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The associations between intakes of one-carbon metabolism-related vitamins and breast density among young women

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Abstract

Background: Folate is the primary methyl donor and B vitamins are cofactors for one-carbon metabolism that maintain DNA integrity and epigenetic signatures implicated in carcinogenesis. Breast tissue is particularly susceptible to stimuli in early life. Only limited data are available on associations of one-carbon metabolism-related vitamin intake during youth and young adulthood with breast density, a strong risk factor for breast cancer.

Methods: Over 18 years in the DISC and DISC06 Follow-up Study, diets of 182 young women were assessed by three 24-hour recalls on five occasions at ages 8–18 years and once at 25–29 years. Multivariable-adjusted linear mixed-effects regression was used to examine associations of intakes of one-carbon metabolism-related vitamins with MRI-measured percent dense breast volume (%DBV) and absolute dense breast volume (ADBV) at ages 25–29 years.

Results: Folate intake in youth was inversely associated with %DBV (P_{trend}=0.006) and ADBV (P_{trend}=0.02). These inverse associations were observed with intake during post-, though not pre-

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Authors' Contributions

S. Jung and J.F. Dorgan designed the research and revised the manuscript. E. Han and S.Jung conducted the research and analyzed the data, and E. Han drafted the manuscript. L.V. Horn, L. Snetselaar, and J.A. Shepherd participated in data collection. S. Jung, J.F. Dorgan, L.V. Horn, L. Snetselaar, S. Jung, Y.J. Park, and H. Kim critically reviewed the manuscript and contributed to interpretation. S. Jung had primary responsibility for final content. All authors read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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menarche. In contrast, premenarche vitamin B2 intake was positively associated with ADBV (P_{trend}<0.001). Young adult folate and vitamin B6 intakes were inversely associated with %DBV (all P_{trend}≤0.04), whereas vitamins B6 and B12 were inversely associated with ADBV (all P_{trend}≤0.04).

Conclusions: Among these DISC participants intakes of one-carbon metabolism-related vitamins were associated with breast density. Larger prospective studies among diverse populations are needed to replicate these findings.

Impact: Our results suggest the importance of one-carbon metabolism-related vitamin intakes early in life with development of breast density and thereby potentially breast cancer risk later in life.

Keywords

one-carbon metabolism; folate; B vitamins; youth; young adulthood; breast density; breast cancer risk

Introduction

Breast density is a measure of the relative proportion of breast glandular and stromal tissue to fatty tissue and a strong risk factor for breast cancer (1–3). A systematic review of 42 studies showed that women with 75% or greater breast density had a four- to six- fold increased risk of breast cancer compared to those with 5% or less breast density (2). Breast tissue undergoes structural changes across a woman's lifespan, rapidly proliferating during puberty (4) and fully differentiating during the first term pregnancy (5). Substantial evidence from animal and epidemiologic studies strongly supports vulnerability of the breasts before full differentiation (6,7). Identification of early life modifiable factors associated with development of breast density may have significant implications for the early prevention of breast cancer.

One-carbon metabolism is a network of biochemical pathways that interconnect multiple B vitamins for maintaining DNA methylation and synthesis (8,9). Folate (vitamin B9) is the primary methyl donor for one-carbon metabolism, whereas vitamins B2, B6, and B12 catalyze this process as essential cofactors. A vitamin B6-dependent enzyme converts tetrahydrofolate (THF), a reduced form of folate, into 5,10-methylene THF. 5,10-Methylene THF is either used for the biosynthesis of purines and pyrimidines, the building blocks of RNA and DNA, or reduced to 5-methyl THF by methylenetetrahydrofolate reductase using vitamin B2 as a cofactor. 5-Methyl THF methylates homocysteine to form methionine by a B12-containing methyltransferase. The methionine is converted to S-adenosyl methionine, a universal donor of methyl groups for DNA and RNA, thereby affecting gene stability and expression (8). Insufficient intake of one-carbon metabolism-related vitamins may, therefore, disrupt DNA synthesis, repair, and methylation, which may contribute to the mechanisms for breast carcinogenesis (10,11).

To our knowledge, no studies have investigated the associations of one-carbon metabolism-related vitamins consumed early in life with breast density. To date, there have been six studies that examined associations between these vitamins (e.g., folate (12–15), vitamins

B12 (16,17)) and breast density. Though one study reported a positive association between vitamin B12 and breast density (17), results of the other studies were null (12–16). These studies included women in midlife or older (12–17), and associations of one-carbon metabolism related vitamins with breast density could differ over the life course. We, therefore, prospectively examined associations of youth and young adult intakes of one-carbon metabolism-related vitamins with breast density measured at 25–29 years in the Dietary Intervention Study in Children (DISC) (18,19) and DISC06 Follow-up Study (20,21), which are among the few long-term cohorts of children with repeated measurement of diet and lifestyle factors.

