (0.50-1.17)	0.89 (0.59-1.34)	
Q4 (≥875.80)	76 (19)	95 (25)	0.52 (0.32-0.84)	0.63 (0.41-0.97)	
Supplemental Calcium (mg)[3]					
Non-users	124 (31)	100 (26)	REF	REF	
Low (>0-<1000)	132 (33)	116 (30)	0.94 (0.65-1.36)	1.06 (0.72-1.56)	0.05
High (≥ 1000)	140 (35)	165 (43)	0.74 (0.52-1.01)	1.02 (0.70-1.50)	

[1] Model 1 adjusted for age (continuous), total energy intake (continuous)
[2] Model 2 adjusted for age (continuous), total energy intake (continuous), parity, BMI, hormone therapy use, oral contraceptive use, menopause status, age at menarche, diabetes, education, race
[3] Based on median among Control users

Table 5. Total vitamin D, total calcium intake and endometrial cancer risk, stratified by BMI, hormone therapy use and menopause, The EDGE Study

Dietary Factor *Population*	Cases/Controls *396/381*	Quartile 1	Quartile 2 OR (95% CI)	Quartile 3 OR (95% CI)	Quartile 4 OR (95% CI)	P for Interaction
Total Vitamin D						
BMI[1]						0.99
<25	101/185	REF	1.36 (0.65-2.86)	0.94 (0.45-1.95)	1.12 (0.54-2.35)	
25-<30	115/114	REF	1.15 (0.54-2.44)	0.94 (0.42-2.13)	1.05 (0.49-2.26)	
≥30	180/82	REF	0.85 (0.42-1.72)	1.19 (0.53-2.69)	1.17 (0.52-2.63)	
HT[2]						0.77
Ever	79/101	REF	0.78 (0.29-2.06)	0.64 (0.26-1.59)	0.83 (0.33-2.06)	
Never	317/280	REF	1.24 (0.77-1.99)	1.22 (0.73-2.03)	1.15 (0.69-1.89)	
Menopause						
Post-menopause	339/333	REF	1.23 (0.77-1.96)	1.08 (0.66-1.77)	1.10 (0.64-1.89)	NA
Total Calcium						
BMI[1]						
<25	101/185	REF	0.86 (0.40-1.87)	1.28 (0.63-2.62)	0.73 (0.35-1.53)	0.77
25-<30	115/114	REF	1.04 (0.50-2.16)	0.74 (0.35-1.56)	0.85 (0.37-1.96)	
≥30	180/82	REF	0.72 (0.36-1.46)	1.24 (0.52-2.97)	0.93 (0.42-2.07)	
HT[2]						
Ever	79/101	REF	1.09 (0.39-3.06)	1.85 (0.77-4.49)	1.65 (0.62-4.39)	0.05
Never	317/280	REF	0.83 (0.52-1.32)	0.98 (0.59-1.63)	0.67 (0.40-1.12)	
Menopause						
Post-menopause	339/333	REF	0.75 (0.46-1.22)	0.89 (0.55-1.47)	0.54 (0.29-0.98)	NA

[1]Model adjusted for age (continuous), total energy intake (continuous), parity, oral contraceptive use, menopause status, age at menarche, diabetes, education, race, HT use
[2]Model further adjusted for BMI, excluding HT use

Chapter 2: Dairy Foods, Related Nutrients and Ovarian Cancer Risk, The NJ Ovarian Cancer Study

Introduction

Ovarian cancer is the most lethal of the female reproductive cancers, and accounts for 5% of cancer deaths among women in the US [42]. Risk increases with age, with 85-90% of cases occurring after menopause [43]. With no currently reliable screening, and early-stage disease often having no symptoms, most cases are advanced when diagnosed with 5 year survival of 46% [32]. Only a few established modifiable risk factors have been identified, among them oral contraceptive use, parity and tubal ligation which are associated with reduced risk [84, 85], and body fatness which has been deemed a 'probable' cause of ovarian cancer [43].

Diet, in particular dairy foods, vitamin D and calcium, is a modifiable risk factor for ovarian cancer, however studies have been inconclusive showing null, positive and negative associations [18, 44, 45, 86, 87]. Recently updated dietary guidelines recommend a minimum of 3 cup equivalents of dairy food per day for women ages 51 years and older [88]. In addition, vitamin D and calcium supplementation is recommended for postmenopausal women who are not meeting minimum dietary requirements [89]. In vitro and in vivo evidence support roles for vitamin D and calcium in regulation of multiple cancer risk and prognosis relevant pathways [14, 90, 91]. However, given the inconclusive epidemiologic evidence, further study is needed to clarify the role that dairy foods, vitamin D and calcium play.

In this study we examined the association between ovarian cancer risk and intake of dairy foods, vitamin D and calcium using data from The New Jersey Ovarian Cancer Study, a population based case-control study.

Subjects and Methods

Study Population

The New Jersey Ovarian Cancer Study is a population-based, case-control study that was conducted in six counties in NJ (Bergen, Essex, Hudson, Middlesex, Morris, Union) and described in detailed elsewhere [92, 93]. Identical data collection instruments, procedures and a shared control group with the Estrogen, Diet, Genetics, and Endometrial Cancer (EDGE) Study [94], were used as described in Chapter 1.

Case definition included newly diagnosed, histologically confirmed invasive epithelial ovarian cancer, identified between January 2004 and May 2008 through rapid case ascertainment conducted by the New Jersey State Cancer Registry (NJSCR). In total, 682 eligible cases were initially identified, however 70 women were not contacted because they were either deceased, or their physicians advised against it. An additional 119 women were ineligible because they could not be reached, no longer met eligibility requirements, or had a communication barrier or medical condition that precluded participation. Of the 493 remaining ovarian cancer cases, 252 gave consent to participate (51%) and 233 ovarian cancer cases completed the study interview (47%). Control ascertainment was previously detailed in Chapter 1 of this dissertation.

Of the 233 cases and 467 controls who participated in the study, 205 cases (88%) and 398 (85%) controls completed both the main questionnaire and the FFQ. Further

exclusions for this analysis included 8 controls that had a double oophorectomy and one case who had missing data on education, resulting in 204 cases and 390 controls for analysis.

Data Collection

Methods used for data collection were described in detail in Chapter 1. Briefly, the study questionnaire was administered through telephone interviews conducted by trained staff. Dietary intake was assessed using a self-administered Block 98.2 Food Frequency Questionnaire (FFQ), which estimated usual intake of each food item over the six months prior to diagnosis for cases and six months prior to the interview date for controls.

Dietary Assessment

Assessment methods for dietary intake were described fully in Chapter 1. The primary exposure variables used in this analysis were dairy foods from major sources (milk, cheese, yogurt, ice cream), vitamin D (total, dietary, supplemental), and calcium (total, dietary, supplemental). Weekly dairy food intake levels were derived from raw dietary data and converted into weekly milk equivalents using the MyPyramid Equivalent system described in Chapter 1; daily intake levels were used for total, dietary, and supplemental intakes of vitamin D (IU/day) and calcium (mg/day).

Data Analysis

The complete statistical analysis plan has been discussed in Chapter 1. Briefly, intake of dairy foods, total vitamin D and total calcium were converted into quartiles for analysis, using cut points based on distributions among controls. Supplemental vitamin D and supplemental calcium were categorized as ‘users’ and ‘non-users’ with the median

intake among user controls as the cut point for 'low' (below median) and 'high' (above median) users. Sub-type analysis for milk was conducted among users, with non-fat as the reference category, excluding 'unknown' responses (i.e., participants who indicated they used milk but didn't indicate the subtype usually consumed). Cheese and ice cream subtype analyses were conducted among users of the foods, with 'usually low fat' as the reference group.

Descriptive statistics were used to compare characteristics between cases and controls, and ANCOVA was used to estimate age adjusted mean intake levels of dairy foods and nutrients. Multivariable regression models were used to estimate associations between all dietary exposures and ovarian cancer risk. Fully adjusted multivariable regression models for this analysis included reference age (continuous), total energy intake (continuous), education (high school or less, college, graduate degree); race (white, black, other); oral contraceptive use (ever, never); hormone therapy use (ever, never); age at menarche (<12, 12-13,>13); menopause (pre/postmenopausal); parity (0, 1-2, >3); BMI (<25, 25-<30, $\geq$30kg/m^2), tubal ligation (yes/no), and first –degree family history of breast/ovarian cancer (yes/no). Smoking (never, past, current), time spent outdoors (hours/week; continuous) and consumption of total added sugars (continuous) were evaluated as potential covariates but were not retained in final models because they resulted in <10% change in the main effect. Tests for linear trend were conducted by including the median of each quartile (continuous) in the fully adjusted logistic regression models. Tests for interaction were performed using the Likelihood Ratio Test; stratified analyses by BMI, HT use and menopause were conducted to assess the presence of effect

modification for associations between total vitamin D and total calcium with ovarian cancer risk. All analyses were performed using SAS software, version 9.4 (Cary, NC).

Results

Descriptive characteristics of the study participants are presented in Table 1. The majority of cases (92%) and controls (92%) were white. On average, compared to controls cases were significantly younger (mean age 57.23 vs. 64.55 years), had lower parity, were less likely to be menopausal, and less likely to spend time outdoors (mean 15.13. vs. 17.71 hrs/wk) (all $p<0.05$). Cases were slightly more likely to have used oral contraceptives compared to controls ($p=0.08$). Education, hormone therapy use, age at menarche, BMI, tubal ligation, smoking, and family history of ovarian cancer did not differ significantly between cases and controls.

Table 2 presents the age adjusted mean and median intakes of total energy, dairy foods and total, dietary, and supplemental intake of vitamin D and calcium. Mean intake of total energy and all dairy foods did not differ significantly between cases and controls. For vitamin D and calcium, mean total and dietary intake were not significantly different between cases and controls. Mean intake of supplemental vitamin D among users (cases 62% , controls 59%), however, was significantly lower for cases than controls (335.75 vs. 361.03 IU/day) ($p=0.02$). Among users of supplemental calcium (72% cases, 74% controls), cases had a lower mean intake than controls (646.82 vs. 735.21; $p=0.07$).

As shown in Table 3, total dairy intake was not associated with ovarian cancer risk. Milk increased risk of ovarian cancer, although the association was not linear (Q3 OR: 1.93; 95% CI:1.12, 3.32). Among milk users, those who consumed reduced fat 2% had a significantly increased risk of ovarian cancer compared to those who consumed

non-fat milk in the base model, which remained statistically significant in the fully adjusted model (OR: 2.01; 95% CI: 1.12, 3.58). Total yogurt, total cheese and total ice cream intakes were not associated with ovarian cancer risk. However, increased risk was suggested for less frequent use of low-fat cheese and ice cream subtypes.

