Research Article

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Adolescent Diet and Subsequent Serum Hormones, Breast Density, and Bone Mineral Density in Young Women: Results of the Dietary Intervention Study in Children Follow-up Study

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Abstract

Background: Adolescent diet is hypothesized to influence breast cancer risk. We evaluated the long-term effects of an intervention to lower fat intake among adolescent girls on biomarkers that are related to breast cancer risk in adults.

Methods: A follow-up study was conducted on 230 girls who participated in the Dietary Intervention Study in Children (DISC), in which healthy, prepubertal, 8 to 10 year olds were randomly assigned to usual care or to a behavioral intervention that promoted a reduced fat diet. Participants were 25 to 29 years old at follow-up visits. All tests of statistical significance are two-sided.

Results: In analyses that did not take account of diet at the time of the follow-up visit, the only statistically significant treatment group difference was higher bone mineral content in intervention group participants compared with usual care group participants; their mean bone mineral contents were 2,444 and 2,377 g, respectively. After adjustment for current diet, the intervention group also had statistically significantly higher bone mineral density and luteal phase serum estradiol concentrations. Serum progesterone concentrations and breast density did not differ by treatment group in unadjusted or adjusted analyses.

Conclusions: Results do not support the hypothesis that consumption of a lower fat diet during adolescence reduces breast cancer risk via effects on subsequent serum estradiol and progesterone levels, breast density, or bone mineral density. It remains unclear, however, if the results are specific to the DISC intervention or are more broadly applicable.

Impact: Modest reductions in fat intake during adolescence are unlikely to lower later breast cancer risk via long-term effects on the biomarkers measured. *Cancer Epidemiol Biomarkers Prev; 19(6); 1545–56. ©2010 AACR.*

Introduction

Animal and ecologic studies strongly support a role for dietary fat in the etiology of breast cancer. Animals whose diets contain a higher percentage of fat develop significantly more tumors (1). International comparisons of breast cancer mortality rates suggest a strong positive association with per capita fat intake, particularly animal fat (2). In contrast, results of observational epidemiologic studies that have evaluated the role of dietary fat in breast cancer etiology are equivocal (3, 4). Furthermore, in the Women's Health Initiative Dietary Modification Randomized Controlled Trial that evaluated the effect of a low-fat dietary pattern, breast cancer incidence was nonsignificantly lower in the intervention group compared with the control group (5). One potential explanation for this disparity is that adult diet is not the most relevant exposure, but rather, diet during adolescence, when most breast development occurs, might be more important in relation to breast cancer etiology. This notion is supported by associations of surrogate markers of childhood diet with breast cancer risk. Specifically, age at menarche and height are established breast cancer risk factors that are influenced by childhood and adolescent diet (6-8). More direct evidence comes from the Nurses' Health Study II, a longitudinal observational study, which reported that adolescent energy intake and meat consumption were significantly and positively

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associated with later breast cancer risk, and vegetable fat was significantly and inversely associated with risk (9, 10). Even so, total fat and animal fat intake during adolescence were not associated with risk. Nonetheless, findings from studies in humans on the association between adolescent diet and breast cancer are inconsistent (11-14), possibly because these studies rely on recall of diet from the distant past, causing some individuals to be misclassified relative to dietary exposure, leading to biased results.

In an attempt to clarify the association of adolescent diet with breast cancer development, we conducted the Hormone Ancillary Study to the Dietary Intervention Study in Children (DISC) between 1990 and 1997. DISC was a multicenter randomized controlled clinical trial to test the safety and efficacy of a dietary intervention to reduce serum low-density lipoprotein cholesterol (LDL-C) in children with elevated LDL-C (15). The Hormone Ancillary Study evaluated the effects of the intervention on serum hormones that have been associated with an increased risk of breast cancer in adults (16). After 5 years on the intervention, at a mean age of 14 years, girls in the intervention group had significantly 20% to 30% lower serum estrogens during the follicular phase of the menstrual cycle compared with girls in the usual care group. Moreover, at the end of the study, which occurred at ~7 years following randomization and at a mean age of 16 years, intervention group girls' luteal phase serum progesterone levels were significantly 53% lower compared with usual care group girls. Estrogens and progesterone are both breast mitogens that have been associated with breast cancer risk in adults (17, 18), and our earlier findings are consistent with a potential protective effect of a low-fat diet during adolescence on development of breast cancer later in life.

Subsequently, we conducted the DISC06 Follow-up Study of female participants from 2006 to 2008 to determine the longer-term effects of the DISC intervention during childhood and adolescence on subsequent biomarkers that have been associated with breast cancer risk in adults, including serum levels of estrogens and progesterone, breast density, and bone mineral density (BMD). Results of that follow-up study are reported here.

Materials and Methods

Design

DISC was a multicenter randomized controlled clinical trial sponsored by the National Heart, Lung, and Blood Institute to test the safety and efficacy of a dietary intervention to reduce serum LDL-C in children with elevated LDL-C. The design and results of the trial have been described previously (15, 19-21). Briefly, between 1988 and 1990, 663 children 8 to 10 years old with elevated LDL-C were recruited into DISC at six clinical centers and randomized to intervention or usual care.[15] In 1990, the National Cancer Institute initiated a study ancillary to DISC to assess the effect of the reduced fat dietary intervention on serum sex hormones during adolescence. The initial DISC protocol was designed for 3 years of intervention and was subsequently extended with planned intervention and follow-up until participants reached 18 years of age. However, because LDL-C did not differ significantly between treatment groups after year-3 follow-up visits, the trial was terminated in 1997 when the mean age of participants was 16.7 years. In 2006 to 2008 when participants were 25 to 29 years old, the DISC06 Follow-up Study was conducted to evaluate the longer-term effects of the diet intervention on biomarkers associated with breast cancer in DISC female participants. Assent was obtained from DISC participants, and informed consent was obtained from their parents/guardians before randomization. Informed consent was obtained from participants again before the DISC06 follow-up visit. The original and follow-up DISC protocols were approved by Institutional Review Boards at all participating centers. A National Heart, Lung, and Blood Institute–appointed independent data and safety monitoring committee provided oversight during the conduct of the original trial.

Participants

Eligibility criteria for girls in the original DISC were as follows: (*a*) 7.8 to 10.1 years old, (*b*) serum LDL-C in the 80th to 98th percentiles (22), (*c*) no major illness or medication that could affect blood lipids or growth, (*d*) height ≥5th percentile and weight for height in the 5th to 90th percentile (according to growth data from the Bogalusa Hearth Study),[16] (*e*) Tanner stage I for breast and pubic hair (23), and (*f*) normal psychosocial and cognitive development as evaluated by progress in school and the Achenbach Child Behavior Checklist (24). Girls were excluded if they or family members were already following a low-fat diet, a parent had a history of early heart disease, the family planned to move within 3 years, or if the child had known behavioral problems. DISC participants were originally recruited through schools, health maintenance organizations, and pediatric practices. Follow-up contacts were initially made with parents or guardians using contact information collected in the original study. Participants who had moved were traced using public records.

