Dietary fat intake during adolescence and breast density among young women

Seungyoun Jung[1], **Olga Goloubeva**[1], **Catherine Klifa**[2], **Erin S LeBlanc**[3], **Linda G Snetselaar**[4], **Linda Van Horn**[5], and **Joanne F. Dorgan**[1]

[1]University of Maryland School of Medicine, Baltimore, MD

[2]Dangeard Group, Luynes, France

[3]Kaiser Permanente Center for Health Research, Portland, OR

[4]University of Iowa, Iowa City, IA

[5]Feinberg School of Medicine, Northwestern University, Chicago, IL

Abstract

BACKGROUND—Lack of association between fat intake and breast cancer risk in cohort studies might be attributed to disregard of temporal effects during adolescence when breasts develop and are particularly sensitive to stimuli. We prospectively examined associations between adolescent fat intakes and breast density.

METHOD—Among 177 women who participated in the Dietary Intervention Study in Children, dietary intakes at ages 10–18 were assessed on five occasions by 24-hour recalls and averaged. We calculated geometric mean and 95% confidence intervals for MRI-measured breast density at ages 25–29 across quartiles of fat intake using linear mixed-effect regression.

RESULTS—Comparing women in the extreme quartiles of adolescent fat intakes, percent dense breast volume (%DBV) was positively associated with saturated fat (mean=16.4% vs. 21.5%; $P_{trend}<0.001$). Conversely, %DBV was inversely associated with monounsaturated fat (25.0% vs. 15.8%; $P_{trend}<0.001$) and the ratio of polyunsaturated fat to saturated fat (P/S ratio; 19.1% vs. 14.3%; $P_{trend}<0.001$). When examining intake by pubertal stages, %DBV was inversely associated with intake of polyunsaturated fat (20.8% vs. 16.4%; $P_{trend}=0.04$), long-chain omega-3 fat (17.8% vs. 15.8%; $P_{trend}<0.001$), and P/S ratio (22.5% vs. 16.1%; $P_{trend}<0.001$) before menarche, but not after. These associations observed with %DBV were consistently observed with absolute dense breast volume but not with absolute non-dense breast volume.

CONCLUSIONS—In our study, adolescent intakes of higher saturated fat and lower mono- and polyunsaturated fat are associated with higher breast density measured approximately 15 years later.

Corresponding author: **Dr. Joanne F. Dorgan**, University of Maryland School of Medicine, Howard Hall 102E, Baltimore, MD 21201, Tel: 410-706-1602, Fax: 410-706-8013, ; Email: jdorgan@som.umaryland.edu

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IMPACT—The fat subtype composition in adolescent diet may be important in early breast cancer prevention.

Keywords

Adolescent diet; Fat intake; Saturated fat; Monounsaturated fat; Polyunsaturated fat; Long-chain omega-3 fat; Breast density; Absolute dense breast volume; Absolute non-dense breast volume; Young adult; Adolescence; Timing of exposure; Breast cancer

Introduction

Though studied extensively, the association between diet and breast cancer remains unsettled. In pooled analyses of eight prospective cohort studies ([1]) and randomized controlled trials ([2], [3]), little association was observed between total and subtypes of fat intakes during adulthood and breast cancer (BRC) risk, despite positive associations observed in ecological ([4], [5]) and animal studies ([6]). Associations with risk of BRC subtypes also have been inconsistent ([7], [8]). However, breast tissue is most sensitive to stimuli during adolescence ([9]), when breasts develop and undergo structural changes ([10]–[12]). Little is known whether early-life fat intake affects breast development or morphology, which may, in part, mediate BRC risk later in life.

Breast density is a measure of the relative amount of glandular and stromal tissue in the breasts, and is a strong risk factor for BRC ([13], [14]). Dietary fat intake was reported to affect endogenous hormones ([15], [16]), which regulates ductal morphogenesis during puberty ([12]). Pubertal high-fat diets in rats altered cellular proliferation and breast tissue composition ([17], [18]), and induced abnormal breast lesions ([18], [19]). The few studies that examined associations of early-life fat intake with breast density reported null results ([20], [21]). Still, these studies were limited by recall of diet in the distant past, and no investigation of fat subtypes and pubertal stages In the Dietary Intervention Study in Children (DISC), children with elevated levels of blood cholesterol who were randomized to a reduced fat diet for 7 years had similar breast density in their 20s to children randomized to the control group who consumed usual diets ([22]), though variation in fat intake between groups was modest.

Therefore, utilizing adolescent dietary data collected repeatedly in the DISC trial ([23], [24]) and breast density measured by magnetic resonance imaging (MRI) at age 25–29 years in the DISC06 Follow-Up Study ([22], [25]), we expanded our initial analyses and prospectively evaluated associations between breast density and reported total and subtypes of fat intakes during adolescence and at specific pubertal stages.

Materials and Methods

Study population

DISC was a two-armed, multicenter, randomized controlled clinical trial to evaluate the efficacy and safety of a lipid-lowering diet in children with elevated low-density lipoprotein cholesterol (LDL-C) ([26], [27]). Between 1988 and 1990, healthy, pre-pubertal, 8–10 year-old girls (N=301) and boys (N=362) with elevated LDL-C were randomized to a reduced-fat diet intervention or a usual-care control group in six clinical centers until 1997 ([27]). Between

2006 and 2008, the DISC06 Follow-Up Study was conducted among female DISC participants aged 25–29 years to assess the long-term effect of the diet intervention during childhood and adolescence on BRC related biomarkers [22]. Assent from participants and informed consent from parents or guardians were obtained prior to randomization and informed consent was obtained from participants before the DISC06 Follow-Up Study. The institutional review boards at participating centers approved original and follow-up DISC protocols.