Materials and Methods

Study design

The DISC was a multicenter, randomized controlled clinical trial to assess the efficacy, safety, and acceptability of a lipid-lowering diet in children with elevated plasma low-density lipoprotein cholesterol (LDL-C) levels (18,22–26). Between 1988 and 1990, 663 pre-pubertal, 8- to 10- year-old children (301 girls and 362 boys) with elevated plasma LDL-C were enrolled in the DISC and randomly assigned by the data coordinating center at Maryland Medical Research Institute (Baltimore, MD) to either a reduced saturated fat diet intervention or a usual-care control group at six clinical centers: Children's Hospital (New Orleans, LA), Johns Hopkins University Hospital (Baltimore, MD), Kaiser Permanente Center for Health Research (Portland, OR), University of Medicine and Dentistry of New Jersey (Newark, NJ), Northwestern University Feinberg School of Medicine (Chicago, IL), and University of Iowa Hospital and Clinics (Iowa City, IA) (24). The trial ended in 1997, when the mean age of participants was 16.7 years, after a mean of 7.4 years intervention that achieved reductions in intakes of dietary total fat, saturated fat and cholesterol among the intervention group (23). Between 2006 and 2008, when DISC participants were 25- to 29 years old, the DISC06 Follow-up Study was conducted among 260 female DISC participants to investigate the long-term effect of the diet intervention in youth on breast cancer related biomarkers (20). Assent from participants and informed consent from parents/guardians were obtained prior to the DISC trial, whereas participants provided informed consent prior to the DISC06 Follow-up Study. Institutional review boards at all participating clinical centers and the data coordinating center approved the protocols of the DISC and the DISC06 Follow-up Study.

Study population

Of the 260 young women who participated in the DISC trial and DISC06 Follow-up Study, 30 who were pregnant or breastfeeding during or within 12 weeks before the follow-up visit, 16 who had breast augmentation or reduction surgery, and 32 who had missing or unacceptable breast density images were excluded. Thus, the present analysis included 182 women (Supplementary Figure 1).

Data collection

Data on covariates used in the current analysis were mostly collected during the DISC06 follow-up visit when participants completed questionnaires on sociodemographic

characteristics, medication use, reproductive and medical history, and health-related behaviors such as alcohol consumption, smoking status, and physical activity. In addition, young adult whole body fat percentage was measured once by dual-energy X-ray absorptiometry (DXA) at the DISC06 follow-up visit (27). Height and weight were measured at the DISC06 follow-up visit as well as at each clinic visit during the DISC trial, and body mass index (BMI) was calculated as weight/height^2 (kg/m^2) (21). Childhood BMI z-scores were calculated using Centers for Disease Control and Prevention 2000 Growth Charts (28). Onset of menses was ascertained annually until menarche during DISC (29).

Dietary assessment

Usual diet was assessed using three 24-hour dietary recalls during DISC at baseline, at year 1, year 3, and year 5, and at the last visit, and at the DISC06 follow-up visit (30,31). At each time point, the first recall was collected in-person by trained nutritionists, while the two subsequent recalls were obtained by phone over two weeks. Two recalls were conducted on non-consecutive weekdays and the third was conducted on a weekend day. Participants' dietary supplement intakes were obtained as part of the 24-hour recall. Nutrient analysis was performed at the University of Minnesota Nutrition Coordinating Center. Intakes from each of the three days were averaged to estimate usual daily intakes separately from food and supplements at each clinic visit (21). Total nutrient intakes were calculated by summing intakes from foods and supplements and energy-adjusted using the residual method (32)

Breast density measurements

Non-contrast magnetic resonance imaging (MRI) was used to quantify participants' breast densities at the DISC06 Follow-up Study visit. A whole-body 1.5 T or higher field-strength MRI scanner with a dedicated breast imaging radiofrequency coil was used for scanning. A standard protocol was followed consisting of a 3D T1-weighted fast gradient echo pulse sequence performed with and without fat-suppression and in transaxial and coronal orientations. All MRI images were processed at the University of California San Francisco using customized software to identify the chest wall-breast tissue boundary and skin surface and to separate fibroglandular tissue from fatty tissue using a segmentation method based on fuzzy *C*-means clustering (FCM) (33). MRI technologists were trained to identify and correct MRI image failures due to motion artifacts, inadequate breast coverage, or imperfect fat suppression. Acceptable image quality on three volunteers was required before the clinic was certified to image DISC participants.

Total breast volume and absolute dense breast volume (ADBV) were obtained for each breast and absolute non-dense breast volume (ANDBV) was calculated by subtraction. Percent dense breast volume (%DBV) was calculated as the percentage of ADBV over total breast volume. All breast density measures on the two breasts were highly correlated ($r > 0.94$). Results for the two breasts were averaged to provide single young adult measures of %DBV, ADBV and ANDBV for each participant.