All female DISC participants were invited to participate in the DISC06 Follow-up Study, and 260 (86.4%) of the 301 females originally randomized in DISC participated. Women who were pregnant or breast-feeding at the time

[15] DISC clinical centers were located at Childrens' Hospital (New Orleans, LA), Johns Hopkins University (Baltimore, MD), Kaiser Permanente Center for Health Research (Portland, OR), New Jersey Medical School (Newark, NJ), Northwestern University Medical School (Chicago, IL), and University of Iowa (Iowa City, IA), and the DISC coordinating center was located at Maryland Medical Research Institute (Baltimore, MD).
[16] L.S. Weber, personal communication.

of the visit completed questionnaires and gave a blood sample for DNA but did not provide blood for hormone analyses or complete dual-energy X-ray absorptiometry (DXA) or breast magnetic resonance imaging (MRI) exams ($n = 25$). Thus, they are not included in results reported here. Women who completed a pregnancy or breast-feeding within 12 weeks before the visit also were not included in analyses ($n = 5$).

Intervention

DISC dietary goals were to limit total fat to 28% of calories with <8% saturated fat, ≤9% polyunsaturated fat, with the remainder from monounsaturated fat. Dietary counseling was focused on reducing saturated fat intake. Cholesterol intake was limited to 75 mg/1,000 kcal, not to exceed 150 mg/d. Dietary fiber intake was encouraged with an emphasis on fruits/vegetables and whole grains. To ensure safety of the intervention diet to promote growth and development, nutritional adequacy for the intervention group in terms of intakes of energy, protein, calcium, iron, zinc, and vitamins A, C, and B_6 was monitored locally during the trial, and deficiencies and excesses were discussed with participants and family members. The intervention was implemented through a series of individual and group sessions led by nutritionists and behaviorists, where participants and their families learned how to achieve the dietary goals (25, 26). The usual care group was given educational materials generally available to the public on heart-healthy eating. The effects of the DISC intervention on intakes of fat, cholesterol, fiber, and selected micronutrients have been reported previously (16, 19-21). There was no programmatic intervention during the 9 years between the end of the original DISC trial and the follow-up visit.

Data collection

Each participant attended a single clinic visit between 2006 and 2008. These follow-up data collection visits were timed to occur in the luteal phase of the menstrual cycle, 1 to 14 days before the anticipated start of next menses. Data were collected at visits by trained staff masked to intervention assignment. Heights and weights were measured using a standardized protocol. Participants completed questionnaires that covered the following topics: demographic characteristics, medical history, reproductive and menstrual histories, prescription and nonprescription drug use including extensive information on past and current hormone use, health habits including smoking and alcohol use, and family history of breast cancer. Leisure physical activity was assessed using a historical version of the Modifiable Activity Questionnaire (27). Data from three nonconsecutive 24-hour dietary recalls collected over 2 weeks were averaged to estimate nutrient intakes based on the Nutrition Data System for Research developed at the University of Minnesota. Participants completed menstrual cycle calendars following visits for up to 6 weeks until the start of their next menses.

A centralized data collection training session was held before initiation of data collection to train and certify individuals responsible for the different types of data collection, except BMD and breast density (described below). Each clinical center had at least one person centrally trained and certified to collect each type of data who trained and certified others at their center locally.

Blood sampling

Hormones were measured in serum that was collected in the morning after an overnight fast by venipuncture using standard procedures. Blood was allowed to stand at room temperature for 45 minutes to allow complete clotting. Blood was then centrifuged and serum was separated and pipetted in 0.5-mL aliquots into cryovials. Cryovials were labeled and stored at −80°C.

Hormone measurements

All hormone analyses were done at the Reproductive Endocrine Research Laboratory, University of Southern California Keck School of Medicine. Serum samples from intervention and usual care group participants were randomly organized within batches to insure balance across batches. The laboratory was blinded to treatment group assignment. Estradiol and progesterone were quantified by specific RIAs following extraction and Celite column partition chromatography, as described previously (28, 29). Sex hormone-binding globulin (SHBG) was measured by a chemiluminescent immunoassay on the Immulite analyzer (Siemens Medical Solutions Diagnostics) to allow calculation of bioavailable (free plus albumin bound) estradiol (30). The coefficients of variation for estradiol, progesterone, and SHBG were 14.7%, 7.8%, and 3.7%, respectively.

Breast density

Breast density was measured using noncontrast MRI. Each subject was imaged in a whole-body 1.5 Tesla or higher field strength MRI scanner using a dedicated breast imaging radiofrequency coil. The following two pulse sequences were done in the transaxial and coronal orientations with a 32- to 40-cm field of view for bilateral coverage: (*a*) three-dimensional fast gradient echo sequence without fat saturation and (*b*) three-dimensional fast gradient echo sequence with fat saturation.

All MRI image data were processed at the University of California at San Francisco (UCSF) by the same investigator (C.K.) using customized image processing software to identify the chest wall–breast tissue boundary and skin surface and to separate breast fibroglandular and fatty tissue (31). Total volumes of fibroglandular and fatty tissue were computed separately for each breast. In problematic cases, such as those with incomplete or failed fat saturation, manual delineation was used. Breast density was expressed either as "percent of dense breast tissue" (ratio fibroglandular volume over total volume of the breast) or as "volume of dense tissue" (fibroglandular volume).

To insure accuracy and uniformity of data acquisition at the different clinical centers, MRI technologists at the sites were individually trained (by C.K.) to recognize and correct failures due to incomplete fat suppression, motion artifacts, and inadequate breast coverage. In addition, acceptable image quality on three volunteers was required for site certification. Participant scans that were inaccurate due to artifacts, motion, or technique were excluded (n = 21).

Bone densitometry

Bone density was measured using clinical DXA protocols. Scans were acquired of the lumbar spine (L1-L4), proximal femur, and whole body at default scan speeds on Hologic (Hologic, Inc.) and GE Lunar (General Electric/Lunar) systems. All DXA image data were processed centrally at the UCSF by trained staff coordinated by one of the investigators (J.A.S.). Centralized analyses were done using the manufacturers' software (Hologic 12.4; Lunar Prodigy 11.4) on each scan following the guidelines of the International Society for Clinical Densitometry. Bone mineral content (BMC; g), bone area (AREA; cm^2), and areal BMD (BMD = BMC/AREA; g/cm^2) were reported for whole body only in this report.

Different DXA systems were used at the six DISC clinical centers. To reduce site-specific bias from pooled results, static calibration objects (i.e., phantoms) were scanned on each system including the Hologic spine, hip, block, and whole-body phantoms. Using this phantom data, systems of the same make and model were cross-calibrated for BMD, BMC, and AREA to one reference study site. Intermanufacturer calibration for spine and hip was accomplished using the *in vivo* universal standardization equations (32, 33). Intermanufacturer whole-body results were cross-calibrated using equations derived by UCSF from unpublished data. Furthermore, because DXA systems can have calibration drifts, device-specific spine and whole-body phantoms were scanned routinely throughout the course of the study to allow correction for any calibration drifts. To insure accuracy and uniformity of data acquisition at the different clinical sites, all DXA personnel were trained by UCSF personnel on the protocol, patient positioning, data transfer, and phantom scanning procedures. Each site then recruited five volunteers who were scanned according to the study protocol. The clinical center was not certified to recruit study participants until their test data met the quality assurance standards of UCSF. Over the course of this study, 10 scans of the total 666 scans acquired were excluded by UCSF because of artifacts, motion, or poor positioning.