No statistically significant associations were observed for vitamin D (total, dietary or supplemental) although an increased risk, albeit non-significant, was observed among supplement users compared to 'non-users' (low: OR: 1.69; 95% CI: 0.98, 2.90; High: OR: 1.17; 95% CI: 0.77, 1.77) (Table 4). For total calcium, a suggestion of reduced risk was observed although statistical significance was not achieved [Q4 vs. Q1, OR: 0.71; 95% CI: 0.40, 1.23]. No significant associations were observed for dietary or supplemental calcium intake with the risk of ovarian cancer. Results were unchanged in sensitivity analyses that tested total dairy, total milk, total vitamin D, dietary vitamin D, supplemental vitamin D, total calcium, dietary calcium, and supplemental calcium as continuous variables to ascertain any differences in their relationship with ovarian cancer risk as a result of categorizing the continuous measures into quartiles (data not shown). No changes in risk estimates were observed in models adjusting for total fat (excluding total energy) (data not shown).

No evidence of effect modification by BMI on the association between total vitamin D and ovarian cancer was found in our study (Table 5). However, a statistically significant interaction by hormone therapy use on the association between total vitamin D and ovarian cancer was observed (p=0.009); 'never' users had ORs >1.0, and 'ever' users had ORs 1.02 (T2) and 0.36 (T3), although the risk estimates were statistically significant. There was no evidence of effect modification by BMI or HT use on the

association between total calcium intake and ovarian cancer risk. Stratified analyses among post-menopausal women showed no evidence of an association for total vitamin D or total calcium with ovarian cancer risk in this subgroup.

Discussion

Our results provide evidence of a non-linear increased risk of epithelial ovarian cancer with milk intake, a finding that is consistent with results from two other case-control studies [45, 86]. Our null results for other dairy foods and ovarian cancer risk are consistent with other case-control studies for yogurt, cheese and ice cream intake [45, 95, 96], and several meta-analyses for total dairy, yogurt [97], and cheese [98] intake; a pooled analysis of 12 cohort studies, and a systematic literature review conducted by WCRF/AICR found no association for any dairy food [18, 99]. In contrast, two population based case-control studies reported a statistically significant inverse association for total dairy and total milk [86] and cheese [47] intake and risk of ovarian cancer, while other evidence supports a significantly increased risk for total dairy and yogurt consumption [44, 47, 100]. More frequent use of reduced fat 2% milk was associated with a significantly greater risk of ovarian cancer compared to non-fat milk, and a suggested increased risk was found with less frequent consumption of low-fat cheese and ice cream. These findings suggests a possible role for dairy fat, however this is not supported by a recent meta-analysis which found no association between ovarian cancer risk and consumption of dietary fat (plant or animal based) or fatty acid intake [101].

We found no evidence for a clear association between any category of vitamin D (total, dietary or supplemental) and ovarian cancer risk which is consistent with a

systematic literature review [102], a pooled analysis of cohort studies [18], and a meta-analysis [98]. Supplemental vitamin D intake was associated with a non-statistically significant increased risk for ovarian cancer in our study, however other studies have reported no association with vitamin D supplements [102] or combined vitamin D/calcium supplements [77]. It should be noted that we were not able to isolate vitamin D supplement use from calcium supplement use in our study. Vitamin D supplement use was assessed through use of multiple vitamins (i.e., Centrum, Regular Once-A-Day) and calcium supplementation was queried as 'calcium alone or in combination with something else'. We found significant effect modification by HT use on the association between total vitamin D and ovarian cancer risk, with lower risk among ever HT users and increased risk among never HT users. No studies were available for direct comparison of this finding.

Our analysis found a non-significant inverse association for total and dietary calcium with epithelial ovarian cancer risk. A recent meta-analysis of 10 studies found a statistically significant decreased risk for the highest vs. lowest quartile of dietary calcium and risk of epithelial ovarian cancer (OR: 0.78; 95% CI: 0.69, 0.88) [103], whereas our results were closer to the null (OR: 0.96; 95% CI: 0.51-1.81) .

There is mounting evidence that the effect of calcium and vitamin D might vary based on epithelial ovarian cancer tumor sub-type. Subgroup analyses have shown stronger inverse associations for dietary calcium with epithelial subtype [103], for total calcium with serous borderline and mucinous subtypes [45] and for total vitamin D with serous borderline and endometrioid subtypes [45]. Further research is warranted to clarify these relationships.

A challenge often encountered in nutritional epidemiology is the inconsistent classification of dairy food and nutrient exposure, which can make direct comparisons to other studies challenging. Differences in exposure measurement (e.g., grams/day; portions/day; cups/day) and exposure period (e.g., daily, weekly, monthly) may contribute to the variability and inconsistency of findings. Our study used standardized milk equivalents as the unit of measure for dairy food intake, thereby reducing some of the variability incurred when comparing nutrient profiles across different food sources of a given nutrient. Few studies have used standardized units of measure in the comparison of different dairy food sources with the risk of ovarian cancer. Because nutrients found in dairy foods can come from different sources simultaneously (i.e., foods, supplements), isolating the effects of individual nutrients or foods can be difficult. Although total, dietary and supplemental sources of vitamin D and calcium were modeled separately in our study, it is possible that differences in effects were masked as a result of the collinear relationship between them.

Findings of the current study should be considered alongside a number of limitations. As a case-control study we cannot rule out potential recall bias, where cases may recall or report dietary intake differently from controls. However, knowledge of the relationship between dairy foods, vitamin D and calcium with ovarian cancer risk in the general population is low therefore awareness of this link among women in this study is unlikely. Furthermore, to reduce the potential that undetected disease influenced dietary recall, cases were asked to estimate their diet 6 months prior to diagnosis, which is beyond the 4-month median pre-diagnostic symptom duration reported for invasive cases [104]. Another limitation is the potential for misclassification of dietary intake due to

measurement error. Our study focused on dietary intake of vitamin D, and as such did not account for vitamin D levels that may have come from exposure to sunlight, thereby potentially introducing error in the dose assessment of this nutrient. Finally, it's possible that the statistically significant findings in our study were due to chance, given the large number of comparisons in our analyses. The risk of ovarian cancer and dairy foods/nutrients by histological subtype could not be examined due to the small number of cases in our study. Another limitation is the inability to quantify intake of specific milk and cheese subtypes. Whole milk has been shown to increase the risk of ovarian cancer in some studies [44, 98, 105] and skim and low-fat milk have shown inverse associations [95], and both hard cheese and cottage cheese have shown to be protective against ovarian cancer at the highest vs. lowest level of intake [87, 105]. The small number of cases and controls in our study may have limited the statistical power for some analyses. The study lacked sufficient sample size to perform more precise analyses of our exposures on the risk of ovarian cancer, for example, by excluding supplement users when examining the effect of dietary vitamin D or calcium.

In conclusion, results of this population based case-control study did not support an association between dairy food, vitamin D and calcium intake with ovarian cancer risk. Existing literature on this relationship is conflicting, and likely reflects differences in study design, populations, exposure measurement and exposure period. However, given the lethality of ovarian cancer and the lack of an effective screening tool, identification of preventative measures remains critical. Dietary and lifestyle modifications represent a low cost, low risk approach. Therefore, larger studies that are

sufficiently powered to measure whether and how other factors such as hormone status and tumor type may influence this association are warranted.

Table 1. Selected characteristics of cases and controls, The NJ Ovarian Cancer Study

	Cases (N=204) *n* (%)	Controls (N=390) *n* (%)	P[1]
Age (y) Mean (SD)	57.23 (10.41)	64.55 (10.92)	<0.0001
Education			
High school or less	60 (29)	132 (34)	0.46
College	93 (46)	159 (41)	
Graduate degree	51 (25)	99 (25)	
Race			
White	187 (92)	356 (92)	0.97
Black	9 (4)	17 (4)	
Other	8 (4)	17 (4)	
Oral contraceptive use			
Ever	119 (58)	198 (51)	0.08
Never	85 (42)	192 (49)	
Hormone therapy use			
Ever	46 (22)	106 (27)	0.22
Never	158 (78)	284 (73)	
Age at menarche (y)			
<12	46 (23)	91 (23)	0.32
12-13	116 (57)	200 (52)	
>13	41 (20)	98 (25)	
Menopause status			
Premenopausal	71 (35)	49 (13)	<.0001
Postmenopausal	133 (65)	338 (87)	
Parity			
0	61 (30)	48 (12)	<.0001
1- 2	94 (46)	178 (46)	
>3	49 (24)	164 (42)	
Body mass index 1y before ref (kg/m^2)[2]			
<25	90 (44)	180 (47)	0.11
25–<30	54 (26)	122 (31)	
≥30	60 (29)	85 (22)	
Tubal Ligation			
Yes	30 (15)	76 (19)	0.15
No	174 (85)	314 (81)	
Smoking			
Never	108 (53)	203 (52)	0.97
Past	77 (38)	149 (38)	
Current	19 (9)	38 (10)	
First Degree Relative with Breast/Ovarian Cancer			
Yes	9 (4)	13 (3)	0.51
No	195 (96)	377 (97)	
Time spent outdoors (hours/wk, mean (SD))	15.13 (11.83)	17.71 (14.82)	0.03

[1]Chi Square Test was used for categorical variables and Student's t-test was used for continuous variables

[2] Before diagnosis for cases, interview date for controls

Table 2. Age adjusted means (± SE) and medians for dietary factors among cases and controls, the NJ Ovarian Cancer Study

Food/Nutrient Intake	Cases (N=204)		Controls (N=390)		P[1]
	Mean (SE)	Median	Mean (SE)	Median	
Total energy intake (kcal/day)	1593.96 (47.81)	1513.85	1602.50 (34.18)	1484.60	0.88
Total dairy (weekly number of servings)	7.60 (0.44)	5.60	7.35 (0.32)	6.30	0.66
Total Milk (weekly milk equivalents)[2,3]	4.08 (0.36)	2.09	3.83 (0.26)	1.56	0.58
Yogurt (weekly milk equivalents)	0.78 (0.11)	0.25	0.93 (0.08)	0.16	0.26
Cheese (weekly milk equivalents)	1.38 (0.12)	0.99	1.31 (0.08)	0.66	0.68
Ice cream (weekly milk equivalents)	1.07 (0.15)	0.23	1.01 (0.10)	0.38	0.76
Total vitamin D (IU/day)	352.94 (16.19)	370.79	348.29 (11.54)	424.19	0.81
Dietary vitamin D (IU/day)	140.58 (7.70)	114.13	137.95 (5.49)	108.80	0.79
Supplemental vitamin D (Users; IU/day)	335.75 (8.49)	400.00	361.03 (6.22)	400.00	0.02
(Cases=127; Controls=229)					
Total calcium (mg/day)	1149.04 (46.99)	1011.95	1201.59 (32.75)	1238.10	0.36
Dietary calcium (mg/day)	675.13 (24.97)	590.50	666.29 (17.77)	600.10	0.78
Supplemental calcium (Users; mg/day)	646.82 (38.68)	807.40	735.21 (27.09)	1000.00	0.07
(*Cases=146; Controls=287)*					

[1] ANCOVA
[2] Milk equivalent= 1 cup milk; 1 cup yogurt; 1 ½ oz. natural cheese, 2 oz. processed cheese
[3] Includes milk as a beverage and on cereal; whole, reduced fat 2%, low fat 1%, non-fat, soy and rice types