Statistical analysis

Intakes of one-carbon metabolism-related vitamins in youth and in young adulthood were estimated separately. Long-term dietary intake during youth, estimated as the average intake

across DISC trial visits, was our primary exposure for youth intake. To investigate specific pubertal stages (34,35), average pre- and post-menarche intakes were calculated. Young adult intakes were estimated as the average intake at the DISC06 follow-up visit.

Young adult breast density measurements were natural log-transformed to improve normality prior to analysis. Associations of one-carbon metabolism-related vitamins with young adult breast density measures were estimated by calculating geometric means and 95% confidence intervals using linear mixed-effects regression models with robust standard errors. Models included, as fixed effects, the following potential confounders associated with breast density (31,36,37) or breast cancer risk (38): childhood BMI z-score at baseline; and DXA measured young adult body fat percentage, number of live births, duration of hormonal contraceptive use, race, education, smoking status, and alcohol intake ascertained at the DISC06 follow-up visit. Energy intake and treatment assignment also were included as fixed effects, and clinic was included as a random effect. A test for trend was performed by modeling the quartile medians for each vitamin as a continuous term and calculating the Wald test statistic. Linear-mixed effects regression models were fit in 25 multiply imputed data sets that estimated missing values for whole-body fat percentage (n=6) from a prediction model that included BMI, a strong correlate of whole-body fat percentage, as an independent variable. Results from each imputed dataset were pooled using Rubin's rule (39).

Several sensitivity analyses were conducted to evaluate robustness of observed results. Adolescent and young adult vitamin intakes were mutually adjusted to control for the correlation of dietary intakes over the lifecourse (40). We conducted analyses restricted to nulliparous women whose breasts had not yet undergone full differentiation and might be more susceptible to dietary exposures. We further performed analyses stratified by alcohol consumption, which impairs folate absorption (41), level of one-carbon metabolism-related vitamin intakes during childhood, and intervention assignment to explore possible effect modification. Statistical significance of effect modification was tested by including the cross-product term between vitamin intake and the stratification factor in the fully adjusted model.

All analyses were performed using STATA (version 13.0) (College Station, Texas, USA). All statistical tests were two-sided and conducted at the 0.05 significance level.

Data availability: Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

Results

The mean age at the DISC06 follow-up visit of the 182 participants included in the current analysis was 27.2 ± 1.0 years (Table 1). Their mean BMI at the visit was 25.4 ± 5.4 kg/m^2, and their mean age at menarche was 12.9 ± 1.3 years. The majority were non-Hispanic white (86%), educated (66% had acquired at least a bachelor's degree), nulliparous (71%), and former or current hormonal contraceptive users (94%). Their reported mean intakes of one-carbon metabolism-related vitamins across DISC trial visits during youth were 225.5

± 58.5 μg/day for folate, 1.7 ± 0.3 mg/day for vitamin B2, 1.3 ± 0.3 μg/day for vitamin B6, and 3.6 ± 1.1 μg/day for vitamin B12. Their reported mean intakes of one-carbon metabolism-related vitamins at the DISC06 follow-up visit were 414.1 ± 160.1 μg/day for folate, 2.0 ± 0.7 mg/day for vitamin B2, 1.8 ± 0.8 μg/day for vitamin B6, and 5.0 ± 3.5 μg/day for vitamin B12. The means and interquartile ranges of young adult breast density measures were 24.5% (9.7%–41.2%) for %DBV, 93.0 cm^3 (50.0 cm^3–140.3 cm^3) for ADBV, and 289.5 cm^3 (157.8 cm^3–485.3 cm^3) for ANDBV.

Table 2 shows the associations of %DBV and ADBV with one-carbon metabolism-related vitamins consumed during youth and at specific pubertal stages defined by menarche status. Folate intake during youth was significantly inversely associated with %DBV and ADBV. Mean %DBV decreased from 19.7% in the lowest quartile of folate intake to 16.9% in the highest quartile (P_{trend}=0.006), while ADBV decreased from 80.3 cm^3 to 70.2 cm^3 (P_{trend}=0.02). Inverse associations of folate with %DBV and ADBV (all $P_{trend}\leq$0.02) were consistently observed with vitamin intakes postmenarche, but not premenarche. Mean %DBV decreased from 21.2% to 15.8% across increasing quartiles of postmenarche folate intake (P_{trend}=0.01), and mean ADBV decreased from 90.2 cm^3 to 68.7 cm^3 (P_{trend}=0.02). The remaining vitamins were not significantly associated with %DBV or ADBV during youth, except premenarche vitamin B2 intake was significantly positively associated with ADBV. Mean ADBV increased monotonically from 69.9 cm^3 to 93.5 cm^3 with increasing quartiles of premenarche vitamin B2 intake (P_{trend}=0.004). This positive association did not change by additional adjustment for intake of saturated fats that are also abundant in major food sources of vitamin B2 (P_{trend}=0.009).