Statistical analysis

All analyses were done by randomized treatment assignment. Serum hormone and breast density data were transformed to the $\log_e$ scale before analysis to improve normality. BMD and BMC data were normally distributed and analyzed on the original scale. Linear mixed-effects models were fit by maximum likelihood separately for each outcome to calculate adjusted mean values and to test the statistical significance of differences between treatment groups. Clinic was included in all models as a random effect; all other variables were included as fixed effects. Adjusted models included a continuous term for body size/composition selected on the basis of prior knowledge and empirical evidence: body mass index (BMI = weight in kg/height in m^2) was included in serum hormone models, whole-body percent fat from DXA was included in breast density models, and height and whole-body total mass from DXA were included in bone density models. All adjusted models also included terms for age at randomization (continuous), age at visit (continuous), BMI at baseline (Z-score, continuous), race (white, non-white), education (high school or vocational school, some college, bachelor's degree, graduate degree), smoking status (never, former, current), leisure physical activity in metabolic equivalents per hour per week (MET-h/wk, continuous) at 14 to 17 years old and separately during the past year, number of full-term pregnancies (continuous), and use of hormonal contraceptives. Duration of hormone use was more strongly related to serum hormone concentrations and to breast density and was included in these models as a continuous term. Status of hormone use (never, former, current) was more strongly related to BMC and BMD and was included in these models as a categorical variable. Serum hormone models also included days until start of next menses modeled using a cubic spline, whereas bone density models included a term for type of DXA machine used (Hologic, GE Lunar). Age at menarche and history of having breast-fed an infant were not associated with serum hormones, breast density, or BMD after adjustment for the aforementioned variables and were not included in final models. Models adjusted for current diet included continuous terms for energy intake (kcal/d), saturated fat (% kcal), monounsaturated fat (% kcal), polyunsaturated fat (% kcal), cholesterol (mg/1,000 kcal), soluble fiber (g/1,000 kcal), and insoluble fiber (g/1,000 kcal). BMC and BMD models also included continuous terms for current calcium (mg) and vitamin D (μg) from food and dietary supplements. Tests of statistical significance were done using two-sided tests. A large number of statistical tests were done and care should be taken in interpretation of P values. All analyses were conducted using STATA10.1 and SAS 9.2.

Results

A total of 260 of the 301 DISC female participants participated in the DISC06 Follow-up Study. Of these, 30 were pregnant or breast-feeding within 12 weeks before or at their visits and were excluded from analyses. Characteristics of the remaining 230 participants are shown by DISC treatment group in Table 1. Approximately 90% of participants were white, 6% were black, and the remainder were other races. Participants' ages ranged from 24.9

Table 1. Characteristics of DISC intervention and usual care group participants at DISC06 follow-up visits

Characteristics	Intervention group (n = 118)	Usual care group (n = 112)	P*
Continuous variables	**Mean ± SD**	**Mean ± SD**	
Age at visit (y)	27.3 ± 1.0	27.2 ± 1.1	0.46
Age at randomization (y)	9.2 ± 0.6	9.2 ± 0.6	0.67
Height (cm)	165.1 ± 6.3	165.2 ± 6.1	0.90
Weight (kg)	69.5 ± 16.1	68.3 ± 15.0	0.54
BMI at visit (kg/m^2)	25.5 ± 5.7	25.0 ± 5.0	0.45
BMI at baseline (Z-score)	0.2 ± 0.9	0.2 ± 0.9	0.84
Age at menarche (y)	12.9 ± 1.2	12.9 ± 1.2	0.83
No. full-term pregnancies (n = 57)	1.7 ± 1.0	1.4 ± 0.6	0.17
Age at first full-term pregnancy (n = 57)	22.9 ± 3.2	22.7 ± 2.8	0.84
Duration breast-fed, wk (n = 49)	29.4 ± 29.7	39.0 ± 31.5	0.27
Duration hormonal contraceptive use (y)	5.5 ± 3.7	5.0 ± 3.6	0.41
Categorical variables	***n* (%)**	***n* (%)**	$P^{\dagger}$
Race			0.58
White	109 (92.4)	99 (88.4)	
Black	6 (5.1)	8 (7.1)	
Other	3 (2.5)	5 (4.5)	
Education			0.19
High school	6 (5.1)	8 (7.1)	
Vocational or technical school	5 (4.2)	6 (5.4)	
Some college	32 (27.1)	17 (15.2)	
Bachelor's degree	54 (45.8)	64 (57.1)	
Graduate degree	21 (17.8)	17 (15.2)	
Marital status			0.47
Single	61 (51.7)	65 (58.0)	
Married or living as married	51 (43.2)	44 (39.3)	
Separated or divorced	6 (5.1)	3 (2.7)	
Ever full-term pregnancy	27 (22.9)	30 (26.8)	0.49
Ever breast-fed	25 (21.2)	24 (21.4)	0.96
Menstrual cycle phase at visit			0.70
Luteal (1-14 d before menses)	99 (83.9)	90 (80.4)	
Follicular (15-34 d before or on day of menses)	9 (7.6)	10 (8.9)	
Long cycle (35+ d before menses)	3 (2.5)	6 (5.4)	
Missing	7 (5.9)	6 (5.4)	
Hormonal contraceptive use			0.76
Never	7 (5.9)	9 (8.0)	
Former	46 (39.0)	40 (35.7)	
Current	65 (55.1)	63 (56.3)	
Smoking status			0.55
Never	59 (50.0)	64 (57.1)	
Former	25 (21.2)	21 (18.8)	
Current	34 (28.8)	27 (24.1)	
Alcohol consumption status			0.38
Never	3 (2.5)	7 (6.2)	
Former	6 (5.1)	5 (4.5)	
Current	109 (92.4)	100 (89.3)	
History of breast cancer in mother	3 (2.7)	5 (4.5)	0.72

*P values from Student's t test.
†P values from Fisher's exact test or χ^2 test.