Of 260 women who participated in the DISC06 Follow-Up Study, women who were pregnant or breastfeeding within 12 weeks before the follow-up visit (N=30), had breast augmentation or reduction surgery (N=16), had unacceptable or missing breast density data (N=32), or were without dietary data during the DISC trial (N=5) were excluded. Consequently, this analysis includes 177 women.

Data collection

Participants completed questionnaires on demographics, lifestyle, physical activity, medication use, and reproductive, menstrual, and medical history at baseline and at follow-up visits in the DISC trial and the DISC06 Follow-Up Study. Height and weight were measured [25]. Childhood BMI z-score was calculated based on Centers for Disease Control and Prevention 2000 Growth Charts [28]. In DISC06, whole-body percent fat was estimated by dual energy x-ray absorptiometry [25]. During the DISC trial, sexual maturation was evaluated annually by ascertaining onset of menses and conducting a physical examination including Tanner staging [29].

Diet at baseline, year 1, 3, and 5 and last visits during the DISC trial and at DISC06 follow-up visits was assessed via three nonconsecutive 24-hour dietary recalls obtained on two weekdays and one weekend day over two weeks [30, 31]. Trained, certified nutritionists conducted face-to-face interviews during clinic visits and two subsequent recalls were obtained via telephone. Parents provided additional details, if needed. Nutrition Coordinating Center at the University of Minnesota estimated nutrient intake from 24-hour recalls using their food and nutrient database (version 20). Nutrient intakes from recalls were averaged to estimate usual dietary intake. Records with implausible energy intakes (<600 kcal/day or >3500 kcal/day; N=7) were set to missing [32]. Energy intakes from each type of fat or protein were divided by total energy intake to calculate percent energy from those nutrients. The correlations between 24-hour recalls and dietary records collected on a subset of participants were 0.73 for total fat, 0.64 for saturated fat, 0.78 for monounsaturated fat, and 0.64 for polyunsaturated fat [33].

Breast density was measured by noncontrast MRI [22], because MRI avoids radiation exposure, is not impaired by high breast density typical of young women, and provides three dimensional volumetric measurements of breast composition reported by some to be more predictive of BRC risk [34]. Even so, percent breast density measured by MRI is highly correlated with mammographic measurements ($r > 0.75$) [35, 36]. Breasts were imaged with a whole-body 1.5 Tesla or higher field-strength MRI scanner with a dedicated breast-imaging radiofrequency coil. All MRI image data were processed by Dr. C. Klifa (University of California, San Francisco), who identified chest wall-breast tissue boundary

and skin surface, and separated breast fibroglandular and fatty tissue via automated fuzzy C-means method ([37]). MRI technologists were trained to recognize and correct failures. Each DISC clinic was certified after obtaining acceptable images from three volunteers.

We measured total breast volume and absolute dense breast fibroglandular volume (ADBV) for each breast. Absolute non-dense breast volume (ANDBV) was estimated by subtracting ADBV from total breast volume. Percent dense breast volume (%DBV) was calculated as the ratio of ADBV to total breast volume. Breast density measures for both breasts were averaged for analysis.

Statistical analyses

Our primary exposure was long-term fat intake during adolescence estimated by averaging reported intakes ([38]) over the course of the DISC trial. We also estimated fat intakes separately by menarche status as breasts' susceptibility to stimuli might vary during pubertal transition ([10], [39]–[41]). Specifically, we averaged fat intakes from age 10 years, the approximate age of onset of breast development ([42], [43]), to the last DISC trial visit (median age=16.6 years) to estimate overall adolescent intake, from age 10 years to the onset of menarche to estimate premenarcheal intake, and thereafter to the last DISC trial visit to estimate postmenarcheal intake.

%DBV, ADBV, and ANDBV values were log-transformed to approximate normality. We imputed missing values of whole-body percent fat (N=6) using values from a prediction model that included adult BMI as an independent variable; this process was repeated 25 times to create 25 multiply imputed datasets. The correlation coefficient between imputed whole-body percent fat and adult BMI ranged from 0.74 to 0.99 across the imputed datasets, which compared favorably to a correlation of 0.82 based on actual data. In each imputed dataset, the geometric mean and the 95% confidence interval for %DBV, ADBV, and ANDBV were calculated across fat intake quartiles using linear mixed-effects regression models with robust standard errors. To adjust for potential confounders known to be associated with breast cancer risk, breast density and fat intake ([1], [25], [44]), fully adjusted multivariable models included the following predictors, race, education, childhood BMI z-score, adulthood whole-body percent fat, duration of hormone use, number of live birth, smoking status, and intakes of total calories, protein, fiber, and alcohol as fixed effects; clinic was included as a random effect; other types of fat were simultaneously adjusted for in analyses of specific types of fat ([38]). Trends across quartile medians modeled as continuous variables were tested using the Wald test statistic. Interactions of fat intake by intervention assignment, hormone use, and whole-body percent fat were tested in the model by including cross-product terms. Results from the fully adjusted multivariable model in each imputed dataset were pooled using Rubin's rule ([45]).

Analyses were conducted by STATA (version 13.0; College Station, TX, USA). All tests were two-sided and done at the 0.05 level of significance.