When associations were examined with young adult one-carbon metabolism-related vitamin intakes (Table 3), significant inverse associations were observed for folate and vitamin B6 with %DBV and for vitamin B6 and vitamin B12 with ADBV (all $P_{trend}\leq$0.04). With increasing quartiles of intake, mean %DBV decreased from 22.0% to 17.0% for folate and from 20.2% to 15.5% for vitamin B6, whereas mean ADBV decreased from 74.0 cm^3 to 66.5 cm^3 for vitamin B6 and from 82.0 cm^3 to 71.6 cm^3 for vitamin B12. No associations were observed for vitamin B2 intake with %DBV and ADBV.

In sensitivity analyses, mutual adjustment for youth and young adult intakes of one-carbon metabolism-related vitamins did not materially change results, except the inverse association between young adult folate intake and %DBV became nonsignificant (Supplementary Tables 1 and 2). There also was no evidence for effect modification of the associations of young adult intakes of one-carbon metabolism-related vitamins with %DBV by youth intakes of these vitamins (Supplementary Table 3). Restricting analyses to nulliparous women attenuated the inverse association for youth intakes of folate with %DBV and ADBV and young adult vitamins B6 and B12 intakes with ADBV (Supplementary Tables 4 and 5). No significant effect modification was observed by alcohol consumption (Supplementary Table 6). However, DISC treatment group assignment significantly modified associations between youth vitamin B2 intake and young adult folate intake with %DBV. Though youth B2 intake was not associated with %DBV overall, in analysis stratified by treatment group, %DBV decreased significantly (P_{trend}=0.006) from 19.5% to 13.4% across increasing quartiles of youth B2 intake in the usual care group but increased non-significantly from 17.9% to

26.4% in the intervention group ($P_{interaction}$=0.01). Furthermore, the inverse association of young adult folate intake with %DBV overall was observed only in the usual care group. %DBV decreased significantly (P_{trend}=0.03) from 20.1% to 15.6% across increasing quartiles of young adult folate intake in the usual care group but decreased non-significantly from 21.6% to 18.3% in the intervention group ($P_{interaction}$=0.01).

In secondary analyses, we evaluated associations of one-carbon metabolism-related vitamins with ANDBV (Supplementary Tables 7 and 8). Folate intake during youth postmenarche was significantly positively associated with ANDBV; mean ANDBV increased from 282.4 cm^3 to 330.5 cm^3 across increasing quartiles of folate (P_{trend}=0.01). Youth vitamin B2, B6, and B12 intakes were not associated with ANDBV regardless of menarche status. Young adult vitamin intakes also were not associated with ANDBV.

Discussion

DISC is the first study to report associations of youth and early adulthood consumption of one-carbon metabolism-related vitamins with young adult measures of breast density. Youth folate intake was inversely associated with %DBV and ADBV. In analysis stratified by menarche status, these associations were only observed postmenarche. Premenarche vitamin B2 intake, in contrast, was positively associated with ADBV. Young adult folate and vitamin B6 intakes also were inversely associated with %DBV, while vitamin B6 and B12 were inversely associated with ADBV.

To date, associations between one-carbon metabolism-related vitamins and breast density have been examined in only a few studies of adult women in midlife or older (12–17), showing weak and inconsistent evidence. Folate intake among women with mean ages of 47 years (12) or older (13–15) was not associated with breast density (12–15) or breast dense area (12) in a cohort study (13) or three cross-sectional studies (12,14,15).Vitamin B12 intake among women with a mean age of 58 years (16,17) also was not associated with breast density (16) or dense breast area (16) in a cross-sectional study, though a significant positive association was observed with breast density in the Minnesota Breast Cancer Family Study (17). To our knowledge, no studies have examined associations of breast density with other B vitamins related to one-carbon metabolism (e.g., vitamins B2 and B6). Circulating homocysteine levels reflect adequacy of dietary intakes of folate and other B-vitamins involved in one-carbon metabolism. Though homocysteine was associated with breast cancer risk in two case-control study (42,43), it was not associated with risk in five prospective nested case-control studies (44–47). We are not aware of any studies that examined associations of homocysteine with breast density.

To the best of our knowledge, there are no studies that investigated associations of intakes of one-carbon metabolism-related vitamins early in life, during youth and young adulthood, when effects could be greatest. Breast tissue undergoes dynamic structural and physiologic changes over a woman’s life, experiencing high cellular proliferation during puberty, differentiating during pregnancy and lactation, and regressing subsequently with aging (48,49). Exposure to carcinogens, particularly during periods of rapid and extensive proliferation before full differentiation, may increase the chance of DNA damage leading

to malignant transformation (50). There is substantial evidence for a greater influence of exposures on the breasts at younger compared to older ages (7,51,52). For example, radiation (53), famine (54), high red meat consumption (55) and low soy diets (56) are more strongly associated with breast cancer risk when the exposure occurs during adolescence compared to adulthood. We also observed stronger associations of sex hormones (34) and saturated fat (57) and carbohydrate intakes (58) during adolescence compared to young adulthood with young adult %DBV in our prior analyses of DISC and the DISC06 Follow-up Study.