Table 2. Median daily nutrient intakes and leisure physical activity levels of DISC intervention and usual care group participants at DISC06 follow-up visits

Nutrient intake	Intervention group (n = 111)	Usual care group (n = 107)	P*
	Median (IQR)	Median (IQR)	
Energy (kcal)	1,602 (1,348-1,989)	1,710 (1,423-2,093)	0.13
Total fat (% kcal)	30.2 (26.2-35.5)	32.2 (28.6-35.9)	0.06
Saturated fat (% kcal)	9.8 (7.9-12.0)	11.3 (9.5-13.3)	<0.001
Polyunsaturated fat (% kcal)	6.6 (5.4-8.2)	6.2 (5.4-7.3)	0.18
Monounsaturated fat (% kcal)	11.1 (9.0-13.0)	11.8 (10.2-13.6)	0.07
Protein (% kcal)	16.4 (13.7-19.8)	16.4 (13.8-19.1)	0.57
Carbohydrate (% kcal)	51.8 (45.5-56.7)	49.9 (42.7-56.3)	0.13
Cholesterol (mg/1,000 kcal)	112.8 (77.7-152.2)	129.5 (87.5-172.6)	0.06
Alcohol (g)	0.4 (0.0-11.0)	0.3 (0.0-14.7)	0.21
Dietary fiber (g/1,000 kcal)	8.9 (6.3-11.2)	7.3 (5.8-10.5)	0.01
Calcium from food (mg)	771.9 (584.5-984.8)	781.5 (607.8-1092.3)	0.40
Calcium from food and supplements (mg)	852.0 (646.0-1060.8)	871.9 (653.6-1185.4)	0.26
Vitamin D from food (μg)	2.9 (1.4-4.9)	3.5 (2.0-5.3)	0.06
Vitamin D from food and supplements (μg)	4.0 (2.1-6.3)	4.6 (2.8-8.2)	0.06
Phytoestrogens from food (mg)	0.6 (0.3-3.2)	0.6 (0.3-2.2)	0.97
Physical activity	**Intervention group (n = 118)**	**Usual care group (n = 112)**	**P***
	Median (IQR)	Median (IQR)	
Leisure physical activity in past year (MET-h/wk)	22.5 (8.4-36.2)	18.5 (8.1-36.7)	0.68
Leisure physical activity at 14-17 y old (MET-h/wk)	40.4 (14.8-75.8)	31.0 (11.0-66.5)	0.29

Abbreviation: IQR, interquartile range.
*P values from Wilcoxon signed-rank test.

to 29.7 years and did not differ by treatment group. Intervention and usual care group participants also did not differ in adiposity, menstrual, and reproductive characteristics, including age at menarche, number of full-term pregnancies, and hormonal contraceptive use, or by cigarette smoking status. Every attempt was made to schedule DISC06 follow-up visits in the luteal phase of the menstrual cycle; 84% of intervention group participants' visits and 80% of usual care group participants' visits took place during the luteal phase.

Current diet composition at the DISC06 follow-up visit for the 218 participants who completed dietary recalls is shown in Table 2. Intervention group participants' median reported energy intake was 1,602 kcal/d, which was slightly, but not statistically significantly, less than the median of 1,710 kcal/d reported by usual care group participants. The intervention group reported consuming a statistically significantly lower percent of calories as saturated fat compared with the usual care group; their median intakes were 9.8% and 11.3%, respectively. The intervention group also reported consuming a slightly lower percent of calories as total fat and monounsaturated fat and slightly less cholesterol/1,000 kcal compared with the usual care group. In contrast, the intervention group reported consuming statistically significantly more dietary fiber compared with the usual care group. Reported intakes of polyunsaturated fat, protein, and carbohydrates as percentages of calories did not differ by treatment group. Self-reported leisure-time physical activity levels at ages 14 to 17 years and over the past 12 months also did not differ.

Estradiol, progesterone, and SHBG were measured in serum from 87 participants who did not take hormonal contraceptives during the month before their DISC06 follow-up visit and who were in the luteal phase of their menstrual cycle. Unadjusted and adjusted mean hormone and SHBG concentrations are shown by treatment group in Table 3. Unadjusted serum estradiol concentrations did not differ substantially by treatment group. After adjustment for nondietary variables, the intervention group's mean estradiol concentration was 131.3 pg/mL [95% confidence interval (CI), 101.8-169.4 pg/mL], which was higher than the mean of 114.6 pg/mL (95% CI, 87.4-150.4 pg/mL) for the usual care group, but the difference was not statistically significant (P = 0.40). The difference became significant (P = 0.02), however, after further adjustment for current dietary fat and fiber intake; diet-adjusted mean luteal phase estradiol concentrations were 141.5 pg/mL (95% CI, 116.1-172.4 pg/mL) and 96.7 pg/mL (95% CI, 77.1-121.2 pg/mL) for the intervention and usual care groups, respectively. A similar pattern was observed for non–SHBG-bound estradiol such that significant treatment

differences were observed only after adjustment for current diet. No treatment group differences were observed for progesterone or SHBG in unadjusted or adjusted analyses.

Treatment group differences in breast density were evaluated after excluding 13 scans on women who had breast implants or breast reduction surgery. Results based on the 182 participants who had scans available for inclusion in analysis are summarized by treatment group in Table 4. Breast density expressed as the percent of breast tissue that was dense or the volume of dense tissue did not differ significantly by treatment group in adjusted or unadjusted analyses. In unadjusted analyses, mean percent densities were 21.2% (95% CI, 17.1-26.3%) and 16.7% (95% CI, 13.6-20.5%) for intervention group and usual care group participants, respectively (P = 0.12). After adjustment for nondietary variables, intervention group participants' mean percent density was 20.2% (95% CI, 17.5-23.3%) compared with 18.3% (95% CI, 16.0-21.0%) for usual care group participants (P = 0.35). Further adjustment for current dietary fat and fiber intake did not alter results in a meaningful way. Results were similar when breast density was expressed as volume of dense tissue.

Whole-body BMC and BMD for 215 participants who had acceptable DXA scans are summarized in Table 5. Whole-body BMC did not differ by treatment group in unadjusted analyses. However, after adjustment for nondietary variables, intervention group participants had statistically significantly (P = 0.02) higher BMC compared with usual care group participants; their mean BMCs were 2,444 g (95% CI, 2,389-2,499 g) and 2,377 g (95% CI, 2,321-2,432 g), respectively. Further adjustment for current dietary fat, fiber, calcium, and vitamin D intake increased the statistical significance of the treatment group difference (P = 0.003); diet-adjusted mean BMC was 2,448 g (95% CI, 2,395-2,502 g) for intervention group participants compared with 2,356 g (95% CI, 2,302-2,410 g) for usual care group participants. Treatment group differences in whole-body BMD generally showed the same pattern observed for BMC.

Women who were pregnant or breast-feeding within 3 months of the visit were not included in the current analysis because of concerns about the potential effects of a recent pregnancy or breast-feeding on biomarkers investigated, particularly serum hormone levels and breast density. To evaluate potential longer-term effects of pregnancy and breast-feeding, we repeated analyses limited

Table 3. Geometric mean and 95% CI for serum hormone concentrations at follow-up in DISC intervention and usual care groups

	Intervention group		Usual care group		*P**
	n	Mean (5-95% CI)	*n*	Mean (5-95% CI)	
Progesterone (ng/mL)					
Unadjusted	47	3.7 (2.3-6.1)	40	4.9 (2.9-8.4)	0.47
Adjusted (excluding current diet)†	46	4.8 (3.2-7.2)	39	4.4 (2.8-6.8)	0.79
Adjusted (including current diet)‡	45	4.7 (3.1-7.1)	36	3.9 (2.4-6.2)	0.57
Total estradiol (pg/mL)					
Unadjusted	47	120.4 (90.1-160.8)	40	115.6 (84.9-157.3)	0.84
Adjusted (excluding current diet)†	46	131.3 (101.8-169.4)	39	114.6 (87.4-150.4)	0.40
Adjusted (including current diet)‡	45	141.5 (116.1-172.4)	36	96.7 (77.1-121.2)	0.02
Non–SHBG-bound estradiol (pg/mL)					
Unadjusted	46	87.9 (71.8-107.5)	40	80.8 (65.4-99.8)	0.55
Adjusted (excluding current diet)†	45	93.2 (79.2-109.7)	39	78.6 (66.0-93.7)	0.18
Adjusted (including current diet)‡	44	97.6 (84.1-113.2)	36	67.1 (56.7-79.4)	0.003
SHBG (nmol/L)					
Unadjusted	46	50.3 (42.6-59.4)	40	50.0 (41.8-59.8)	0.97
Adjusted (excluding current diet)†	45	52.8 (46.0-60.5)	39	47.1 (40.7-54.6)	0.29
Adjusted (including current diet)‡	44	51.7 (44.9-59.4)	36	48.1 (41.0-56.4)	0.55

*P values from Wald test using linear mixed-effects regression.