Results

In our analyses of 177 women, the mean percent energy from total and major subtypes of fat intakes during adolescence at age 10–18 years were 29.7% for total fat, 10.8% for saturated fat, 10.9% for monounsaturated fat, and 5.8% for polyunsaturated fat (Table 1). In the DISC06 Follow-Up Study when breast density was measured, participants' mean age was 27.2 years and their mean BMI was 25.4kg/m^2. The majority of women were White (91%) and nulliparous (72%), had attended at least college (91%), and ever used hormonal contraceptives (94%). The median of breast density measures (interquartile ranges) were 24.3% (9.7%–41.2%) for %DBV, 93.6 cm^3 (50.0 cm^3 – 140.3 cm^3) for ADBV, and 299.2 cm^3 (157.8 cm^3 – 484.9 cm^3) for ANDBV. Fat intakes at the DISC06 follow-up visit are shown in Supplementary Table 1.

We present results only from the fully adjusted multivariable model because associations of fat intakes with breast density measures were similar from the simpler model adjusting for adult whole body percent fat, treatment assignment, and intake of total energy and protein (data not shown). Intake of saturated fat was significantly positively, whereas monounsaturated fat and the ratio of polyunsaturated fat to saturated fat (P/S ratio) were significantly inversely associated with %DBV (all P_{trend}<0.001) (Table 2). Comparing women in the extreme quartiles of each fat subtype or P/S ratio (Q1-Q4), the multivariable-adjusted mean %DBV increased from 16.4% to 21.5% for saturated fat, while it decreased from 25.0% to 15.8% for monounsaturated fat and from 19.1% to 14.3% for the P/S ratio.

When we examined intakes before and after menarche ([10, 39–41]) (Table 2), premenarcheal, but not postmenarcheal, intakes of polyunsaturated fat (P_{trend}=0.04) and long-chain omega-3 fat (P_{trend}<0.001) and the P/S ratio (P_{trend}<0.001) were significantly inversely associated with %DBV. The multivariable-adjusted mean %DBV decreased from 20.8% to 16.4% for polyunsaturated fat, from 17.8% to 15.8% for long-chain omega-3 fat, and from 22.5% to 16.1% for the P/S ratio when comparing women in the lowest and the highest quartiles for each fat. Total fat and linoleic acid intakes during adolescence or during pre- or postmenarche periods were not associated with %DBV.

Because variation of fat intakes and the P/S ratio was relatively modest, particularly among women in the lower quartiles, we conducted sensitivity analyses categorizing fat intakes and the P/S ratio into tertiles to improve contrasts (data not shown). The linear inverse trend with %DBV became clearer. For example, the multivariable-adjusted mean %DBV decreased monotonically from 20.9% to 18.4% and 17.0% for monounsaturated fat and from 21.8% to 18.6% and 16.2% for the P/S ratio with increasing tertiles.

Additional adjustment for adult fat intakes or restricting analyses to nulliparous women did not change results substantially (data not shown). Treatment assignment, whole-body percent fat, and hormone use did not statistically significantly modify associations between total or subtypes of fat intakes and %DBV (all $P_{interaction}$ 0.11).

When examining associations separately for individual components of breast tissue that compose %DBV (Table 3–4), the associations observed with %DBV were generally seen with ADBV, although results for polyunsaturated fat were not significant. Comparing

extreme quartiles (Q1-Q4) of each subtype of fat intake or the P/S ratio, the multivariable-adjusted mean ADBV increased from 65.6 cm^3 to 97.4 cm^3 for saturated fat (P$_{trend}$=0.03), whereas it decreased from 105.4 cm^3 to 69.0 cm^3 for monounsaturated fat (P$_{trend}$=0.05) as well as for premenarcheal long-chain omega-3 fat (from 76.6 cm^3 to 65.5cm^3; P$_{trend}$<0.001) and P/S ratio (from 85.1cm^3 to 71.8cm^3; P$_{trend}$<0.002). In contrast, ANDBV was significantly positively associated with premenarcheal intakes of polyunsaturated fat (P$_{trend}$=0.01), linoleic acid (P$_{trend}$=0.02) and the P/S ratio (P$_{trend}$<0.001). The multivariable-adjusted mean ANDBV increased from 260.8 cm^3 to 324.1 cm^3 for polyunsaturated fat, from 280.2 cm^3 to 320.0 cm^3 for linoleic acid, and from 241.2 cm^3 to 313.7 cm^3 for the P/S ratio between the extreme quartiles.

Discussion

In this prospective analysis adolescent total fat intake was not significantly associated with %DBV, ADBV, or ANDBV. However, adolescent intake of saturated fat was significantly positively associated, while monounsaturated fat was significantly inversely associated with %DBV and ADBV but not with ANDBV. When examining intakes by pubertal stages, premenarcheal intakes of polyunsaturated fat and long-chain omega-3 fat, and the P/S ratio were significantly inversely associated with %DBV. Intake of long-chain omega-3 fat also was significantly inversely associated with ADBV, whereas polyunsaturated fat was significantly positively associated with ANDBV. The P/S ratio was significantly inversely associated with ADBV and positively with ANDBV. No postmenarcheal fat intakes were associated with %DBV, ADBV, or ANDBV.

The null association we observed between total fat intake and %DBV is largely consistent with previous evidence. Of eight cross-sectional studies of adult women [46–53], total fat intake was not associated with breast density in six [46, 47, 50–53], whereas two found significant positive associations [48, 49]. Similarly, high-fat food consumption during childhood was not associated with breast density in adulthood [20, 21]. In two [22, 54] of three randomized controlled trials [22, 54, 55], women assigned to a low-fat diet in adolescence [22] or midlife [54] had similar breast density to women assigned to a usual diet, although another found a significant difference in breast density [55].