Table 3. Association between dairy food intake and ovarian cancer risk, The NJ Ovarian Cancer Study

Dairy Food Intake (weekly milk equivalents)	Cases N=204 *n(%)*	Controls N=390 *n(%)*	OR[1] (95% CI)	OR[2] (95% CI)	P for Trend
Total dairy (servings/week)					
Q1 (<2.8)	45 (22)	91 (23)	REF	REF	0.93
Q2 (2.8-<6.3)	59 (29)	99 (26)	1.14 (0.69-1.90)	1.06 (0.63-1.81)	
Q3 (6.3-<10.5)	46 (23)	95 (24)	0.91 (0.52-1.55)	0.82 (0.47-1.44)	
Q4 (≥10.5)	54 (26)	105 (27)	1.10 (0.64-1.90)	1.06 (0.61-1.87)	
Total milk[3]					
Q1 (<0.15)	37 (18)	99 (25)	REF	REF	0.37
Q2 (0.15-<1.56)	53 (26)	96 (25)	1.54 (0.90-2.62)	1.41 (0.81-2.45)	
Q3 (1.56-<7.0)	68 (33)	92 (24)	1.91 (1.17-3.31)	1.93 (1.12-3.32)	
Q4 (≥ 7.0)	46 (23)	103 (26)	1.50 (0.86-2.63)	1.47 (0.82-2.63)	
Milk Type Usually Consumed *(Users: cases=160; controls=287)*					
Non-fat	45 (29)	105 (37)	REF	REF	NA
Low-fat 1%	39 (24)	67 (23)	1.45 (0.83-2.50)	1.47 (0.83-2.61)	
Reduced fat 2%	51 (32)	61 (21)	1.84 (1.07-3.17)	2.01 (1.12-3.58)	
Whole	18 (11)	37 (13)	1.25 (0.62-2.54)	1.13 (0.53-2.40)	
Other (soy & rice)	7 (4)	17 (6)	0.99 (0.37-2.64)	0.97 (0.35-2.68)	
Yogurt					
Q1 (0)	42 (21)	99 (25)	REF	REF	0.53
Q2 (>0-<0.16)	56 (27)	90 (23)	0.81 (0.47-1.41)	0.89 (0.50-1.56)	
Q3 (0.16-<1.0)	67 (33)	89 (23)	1.35 (0.81-2.45)	1.41 (0.83-2.40)	
Q4 (≥1.0)	39 (19)	127 (28)	0.90 (0.53-1.51)	0.91 (0.53-1.56)	
Total Cheese					
Q1 (<0.21)	42 (21)	84 (22)	REF	REF	0.81
Q2 (0.21-<0.66)	30 (15)	80 (21)	0.63 (0.34-1.14)	0.63 (0.34-1.16)	
Q3 (0.66-<1.98)	79 (38)	127 (32)	1.19 (0.73-1.94)	1.18 (0.71-1.96)	
Q4 (≥1.98)	53 (26)	99 (25)	0.91 (0.53-1.57)	0.88 (0.50-1.55)	
Cheese Type Usually Consumed *(Users: cases=159;controls=319)*					NA
Usually low-fat	19 (9)	63 (16)	REF	REF	
Sometimes low-fat	50 (25)	86 (22)	1.61 (0.84-3.09)	1.66 (0.84-3.27)	
Hardly ever low-fat	90 (44)	170 (44)	1.50 (0.82-2.74)	1.52 (0.81-2.85)	
Total Ice Cream					0.51
Q1 (<0.09)	40 (20)	69 (19)	REF	REF	
Q2 (0.09-<0.23)	64 (31)	101 (27)	0.72 (0.43-1.22)	1.14 (0.57-2.31)	
Q3 (0.23-<0.94)	46 (23)	114 (29)	0.67 (0.38-1.18)	0.88 (0.42-1.87)	
Q4 (≥0.94)	54 (26)	97 (25)	0.87 (0.49-1.52)	1.09 (0.54-2.24)	
Ice Cream Type *(Users: cases=174; controls=325)*					
Usually low-fat	32 (16)	70 (18)	REF	REF	NA
Sometimes low-fat	57 (28)	88 (23)	1.30 (0.74-2.28)	1.25 (0.70-2.24)	
Hardly ever low-fat	85 (42)	167 (43)	1.12 (0.66-0.88)	1.16 (0.68-1.99)	

[1] Model 1 adjusted for age (continuous), total energy intake (continuous)

[2] Model 2 adjusted for age (continuous), total energy intake (continuous), parity, BMI, hormone therapy use, oral contraceptive use, menopause status, age at menarche, education, race, family history of OC, tubal ligation

[3] Includes milk as beverage and on cereal; whole, reduced fat 2%, low fat 1%, non-fat, soy and rice types

Table 4. Association between vitamin D and calcium intakes with ovarian cancer risk, the NJ Ovarian Cancer Study

Daily Nutrient Intake	Cases N=204 *n(%)*	Controls N=390 *n(%)*	OR[1] (95% CI)	OR[2] (95% CI)	P for Trend
Total Vitamin D (IU)					
Q1 (<122.18)	44 (22)	97 (25)	REF	REF	0.92
Q2 (122.18-<428.06)	72 (35)	98 (25)	1.54 (0.94-2.51)	1.47 (0.88-2.47)	
Q3 (428.06-<522.56)	38 (19)	97 (25)	0.97 (0.57-1.68)	0.97 (0.55-1.71)	
Q4 (≥522.56)	50 (25)	98 (25)	1.29 (0.76-2.20)	1.28 (0.74-2.22)	
Dietary Vitamin D (IU)					
Q1 (<60.94)	45 (22)	99 (26)	REF	REF	0.97
Q2 (60.94-<109.02)	54 (26)	96 (24)	1.11 (0.67-1.87)	1.19 (0.69-2.04)	
Q3 (109.02-<185.56)	58 (28)	97 (24)	1.16 (0.69-1.94)	1.15 (0.67-1.97)	
Q4 (≥185.56)	47 (24)	98 (26)	1.09 (0.62-1.94)	1.09 (0.60-1.95)	
Supplemental Vitamin D (IU)[4]					
Non-users	77 (38)	161 (41)	REF	REF	0.32
Low (>0-<400.0)	43 (21)	45 (12)	1.69 (0.99-2.84)	1.69 (0.98-2.90)	
High (≥ 400.0)	84 (41)	184 (47)	1.12 (0.75-1.66)	1.17 (0.77-1.77)	
Total Calcium (mg)					
Q1 (<659.60)	67 (33)	97 (25)	REF	REF	0.41
Q2 (659.60-<1238.10)	51 (25)	98 (25)	0.67 (0.40-1.1)	0.71 (0.42-1.21)	
Q3 (1238.10-<1715.60)	50 (25)	97 (25)	0.78 (0.47-1.27)	0.83 (0.49-1.40)	
Q4 (≥1715.)	36 (17)	98 (25)	0.63 (0.36-1.10)	0.71 (0.40-1.23)	
Dietary Calcium (mg)					
Q1 (<420.50)	55 (27)	98 (25)	REF	REF	0.94
Q2 (420.50-<600.0)	49 (24)	98 (25)	0.83 (0.48-1.34)	0.79 (0.46-1.35)	
Q3 (600.0-<875.80)	51 (25)	97 (25)	0.88 (0.52-1.51)	0.86 (0.49-1.15)	
Q4 (≥875.80)	49 (24)	97 (25)	0.97 (0.53-1.78)	0.96 (0.51-1.81)	
Supplemental Calcium (mg)[3]					
Non-users	58 (28)	103 (26)	REF	REF	0.80
Low (>0-<1000)	77 (38)	120 (31)	1.19 (0.75-1.87)	1.34 (0.84-2.16)	
High (≥ 1000)	69 (34)	167 (43)	0.95 (0.60-1.49)	1.09 (0.68-1.79)	

[1] Model 1 adjusted for age (continuous), total energy intake (continuous)

[2] Model 2 adjusted for age (continuous), total energy intake (continuous), parity, BMI, hormone therapy use, oral contraceptive use, menopause status, age at menarche, education, race, family history of OC, tubal ligation

[3] Based on median among Control users

Table 5. Dietary factors and ovarian cancer risk by selected characteristics, The NJ Ovarian Cancer Study

Dietary Factor *Population*	Cases/Controls *203/383*	Tertile 1	Tertile2 OR (95% CI)	Tertile 3 OR (95% CI)	P for Interaction
Total Vitamin D					
BMI[1]					0.41
<25	90/179	REF	1.66 (0.84-3.31)	1.01 (0.49-2.07)	
25-<30	53/119	REF	0.70 (0.29-1.70)	1.00 (0.38-2.61)	
≥30	60/85	REF	1.36 (0.52-3.55)	0.74 (0.25-2.14)	
HT[2]					.009
Ever	46/105	REF	1.02 (0.38-2.74)	0.36 (0.13-1.00)	
Never	157/281	REF	1.23 (0.74-2.06)	1.43 (0.82-2.51)	
Menopause					
Post-menopause	133/338	REF	1.11 (0.66-1.86)	0.81 (0.47-1.40)	NA
Total Calcium					
BMI[1]					0.48
<25	90/179	REF	0.88 (0.43-1.78)	0.83 (0.40-1.71)	
25-<30	53/119	REF	0.89 (0.38-2.11)	0.65 (0.24-1.73)	
≥30	60/85	REF	1.39 (0.47-4.09)	1.43 (0.46-4.39)	
HT[2]					0.94
Ever	46/105	REF	1.44 (0.54-3.86)	1.09 (0.39-3.02)	
Never	157/278	REF	0.95 (0.55-1.63)	0.87 (0.48-1.57)	
Menopause					
Post-menopause	133/338	REF	0.85 (0.49-1.44)	0.80 (0.46-1.41)	NA

[1]Model adjusted for adjusted for age (continuous), total energy intake (continuous), parity, oral contraceptive use, menopause status, age at menarche, education, race, family history of OC, tubal ligation
[2]Model further adjusted for BMI, excluding HT use

Chapter 3: Dairy Foods, Related Nutrients and Sexual Development, Growth and Obesity in New Jersey Girls

Introduction

Girls in the US are reaching sexual maturity earlier, and there is evidence suggesting a downward trend in the age of thelarche (i.e., Tanner breast stage ≥2) among females in the US [49, 106-108]. Among girls ages 9 and 10 years, the prevalence of breast development at Tanner stage 2 or greater has been reported as 32% and 62%, respectively [49]. In 1999 the Lawson Wilkins Pediatric Endocrine Society recommended lowering the normal age of puberty onset in White girls from 8 to 7 years of age, based on a study of 17,077 girls seen in pediatric practices with pubertal characteristics earlier than expected [49, 109]. More recently, in 2007 a consensus panel reported that sufficient data were available to suggest a trend toward early breast development in the US, over the last half of the 20th century [110]. Puberty is a period of particular vulnerability in a woman's development when nutritional and environmental exposures can impact later risk of cancer; characteristics of early maturity such as early age at menarche and accelerated linear growth velocity have been found to increase the risk of breast cancer [48, 111]. Body size has been linked to onset of puberty with taller, heavier girls reaching puberty faster than smaller, leaner girls [52]. Obesity in particular is a strong predictor of early onset of puberty, possibly attributed to increased estrogen levels through aromatization of androgens in adipose tissue, and the latest estimates suggest that 17.5% of females between 6 and 11 years are obese, defined as BMI-for-age at or above the 95th percentile [52, 112, 113].