†Adjusted for BMI at visit, BMI at baseline (Z-score), age at visit, age at randomization, race, smoking status, education, leisure physical activity at 14 to 17 y old and during the past year, days until next menses (cubic spline), duration of hormonal contraceptive use, and number of full-term pregnancies.

‡Adjusted for BMI at visit, BMI at baseline (Z-score), age at visit, age at randomization, race, smoking status, education, leisure physical activity at 14 to 17 y old and during the past year, days until next menses (cubic spline), duration of hormonal contraceptive use, number of full-term pregnancies, current dietary intake of energy (kcal), % saturated fat, % monounsaturated fat, % polyunsaturated fat, cholesterol (mg/1,000 kcal), insoluble fiber (g/1,000 kcal), and soluble fiber (g/1,000 kcal).

Table 4. Geometric mean and 95% CI for breast density at follow-up in DISC intervention and usual care groups

	Intervention group		Usual care group		*P**
	n	Mean (5-95% CI)	*n*	Mean (5-95% CI)	
Percent dense tissue (%)					
Unadjusted	87	21.2 (17.1-26.3)	95	16.7 (13.6-20.5)	0.12
Adjusted (excluding current diet)†	84	20.2 (17.5-23.3)	92	18.3 (16.0-21.0)	0.35
Adjusted (including current diet)‡	82	19.7 (17.0-22.7)	89	18.3 (15.9-21.0)	0.51
Volume of dense tissue (mm^3)					
Unadjusted (mm^3)	87	86,046 (70,990-104,296)	95	73,338 (60,801-88,460)	0.20
Adjusted (excluding current diet)†	84	83,307 (70,138-98,947)	92	74,788 (63,351-88,289)	0.34
Adjusted (including current diet)‡	82	79,460 (67,882-93,012)	89	75,382 (64,848-87,626)	0.65

*P values from Wald test using linear mixed-effects regression.
†Adjusted for % body fat from DXA, BMI at baseline (Z-score), age at visit, age at randomization, race, smoking status, education, leisure physical activity at 14 to 17 y old and during the past year, duration of hormonal contraceptive use, and number of full-term pregnancies.
‡Adjusted for % body fat from DXA, BMI at baseline (Z-score), age at visit, age at randomization, race, smoking status, education, leisure physical activity at 14 to 17 y old and during the past year, duration of hormonal contraceptive use, number of full-term pregnancies, current intake of energy (kcal), % saturated fat, % monounsaturated fat, % polyunsaturated fat, cholesterol (mg/1,000 kcal), insoluble fiber (g/1,000 kcal), and soluble fiber (g/1,000 kcal).

to women who had not been pregnant or breast-feeding within 6 months before the visit and again restricted to women who had not been pregnant or breast-feeding within 12 months before the visit. Results of these subset analyses did not differ substantially from those presented for any of the biomarkers.

Discussion

Adolescent diet has been hypothesized to influence breast cancer risk. Methodologic difficulties due to the long duration between exposure and disease onset and problems with recall of diet in the distant past have hindered direct assessment of the association. As an alternative strategy, we conducted the DISC06 Follow-up Study to determine the long-term effects of the DISC intervention to reduce fat intake during childhood and adolescence on breast cancer–related biomarkers in female participants at 25 to 29 years of age. In analyses that did not take account of diet at the time of the follow-up visit, the only statistically significant treatment group difference was higher mean whole-body BMC in intervention group participants compared with usual care group participants. After adjustment for current dietary intakes of fat, fiber, calcium, and vitamin D, intervention group participants also had significantly higher mean whole-body BMD compared with usual care group participants. Furthermore, mean luteal phase serum total estradiol and non–SHBG-bound estradiol concentrations were statistically significantly higher in intervention group participants compared with usual care group participants after adjustment for current dietary fat and fiber intake. Serum progesterone concentrations and breast density did not differ by treatment group in unadjusted or adjusted analyses. Results do not provide strong evidence to support positive associations of adolescent diet with biomarkers related to increased breast cancer risk in young adults.

We investigated the long-term effects of childhood and adolescent diet on serum biomarkers associated with breast cancer by conducting a follow-up study of young women who as children were randomly assigned to a fat-modified dietary intervention or to usual care as part of the DISC clinical trial. Because diet is related to a plethora of factors, the randomized prospective design was an important strength of our study that was further enhanced by an 86% participation rate. Data collection was done using standardized procedures by trained personnel masked to treatment group assignment, and numerous quality controls were in place to ensure data integrity. DISC participants were enrolled at 8 to 10 years of age and continued on the trial for a median of 7 years. The intensive intervention during the first 3 years achieved a significant decrease in girls' total fat intakes from 34% kcal at baseline to 29% kcal at the year-1 visit that further declined to 28% kcal over the remainder of the trial.[17] However, after the year-3 visit, as a consequence of maturational and/or secular changes, usual care group participants also reported reduced fat intakes such that by the end of

[17] Unpublished data for girls only.

the trial there was not a statistically significant treatment group difference in total fat intake. Treatment group differences in saturated fat intake observed at year-1 visits also diminished over the course of the trial. Even so, the limited exposure difference that was maximal at possibly a younger than optimal age may not have been adequate to have lasting effects on some biomarkers.

We attempted to schedule visits to the luteal phase of the menstrual cycle to maximize statistical power to observe differences in luteal phase progesterone levels similar to those seen at the end of the DISC intervention. But as a consequence, we were not able to compare treatment groups in the follicular phase of the menstrual cycle. Eighty-six percent of DISC female participants attended the DISC06 follow-up visit. However, the effective sample was smaller because participants who were pregnant or breast-feeding just before or at the visit were excluded from analysis. Furthermore, sample sizes differed across the outcomes evaluated because participants who had breast implants or breast reduction also were excluded from breast density analyses, and participants who were using hormonal contraceptives or who were not in the luteal phase of their menstrual cycles were excluded from hormone analyses. Thus, participants included in analyses may not have been representative of all DISC female participants, particularly for hormone analyses where sample size was smallest. All DISC participants had elevated LDL-C at randomization and met several additional eligibility criteria, which could reduce the generalizability of findings. Whether results for a random sample of girls would be similar to those reported here is unknown.