Nonetheless, fatty acids have unique chemical and biophysical properties [56, 57] and varying physiological functions [58–61]. Examining only total fat intakes may mask specific effects of each subtype of fat on breasts. Indeed, in our data, saturated fat and unsaturated fat intakes, such as mono- and polyunsaturated fat, particularly from long-chain omega-3 fat, had opposing associations with %DBV. Our significant positive association of saturated fat with %DBV is consistent with three studies [46, 48, 62], although others have reported no associations [47, 50, 52] or significant inverse associations [53]. Contrary to the inverse associations we observed with unsaturated fat intake, most studies of adult women found no significant association between breast density and intakes of monounsaturated fat [47, 48, 50, 53, 62], polyunsaturated fat [46–48, 50, 53], or omega-3 fat [48], while one found significant positive association with monounsaturated fat [49].

The stronger associations observed for specific subtypes of fat in our study, compared to previous studies of adult women, might be attributed to the early-life dietary assessment. Adolescence is critical periods when mammary ducts elongate and branch ([10]). These structural changes accompany rapid proliferation of undifferentiated cells, which may render breasts particularly vulnerable to any effects of fat. The degree of saturation in fatty acids influences fluidity and structure of cell membranes, which affects cell receptors, membrane transporters and cellular signaling pathways ([56], [57], [63]). Increasing saturated fat while decreasing monounsaturated fat and polyunsaturated fat, and particularly omega-3 fat, were shown to promote proliferation, insulin resistance, and inflammation, and to depress cellular responses to DNA damage and apoptosis ([60], [61], [64]–[67]). The P/S ratio was also inversely associated with estrogens that promote ductal growth and proliferation ([68], [69]). The opposing associations of saturated and unsaturated fats we observed with %DBV may reflect different roles of these fats in formation and maintenance of breast tissue.

Early-life timing when fat would most influence breast morphology is unknown. Nonetheless, breast maturation during adolescence consists of a sequence of physical changes, in which ductal outgrowth is followed by accumulation of breast fat ([10]). Recent studies underscore earlier prolonged exposures at the time of breasts' ductal expansion, regarding early BRC initiation ([39], [40], [70]). In prospective analyses, an earlier age at thelarche (onset of breast development) ([39]), a longer interval between thelarche and menarche ([39]), and higher premenarcheal adrenal hormone levels ([40]) were significantly associated with higher breast density ([40]) and increased BRC risk ([39]). In this first analysis examining fat intakes before and after menarche with %DBV, stronger associations observed between %DBV and polyunsaturated fat and omega-3 fat intakes before menarche, but not after, align with previous evidence ([39], [40], [70]). However, the significant associations of saturated and monounsaturated fat intakes with %DBV in our data were from cumulative intakes during both pre- and post-menarche periods. Further investigation is warranted to examine whether our results reflect true fat-induced dynamics occurring with breast maturation.

With regard to whether our findings can be translated to later BRC risk, it is worth noting that significant associations observed between subtypes of fat and %DBV were generally also seen with ADBV. ADBV reflects fibroglandular tissue amount, which comprises highly proliferative structures like mammary ducts where most tumors arise. Indeed, ADBV was more strongly associated with BRC risk than %DBV in a recent cohort study ([34]). Our significant results for ADBV may indicate that fat intake alters breast tissue composition during adolescence and potentially could influence later BRC risk.

Our study has some limitations. We could not examine associations with the main food sources of saturated fat (e.g., cheese, milk, meat) and unsaturated fats (e.g., fish, nuts, seeds) ([71]–[75]) or methods used to prepare food ([76]), because individual foods and cooking methods data are not available. We had limited intake variations for total and subtypes of fat. Nonetheless, percent energy range for each fat type in our study was comparable with national data for girls of a similar age ([30]). Significant inverse associations observed for unsaturated fats and the P/S ratio with %DBV was nonlinear, possibly due to modest intake variations in lower quartiles; for example, median daily percent energy intakes from long-

chain omega-3 fat were 0.01% and 0.03% in second quartile and third quartile, respectively, while the corresponding value in the highest quartile was 0.09%. We had adequate power to detect modest associations of total fat, saturated fat, monounsaturated fat, polyunsaturated fat and linoleic acid intakes during adolescence with breast density measures, but power was more limited for omega-3 fatty acids and the P:S ratio. Although analyses and interpretation were conducted cautiously with consideration of prior findings and biological relevance ([77]), concern remains about multiple testing. Our results could be due to chance and warrant replication in a separate cohort. Unknown residual confounding cannot be ruled out; however, we comprehensively adjusted for the known possible confounders associated with both breast density and intake of fat such as number of live births, duration of hormonal contraceptive use, educational attainment, smoking status and alcohol consumption in our full multivariable model. Similar results between simpler and fully adjusted models suggest little confounding by known covariates.

Nevertheless, this is the first comprehensive prospective analysis that examined the long-term effect of adolescent fat intakes on breast density, measured approximately 15 years later. The prospective design minimized recall bias of early life diet. With multiple diet assessments, we estimated fat intakes at specific pubertal stages, as well as long-term intake during adolescence; averaging intake also dampened potential random measurement errors. MRI-measured breast density is more accurate than mammography for dense breasts typical of young women. We measured breast density at ages 25–29 years, which might be the most relevant marker to assess the effect of adolescent fat intake on breasts, because it precedes natural involution of breast tissue that occurs with aging, and decreases breast density ([78]). We adjusted for both childhood and adulthood potential confounders.