Diet and nutritional status are major determinants of growth, and may influence the onset of puberty directly or indirectly through effects on energy balance, or hormonal

responses to specific food components [52, 53]. Dairy foods, vitamin D and calcium have been implicated as having a role in body weight/adiposity, fat regulation, growth, and early sexual development although evidence is limited and results are mixed [23, 25, 59-62, 114, 115]. Milk contains a number of components capable of affecting growth and body size including essential nutrients, as well as other growth-enhancing molecules such as fat and IGF-1, which can potentially alter development parameters at critical biological transition periods [13, 20, 54]. Milk and dairy products are the major source of animal derived estrogens in the human diet, accounting for 60-80% of estrogens consumed [55]. Estrone sulfate, the main estrogen in milk, has high bioavailability and once inside the body can be converted to estrone and estradiol; an estimated 47% of estrone found in a standard diet is thought to originate from dairy sources [55].

Consumption of dairy foods is deeply embedded in the dietary practices of children in the US. The Dietary Guidelines for Americans recommends three servings of dairy per day for individuals 9 years and older, and considers dairy foods a key element of a healthy eating pattern [88]. Moreover, these guidelines further influence dairy consumption since they serve as the evidence base for informing large, national supplemental nutrition programs for children including the National School Lunch Program and School Breakfast Program, which together feed more than 30 million children each school day [88]. The Special Supplemental Nutrition Program for Women, Infants and Children also uses the Dietary Guidelines as the foundation for its food packages and nutrition education program, which serves approximately 8 million beneficiaries. Beyond these programs, the Dietary Guidelines influence food choices by informing programs, policies, and education that target other audiences including the

general public, schools, and community groups. Given the widespread recommendation that children consume dairy products every day, it is essential to understand the impact of these foods on normal growth and development as well as disease risk in this vulnerable population.

Our study examined the relationship between dairy foods, vitamin D and calcium intake with early breast development (thelarche), prevalence of overweight and above, and body size among pre-menarcheal girls in The Jersey Girl Study.

Subjects and Methods

Study Population

This was a cross-sectional analysis of the baseline assessment from The Jersey Girl Study, an ongoing prospective cohort study of girls aged 9-10 years (baseline) residing in New Jersey. Methods for the Jersey Girl Study have been described in detail elsewhere [116] . In brief, healthy girls aged 9-10 years residing with their biological mothers in New Jersey with no cognitive impairments, and who could speak English, were eligible. Study participants were obtained through pediatric practices, media advertisement and community recruitment efforts. Data collection occurred by mail, phone and a clinic visit. Questionnaires completed by mothers collected information on factors considered or known to affect the onset of puberty, which included demographic characteristics (i.e., race), environmental exposures, medical history, physical activity, prenatal and early childhood factors as well as maternal and paternal factors. Trained personnel collected anthropometric measures during the clinic visit using methods adapted from National Health and Nutrition Examination Survey III Study Procedures, National Health and Nutrition Examination Survey, 1988, including height, waist and hip

circumferences, weight, and body composition (fat mass, fat free mass, and % body fat) using bioelectrical impedance analysis with a Tanita ® RBF-300A scale. Puberty staging used the Tanner scale for breast and pubic hair development, which ranges from Tanner stage 1 (pre-pubertal) to 5 (adult), with Tanner stage 2+ marking the onset of breast development or pubic hair. Tanner stage was collected using standardized forms that included pictures representing each stage. Girls were given the choice of having the staging assessment done by their own pediatricians at a physical exam, or by one of the study-affiliated female physicians. Study-affiliated physicians were either pediatric residents or breast fellows who were trained by the same pediatric endocrinologist, and used palpation to assess staging. Mothers were also requested to report staging using the same forms for breast and pubic hair development. Good agreement between physicians' and mothers' assessment of breast development was revealed through a preliminary analysis (kappa: 0.7, 95% CI: 0.6-0.8), making it possible to rely on the Tanner stage assessed by mothers when physician assessment was not available [117].

Two hundred five (205) girls completed the interview and provided dietary data. Three (3) girls were excluded from analyses due to missing data on Tanner stage, menarche status, BMI or age, with a final sample size of 202 for this analysis.

Dietary Assessment

Dietary intake was assessed using 24-hr recalls over a three day period (including one weekend day) from girls and their mothers, conducted by the Cincinnati Center for Nutritional Research and Analysis. Mean intake over the 3-day dietary recall period was calculated and used to represent 'usual intake'. The multiple 24-hour recall method, including parents as proxy reporters, has been shown to be the most accurate for

assessing total energy in children ages 4-11 years [118]. Nutrient analysis for the present study was performed using The Nutrition Data System (NDS-R), developed by and maintained by the Nutrition Coordinating Center at the University of Minnesota [119, 120].

Data Analysis

The primary outcome variables for this analysis were Tanner stage for breast development evaluated as Tanner Stage 1 (reference) or Tanner Stage $\geq$2, and prevalence of overweight and above, evaluated as underweight/healthy (BMI-for-age <85th percentile; reference) and overweight/obese (at or above 85th percentile) using BMI-for-age percentiles [113, 121]. Nutrient density variables were created for dairy foods as grams/day/1,000 kcal and included total dairy (total milk, total cheese, yogurt, frozen dairy desserts), total milk (whole, reduced fat 2%, low-fat 1%, non-fat), and total cheese (full-, low-, reduced-fat, fat-free). There were too few users of yogurt and frozen dairy desserts to assess these foods individually. Daily vitamin D and calcium intakes were analyzed using dietary sources only, due to the small number of supplement users in this cohort and were analyzed as mcg/day/1,000 kcal and mg/day/1,000 kcal, respectively.

Extreme energy intake was defined using the interquartile range (IQR) of total energy intake. Energy intake levels greater/less than 1.5 times the IQR were considered outliers, however none of the girls met that criterion. Selected sample characteristics were summarized using mean and standard deviation for continuous variables, and frequencies/proportions for categorical variables. For analysis, tertiles of nutrient density values were created for each food and nutrient intake. Age adjusted means of

anthropometric measures BMI (kg/m^2), weight (kg), height (cm), fat mass (kg), percent body fat (%), waist circumference (cm), and hip circumference (cm) were compared across levels of nutrient/food intake using ANCOVA. Chi square tests were conducted for associations between Tanner stage and tertiles of intake.

Because the outcomes examined in this analysis are not rare in the population that gave rise to this sample, prevalence ratios (PR) using a Poisson model with robust variance analysis [122, 123] rather than odds ratios using logistic regression models, were used to estimate associations between the exposures and outcomes; odds ratios are known to overestimate effect sizes for outcomes that are not rare [124]. Covariates considered included girl's age, total energy, family income, race, and mother's education; however, due to the homogeneous sample and little variation in participants' demographic characteristics, only age and total energy were included in the final models; models for Tanner stage were further adjusted for BMI (continuous). In all analyses, the lowest tertile of intake was used as the reference. Linear trend tests were conducted by creating a variable based on the median value of each food and nutrient tertile among controls and testing it as a continuous variable in the regression model. All analyses were conducted using SAS, version 9.3 (SAS Institute, Cary NC).

Results

Table 1 presents demographic and descriptive characteristics of study participants. A total of 202 girls participated in our study. At baseline, 57% of girls were 9 years of age, and 43% were 10 years. A majority were white (88%), of healthy weight (73%) and had not reached menarche (97%). The education level of the girls' mothers was Bachelor's degree or higher in 80% of the sample. Fifty-three percent (53%) of 9 year

olds and 69% of 10 year olds had reached Tanner stage 2 or greater for breast development at baseline, and on average girls spent just over 5 hours per week outdoors.

We compared age-adjusted mean anthropometric measures according to levels of total dairy, total milk, dietary vitamin D and dietary calcium intake. As shown in Table 2, a statistically significant, non-linear relationship was found between body weight and total dairy intake (p=0.03), with girls in the middle tertile having higher mean body weight than girls in the lowest or highest tertile after adjusting for age. Other measures of body size and adiposity were also higher among girls in the middle versus the lowest and highest tertile of dairy intake, although differences were not statistically significant (i.e., BMI, height, fat mass, waist and hip circumference). Total milk intake was significantly related to several adiposity measures (Table 3), including higher BMI (p=0.02), higher mean body weight (p=0.005), higher mean fat mass (p=0.006), higher percent body fat (p=0.008), higher waist (p=0.01) and hip (p=0.008) circumference. Chi square analysis showed a statistically significant difference in proportion of girls at Tanner stage $\geq$2 across categories of total milk intake with 29%, 40% and 31% of girls at low, medium, and high milk intake respectively.

Anthropometric measures were not significantly related to dietary vitamin D intake, as shown in Table 4. Examining differences in mean anthropometric measures by calcium intake (Table 5) showed that girls with higher dietary calcium intake were significantly more likely to have lower mean BMI, fat mass, and percent body fat compared to girls with low dietary calcium intake, after adjusting for age. Sensitivity analysis further adjusting all models for total energy intake did not alter any of the findings (data not shown).

Multivariable analyses found no association between achieving Tanner stage ≥2 and total dairy or total cheese intake (Table 6). A non-linear association was found for girls in the middle tertile of total milk intake, but not the highest, having a significantly greater unadjusted (crude) PR compared to girls in the lowest tertile (PR:1.56; 95%CI: 1.13, 2.15), however this result was attenuated after adjusting for age, total energy and BMI. Neither dietary calcium nor dietary vitamin D were associated with reaching Tanner stage ≥2 in our analysis (Table 6).

As shown in Table 7, crude and adjusted prevalence ratios for the highest category of total milk intake showed a statistically significant positive association with prevalence of overweight and above compared to the lowest category (crude PR: 1.15; 95% CI:1.02, 1.28; adjusted PR: 1.34; 95% CI: 1.02, 1.27). Neither total dairy nor total cheese intake was associated with prevalence of overweight. Shown in Table 7, a non-linear, statistically significant positive association was found for dietary calcium with girls in the middle tertile, but not the highest having an increased prevalence of overweight and above versus the lowest tertile, although the association was weak and marginally significant (PR: 1.09; 95% CI: 1.02, 1.16). Our evidence did not support an association between dietary vitamin D and prevalence of overweight and above.