Diet and serum hormones

One mechanism by which adolescent diet could influence breast cancer risk is by altering circulating levels of estrogens and progesterone, which are breast mitogens that have been associated with breast cancer risk in adults (17, 18). As previously reported, the DISC intervention lowered serum estradiol and progesterone levels during adolescence (16). However, serum estradiol and progesterone concentrations did not differ between the DISC intervention and usual care group participants at the DISC06 follow-up visit in analyses that did not take into account current diet. In current diet–adjusted analyses, intervention group participants had significantly higher serum total estradiol and non–SHBG-bound estradiol concentrations compared with usual care group participants. To our knowledge, no previous studies have evaluated associations of childhood and adolescent diet with serum hormone levels in young adulthood. Current dietary fat intake by premenopausal women is not associated with serum progesterone levels in most studies (34, 35), and evidence for an association with serum estradiol is inconsistent (34-37). In a meta-analysis of dietary fat intervention studies, consumption of a low-fat diet lowered serum estradiol levels by ~7.4% in premenopausal women (38). The change in mean estradiol concentrations of DISC intervention and usual care group participants at the DISC06 follow-up visit after adjustment for current dietary fat and fiber intake is consistent with an effect of current diet on serum estradiol concentration. But the etiology and clinical significance of higher current diet–adjusted luteal phase estradiol levels in intervention group participants are unclear. All DISC participants' serum estradiol values were within the

Table 5. Mean and 95% CI for BMC and BMD at follow-up in DISC intervention and usual care groups

	Intervention group		Usual care group		*P**
	n	Mean (5-95% CI)	*n*	Mean (5-95% CI)	
BMC (g)					
Unadjusted	109	2,450 (2,352-2,548)	106	2,378 (2,279-2,477)	0.13
Adjusted (excluding current diet)†	109	2,444 (2,389-2,499)	106	2,377 (2,321-2,432)	0.02
Adjusted (including current diet)‡	102	2,448 (2,395-2,502)	101	2,356 (2,302-2,410)	0.003
BMD (g/cm^2)					
Unadjusted	109	1.18 (1.16-1.21)	106	1.16 (1.14-1.19)	0.08
Adjusted (excluding current diet)†	109	1.18 (1.16-1.21)	106	1.16 (1.14-1.19)	0.07
Adjusted (including current diet)‡	102	1.19 (1.16-1.21)	101	1.16 (1.14-1.18)	0.01

*P values from Wald test using linear mixed-effects regression.

†Adjusted for height, total body mass from DXA, type of DXA machine, BMI at baseline (Z-score), age at visit, age at randomization, race, smoking status, education, leisure physical activity at 14 to 17 y old and during the past year, hormonal contraceptive use, and number of full-term pregnancies.

‡Adjusted for height, total body mass from DXA, type of DXA machine, BMI at baseline (Z-score), age at visit, age at randomization, race, smoking status, education, leisure physical activity at 14 to 17 y old and during the past year, hormonal contraceptive use, number of full-term pregnancies, current intake of energy (kcal), % saturated fat, % monounsaturated fat, % polyunsaturated fat, cholesterol (mg/1,000 kcal), insoluble fiber (g/1,000 kcal), soluble fiber (g/1,000 kcal), calcium (mg), and vitamin D (μg).

reference range for the luteal phase of the menstrual cycle. Furthermore, although follicular phase estradiol levels were related to breast cancer risk in the Nurses' Health Study II (39), luteal phase concentrations were not related to risk in that study or in the European Prospective Investigation into Cancer and Nutrition (40). It seems that current diet is more important than adolescent diet in determining serum estradiol levels in young adulthood, but their relationship to breast cancer risk remains unclear.

Diet and breast density

Breast density is one of the strongest known breast cancer risk factors; women with dense breasts are at a 4-fold excess risk of developing breast cancer (41). Estrogens and progesterone regulate elongation and branching of breast ducts (42), and we hypothesized that DISC intervention group participants, who had lower serum estradiol and progesterone levels during adolescence compared with usual care group participants, also would have lower breast density at DISC06 follow-up visits. However, neither breast density nor volume of dense breast tissue measured at follow-up visits differed by treatment group in unadjusted or adjusted analyses.

We measured breast density by MRI, whereas most studies on the association of breast density with breast cancer measured breast density by mammography. Because MRI measures the three-dimensional volume of breast tissue, whereas mammography estimates breast density from a two-dimensional projected image of the breast, absolute values of density differ by modality, with breast density measured by MRI being ~1.5 times lower than that measured using mammography (43). Nevertheless, breast density measured by MRI and mammography is highly correlated (31, 44), and the association of adolescent diet with breast density would not be expected to differ depending on the modality used to measure density. In a study of young women's breast tissue composition by Boyd et al. (44), the median percent water was 45%, which is substantially larger than the percent dense breast tissue that we observed. Thicker MRI sections used in the Boyd study were more likely to contain mixtures of water and fat, which may have contributed to higher overall percent water values.

Our results are consistent with results of the Minnesota Breast Cancer Family Cohort Study in which recalled intake of fatty foods at 12 to 13 years of age was not associated with breast density measured at 47 to 64 years of age (45). They also agree with most studies that have evaluated associations of recent fat intake during adulthood with breast density. These studies generally do not support a positive association of total dietary fat with breast density in premenopausal or postmenopausal women (46-48). Results for exposures to intakes of specific fatty acids (e.g., saturated fatty acids, polyunsaturated fatty acid, and monounsaturated fatty acids) are inconsistent (47-50), which may reflect imprecise estimation of the fatty acid contents of foods or lack of a true underlying association. The report by Boyd et al. (51) of a greater decrease in area of dense breast tissue after 2 years among women assigned to a low-fat diet in a controlled clinical trial led to the initial excitement about a possible effect of dietary fat intake on breast density. However, consistent with observational studies, another larger low-fat diet intervention trial that aimed to confirm these results (52) failed to detect any treatment group differences in changes in area of dense tissue or breast density. Taken together, these results do not support an effect of adolescent or adult dietary fat intake on breast density.

Diet and BMD

Women with higher BMD have been shown to be at an increased risk of breast cancer in several studies (53-55). The mechanism underlying this association is unclear, but it has been hypothesized that higher BMD is a time-integrated marker of higher circulating estrogens and androgens that are positively associated with risk (56). It was hypothesized that the DISC intervention group participants would have lower BMDs compared with the usual care group participants. However, DISC intervention group participants had higher whole-body BMC and BMD compared with usual care group participants.

DISC participants' BMDs at 25 to 29 years of age represent peak BMDs that are related to genetics and earlier environmental exposures (57). In older women, such as those who participated in studies of the BMD–breast cancer association, the rate of bone mineral loss also contributes to BMD (58). Thus, the association of higher BMD with increased breast cancer risk could be due to genetic and environmental factors that alter the rate of bone mineral loss, which would not be reflected in peak BMD of DISC participants. Furthermore, we report results for whole-body BMD, whereas studies of BMD and breast cancer generally report densities at specific sites (e.g., hip and lumbar spine). The association of the DISC intervention with BMD varied depending on whether whole-body, hip, femoral neck, or apical spine BMD was the outcome (data not shown). However, the intervention group had similar or higher mean BMDs compared with the usual care group across all measures.