Several lines of evidence support the inverse associations of early life folate and vitamin B6 intakes with %DBV and ADBV that we observed. In a study of children aged 6–18 years, high folate consumption was associated with less DNA damage (59) that is positively associated with breast density (60–62). There also is increasing evidence for epigenetic control of sex hormones and growth hormone (GH)/insulin-like growth factors (IGF) (63,64). These hormones regulate breast ductal outgrowth (65–67) that increases dense breast area and susceptibility to inflammation (68–70), both of which are positively associated with breast density (61,62,71,72). Aberrant DNA repair or DNA methylation resulting from lower intakes of one-carbon metabolism-related vitamins during youth or young adulthood could potentially affect breast density by altering breast gene stability or expression or by modifying early-life reprograming of peripheral hormonal signals and physiology.

Puberty is a time of rapid physical growth and sexual maturation (65–67). Large hormonal changes and breast development occur over the course of specific transitional stages at puberty. In particular, ovarian hormonal surges become detectable at menarche (35). Accumulating evidence indicates that exposures during youth associated with breast cancer risk may differ before and after menarche (34,35,57,58,73). Earlier onset of menarche is an established breast cancer risk factor (74). Recent studies also have shown positive associations between a longer time interval between thelarche (the onset of breast development) and menarche with breast cancer risk (35) and breast density (73,75). Similarly, in our previous analyses from DISC and the DISC06 Follow-up Study, associations of dietary fat (57), carbohydrates (58) and sex hormones (34) with breast density varied depending on pubertal timing.

Some differential associations of one-carbon metabolism-related vitamins with %DBV and ADBV also were observed depending on pubertal timing in the current analysis. Youth folate intake was strongly inversely associated with %DBV and ADBV when it was consumed after, but not before, menarche. In contrast, vitamin B2 intake was positively associated with ADBV when it was consumed before, but not after, menarche. The cause for these different associations is unclear. However, major food sources of vitamin B2 and folate differ. Vitamin B2 is plentiful in beef, pork, cheese, and cow’s milk, which are major sources of saturated fat that, when consumed during youth, has been positively associated with breast density (57,76). In contrast, folate is rich in green leafy vegetables, fruits, and whole grains. Our results may have arisen due to associations with breast density measures of other highly correlated nutrients within distinct food sources of folate and vitamin B2, though adding saturated fat intake to models in sensitivity analysis did not change results.

Alternatively, given the relatively small sample size of our study, this result could be a chance finding. Nonetheless, our results add to the growing evidence that susceptibility of the breasts to exposures may vary over the pubertal transition.

Breast tissue is fully differentiated after a first full-term pregnancy when it is considered less susceptible to stimuli (48–50). Thus, we hypothesized *a priori,* a greater effect of one-carbon metabolism-related vitamins among nulliparous women. In sensitivity analyses restricted to nulliparous women, we generally observed patterns of associations consistent with those seen in the entire study population. However, associations of youth folate intake with %DBV and ADBV and associations of young adult vitamins B6 and B12 intakes with ADBV were attenuated and no longer significant, possibly related, in part, to the smaller sample size.

In analysis stratified by treatment group, youth vitamin B2 and young adult folate intakes were significantly inversely associated with %DBV in women who were in the DISC usual care control group as children but not in those who received the lipid-lowering diet intervention. The DISC intervention was a multi-faceted behavioral intervention that may have altered the association of one-carbon metabolism and breast density, possibly via epigenetic modification of genes associated with breast density. Alternatively, our results could be due to chance especially considering the multiple comparisons made. We are not aware of any previous reports of similar one-carbon metabolism-related nutrient interactions in relation to breast density or breast cancer risk and urge caution in interpretation of these findings.

We evaluated associations of one-carbon metabolism-related vitamins with ANDBV in secondary analysis. The role of breast adipose tissue in breast cancer risk has been controversial (77), though accumulating evidence supports an inverse association of fatty non-dense breast area, independent of percent density or dense breast area, with breast cancer risk (78–80). The breast microenvironment in fatty compared to dense breasts has been hypothesized to reduce breast cancer risk by promoting normal mammary development and homeostasis (77). Our significant positive association of postmenarche folate intake with ANDBV is consistent with increased weight gain in juvenile rats supplemented with folate that the authors speculated may have been due to increased adipogenesis (81). We are aware of only one cross-sectional study that evaluated the association of adult folate intake and non-dense breast area and results were null (12). Additional studies are needed to determine if timing of exposure to folate over the lifecourse modifies epigenomic signatures in the breast and their associations with breast density measures including ANDBV.

The longitudinal design of this study was a major strength that facilitated prospective evaluation of associations between youth diet and development of breast density while minimizing potential for recall bias. All data were collected by trained personnel following a standardized protocol to ensure data quality. Volumetric breast density was measured by MRI, which provides accurate breast composition data for young women not impaired by high parenchymal breast density (82). We could comprehensively adjust for potential confounders from youth and young adulthood. Finally, because of the natural involution of the breast tissue that occurs with aging (83), measurement of breast density in younger

women, may better capture the effects of early dietary intake and other early life exposures on subsequent outcomes.