Discussion

In our study, girls with higher total dairy and total milk tended to have higher body size and adiposity, two known risk factors for early sexual development in girls [125], and girls with higher calcium tended to have lower measures of adiposity. Girls with higher dairy intake weighed more than girls with lower intake, and had the lowest fat-free mass. Higher milk intake was associated with higher body weight, fat mass, and

BMI, as well as greater percent body fat, waist and hip circumference. In contrast, higher dietary calcium intake was associated with lower BMI, lower fat mass, and lower percent body fat. Only total milk and dietary calcium were significantly associated with increased prevalence of overweight and above in multivariable analyses.

Evidence of a relationship between dairy intake and body size among girls 9-10 years of age is lacking, although some authors have reported effects similar to ours in groups that differ in age and composition. Similar to our results, a longitudinal study found no association between total dairy intake (servings/day) and BMI or percent body fat among girls followed from preadolescence through adolescence [126]. Carruth and Skinner reported a 3.5% decrease in body fat percentage with one serving of dairy per day in a longitudinal study among preschoolers [127]. In contrast, a cross-sectional analysis of 24-hour recall dietary data collected from 11 year old girls showed that those who consumed at least 3 servings of dairy per day had significantly lower BMI and percentage of body fat compared to girls who consumed fewer than 3 servings [128]. Another longitudinal study among pubertal girls ages 9 to 13 years found no difference in body weight or percent body fat between a group consuming a high-dairy diet and one consuming a usual diet [63]. Similarly, Wiley et al. reported no differences in BMI across quartiles of dairy intake (measured in kJ) among children 5-10 years of age [129]. These discrepancies may be due to inconsistencies in the definition of ‘dairy’ across studies with different combinations of dairy foods contributing disparate nutrient profiles, or differences in quantifying dairy exposure (i.e., grams/day, number of servings/day) [62].

Milk intake was significantly related to increased body size and higher measures of adiposity in our study, independent of total energy intake. This finding is supported by

Wiley et al., who found that children 5-10 years of age in the highest quartile of milk intake had BMI values that were significantly higher than children at the mid-quartile intake [129]. However, these results are in contrast to other studies that have shown an inverse association between milk consumption and BMI [25, 61] and a null relationship in children and adolescents [130]. Different measures of milk intake (grams/day vs. milk drinking status), differences in age and characteristics of study participants and control of different covariates across studies could be responsible for the differences in results.

We found dietary calcium intake to be associated with lower adiposity, similar to a finding by Novotny et al., who found that higher calcium intakes were associated with lower body fat among females ages 9-14 years of age; specifically, the authors found dairy calcium intake to be associated with a statistically significant decrease in iliac skinfold thickness [61]. Similarly, a longitudinal study that included girls 2 months to 8 years of age found a negative association between calcium and body fat as measured by DEXA. However, other studies reported no association between dairy calcium and BMI among children 5-10 yrs of age or adolescents [126, 129]. While a specific mechanism for calcium's role in obesity has not been elucidated, experimental evidence has suggested several possible hypotheses including regulation of energy metabolism, attenuation of adipocyte lipid accretion and weight gain in the presence of overconsumption of an energy-dense diet, and increased lipolysis and thermogenesis preservation during caloric restriction, potentially accelerating weight loss [131]. Our study found a weak, albeit statistically significant, increased risk for prevalence of overweight and above for the middle quartile of dietary calcium vs. the lowest which is inconsistent with other evidence [62].

The contradictory findings of higher adiposity with milk intake and lower adiposity with dietary calcium may be explained by several factors. There may have been unmeasured sources of dietary calcium from non-dairy foods (e.g., vegetables, fortified foods) among some girls in our study, which we were not able to capture since we did not quantify dairy versus non-dairy dietary calcium intake. Another factor may be unreported use of supplemental calcium among some girls, influencing results attributed to dietary calcium. Finally, given the large number of statistical comparisons in our analyses our results may be due to chance.

A primary strength of our study is the wide array of anthropometric measures collected by trained research staff, which enabled targeted estimation of associations between dairy food, vitamin D and calcium with specific measures of adiposity and body size, since body fat distribution rather than total body mass has been found to affect sex hormones in girls and subsequently puberty onset [125]. There is a dearth of research examining the association between dietary factors and risk for early sexual development in young girls, and to our knowledge ours is the first to study the association between dairy foods, dietary vitamin D and dietary calcium with thelarche in this US population.

There are several important limitations to our study. Our data are cross-sectional, and while statistical associations were found between dairy, milk, and dietary calcium with adiposity measures, causality cannot be assumed. Food and nutrient intakes assessed in our study were based on current consumption, and the extent to which they are representative of earlier dietary patterns that might have contributed to current body size is unknown. In some cases, intake was measured in units not commonly used (i.e., vitamin D in mcg/day vs. IU/day). Girls in our study were mainly from upper

socioeconomic levels and were majority white therefore our findings may not be generalizable beyond this population. Finally, our study included a sample of 200 girls and may have been too small to detect some associations.

In conclusion, this cross-sectional study among 9-10 year old girls suggests that dairy foods, particularly milk and dietary calcium, may affect body size and adiposity in this population which are well known risk factors for early puberty. As this study was cross-sectional, further longitudinal studies are needed for more definitive conclusions regarding the role of dairy foods and related nutrients in normal growth and development in young girls.

Table 1. Selected characteristics of participants, The Jersey Girl Study

	N=202 *n (%)*
Age at recruitment	
9 yrs	115 (57)
10 yrs	87 (43)
Mother's Education	
High school/some college	41 (20)
Bachelor's degree or higher	161 (80)
Girls' Race	
White	177 (88)
Other	25 (12)
Family's Income	
<100,000	47 (25)
≥100,000	140 (75)
BMI-for-age and gender (CDC definition)	
Underweight (<5th percentile)	12 (6)
Healthy weight (5th - <85th percentile)|	147 (73)
Overweight (85th percentile - <95th percentile)	21 (10)
Obese (> 95th percentile)	22 (11)
Physical Activity (hrs/week)	
0-< 3	19 (10)
≥3-<5	44 (22)
≥5	137 (68)
Puberty Stage at Recruitment	
Breast: Tanner B2+	
Age 9 yrs	
Yes	61 (53)
No	54 (47)
Age 10 yrs	
Yes	59 (69)
No	28 (31)
Menarche at recruitment	
Started menarche	5 (3)
Not started menarche	197 (97)
Hours per week spent outdoors, mean (±SD)	5.48 (±3.60)

Table 2. Participant characteristics by total dairy intake, The Jersey Girl Study

Total Dairy Intake[1] (g/day/1,000 kcal)	Low (n=67) <74.86	Medium (n=69) 74.86 -<172.13	High (n=66) >172.13	P Value[2]
Total energy (kcal/day) (mean ± SE)	1761.17 (47.01)	1691.06 (46.32)	1704.70 (47.36)	0.53
Age (yrs)	9.46	9.42	9.41	0.86
Tanner stage B2+(%)	63%	51%	65%	0.19
Anthropometrics (age adjusted means ±SE)				
BMI (kg/m^2)	17.78 (0.37)	18.29 (0.38)	18.07 (0.38)	0.64
Weight (kg)	34.49 (1.05)	36.41 (1.06)	35.63 (1.06)	0.03
Height (cm)	138.95 (0.92)	140.31 (0.94)	139.29 (0.94)	0.57
Fat mass (kg)	7.18 (0.65)	8.09 (0.65)	8.02 (0.65)	0.83
Fat-free mass (kg)	27.68 (0.49)	28.49 (0.49)	27.58 (0.48)	<0.001
Percent body fat (%)	19.72 (1.09)	20.21 (1.09)	20.46 (1.09)	0.94
Waist circumference (cm)	64.97 (1.08)	66.50 (1.09)	66.20 (1.09)	0.58
Hip Circumference (cm)	76.44 (0.94)	78.15 (0.95)	77.43 (0.95)	0.44
Waist-to-hip ratio	0.85 (0.006)	0.85 (0.006)	0.85 (0.006)	0.88

[1] Includes milk (whole, low fat and fat free), cheese (regular, low fat, reduced fat and fat free), yogurt (regular, low fat and fat free), frozen dairy dessert

[2] P values based on ANCOVA analyses for anthropometric measures and chi square for Tanner stage B2+

Table 3. Participant characteristics by total milk intake, The Jersey Girl Study

Total Milk Intake [1] (g/day/1,000 kcal)	Low (n=67) <35.05	Med (n=67) 35.05-<133.78	High (n=68) ≥133.78	P Value[2]
Total energy (kcal/day) (mean ± SE)	1698 (46.47)	1807.05 (46.47)	1651 .87 (46.13)	0.05
Age (yrs)	9.46	9.42	9.41	0.81
Tanner stage B2+ (%)	29%	40%	31%	0.04
Anthropometrics (age adjusted means ±SE)				
BMI (kg/m^2)	17.23 (0.37)	18.17 (0.37)	18.72 (0.37)	0.02
Weight (kg)	33.07 (1.02)	35.51 (1.03)	37.84 (1.02)	0.005
Height (cm)	138.06 (0.92)	139.25 (0.92)	141.16 (0.91)	0.06
Fat mass (kg)	6.41 (0.64)	7.54 (0.63)	9.25 (0.62)	0.006
Fat-free mass (kg)	26.99 (0.48)	28.13 (0.48)	28.56 (0.47)	0.06
Percent body fat (%)	17.76 (1.07)	19.80 (1.05)	22.94 (1.05)	0.008
Waist circumference (cm)	63.67 (1.07)	65.65 (1.07)	68.19 (1.06)	0.01
Hip Circumference (cm)	75.04 (0.93)	77.76 (0.92)	79.09 (0.92)	0.008
Waist-to-hip ratio	0.85 (0.006)	0.84 (0.006)	0.86 (0.006)	0.14

[1]Includes regular, low fat and fat free

[2]P values based on ANCOVA analyses for anthropometric measures and chi square for Tanner stage B2+

Table 4. Participant characteristics by dietary vitamin D intake, The Jersey Girl Study

Dietary vitamin D intake (mcg/day/1,000 kcal)	Low (n=70) <2.33	Medium (n=79) 2.33-<3.56	High (n=53) ≥3.56	P Value[1]
Total energy (kcal/day) (mean ± SE)	1748.44 (47.08)	1697.98 (46.39)	1710.39 (47.43)	0.73
Age (yrs)	9.57	9.28	9.44	0.003
Tanner stage B2+ (%)	56%	67%	52%	0.19
Anthropometrics (age adjusted means ±SE)				
BMI (kg/m^2)	17.78 (0.65)	18.56 (0.38)	17.78 (0.39)	0.27
Weight (kg)	35.00 (1.06)	37.04 (1.005)	34.39 (1.07)	0.19
Height (cm)	139.53 (0.94)	140.74 (0.93)	138.22 (0.94)	0.17
Fat mass (kg)	7.41 (0.65)	8.75 (0.65)	7.09 (0.65)	0.17
Fat-free mass (kg)	27.72 (0.49)	28.54 (0.49)	27.47 (0.49)	0.28
Percent body fat (%)	19.51 (1.09)	21.79 (1.09)	18.71 (1.09)	0.12
Waist circumference (cm)	64.81 (1.09)	67.99 (1.09)	64.78 (1.09)	0.06
Hip Circumference (cm)	76.73 (0.95)	78.91 (0.98)	76.30 (0.95)	0.12
Waist-to-hip ratio	0.84 (0.006)	0.86 (0.006)	0.85 (0.006)	0.15