In conclusion, we observed that types of dietary fat during adolescence were associated with adult breast density. Higher intakes of saturated fats and lower intakes of unsaturated fats were associated with higher %DBV and ADBV, whereas higher intakes of unsaturated fats were associated with lower ANDBV. These findings emphasize fat subtype composition in adolescent diets in determining breast's morphologic characteristics and support early risk accumulation of BRC. Further cohort studies are warranted to replicate our findings, validate whether they are independent of other components in food sources for fat, and identify underlying mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

ADBV	absolute dense breast volume
ANDBV	absolute non-dense breast volume
BMI	body mass index
BRC	breast cancer
DISC	Dietary Intervention Study in Children
DISC06	Dietary Intervention Study in Children 2006 Follow-up Study
IQR	interquartile range
MRI	magnetic resonance imaging
%DBV	percent dense breast volume

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Table 1

Study population characteristics at the DISC06 follow-up visit

Characteristics	N	Mean ± SD
Age (yrs)	177	27.2 ± 1.0
BMI	177	25.4 ± 5.4
Physical activity (Mets-hrs/week)	177	309.4 ± 54.5
Age at menarche (yrs)	177	12.9 ± 1.2
Duration of hormonal contraceptive use (yrs)[a]	166	5.6 ± 3.5
Dietary intake during adolescence		
Total energy intake (kcal/day)	177	1611.2 ± 350.8
Percentage of energy from		
Total fat (%)	177	29.7 ± 4.8
Saturated fat (%)	177	10.8 ± 2.2
Monounsaturated fat (%)	177	10.9 ± 1.9
Polyunsaturated fat (%)	177	5.8 ± 1.3
Long-chain omega-3 fat (%)	177	0.04 ± 0.06
Linoleic acid (%)	177	5.2 ± 1.2

	N	Percentage
Race		
White	161	91%
Non-white	16	9%
Education		
Some college	43	24%
Bachelor's degree	93	53%
Graduate degree	24	14%
Other[b]	17	9%
Number of live births		
0	127	72%
1	50	28%
Family history of breast cancer		
No	167	97%
Yes	6	3%
Hormonal contraceptive use		
Never	11	6%
Former	62	35%
Current	104	59%

	N	Percentage
Smoking status		
Never	97	55%
Former	38	21%

Characteristics	N	Mean ± SD
Current	42	24%
Alcohol consumption		
Never/former	16	9%
Current, <3 per week	67	38%
Current, 3-<6 per week	33	19%
Current, 6-<10 per week	40	22%
Current, 10 per week	21	12%
Treatment assignment		
Intervention	86	49%
Usual care	91	51%

Breast density measures	N	Median (IQR)
Percent dense breast volume (%)	177	24.3 (9.7–42.5)
Absolute dense breast volume (cm^3)	177	93.6 (50.0–140.3)
Absolute non-dense breast volume (cm^3)	177	299.2 (157.8–484.9)

Abbreviations: SD, standard deviation; BMI; body mass index; IQR, interquartile range

[a] Mean duration of hormonal use was calculated among past and current hormone users.

[b] Other education includes education until 8–11 years, completion of high school and vocational or technical school.

Table 2

Multivariable[a] adjusted geometric mean and 95% confidence interval (95% CI) of percent dense breast volume (%) (%DBV) according to quartiles of intakes of total and subtypes of fat during adolescence

| Quartiles of intake | All periods (N=177) | | By menarcheal status | | | |
| | | | Before menarche (N=160) | | After menarche (N=163) | |
	Median intake, % kcal	%DBV mean (95% CI)	Median intake, % kcal	%DBV mean (95% CI)	Median intake, % kcal	%DBV mean (95% CI)
Total fat						
Q1	24.4	21.4 (20.0–23.0)	24.6	17.1 (14.9–19.7)	22.5	16.6 (14.0–19.7)
Q2	28.0	16.8 (14.1–20.0)	29.5	22.7 (19.0–27.2)	27.2	19.3 (17.6–21.2)
Q3	31.3	18.4 (15.5–21.8)	33.1	18.1 (13.6–24.2)	30.3	17.4 (14.8–20.5)
Q4	35.3	18.6 (14.5–23.9)	37.4	16.5 (12.7–21.4)	35.4	20.7 (16.9–25.4)
P_{trend}[b]		0.50		0.61		0.22
Saturated fat						
Q1	8.2	16.4 (14.1–19.0)	8.5	16.2 (14.0–18.8)	7.6	18.4 (17.2–19.6)
Q2	10.0	17.7 (16.8–18.6)	10.4	17.1 (14.2–20.7)	9.6	16.8 (13.2–21.4)
Q3	11.6	19.9 (16.7–23.6)	12.1	20.4 (17.5–23.8)	11.3	17.9 (12.8–25.0)
Q4	13.4	21.5 (16.6–27.8)	14.1	20.5 (15.5–27.3)	13.3	21.1 (16.2–27.4)
P_{trend}[b]		<0.001		0.22		0.37
Monounsaturated fat						
Q1	8.8	25.0 (18.3–34.1)	9.1	18.7 (14.3–24.6)	8.0	19.4 (10.6–35.5)
Q2	10.4	16.8 (13.5–20.8)	10.9	18.9 (15.2–23.4)	9.7	20.4 (15.8–26.2)
Q3	11.5	18.6 (13.2–26.4)	12.2	17.3 (12.8–23.4)	11.4	17.0 (12.3–23.5)
Q4	13.2	15.8 (13.3–18.8)	14.2	19.0 (15.9–22.7)	13.3	17.3 (13.0–23.0)
P_{trend}[b]		<0.001		0.88		0.74
Polyunsaturated fat						
Q1	4.3	21.4 (18.3–25.1)	4.1	20.8 (17.0–25.5)	4.1	17.2 (14.7–20.1)
Q2	5.2	18.6 (14.3–24.2)	5.2	20.4 (18.5–22.3)	5.0	18.7 (15.0–23.3)
Q3	6.1	17.5 (12.7–24.0)	6.3	16.7 (13.6–20.6)	6.0	19.0 (16.6–21.7)
Q4	7.3	17.6 (16.0–19.4)	7.9	16.4 (13.3–20.4)	7.6	19.0 (15.0–24.1)