Diet is one of the primary environmental determinants of bone density, and childhood and adolescent diet is related to bone density in adulthood. In particular, higher intakes of milk and calcium during adolescence have been associated with higher BMD in adulthood (59-61). DISC intervention group participants' calcium intakes were monitored throughout the intervention to ensure that they were adequate to promote growth. Although absolute intakes of calcium and vitamin D did not differ by treatment group during the intervention (20), the intervention group reported consuming more calcium and vitamin D per 1,000 kcal than the usual care group.[18]

[18] Unpublished data.

Conversely, at the DISC06 follow-up visit, the intervention group reported consuming slightly less vitamin D compared with the usual care group, but their reported calcium intakes did not differ. Thus, DISC nutritionists' efforts to ensure adequate calcium intake by intervention group participants may have contributed to their higher BMC and BMD at the DISC06 follow-up visit compared with usual care group participants. Although treatment group differences were not statistically significant, the intervention group reported higher levels of leisure-time physical activity during adolescence compared with the usual care group, which also may have contributed to the observed differences in BMC and BMD.

The association of dietary fat with BMD has been evaluated in several studies with mixed results. In a cross-sectional study of children and adolescents, BMD measured at the distal forearm was significantly and positively associated with saturated fat intake (62). However, total fat consumption during adolescence was not associated with BMD of the lumbar spine or hip femoral neck at 20 to 25 years of age in female participants in the prospective Northern Ireland Young Hearts Project (59). Similarly, current intakes of total fat and saturated fatty acids were not associated with total hip BMD in adult females in the National Health and Nutrition Examination Survey III (63). Thus, although there is some limited support for an effect of fat intake during childhood and adolescence on BMD, additional research is needed to clarify this association.

In summary, findings from the DISC06 follow-up study on the long-term effects of a fat-modified diet intervention during childhood and adolescence on subsequent serum estradiol and progesterone levels, breast density, and BMD do not support the hypothesis that dietary fat intake during adolescence influences later breast cancer risk via effects on these biomarkers. Additional research is needed to determine if these results reflect the rather modest treatment group differences in dietary fat intake achieved in DISC, the timing of the intervention, or other factors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Welsch CW. Relationship between dietary fat and experimental mammary tumorigenesis: a review and critique. Cancer Res 1992; 52:2040–8s.
2. Hebert JR, Rosen A. Nutritional, socioeconomic, and reproductive factors in relation to female breast cancer mortality: findings from a cross-national study. Cancer Detect Prev 1996;20:234–44.
3. Howe GR, Hirohata T, Hislop TG, et al. Dietary factors and risk of breast cancer: combined analysis of 12 case-control studies. J Natl Cancer Inst 1990;82:561–9.
4. Michels KB, Mohllajee AP, Roset-Bahmanyar E, et al. Diet and breast cancer: a review of the prospective observational studies. Cancer 2007;109:2712–49.
5. Prentice RL, Thomson CA, Caan B, et al. Low-fat dietary pattern and cancer incidence in the Women's Health Initiative Dietary Modification Randomized Controlled Trial. J Natl Cancer Inst 2007;99:1534–43.
6. Velie EM. Lifetime reproductive and anthropometric risk factors for breast cancer in postmenopausal women. Breast Dis 2006;26:1–19.
7. Merzenich H, Boeing H, Wahrendorf J. Dietary fat and sports activity as determinants for age at menarche. Am J Epidemiol 1993;138: 217–24.
8. Berkey CS, Colditz GA, Rockett HR, Frazier AL, Willett WC. Dairy consumption and female height growth: prospective cohort study. Cancer Epidemiol Biomarkers Prev 2009;18:1881–7.
9. Frazier AL, Li L, Cho E, Willett WC, Colditz GA. Adolescent diet and risk of breast cancer. Cancer Causes Control 2004;15:73–82.
10. Linos E, Willett WC, Cho E, et al. Red meat consumption during adolescence among premenopausal women and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2008;17:2146–51.
11. Hislop TG, Coldman AJ, Elwood JM, Brauer G, Kan L. Childhood and recent eating patterns and risk of breast cancer. Cancer Detect Prev 1986;9:47–58.
12. Pryor M, Slattery ML, Robison LM, Egger M. Adolescent diet and breast cancer in Utah. Cancer Res 1989;49:2161–7.
13. Potischman N, Weiss HA, Swanson CA, et al. Diet during adolescence and risk of breast cancer among young women. J Natl Cancer Inst 1998;90:226–33.
14. Frazier AL, Ryan CT, Rockett H, Willett WC, Colditz GA. Adolescent diet and risk of breast cancer. Breast Cancer Res 2003;5:R59–64.
15. DISC Collaborative Research Group. Dietary intervention study in children (DISC) with elevated low-density-lipoprotein cholesterol. Design and baseline characteristics. Ann Epidemiol 1993;3:393–402.
16. Dorgan JF, Hunsberger SA, McMahon RP, et al. Diet and sex hormones in girls: findings from a randomized controlled clinical trial. J Natl Cancer Inst 2003;95:132–41.
17. The Endogenous Hormones and Breast Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 2002; 94:606–16.
18. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 2002;288:321–33.
19. The Writing Group for the DISC Collaborative Research Group The Dietary Intervention Study in Children (DISC). Efficacy and safety of lowering dietary intake of fat and cholesterol in children with elevated low-density lipoprotein cholesterol. JAMA 1995;273:1429–35.
20. Obarzanek E, Hunsberger SA, Van Horn L, et al. Safety of a fat-reduced diet: the Dietary Intervention Study in Children (DISC). Pediatrics 1997;100:51–9.
21. Obarzanek E, Kimm SY, Barton BA, et al. Long-term safety and efficacy of a cholesterol-lowering diet in children with elevated low-density lipoprotein cholesterol: seven-year results of the