[1]P values based on ANCOVA analyses for anthropometric measures and chi square for Tanner stage B2+

Table 5. Participant characteristics by dietary calcium intake, The Jersey Girl Study

Dietary calcium intake (mg/day/1,000 kcal)	Low (n=70) <519.94	Medium (n=65) 519.94-<655.83	High (n=67) ≥655.83	P Value[1]
Total energy (kcal/day) (mean ± SE)	1728.28 (46.11)	1724.97 (47.85)	1702.82 (47.13)	0.92
Age (yrs)	9.47	9.40	9.42	0.69
Tanner stage B2+ (%)	57%	61%	60%	0.87
Anthropometrics (age adjusted means ±SE)				
BMI (kg/m^2)	18.89 (0.37)	17.42 (0.38)	17.80 (0.37)	0.02
Weight (kg)	37.34 (1.04)	34.44 (1.06)	34.64 (1.05)	0.09
Height (cm)	140.07 (0.93)	139.72 (0.95)	138.75 (0.93)	0.59
Fat mass (kg)	9.05 (0.64)	7.13 (0.65)	7.10 (0.64)	0.05
Fat-free mass (kg)	28.72 (0.48)	27.51 (0.49)	27.51 (0.48)	0.13
Percent body fat (%)	22.48 (1.07)	18.83 (1.08)	18.80 (1.06)	0.02
Waist circumference (cm)	62.89 (1.07)	64.52 (1.09)	65.16 (1.08)	0.07
Hip Circumference (cm)	78.94 (0.93)	76.60 (0.95)	76.40 (0.94)	0.11
Waist-to-hip ratio	0.86 (0.006)	0.84 (0.006)	0.85 (0.006)	0.22

[1]P values based on ANCOVA analyses for anthropometric measures and chi square for Tanner stage B2+

Table 6. Prevalence ratios and 95% confidence intervals (CI) for breast Tanner Stage ≥2, dairy food and nutrient intake, The Jersey Girl Study

Dairy Food Intake (g/day/1,000 kcal)	BR1[1] N=82 *n(%)*	BR2+[1] N=120 *n(%)*	Crude PR (95% CI)	PR[2] (95% CI)
Total Dairy				
Low (<83.21)	28 (33)	44 (37)	REF	REF
Med (83.21-<167.51)	28 (34)	33 (28)	0.89 (0.66-1.89)	0.98 (0.75-1.30)
High (≥167.51)	26 (33)	43 (35)	1.01 (0.78-1.32)	0.99 (0.77-1.27)
P for trend				*0.13*
Total Milk				
Low (<8.91)	27 (33)	24 (20)	REF	REF
Med (8.91-155.60)	26 (32)	67 (56)	1.56 (1.13-2.15)	1.34 (0.99-1.81)
High (≥155.60)	29 (35)	29 (24)	1.10 (0.75-1.63)	0.96 (0.67-1.38)
P for trend				*0.15*
Total Cheese				
Low (<10.67)	27 (33)	42 (35)	REF	REF
Med (10.67-<22.30)	27 (33)	35 (29)	0.92 (0.68-1.23)	0.91 (0.69-1.18)
High (≥ 22.30)	28 (34)	43 (36)	1.00 (0.77-1.32)	1.03 (0.80-1.32)
P for trend				*0.71*
Dietary calcium (mg/day/1,000 kcal)				
Low (<543.89)	29 (35)	57 (47)	REF	REF
Med (≥543.89-<665.78)	26 (32)	29 (24)	0.79 (0.59-1.05)	0.99 (0.74-1.32)
High (≥665.78)	27 (33)	34 (29)	0.84 (0.64-1.09)	0.91 (0.70-1.16)
P for trend				*0.41*
Dietary vitamin D (mcg/day/1,000 kcal)				
Low (<2.34)	29 (35)	43 (35)	REF	REF
Med (2.34- <4.06)	26 (32)	50 (42)	1.09 (0.85-1.41)	1.13 (0.89-1.45)
High (≥4.06)	27 (33)	27 (23)	0.83 (0.60-1.16)	0.86 (0.64-1.16)
P for trend				*0.22*

PR, prevalence ratio

[1]Tanner stage for breast development at baseline

[2]Adjusted for age at recruitment (continuous), total energy (continuous), BMI (continuous)

Table 7. Prevalence ratios and 95% confidence intervals (CI) for overweight and above, dairy food and nutrient intake, The Jersey Girl Study

Dairy Food Intake (g/day/1,000 kcal)	Underweight/ Normal Weight N=159 *n(%)*	Overweight and Above[1] N=43 *n(%)*	Crude PR (95% CI)	PR[2] (95% CI)
Total Dairy				
Low (<73.22)	52 (33)	10 (23)	REF	REF
Med (73.22-<162.75)	54 (34)	12 (28)	1.02 (0.91-1.38)	1.00 (0.89-1.11)
High (≥162.75)	53 (33)	21 (49)	1.01 (0.99-1.24)	1.09 (0.97-1.21)
P for trend				*0.67*
Total Milk				
Low (<17.49)	52 (33)	7 (16)	REF	REF
Med (17.49-<124.65)	54 (34)	15 (35)	1.09 (0.98-1.21)	1.10 (0.99-1.22)
High (≥124.65)	53 (33)	21 (49)	1.15 (1.02-1.28)	1.34 (1.02-1.27)
P for trend				*0.05*
Total Cheese				
Low (<9.48)	52 (33)	14 (33)	REF	REF
Med (9.48-<22.43)	55 (34)	13 (30)	0.98 (0.88-1.11)	0.99 (0.89-1.11)
High (≥ 22.43)	52 (33)	16 (37)	1.02 (0.91-1.14)	1.03 (0.92-1.15)
P for trend				*0.58*
Dietary calcium (mg/day/1,000 kcal)				
Low (<530.04)	52 (33)	19 (44)	REF	REF
Med (≥530.04-<662.44)	52 (33)	13 (30)	0.95 (0.84-1.06)	1.09 (1.02-1.16)
High (≥662.44)	55 (34)	11 (26)	0.92 (0.03-1.04)	1.02 (0.96-1.08)
P for trend				*0.26*
Dietary vitamin D (mcg/day/1,000 kcal)				
Low (<2.26)	52 (33)	13 (30)		REF
Med (≤2.26- <3.07)	55 (34)	19 (44)	REF	1.02 (0.91-1.15)
High (≥3.07)	52 (33)	11 (26)	1.05 (0.94-1.18)	0.96 (0.93-1.16)
P for trend			0.98 (0.87-1.09)	*0.96*

[1] ≥85th percentile

[2] Adjusted for age at recruitment (continuous), total energy (continuous)

Summary and Public Health Implications

In this dissertation the relationship between dairy food and nutrient intake with the risk of ovarian and endometrial cancers among mostly post-menopausal women, and early sexual development in young girls, was evaluated. We found evidence to suggest that calcium may be protective against endometrial cancer risk and measures of adiposity in young girls. Ovarian and endometrial cancers are estrogen related cancers with few known modifiable risk factors, but diet and obesity may possibly play important roles in their onset [33, 43]. Diet may act directly on the risk of these diseases, or indirectly through its relationship with body weight. Dairy foods, calcium and vitamin D, in particular have been investigated for their association with risk of ovarian and endometrial cancers, producing mixed results. Dairy is a complex class of foods that contains bioactive substances capable of affecting growth and development, such as fats and IGF-1. They are a rich source of essential nutrients including vitamin D and calcium, and are hypothesized to reduce the risk of several cancers [13]. Dairy foods are also a source of animal derived dietary steroids and growth factors, which might have physiologic effects in humans [79]. Different components in dairy foods may target different physiologic systems with their actions, producing different effects at different points during a woman's life, and there is mounting evidence that environmental exposures including diet, at vulnerable periods of life such as puberty, may affect risk of disease in adulthood [111]. Diet and obesity have been implicated in early sexual development among girls, which itself is a risk factor for breast, ovarian and endometrial cancers later in life [48, 50, 111, 125, 132].

There are several public health implications of our findings. Dairy foods are a staple of the American diet, with per capita consumption in 2012 reaching 276 pounds per year [133]. More recently, the *2015-2020 Dietary Guidelines* reaffirmed the recommendation to consume 3 servings of dairy per day for persons 9 years of age and older, and 2 servings per day for children ages 1 to 8 years [88]. With national endorsement to consume these products for a lifetime, understanding their effects on normal growth and development as well as disease risk is important. While our results did not support an association between dairy foods or vitamin D with risk of endometrial cancer or onset of early sexual development among young girls, we confirmed associations between calcium and decreased risk of endometrial cancer, and reduced adiposity in young girls.

This dissertation represents an important addition to the literature: to my knowledge, it is the first to examine the association between dairy foods and nutrients with thelarche among young girls in the US. Future research needs to elucidate calcium's role in ovarian and endometrial cancer risk through studies that will inform nutritional interventions aimed at reducing obesity and cancer risk in later life. Additional studies examining calcium's possible role in mediating onset of puberty through maintenance of a healthy body weight are also warranted.

References

1. Barainca P, R.D., Clemens J, Moshfegh A *Vitamin D: intake of Americans from diet and supplements (632.10).* The FASEB Journal 28(1 Supplement), 2014.
2. Hoy MK, G.J., *Calcium intake of the U.S. population:What We Eat in America, NHANES 2009-2010.* 2014.
3. Garland, C.F., et al., *Role of ultraviolet B irradiance and vitamin D in prevention of ovarian cancer.* Am J Prev Med, 2006. **31**(6): p. 512-4.
4. Giovannucci, E., *The epidemiology of vitamin D and cancer incidence and mortality: a review (United States).* Cancer Causes Control, 2005. **16**(2): p. 83-95.
5. Tsiaras, W.G. and M.A. Weinstock, *Factors influencing vitamin D status.* Acta Derm Venereol, 2011. **91**(2): p. 115-24.
6. American Cancer Society , A.G. *Skin Cancer Protection and Early Prevention*. 2017; Available from: https://www.cancer.org/cancer/skin-cancer/prevention-and-early-detection/uv-protection.html.
7. Organization, W.H. *Sun Protection*. 2017; Available from: http://www.who.int/uv/sun_protection/en/.
8. Fulgoni, V., 3rd, et al., *Dairy consumption and related nutrient intake in African-American adults and children in the United States: continuing survey of food intakes by individuals 1994-1996, 1998, and the National Health And Nutrition Examination Survey 1999-2000.* J Am Diet Assoc, 2007. **107**(2): p. 256-64.
9. Nesby-O'Dell, S., et al., *Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994.* Am J Clin Nutr, 2002. **76**(1): p. 187-92.
10. Guéguen, L., *The Bioavailability of Calcium.* Journal of the American College of Nutrition, 2000. **19**(2).
11. Ross, C.A., *The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know.* J Clin Endocrinol Metab., 2011. **96**(1).
12. ARS. *What We Eat in America, NHANES 2011-2012*. 2015 [cited 2015 10/03/15]; Available from: http://www.ars.usda.gov/disclaim.html#access.
13. Pereira, P.C., *Milk nutritional composition and its role in human health.* Nutrition, 2014. **30**(6): p. 619-27.
14. Peterlik, M., *Calcium, vitamin D and Cancer.* ANTICANCER RESEARCH, 2009. **29**.
15. Krishnan, A.V. and D. Feldman, *Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D.* Annu Rev Pharmacol Toxicol, 2011. **51**: p. 311-36.
16. Ingraham, B., *Molecular basis of the potential of vitamin D to prevent cancer.* Current Medical Research and Opinions, 2008. **24**(1).
17. Lundqvist, J., *Vitamin D as a regulator of steriodogenic enzymes.* F1000Research, 2014. **3**(155): p. 1-10.
18. Genkinger, J.M., et al., *Dairy products and ovarian cancer: a pooled analysis of 12 cohort studies.* Cancer Epidemiol Biomarkers Prev, 2006. **15**(2): p. 364-72.
19. Peterlik, G., Cross, *Vitamin D and calcium in breast cancer risk.* Anticancer Research, 2009. **29**.
20. Davoodi, H., *Effects of milk and milk product consumption on cancer: a review.* Comprehensive Reviews in Food Science and Food Safety, 2013. **12**.
21. Shanmugalingam, T., et al., *Obesity and cancer: the role of vitamin D.* BMC Cancer, 2014. **14**: p. 712.