| Quartiles of intake | All periods (N=177) | | By menarcheal status | | | |
| | | | Before menarche (N=160) | | After menarche (N=163) | |
	Median intake, % kcal	%DBV mean (95% CI)	Median intake, % kcal	%DBV mean (95% CI)	Median intake, % kcal	%DBV mean (95% CI)
P_{trend}[b]		0.28		0.04		0.67
Long-chain omega-3 fat						
Q1	0.007	20.9 (17.4–25.0)	0.005	17.8 (13.8–22.8)	0.004	17.9 (16.0–20.0)
Q2	0.01	20.8 (16.9–25.5)	0.01	21.0 (17.6–25.1)	0.01	18.1 (14.2–23.2)
Q3	0.03	17.7 (14.5–21.6)	0.03	19.8 (15.1–25.9)	0.02	17.8 (15.4–20.5)
Q4	0.08	15.7 (11.2–22.0)	0.09	15.8 (14.2–17.5)	0.09	20.2 (15.9–25.6)
P_{trend}[b]		0.24		<0.001		0.45
Linoleic acid						
Q1	3.8	21.2 (18.6–24.2)	3.7	20.3 (16.1–25.4)	3.51	20.3 (16.1–25.4)
Q2	4.7	17.7 (13.5–23.3)	4.6	19.6 (17.2–22.3)	4.52	19.6 (17.2–22.3)
Q3	5.5	17.4 (14.0–21.8)	5.6	18.0 (16.4–19.8)	5.36	18.0 (16.4–19.8)
Q4	6.7	18.7 (16.3–21.5)	7.1	16.3 (12.7–20.8)	6.74	16.3 (12.7–20.8)
P_{trend}[b]		0.48		0.14		0.14
P/S ratio						
Q1	0.4	19.1 (15.8–23.1)	0.3	22.5 (17.6–28.8)	0.4	17.6 (15.9–19.6)
Q2	0.5	22.1 (17.3–28.2)	0.5	20.3 (16.2–25.6)	0.5	22.4 (18.8–26.6)
Q3	0.6	20.4 (17.3–24.1)	0.6	15.8 (12.8–19.5)	0.6	18.3 (14.7–22.9)
Q4	0.8	14.3 (11.5–17.7)	0.8	16.1 (12.9–20.1)	0.8	16.0 (12.8–19.9)
P_{trend}[b]		<0.001		<0.001		0.12

[a] Geometric means and 95% CI are estimated from linear mixed effects models including clinic as a random effect and including treatment group (diet intervention group and usual care-control group), childhood BMI z-score, current adult percent body fat from DXA (%, continuous), number of live births (0 and >0), duration of hormone use (yrs, continuous), race (White and non-White), education (bachelor's degree, graduate school and other), status of smoking (never, former and current), alcohol consumption (never/former, <3 drinks/week, 3–<6 drinks/week, 6–<10 drinks/week, 10 drinks/week, total energy intake (kcal/day, continuous), fiber intake (g/day, continuous), and percent of energy from protein (%, continuous); in analyses of specific types of fat, percent of energy from saturated fat (%, continuous), polyunsaturated fat (%, continuous), and monounsaturated fats (%, continuous) were mutually adjusted for each another.

[b] P-test for trend was conducted by modeling the quartile medians of intakes of each fat as a continuous term in linear mixed effects models and calculating the Wald test statistic.

Table 3

Multivariable[a] adjusted geometric mean and 95% confidence interval (95% CI) of absolute dense breast volume (ADBV) (cm^3) according to quartiles of total and subtypes of fat intake during adolescence

| | All periods (N=177) | | By menarcheal status | | | |
| | | | Before menarche (N=160) | | After menarche (N=163) | |
Quartiles of intake	Median intake, % kcal	ADBV mean (95% CI)	Median intake, % kcal	ADBV mean (95% CI)	Median intake, % kcal	ADBV mean (95% CI)
Total fat						
Q1	24.4	89.3 (73.5–108.7)	24.6	71.7 (59.9–85.7)	22.5	69.0 (49.7–95.9)
Q2	28.0	63.1 (52.0–76.5)	29.5	90.4 (76.7–106.5)	27.2	78.4 (70.8–86.8)
Q3	31.3	81.8 (69.3–96.7)	33.1	80.8 (64.8–100.8)	30.3	78.2 (62.2–98.2)
Q4	35.3	84.3 (65.5–108.6)	37.4	69.5 (50.6–95.6)	35.4	90.5 (74.5–109.8)
P_{trend}[b]		0.82		0.67		0.19
Saturated fat						
Q1	8.2	65.6 (45.8–94.1)	8.5	67.5 (59.4–76.7)	7.6	74.9 (54.5–103.0)
Q2	10.0	72.0 (66.4–78.1)	10.4	67.4 (53.9–84.3)	9.6	78.3 (63.9–96.0)
Q3	11.6	85.1 (71.3–101.5)	12.1	87.1 (80.6–94.1)	11.3	71.1 (47.9–105.8)
Q4	13.4	97.4 (73.5–129.0)	14.1	91.9 (65.4–129.3)	13.3	91.8 (69.6–120.7)
P_{trend}[b]		0.03		0.08		0.45
Monounsaturated fat						
Q1	8.8	104.5 (76.7–142.4)	9.1	81.6 (70.2–94.9)	8.0	71.7 (34.9–147.4)
Q2	10.4	66.4 (58.2–75.9)	10.9	76.7 (60.4–97.4)	9.7	84.4 (67.5–105.7)
Q3	11.5	80.9 (57.6–113.6)	12.2	76.6 (52.0–112.7)	11.4	75.9 (52.1–110.6)
Q4	13.2	69.0 (57.4–82.9)	14.2	76.0 (60.4–95.5)	13.3	83.1 (56.1–123.0)
P_{trend}[b]		0.05		0.71		0.82
Polyunsaturated fat						
Q1	4.3	86.5 (75.8–98.9)	4.1	85.1 (78.3–92.5)	4.1	69.3 (55.6–86.4)
Q2	5.2	76.9 (54.5–108.4)	5.2	80.2 (67.7–95.0)	5.0	81.7 (65.1–102.6)
Q3	6.1	68.9 (44.0–108.0)	6.3	71.3 (56.5–90.0)	6.0	84.5 (63.4–112.6)
Q4	7.3	84.9 (70.8–101.7)	7.9	74.8 (54.8–102.0)	7.6	79.7 (59.5–106.9)