Our study also has some limitations. The sample size of 182 participants limited ability to detect even moderate associations that require larger studies. Several additional factors could limit generalizability of these results; all participants were aged 25–29 years, most were Caucasian and well-educated with mean BMI in the normal range (84). Folate fortification of grains in the US was initiated in 1998, after the DISC trial was completed, limiting the generalizability of these folate results to US girls' current diet. However, even in the folate-fortification era, 19% of US girls do not meet the estimated average requirement for folate intake (85). We measured breast density by MRI, though breast density is more commonly measured by mammography. Even so, MRI- and mammography-measured breast density are highly correlated ($r \geq 0.75$) (86,87) and similarly related to breast cancer risk (88). Breast density was measured on a single occasion, which may be inadequate for characterization. Even so, breast density tracks over time (89), and positive associations between breast density measured in older premenopausal women and breast cancer risk supports the translation of our results to the potential future risk of breast cancer (90–92). Not all nutrients involved in one carbon metabolism were assessed and results might be susceptible to multiple hypothesis testing. Nonetheless, analyses were conducted based on an *a priori* hypothesis and results were interpreted cautiously considering previous evidence and biological relevance (93). Possible effect modification by genetic predisposition associated with one-carbon metabolism was not explored due to lack of data on genetic variants in our study. Finally, we cannot rule out unknown or residual confounding.

In summary, dietary intakes of vitamins associated with one-carbon metabolism during youth and young adulthood were associated with young adult breast density in DISC, with results differing by menarche status during youth. These findings add to the growing body of literature supporting associations of early life exposures with development of breast density and thereby have potential implications for breast cancer risk later in life. Additional large cohort studies with repeated measurements of breast density in diverse populations are needed to confirm these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ADBV	absolute dense breast volume

ANDBV	absolute non-dense breast volume
BMI	body mass index
%DBV	percentage dense breast volume
DISC	Dietary Intervention Study in Children
DISC06	Dietary Intervention Study in Children 2006 Follow-up Study
IQR	interquartile range
LDL-C	low-density lipoprotein cholesterol
MRI	magnetic resonance imaging
DXA	dual-energy X-ray absorptiometry

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Table 1.

Participant characteristics at the DISC06 follow-up visit (n= 182)

Characteristics	*N*	Mean ± SD
Age (years)	182	27.2 ± 1.0
Body mass index (kg/m^2)	182	25.4 ± 5.4
BMI-z score[a, b]	182	0.2 ± 0.9
Physical activity (METh/week)	182	310.1 ± 55.5
Age at menarche (years) [b]	182	12.9 ± 1.3
Duration of hormonal contraceptive use among users (years)[c]	171	5.6 ± 3.5
Dietary intake		
Young adult intakes		
Total energy intake (kcal/day)	176	1731.3 ± 469.9
Folate (μg/day)	176	414.1 ± 160.1
Vitamin B2 (mg/day)	176	2.0 ± 0.7
Vitamin B6 (μg/day)	176	1.8 ± 0.8
Vitamin B12 (μg/day)	176	5.0 ± 3.5
Youth intakes[b]		
Total energy intake (kcal/day)	181	1616.4 ± 302.4
Folate (μg/day)	181	225.5 ± 58.5
Vitamin B2 (mg/day)	181	1.7 ± 0.3
Vitamin B6 (μg/day)	181	1.3 ± 0.3
Vitamin B12 (μg/day)	181	3.6 ± 1.1
	N	**Percentage**
Race/Ethnicity [b]		
Non-hispanic/white	156	86%
Hispanic white	8	4%
Non-hispanic/other races	13	7%
Hispanic/other races	5	3%
Education		
Some college or less	62	34%
Bachelor’s degree	95	52%
Graduate degree	25	14%
Hormonal contraceptive use		
Never	11	6%
Former	66	36%
Current	105	58%
Number of live births		
0	129	71%
≥1	53	29%
Smoking status		

Characteristics	*N*	Mean ± SD
Never	100	55%
Former	38	21%
Current	44	24%
Alcoholic drink status		
Never/Former	16	9%
Current, < 3 drinks/week	71	39%
Current, ≥3-< 6 drinks/week	33	18%
Current, ≥6-< 10 drinks/week	40	22%
Current, ≥ 10 drinks/week	22	12%
Treatment [b]		
Intervention	87	48%
Usual care	95	52%
Breast density measures	***N***	**Median (IQR)**
Percent dense breast volume (%)	182	24.5 (9.7–41.2)
Absolute dense breast volume (cm^3)	182	93.0 (50.0–140.3)
Absolute nondense breast volume (cm^3)	182	289.5 (157.8–485.3)

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

[a] BMI-z score is calculated at baseline during the DISC trial

[b] Data on these variables were collected during the DISC trial when participants were aged 8–10 years, all other variables were collected at the DISC06 Follow-up visit.