22. Simpson, E., *Aromatization of androgens in women: current concepts and findings.* Fertility and Sterility, 2002. **77**(4).
23. Zemel, Z., *Regulation of Adiposity and Obesity Risk By Dietary Calcium: Mechanisms and Implications.* Journal of the American College of Nutrition, 2002. **21**(2).
24. Harkness, L.S. and A.E. Bonny, *Calcium and vitamin D status in the adolescent: key roles for bone, body weight, glucose tolerance, and estrogen biosynthesis.* J Pediatr Adolesc Gynecol, 2005. **18**(5): p. 305-11.
25. Barba, G. and P. Russo, *Dairy foods, dietary calcium and obesity: a short review of the evidence.* Nutr Metab Cardiovasc Dis, 2006. **16**(6): p. 445-51.
26. Chen, M., et al., *Effects of dairy intake on body weight and fat: a meta-analysis of randomized controlled trials.* Am J Clin Nutr, 2012. **96**(4): p. 735-47.
27. Li, P., et al., *Effects of calcium supplementation on body weight: a meta-analysis.* Am J Clin Nutr, 2016.
28. Major, G., *Calcium plus vitamin D supplementation and fat mass loss in female very low-calcium consumers: potential link with a calcium-specific appetite control.* British Journal of Nutrition, 2009. **101**: p. 659-663.
29. Chandler, P.D., et al., *Effect of vitamin D supplementation alone or with calcium on adiposity measures: a systematic review and meta-analysis of randomized controlled trials.* Nutr Rev, 2015. **73**(9): p. 577-93.
30. Wang, W., Y. Wu, and D. Zhang, *Association of dairy products consumption with risk of obesity in children and adults: a meta-analysis of mainly cross-sectional studies.* Ann Epidemiol, 2016. **26**(12): p. 870-882 e2.
31. Lu, L., et al., *Long-term association between dairy consumption and risk of childhood obesity: a systematic review and meta-analysis of prospective cohort studies.* Eur J Clin Nutr, 2016. **70**(4): p. 414-23.
32. American Cancer Society , A.G. *Cancer Facts & Figures, 2017*. 2017 [cited 2017 July]; Available from: https://www.cancer.org/cancer/endometrial-cancer/about/key-statistics.html.
33. WCRF/AICR, *Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Endometrial Cancer.* 2013.
34. La Vecchia, C., et al., *Nutrition and diet in the etiology of endometrial cancer.* Cancer, 1986. **57**(6): p. 1248-53.
35. McCann, S.E., et al., *Diet in the epidemiology of endometrial cancer in western New York (United States).* Cancer Causes Control, 2000. **11**(10): p. 965-74.
36. Potischman, N., et al., *Dietary associations in a case-control study of endometrial cancer.* Cancer Causes Control, 1993. **4**(3): p. 239-50.
37. Salazar-Martinez, E., et al., *Dietary factors and endometrial cancer risk. Results of a case-control study in Mexico.* Int J Gynecol Cancer, 2005. **15**(5): p. 938-45.
38. Terry, P., et al., *Dietary factors in relation to endometrial cancer: a nationwide case-control study in Sweden.* Nutr Cancer, 2002. **42**(1): p. 25-32.
39. Tzonou, A., et al., *Dietary factors and the risk of endometrial cancer: a case--control study in Greece.* Br J Cancer, 1996. **73**(10): p. 1284-90.
40. Yeh, M., et al., *Higher intakes of vegetables and vegetable-related nutrients are associated with lower endometrial cancer risks.* J Nutr, 2009. **139**(2): p. 317-22.
41. Biel, R.K., et al., *Risk of endometrial cancer in relation to individual nutrients from diet and supplements.* Public Health Nutr, 2011. **14**(11): p. 1948-60.
42. ACS, *Cancer Facts and Figures.* 2016. **Atlanta: American Cancer Society; 2016**.

43. WCRF/AICR. *Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Ovarian Cancer 2014.* 2014; Available from: Available at http://www.dietandcancerreport.org/cup/cup_resources.php.
44. Larsson, S.C., N. Orsini, and A. Wolk, *Milk, milk products and lactose intake and ovarian cancer risk: a meta-analysis of epidemiological studies.* Int J Cancer, 2006. **118**(2): p. 431-41.
45. Merritt, M.A., et al., *Dairy foods and nutrients in relation to risk of ovarian cancer and major histological subtypes.* Int J Cancer, 2013. **132**(5): p. 1114-24.
46. Merritt, M.A., et al., *Dairy food and nutrient intake in different life periods in relation to risk of ovarian cancer.* Cancer Causes Control, 2014. **25**(7): p. 795-808.
47. Faber, M. and *Use of dairy products, lactose, and calcium and risk of ovarian cancer - results from a Danish case-control study.* Acta Oncologica, 2012. **51**.
48. De Stavola, B.L., et al., *Childhood growth and breast cancer.* Am J Epidemiol, 2004. **159**(7): p. 671-82.
49. Herman-Giddens, M.E., et al., *Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network.* Pediatrics, 1997. **99**(4): p. 505-12.
50. Jordan, S., *Height, Age at Menarche, and Risk of Epithelial Ovarian Cancer.* Cancer Epidemiol Biomarkers Prev, 2006. **14**(8).
51. Stoll, B.A., L.J. Vatten, and S. Kvinnsland, *Does early physical maturity influence breast cancer risk?* Acta Oncol, 1994. **33**(2): p. 171-6.
52. Uauy, R. and N. Solomons, *Diet, nutrition, and the life-course approach to cancer prevention.* J Nutr, 2005. **135**(12 Suppl): p. 2934S-2945S.
53. Rogol, A., *Growth and pubertal development in children and adolescents: effects of diet and physical activity.* Am J Clin Nutr, 2000. **72**.
54. Wiley, S., *Does milk make children grow? Relationships Between Milk Consumption and Height in NHANES 1999-2002.* AMERICAN JOURNAL OF HUMAN BIOLOGY, 2005. **17**: p. 425-441.
55. Ganmaa, D. and A. Sato, *The possible role of female sex hormones in milk from pregnant cows in the development of breast, ovarian and corpus uteri cancers.* Med Hypotheses, 2005. **65**(6): p. 1028-37.
56. Rich-Edwards, J.W., et al., *Milk consumption and the prepubertal somatotropic axis.* Nutr J, 2007. **6**: p. 28.
57. Barba, G., et al., *Inverse association between body mass and frequency of milk consumption in children.* Br J Nutr, 2005. **93**(1): p. 15-9.
58. Carwile, J.L., et al., *Milk Consumption after Age 9 Years Does Not Predict Age at Menarche.* J Nutr, 2015. **145**(8): p. 1900-8.
59. Gaskins, A.J., et al., *Dairy intake in relation to breast and pubertal development in Chilean girls.* Am J Clin Nutr, 2017. **105**(5): p. 1166-1175.
60. Berkey, C.S., et al., *Dairy consumption and female height growth: prospective cohort study.* Cancer Epidemiol Biomarkers Prev, 2009. **18**(6): p. 1881-7.
61. Novotny, R., et al., *Dairy intake is associated with lower body fat and soda intake with greater weight in adolescent girls.* Journal of Nutrition, 2004. **134**(8): p. 1905-1909.
62. Skinner, J., *Longitudinal calcium intake is negatively related to children's body fat indexes.* Am Diet Assoc, 2003. **103**: p. 1626-1631.
63. Chan, G., *Effects of dairy products on bone and body composition in pubertal girls.* J Pediatr Adolesc Gynecol, 1995. **126**: p. 551-556.

64. Lappe, J.M., et al., *Girls on a high-calcium diet gain weight at the same rate as girls on a normal diet: A pilot study.* Journal of the American Dietetic Association, 2004. **104**(9): p. 1361-1367.
65. Lorenzen, J., *Calcium supplementation for 1 y does not reduce body weight or fat mass in young girls.* Am J Clin Nutr, 2006. **86**: p. 18-23.
66. USDA. *HHS and USDA Release New Dietary Guidelines to Encourage Healthy Eating Patterns to Prevent Chronic Diseases*. 2016 Nov 2, 2016]; Available from: http://www.hhs.gov/about/news/2016/01/07/hhs-and-usda-release-new-dietary-guidelines-encourage-healthy-eating-patterns-prevent-chronic.html#.
67. NCI. *Cancer Stat Facts: Endometrial Cancer*. 2017; Available from: https://seer.cancer.gov/statfacts/html/corp.html.
68. Flegal, K.M., et al., *Trends in Obesity Among Adults in the United States, 2005 to 2014.* JAMA, 2016. **315**(21): p. 2284-91.
69. Finkelstein, E.A., et al., *Obesity and severe obesity forecasts through 2030.* Am J Prev Med, 2012. **42**(6): p. 563-70.
70. Research, W.C.R.F.A.I.o.C., *WCRF/AICR Systematic Literature Review Continuous Update Project: The Associations between Food, Nutrition and Physical Activity and the Risk of Endometrial Cancer.* 2012.
71. Bandera, E.V., et al., *Phytoestrogen consumption and endometrial cancer risk: a population-based case-control study in New Jersey.* Cancer Causes Control, 2009. **20**(7): p. 1117-27.
72. Block, G., *A DATA-BASED APPROACH TO DIET QUESTIONNAIRE DESIGN AND TESTING.* American Journal of Epidemiology, 1986. **124**(3).
73. USDA. *US Department of Agriculture National Nutrient Database for Standard Reference*. Available from: https://ndb.nal.usda.gov/ndb/.
74. FDA. *Title 21-Food and Drugs, Chapter 1- Food and Drug Administration, Department of Health and Human Services, Subchapter B-Food for Human Consumption, Part 101-Food Labeling, Section 101.12-Reference amounts customarily consumed per eating occasion. In: Food and Drug Administration Department of Health and Human Services, editor. CFR Title 21. USA.* 21 2017; Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.12; Revised as of April 1, 2017.
75. Bowman S, e.a., *My Pyramid Equivalents Database 2.0, for USDA Survey Foods 2003-2004: Documentation and User Guide. .* 2008.
76. Willett, W.C., *Nutritional Epidemiology*. Vol. second ed. 1998, New York, NY 10016: Oxford University Press.
77. Brunner, R.L., et al., *The effect of calcium plus vitamin D on risk for invasive cancer: results of the Women's Health Initiative (WHI) calcium plus vitamin D randomized clinical trial.* Nutr Cancer, 2011. **63**(6): p. 827-41.
78. Filomeno, M., et al., *Mediterranean diet and risk of endometrial cancer: a pooled analysis of three Italian case-control studies.* Br J Cancer, 2015. **112**(11): p. 1816-21.
79. Ganmaa, D., et al., *Milk, dairy intake and risk of endometrial cancer: a 26-year follow-up.* Int J Cancer, 2012. **130**(11): p. 2664-71.
80. Barbone, F., H. Austin, and E.E. Partridge, *Diet and endometrial cancer: a case-control study.* Am J Epidemiol, 1993. **137**(4): p. 393-403.
81. Goodman, M., *Diet, Body Size, Physical Activity, and the Risk of Endometrial Cancer.* Cancer Research, 1997. **57**.