Quartiles of intake	All periods (N=177)		By menarcheal status			
			Before menarche (N=160)		After menarche (N=163)	
	Median intake, % kcal	ADBV mean (95% CI)	Median intake, % kcal	ADBV mean (95% CI)	Median intake, % kcal	ADBV mean (95% CI)
P_{trend}[b]		0.93		0.36		0.73
Long-chain omega-3 fat						
Q1	0.007	89.3 (76.2–104.7)	0.005	76.6 (62.5–93.7)	0.004	71.4 (56.4–90.4)
Q2	0.01	81.9 (65.5–102.3)	0.01	87.7 (68.5–112.2)	0.01	77.0 (54.3–109.1)
Q3	0.03	76.6 (59.1–99.3)	0.03	82.8 (62.4–109.9)	0.02	79.2 (58.9–106.5)
Q4	0.08	69.4 (46.0–104.6)	0.09	65.5 (54.9–78.2)	0.09	87.8 (59.0–130.6)
P_{trend}[b]		0.35		<0.001		0.53
Linoleic acid						
Q1	3.8	83.0 (73.9–93.3)	3.7	86.8 (78.7–95.7)	3.51	78.0 (64.2–94.8)
Q2	4.7	72.7 (54.0–97.9)	4.6	72.1 (57.7–90.2)	4.52	74.8 (57.4–97.5)
Q3	5.5	74.3 (54.6–102.9)	5.6	79.9 (67.9–94.0)	5.36	84.0 (66.1–106.8)
Q4	6.7	85.2 (68.6–105.8)	7.1	72.8 (53.4–99.2)	6.74	77.8 (58.3–103.7)
P_{trend}[b]		0.68		0.40		0.92
P/S ratio						
Q1	0.4	76.6 (62.6–93.8)	0.3	85.1 (64.9–111.5)	0.4	71.1 (62.7–80.7)
Q2	0.5	96.5 (66.8–139.2)	0.5	88.2 (69.5–111.8)	0.5	100.7 (73.7–137.6)
Q3	0.6	82.2 (66.4–101.8)	0.6	67.6 (48.9–93.5)	0.6	73.4 (52.7–102.2)
Q4	0.8	64.2 (49.8–82.8)	0.8	71.8 (58.5–88.1)	0.8	72.4 (54.3–96.5)
P_{trend}[b]		0.19		<0.001		0.54

[a] Geometric means and 95% CI are estimated from linear mixed effects models including clinic as a random effect and including treatment group (diet intervention group and usual care-control group), childhood BMI z-score, current adult percent body fat from DXA (%, continuous), number of live births (0 and >0), duration of hormone use (yrs, continuous), race (White and non-White), education (bachelor's degree, graduate school and other), status of smoking (never, former and current), alcohol consumption (never/former, <3 drinks/week, 3-<6 drinks/week, 6-<10 drinks/week, 10 drinks/week), total energy intake (kcal/day, continuous), fiber intake (g/day, continuous), and percent of energy from protein (%, continuous) as fixed effects; in analyses of specific types of fat, percent of energy from saturated fat (%, continuous), polyunsaturated fat (%, continuous), and monounsaturated fats (%, continuous) were mutually adjusted for each another.

[b] P-test for trend was conducted by modeling the quartile medians of intakes of each fat as a continuous term in linear mixed effects models and calculating the Wald test statistic.

Table 4

Multivariable[a] adjusted geometric mean and 95% confidence interval (95% CI) of absolute nondense breast volume (ANDBV) (cm³) according to quartiles of total and subtypes of fat intake during adolescence