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

Table 2.

Multivariable adjusted[a] geometric means and 95% confidence intervals (95% CIs) of percent dense breast volume (%DBV) and absolute dense breast volume (ADBV, cm^3) according to quartiles of one-carbon metabolism-related vitamin intakes during youth and at specific pubertal stages

Quartiles of intake	Overall youth intake (n=181)			By pubertal stages					
				Premenarcheal intake (n=181)			Postmenarcheal intake (n=163)		
	Median Intakes	%DBV mean (95% CI)	ADBV (cm^3) mean (95% CI)	Median intakes	%DBV mean (95% CI)	ADBV (cm^3)- mean (95% CI)	Median intakes	%DBV mean (95% CI)	ADBV (cm^3) mean (95% CI)
Folate, μg/day									
Q1	162.7	19.7 (17.7, 21.9)	80.3 (65.4, 98.7)	156.5	18.3 (16.7, 20.1)	74.4 (66.3, 83.5)	134.4	21.2 (15.5, 28.8)	90.2 (67.5, 120.5)
Q2	200.6	22.3 (19.8, 25.1)	99.4 (80.6, 122.6)	194.3	22.0 (18.8, 25.8)	87.9 (73.0, 106.0)	189.6	21.3 (17.1, 26.6)	95.1 (84.0, 107.6)
Q3	238.4	16.3 (13.0, 20.5)	69.4 (53.8, 89.5)	232.9	16.5 (12.9, 21.0)	78.6 (61.8, 99.9)	248.2	16.2 (12.4, 21.2)	64.5 (46.0, 90.3)
Q4	294.0	16.9 (14.2, 20.1)	70.2 (59.4, 83.0)	291.8	18.4 (13.7, 24.6)	75.8 (54.6, 105.2)	343.7	15.8 (13.7, 18.3)	68.7 (55.6, 85.0)
P_{trend}[b]		0.006	0.02		0.53	0.87		0.01	0.02
Vitamin B2,mg/day									
Q1	1.4	18.6 (16.6, 20.9)	77.9 (72.3, 83.9)	1.4	17.4 (15.8, 19.1)	69.9 (64.1, 76.3)	1.2	17.5 (14.2, 21.6)	75.0 (56.2, 100.1)
Q2	1.6	18.2 (14.9, 22.2)	71.8 (52.5, 98.1)	1.6	18.7 (15.3, 23.0)	76.2 (62.0, 93.7)	1.5	20.8 (15.8, 27.3)	85.6 (64.8, 113.0)
Q3	1.8	19.4 (16.5, 22.8)	83.3 (73.0, 95.1)	1.8	18.8 (15.4, 23.0)	78.3 (60.9, 100.7)	1.7	20.3 (16.8, 24.5)	86.2 (68.3, 108.8)
Q4	2.0	18.5 (15.8, 21.8)	83.6 (72.2, 96.8)	2.1	19.9 (17.5, 22.6)	93.5 (81.8, 106.8)	2.2	15.6 (12.2, 20.0)	68.6 (51.6, 91.2)
P_{trend}[b]		0.88	0.18		0.16	0.004		0.39	0.56
Vitamin B6, μg/day									
Q1	1.0	19.1 (13.7, 26.7)	78.1 (51.7, 117.9)	1.0	18.8 (16.5, 21.4)	82.0 (64.2, 104.6)	0.9	21.0 (14.8, 29.8)	87.8 (68.5–112.5)
Q2	1.2	20.0 (16.3, 24.6)	87.2 (73.2, 103.9)	1.2	21.5 (18.0, 25.7)	83.6 (70.1, 99.9)	1.1	18.7 (16.6, 21.0)	72.3 (55.1–94.7)
Q3	1.4	18.6 (15.9, 21.8)	75.5 (64.8, 88.1)	1.4	16.9 (15.0, 19.1)	70.8 (61.6, 81.4)	1.4	19.2 (15.2, 24.1)	85.7 (72.2–101.6)
Q4	1.6	17.0 (13.2, 22.0)	75.7 (54.5, 105.1)	1.6	17.8 (13.8, 23.0)	80.1 (60.4, 106.3)	1.8	15.3 (12.2, 19.3)	70.0 (52.8–92.7)
P_{trend}[b]		0.52	0.74		0.54	0.78		0.23	0.36
Vitamin B12, μg/day									
Q1	2.6	18.3 (14.9–22.3)	75.7 (58.9, 97.3)	2.6	20.8 (16.3, 26.6)	79.9 (61.4, 104.0)	2.0	19.0 (16.8, 21.5)	85.5 (75.8–96.5)
Q2	3.2	20.4 (14.0–29.7)	82.5 (49.4, 138.0)	3.3	18.0 (13.8, 23.5)	79.3 (56.3, 111.7)	2.7	16.4 (12.0, 22.4)	65.0 (46.7–90.4)
Q3	3.8	18.6 (14.5–23.8)	74.8 (61.1, 91.8)	3.9	17.7 (14.7, 21.3)	74.4 (56.2, 98.4)	3.6	20.5 (17.4, 24.2)	80.0 (63.9–100.2)