82. Redaniel, M.T., et al., *The association of vitamin D supplementation with the risk of cancer in postmenopausal women.* Cancer Causes Control, 2014. **25**(2): p. 267-71.
83. Fader, A.N., et al., *Endometrial cancer and obesity: epidemiology, biomarkers, prevention and survivorship.* Gynecol Oncol, 2009. **114**(1): p. 121-7.
84. Kushi, L.H., *Prospective study of diet and ovarian cancer.* American Journal of Epidemiology, 1999. **149**(1).
85. Whittemore, A.S., *Characteristics relating to ovarian cancer risk: implications for prevention and detection.* Gynecol Oncol, 1994. **55**(3 Pt 2): p. S15-9.
86. Goodman, M.T., et al., *Association of dairy products, lactose, and calcium with the risk of ovarian cancer.* Am J Epidemiol, 2002. **156**(2): p. 148-57.
87. Koralek, D.O., et al., *Relationship between calcium, lactose, vitamin D, and dairy products and ovarian cancer.* Nutr Cancer, 2006. **56**(1): p. 22-30.
88. USDA, *Dietary Guidelines For Americans: 2015-2020.* 2016.
89. Endocrinologists, A.A.o., *AACE Postmenopausal Osteoporosis Guidelines.* Endocr Pract, 2016. **16**((Supp 3)).
90. Krishnan, A.V., *Mechanisms of the Anti-Cancer and Anti-Inflammatory Actions of Vitamin D.* Annu. Rev. Pharmacol. Toxicol, 2011. **51**.
91. Moukayed, M. and W.B. Grant, *Molecular link between vitamin D and cancer prevention.* Nutrients, 2013. **5**(10): p. 3993-4021.
92. Chandran, U., et al., *Healthy eating index and ovarian cancer risk.* Cancer Causes Control, 2011. **22**(4): p. 563-71.
93. Bandera, E.V., et al., *Phytoestrogen consumption from foods and supplements and epithelial ovarian cancer risk: a population-based case control study.* BMC Womens Health, 2011. **11**: p. 40.
94. Olson, S.H., et al., *Variants in hormone biosynthesis genes and risk of endometrial cancer.* Cancer Causes Control, 2008. **19**(9): p. 955-63.
95. Goodman, M.T., *Association of Dairy Products, Lactose, and Calcium with the Risk of Ovarian Cancer.* American Journal of Epidemiology, 2002. **156**(2): p. 148-157.
96. Pan, S.Y., et al., *A case-control study of diet and the risk of ovarian cancer.* Cancer Epidemiol Biomarkers Prev, 2004. **13**(9): p. 1521-7.
97. Merritt, M.A., et al., *Investigation of dietary factors and endometrial cancer risk using a nutrient-wide association study approach in the EPIC and Nurses' Health Study (NHS) and NHSII.* Cancer Epidemiol Biomarkers Prev, 2015. **24**(2): p. 466-71.
98. Qin, L.Q., *Milk/dairy products consumption, galactose metabolism and ovarian cancer: meta-analysis of epidemiological studies.* European Journal of Cancer Prevention, 2005. **14**(1).
99. WCRF/AICR, *Systematic Literature Review Continuous Update Project Report: The Associations between Food, Nutrition and Physical Activity and the Risk of Ovarian Cancer.* 2013.
100. Qin, B., et al., *Dairy, calcium, vitamin D and ovarian cancer risk in African-American women.* Br J Cancer, 2016. **115**(9): p. 1122-1130.
101. Hou, R., et al., *Dietary fat and fatty acid intake and epithelial ovarian cancer risk: evidence from epidemiological studies.* Oncotarget, 2015. **6**(40): p. 43099-119.
102. Cook, L.S., et al., *A systematic literature review of vitamin D and ovarian cancer.* Am J Obstet Gynecol, 2010. **203**(1): p. 70 e1-8.
103. Song, X., et al., *Calcium Intake and the Risk of Ovarian Cancer: A Meta-Analysis.* Nutrients, 2017. **9**(7).

104. Vine, M.F., et al., *Types and duration of symptoms prior to diagnosis of invasive or borderline ovarian tumor.* Gynecol Oncol, 2001. **83**(3): p. 466-71.
105. Fairfield, K.M., et al., *A prospective study of dietary lactose and ovarian cancer.* Int J Cancer, 2004. **110**(2): p. 271-7.
106. Cabrera, S.M., et al., *Age of thelarche and menarche in contemporary US females: a cross-sectional analysis.* J Pediatr Endocrinol Metab, 2014. **27**(1-2): p. 47-51.
107. Biro, F.M., et al., *Onset of breast development in a longitudinal cohort.* Pediatrics, 2013. **132**(6): p. 1019-27.
108. Berberoglu, M., *Precocious puberty and normal variant puberty: definition, etiology, diagnosis and current management.* J Clin Res Pediatr Endocrinol, 2009. **1**(4): p. 164-74.
109. Cesario, S.K. and L.A. Hughes, *Precocious puberty: a comprehensive review of literature.* J Obstet Gynecol Neonatal Nurs, 2007. **36**(3): p. 263-74.
110. Euling, S.Y., et al., *Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings.* Pediatrics, 2008. **121 Suppl 3**: p. S172-91.
111. Colditz, G.A. and A.L. Frazier, *Models of breast cancer show that risk is set by events of early life: prevention efforts must shift focus.* Cancer Epidemiol Biomarkers Prev, 1995. **4**(5): p. 567-71.
112. CDC, *Prevalence of Obesity Among Adults and Youth: United States, 2011–2014.* NCHS Data Brief, 2015. **219**.
113. Ogden, C.L. and K.M. Flegal, *Changes in terminology for childhood overweight and obesity.* Natl Health Stat Report, 2010(25): p. 1-5.
114. Gilbert-Diamond, D., *Vitamin D deficiency and anthropometric indicators of adiposity in school-age children: a prospective study.* Am J Clin Nutr. **2010**: p. 1446–51.
115. Maclure, M., et al., *A prospective cohort study of nutrient intake and age at menarche.* Am J Clin Nutr, 1991. **54**(4): p. 649-56.
116. Bandera, E.V., et al., *Urinary mycoestrogens, body size and breast development in New Jersey girls.* Sci Total Environ, 2011. **409**(24): p. 5221-7.
117. Bandera, E.V., *Assessing breast development in The Jersey Girl Study: agreement between physician and mom assessment.* American Journal of Epidemiology, 2010. **171**(S12).
118. Burrows, T.L., R.J. Martin, and C.E. Collins, *A systematic review of the validity of dietary assessment methods in children when compared with the method of doubly labeled water.* J Am Diet Assoc, 2010. **110**(10): p. 1501-10.
119. http://www.ncc.umn.edu/products/ndsr.html. *Nutrition Data System*. [cited 2015 October 9, 2015].
120. Harnack, L.J., *A computer-based approach for assessing dietary supplement use in conjunction with dietary recalls.* Food Compost Anal, 2008. **21**(S78–S82).
121. CDC. *BMI-for-Age Growth Charts*. 2000 [cited 2017 3/26/17]; Available from: http://www.cdc.gov/growthcharts.
122. Deddens, J.A. and M.R. Petersen, *Approaches for estimating prevalence ratios.* Occup Environ Med, 2008. **65**(7): p. 481, 501-6.
123. Spiegelman, D. and E. Hertzmark, *Easy SAS calculations for risk or prevalence ratios and differences.* Am J Epidemiol, 2005. **162**(3): p. 199-200.
124. Coutinho, L.M., M. Scazufca, and P.R. Menezes, *Methods for estimating prevalence ratios in cross-sectional studies.* Rev Saude Publica, 2008. **42**(6): p. 992-8.
125. Kaplowitz, P., *Earlier Onset of Puberty in Girls: Relation to Increased Body Mass Index and Race.* PEDIATRICS, 2001. **108**.

126. Phillips, S.M., et al., *Dairy food consumption and body weight and fatness studied longitudinally over the adolescent period.* Int J Obes Relat Metab Disord, 2003. **27**(9): p. 1106-13.
127. Carruth, B.R. and J.D. Skinner, *The role of dietary calcium and other nutrients in moderating body fat in preschool children.* Int J Obes Relat Metab Disord, 2001. **25**(4): p. 559-66.
128. Fiorito, L., *Girl's Dairy Intake, Energy Intake, and Weight Status.* J Am Diet Assoc., 2006. **106**(11): p. 1851-1855.
129. Wiley, A.S., *Dairy and milk consumption and child growth: Is BMI involved? An analysis of NHANES 1999-2004.* Am J Hum Biol, 2010. **22**(4): p. 517-25.
130. Murphy, M.M., et al., *Drinking flavored or plain milk is positively associated with nutrient intake and is not associated with adverse effects on weight status in US children and adolescents.* J Am Diet Assoc, 2008. **108**(4): p. 631-9.
131. Zemel, M.B., *Regulation of adiposity and obesity risk by dietary calcium: mechanisms and implications.* J Am Coll Nutr, 2002. **21**(2): p. 146S-151S.
132. Forman, M.R., et al., *Through the looking glass at early-life exposures and breast cancer risk.* Cancer Invest, 2005. **23**(7): p. 609-24.
133. USDA, E., *Trends in U.S. Per Capita Consumption of Dairy Products, 1970-2012.* Amber Waves, 2014.