| | All periods (N=177) | | By menarcheal status | | | |
| | | | Before menarche (N=160) | | After menarche (N=163) | |
Quartiles of intake	Median intake, % kcal	ANDBV mean (95% CI)	Median intake, % kcal	ANDBV mean (95% CI)	Median intake, % kcal	ANDBV mean (95% CI)
Total fat						
Q1	24.4	271.0 (226.5–324.4)	24.6	276.1 (222.9–341.9)	22.5	293.5 (240.5–358.1)
Q2	28.0	242.9 (242.9–301.2)	29.5	267.4 (245.2–291.6)	27.2	275.4 (233.9–324.4)
Q3	31.3	288.0 (240.9–344.4)	33.1	305.2 (254.7–365.6)	30.3	301.9 (257.5–354.0)
Q4	35.3	326.6 (257.1–414.8)	37.4	312.1 (256.1–380.5)	35.4	298.5 (253.4–351.6)
P_{trend}[b]		0.25		0.33		0.80
Saturated fat						
Q1	8.2	297.1 (232.5–379.7)	8.5	288.3 (259.4–320.3)	7.6	288.1 (219.5–378.1)
Q2	10.0	274.5 (252.2–298.6)	10.4	282.8 (211.9–377.4)	9.6	318.4 (261.0–388.3)
Q3	11.6	281.5 (230.2–344.2)	12.1	281.9 (253.7–313.2)	11.3	270.9 (235.0–312.4)
Q4	13.4	299.7 (265.0–339.1)	14.1	306.0 (276.2–339.0)	13.3	293.1 (259.3–331.3)
P_{trend}[b]		0.78		0.55		0.80
Monounsaturated fat						
Q1	8.8	267.1 (215.2–331.4)	9.1	283.1 (208.2–385.0)	8.0	247.7 (187.7–326.7)
Q2	10.4	268.5 (217.0–332.2)	10.9	289.5 (260.5–321.8)	9.7	280.6 (241.6–325.8)
Q3	11.5	301.2 (267.4–339.3)	12.2	311.2 (269.5–359.3)	11.4	303.3 (252.2–364.7)
Q4	13.2	319.4 (270.3–377.4)	14.2	275.6 (250.3–303.5)	13.3	346.8 (293.7–409.7)
P_{trend}[b]		0.24		0.66		0.10
Polyunsaturated fat						
Q1	4.3	274.8 (242.3–311.7)	4.1	260.8 (217.4–312.9)	4.1	289.2 (260.7–320.8)
Q2	5.2	275.1 (249.6–303.1)	5.2	271.7 (236.9–311.5)	5.0	303.7 (270.6–340.7)
Q3	6.1	278.6 (226.6–342.5)	6.3	306.3 (263.0–356.6)	6.0	302.2 (267.7–341.2)
Q4	7.3	327.3 (254.7–420.8)	7.9	324.1 (291.8–359.8)	7.6	274.0 (218.0–344.3)

| Quartiles of intake | All periods (N=177) | | By menarcheal status | | | |
| | | | Before menarche (N=160) | | After menarche (N=163) | |
	Median intake, % kcal	ANDBV mean (95% CI)	Median intake, % kcal	ANDBV mean (95% CI)	Median intake, % kcal	ANDBV mean (95% CI)
P_{trend} [b]		0.30		0.01		0.49
Long-chain omega-3 fat						
Q1	0.007	297.8 (262.0–338.6)	0.005	297.9 (269.1–329.7)	0.004	281.3 (246.1–321.5)
Q2	0.01	276.6 (233.3–328.1)	0.01	278.6 (260.5–297.9)	0.01	289.1 (262.8–318.0)
Q3	0.03	300.5 (245.9–367.2)	0.03	289.4 (232.4–360.4)	0.02	327.0 (288.2–371.0)
Q4	0.08	277.9 (217.2–355.6)	0.09	292.8 (248.2–345.4)	0.09	273.4 (217.5–343.6)
P_{trend} [b]		0.75		0.87		0.55
Linoleic acid						
Q1	3.8	275.1 (249.3–303.5)	3.7	280.2 (222.3–353.0)	3.51	296.9 (274.3–321.4)
Q2	4.7	276.7 (256.3–298.8)	4.6	249.0 (208.6–297.3)	4.52	292.6 (251.5–340.5)
Q3	5.5	295.8 (233.5–374.6)	5.6	315.0 (276.5–358.8)	5.36	293.8 (240.2–359.4)
Q4	6.7	306.1 (235.7–397.6)	7.1	320.0 (284.7–359.7)	6.74	285.0 (220.7–368.1)
P_{trend} [b]		0.48		0.02		0.78
P/S ratio						
Q1	0.4	280.2 (254.8–308.1)	0.3	241.2 (222.4–261.5)	0.4	274.3 (254.3–296.0)
Q2	0.5	272.5 (219.9–337.7)	0.5	304.3 (253.3–365.6)	0.5	297.0 (251.4–350.8)
Q3	0.6	284.9 (252.8–321.0)	0.6	305.5 (265.7–351.2)	0.6	287.5 (239.6–345.0)
Q4	0.8	316.8 (281.4–354.7)	0.8	313.7 (285.3–344.9)	0.8	311.3 (240.0–403.7)
P_{trend} [b]		0.07		<0.001		0.42

[a] Geometric means and 95% CI are estimated from linear mixed effects models including clinic as a random effect and including treatment group (diet intervention group and usual care-control group), childhood BMI z-score, current adult percent body fat from DXA (%, continuous), number of live births (0 and >0), duration of hormone use (yrs, continuous), race (White and non-White), education (bachelor's degree, graduate school and other), status of smoking (never, former and current), alcohol consumption (never/former, <3 drinks/week, 3–<6 drinks/week, 6–<10 drinks/week, 10 drinks/week), total energy intake (kcal/day, continuous), fiber intake (g/day, continuous), and percent of energy from protein (%, continuous) as fixed effects; in analyses of specific types of fat, percent of energy from saturated fat (%, continuous), polyunsaturated fat (%, continuous), and monounsaturated fats (%, continuous) were mutually adjusted for each another.

[b] P-test for trend was conducted by modeling the quartile medians of intakes of each fat as a continuous term in linear mixed effects models and calculating the Wald test statistic.