Quartiles of intake	Overall youth intake (n=181)			By pubertal stages					
				Premenarcheal intake (n=181)			Postmenarcheal intake (n=163)		
	Median Intakes	%DBV mean (95% CI)	ADBV (cm^3) mean (95% CI)	Median intakes	%DBV mean (95% CI)	ADBV (cm^3)- mean (95% CI)	Median intakes	%DBV mean (95% CI)	ADBV (cm^3) mean (95% CI)
Q4	4.6	17.6 (14.8–20.9)	83.3 (67.4, 103.0)	4.9	18.3 (15.2, 22.0)	82.6 (66.5, 102.6)	4.8	18.1 (15.9, 20.7)	85.8 (76.5–96.4)
P_{trend} [b]		0.61	0.58		0.45	0.88		0.90	0.44

[a] Geometric means and 95% CIs were estimated from linear mixed effects models including clinic as a random effect and the following variables as fixed effects: treatment group (diet intervention group and usual care-control group), childhood BMI z-score at baseline (continuous), young adult body fat percent from DXA (%, continuous), number of live births (0 and ≥ 1), duration of hormonal contraceptive use (years, continuous), race (White and non-White), education (some college or less, bachelor's degree, graduate degree), smoking status (never, former, and current), alcohol consumption (never/former, < 3drinks/week, ≥3-<6 drinks/ week, ≥6-<10 drinks/week, and ≥10 drinks/week), and total energy intake (kcal/day, continuous).

[b] Test for trend was conducted by modeling the quartile medians of each one-carbon metabolism-related vitamins intakes as a continuous term and calculating the Wald test statistic.

Table 3.

Multivariable adjusted[a] geometric mean and 95% confidence interval (95% CIs) of percent dense breast volume (%DBV) and absolute dense breast volume (ADBV, cm^3) according to quartiles of one-carbon metabolism-related vitamin intakes during young adulthood

Quartiles of intake	Young adulthood (n=176)		
	Median intake	%DBV mean (95% CI)	ADBV (cm^3) mean (95% CI)
Folate, μg/day			
Q1	260.6	22.0 (17.7, 27.3)	85.8 (68.5, 107.4)
Q2	346.9	20.8 (16.9, 25.7)	87.1 (74.9, 101.3)
Q3	437.4	15.7 (12.25, 19.7)	68.1 (53.4, 86.8)
Q4	569.7	17.0 (12.8, 22.5)	73.6 (51.0, 106.4)
P_{trend}[b]		0.04	0.39
Vitamin B2, mg/day			
Q1	1.3	19.8 (16.5, 23.6)	80.6 (71.1, 91.5)
Q2	1.8	16.7 (13.8, 20.2)	73.5 (61.6, 87.7)
Q3	2.0	20.0 (15.5, 25.9)	82.0 (63.6, 105.8)
Q4	2.8	18.5 (16.0, 21.2)	77.1 (67.5, 88.0)
P_{trend}[b]		0.93	0.91
Vitamin B6, μg/day			
Q1	1.2	20.2 (16.4, 24.8)	74.0 (61.1, 89.6)
Q2	1.4	22.9 (20.0, 26.2)	99.6 (92.4, 107.2)
Q3	1.8	17.1 (12.9, 22.6)	76.5 (54.0, 108.4)
Q4	2.5	15.5 (13.2, 18.1)	66.5 (57.1, 77.5)
P_{trend}[b]		0.02	0.04
Vitamin B12, μg/day			
Q1	2.3	18.9 (16.0, 22.5)	82.0 (67.5, 99.7)
Q2	3.5	20.4 (18.1, 23.0)	80.6 (71.6, 90.7)
Q3	4.9	18.9 (16.6, 21.4)	79.1 (67.9, 92.2)
Q4	8.3	16.7 (14.7, 19.0)	71.6 (59.3, 86.4)
P_{trend}[b]		0.16	0.02

[a]Geometric means and 95% CIs were estimated from linear mixed effects models including clinic as a random effect and the following variables as fixed effects: treatment group (diet intervention group and usual care-control group), childhood BMI z score at baseline (continuous), young adult body fat percent from DXA (%, continuous), number of live births (0 and ≥ 1), duration of hormonal contraceptive use (years, continuous), race (White and non-White), education (some college or less, bachelor's degree, graduate degree), smoking status (never, former, and current), alcohol consumption (never/former, < 3drinks/week, ≥3-<6 drinks/ week, ≥6-<10 drinks/week, and ≥10 drinks/week), and total energy intake (kcal/day, continuous).

[b]Test for trend was conducted by modeling the quartile medians of each one-carbon metabolism-related vitamins intake as a continuous term and calculating the Wald test statistic.