Dietary Intervention Study in Children (DISC). Pediatrics 2001;107:256–64.
22. Lipid Research Clinics population studies data book. I. The prevalence study. Bethesda (MD): U.S. Department of Health and Human Services, Public Health Service; 1980.
23. Tanner JM. Growth at adolescence. 2nd ed. Oxford (UK): Blackwell Scientific; 1962.
24. Achenbach TM, Edelbrook C. Manual for the child behavior checklist. Burlington (VT): University of Vermont; 1983.
25. Hartmuller VW, Snetselaar L, Van Horn L, Steinmuller P, Smith K, Gernhofer N. Creative approaches to cholesterol lowering used in the dietary intervention study in children. Topics Clin Nutr 1994;10:71–8.
26. Stevens VJ, Obarzanek E, Franklin FA, Steinmuller P, Snetselaar L, Lavigne J. Dietary Intervention Study in Children (DISC): intervention design and participation. J Nutr Educ 1995;27:133–40.
27. Kriska AM. Historical leisure activity questionnaire. Med Sci Sports Exerc 1997;29:S43–5.
28. Probst-Hensch NM, Ingles SA, Diep AT, et al. Aromatase and breast cancer susceptibility. Endocr Relat Cancer 1999;6:165–73.
29. Scott JZ, Stanczyk F, Goebelsmann U, Mishell DR, Jr. A double-antibody radioimmunoassay for serum progesterone using progesterone-3-(o-carboxymethyl)oximino-[125I]-iodohistamine as radioligand. Steroids 1978;31:393–405.
30. Rinaldi S, Geay A, Dechaud H, et al. Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. Cancer Epidemiol Biomarkers Prev 2002;11:1065–71.
31. Klifa C, Carballido-Gamio J, Wilmes L, et al. Quantitation of breast tissue index from MR data using fuzzy clustering. Conf Proc IEEE Eng Med Biol Soc 2004;3:1667–70.
32. Genant HK, Grampp SS, Gluer CC, et al. Universal standardization for dual x-ray absorptiometry: patient and phantom cross-calibration results. J Bone Miner Res 1994;9:1503–14.
33. Lu Y, Fuerst T, Hui S, Genant HK. Standardization of bone mineral density at femoral neck, trochanter and Ward's triangle. Osteoporos Int 2001;12:438–44.
34. Gann PH, Chatterton RT, Gapstur SM, et al. The effects of a low-fat/high-fiber diet on sex hormone levels and menstrual cycling in premenopausal women: a 12-month randomized trial (The Diet and Hormone Study). Cancer 2003;98:1870–9.
35. Bagga D, Ashley JM, Geffrey SP, et al. Effects of a very low fat, high fiber diet on serum hormones and menstrual function. Implications for breast cancer prevention. Cancer 1995;76:2491–6.
36. Dorgan JF, Reichman ME, Judd JT, et al. Relation of energy, fat, and fiber intakes to plasma concentrations of estrogens and androgens in premenopausal women. Am J Clin Nutr 1996;64:25–31.
37. Woods MN, Barnett JB, Spiegelman D, et al. Hormone levels during dietary changes in premenopausal African-American women. J Natl Cancer Inst 1996;88:1369–74.
38. Wu AH, Pike MC, Stram DO. Meta-analysis: dietary fat intake, serum estrogen levels, and the risk of breast cancer. J Natl Cancer Inst 1999;91:529–34.
39. Eliassen AH, Missmer SA, Tworoger SS, et al. Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. J Natl Cancer Inst 2006;98:1406–15.
40. Kaaks R, Berrino F, Key T, et al. Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). J Natl Cancer Inst 2005;97:755–65.
41. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2006;15:1159–69.
42. Atwood CS, Hovey RC, Glover JP, et al. Progesterone induces side-branching of the ductal epithelium in the mammary glands of peripubertal mice. J Endocrinol 2000;167:39–52.
43. Klifa C, Carballido-Gamio J, Wilmes L, et al. Magnetic resonance imaging for secondary assessment of breast density in a high-risk cohort. Magn Reson Imaging 2010;28:8–15.
44. Boyd N, Martin L, Chavez S, et al. Breast-tissue composition and other risk factors for breast cancer in young women: a cross-sectional study. Lancet Oncol 2009;10:569–80.
45. Sellers TA, Vachon CM, Pankratz VS, et al. Association of childhood and adolescent anthropometric factors, physical activity, and diet with adult mammographic breast density. Am J Epidemiol 2007;166:456–64.
46. Sala E, Warren R, Duffy S, Welch A, Luben R, Day N. High risk mammographic parenchymal patterns and diet: a case-control study. Br J Cancer 2000;83:121–6.
47. Vachon CM, Kushi LH, Cerhan JR, Kuni CC, Sellers TA. Association of diet and mammographic breast density in the Minnesota breast cancer family cohort. Cancer Epidemiol Biomarkers Prev 2000;9:151–60.
48. Masala G, Ambrogetti D, Assedi M, et al. Dietary and lifestyle determinants of mammographic breast density. A longitudinal study in a Mediterranean population. Int J Cancer 2006;118:1782–9.
49. Brisson J, Verreault R, Morrison AS, Tennina S, Meyer F. Diet, mammographic features of breast tissue, and breast cancer risk. Am J Epidemiol 1989;130:14–24.
50. Nagata C, Matsubara T, Fujita H, et al. Associations of mammographic density with dietary factors in Japanese women. Cancer Epidemiol Biomarkers Prev 2005;14:2877–80.
51. Boyd NF, Greenberg C, Lockwood G, et al, Canadian Diet and Breast Cancer Prevention Study Group. Effects at two years of a low-fat, high-carbohydrate diet on radiologic features of the breast: results from a randomized trial. J Natl Cancer Inst 1997;89:488–96.
52. Martin LJ, Greenberg CV, Kriukov V, et al. Effect of a low-fat, high-carbohydrate dietary intervention on change in mammographic density over menopause. Breast Cancer Res Treat 2009;113:163–72.
53. Nguyen TV, Center JR, Eisman JA. Association between breast cancer and bone mineral density: the Dubbo Osteoporosis Epidemiology Study. Maturitas 2000;36:27–34.
54. van der Klift M, de Laet CE, Coebergh JW, Hofman A, Pols HA. Bone mineral density and the risk of breast cancer: the Rotterdam Study. Bone 2003;32:211–6.
55. Chen Z, Arendell L, Aickin M, Cauley J, Lewis C, Chlebowski R. Hip bone density predicts breast cancer risk independently of Gail score: results from the Women's Health Initiative. Cancer 2008;113:907–15.
56. Kuller LH, Cauley JA, Lucas L, Cummings S, Browner WS. Sex steroid hormones, bone mineral density, and risk of breast cancer. Environ Health Perspect 1997;105:593–9.
57. Bonjour JP, Chevalley T, Rizzoli R, et al. Gene-environment interactions in the skeletal response to nutrition and exercise during growth. Med Sport Sci 2007;51:64–80.
58. Riggs B, Melton L, Robb R, et al. A population-based assessment of rates of bone loss at multiple skeletal sites: evidence for substantial trabecular bone loss in young adult women and men. J Bone Miner Res 2008;23:205–14.
59. Neville CE, Robson PJ, Murray LJ, et al. The effect of nutrient intake on bone mineral status in young adults: the Northern Ireland young hearts project. Calcif Tissue Int 2002;70:89–98.
60. Wang MC, Crawford PB, Hudes M, et al. Diet in midpuberty and sedentary activity in prepuberty predict peak bone mass. Am J Clin Nutr 2003;77:495–503.
61. Vatanparast H, Whiting SJ, Vatanparast H, Whiting SJ. Early milk intake, later bone health: results from using the milk history questionnaire. Nutr Rev 2004;62:256–60.
62. Gunnes M, Lehmann EH. Dietary calcium, saturated fat, fiber and vitamin C as predictors of forearm cortical and trabecular bone mineral density in healthy children and adolescents. Acta Paediatr 1995;84:388–92.
63. Corwin RL, Hartman TJ, Maczuga SA, et al. Dietary saturated fat intake is inversely associated with bone density in humans: analysis of NHANES III. J Nutr 2006;136:159–65.