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Prediagnostic plasma pyridoxal 5'-phosphate (vitamin B6) levels and invasive breast carcinoma risk: the Multiethnic Cohort

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Abstract

Background—Evidence from experimental and epidemiological studies suggests that vitamin B6 may reduce the risk of breast cancer.

Methods—We examined the association of prediagnostic plasma concentrations of pyridoxal-5'-phosphate (PLP), an active form of vitamin B6, with postmenopausal breast cancer risk in a case-control study nested in the Multiethnic Cohort in Hawaii and Southern California, including 706 cases and 706 controls matched on date of birth, ethnicity, study site, date of blood draw, time of blood draw, hours of fasting prior to blood draw, and use of menopausal hormones. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using conditional logistic regression models.

Results—Women with plasma PLP concentrations in the highest quartile had a 30% reduced risk of invasive breast cancer (CI: 0.50–0.98) compared to the women in the lowest PLP quartile (P for trend=0.02). The association appeared to be limited to cases with hormone receptor-positive tumors (P for heterogeneity=0.04); and remained unchanged in the analysis restricted to women with blood samples collected more than one year prior to cancer diagnosis (OR=0.69; CI: 0.48–0.99; P for trend=0.03).

Conclusions—These data suggest that higher circulating levels of vitamin B6 are associated with a reduced risk of invasive postmenopausal breast cancer.

Impact—These results, in combination with information from two other prospective studies, suggest a role for vitamin B6 in the prevention of postmenopausal breast cancer. Additional studies are needed to further investigate potential heterogeneity of the vitamin B6 association with breast cancer risk by tumor hormone receptor status.

Keywords

invasive breast carcinoma; pyridoxal 5'-phosphate; nested case-control study

Introduction

Identification of molecular mechanisms underlying breast carcinogenesis is fundamental to the prevention of breast cancer, the most common malignancy among women (1). While

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many of the accepted breast cancer risk factors, such as age at menarche, parity, age at first full-term pregnancy, and age at menopause are difficult to modify, diet is a potentially modifiable factor. However, aside from alcohol intake, no association of diet with postmenopausal breast cancer risk has been established (2).

Pyridoxal 5'-phosphate (PLP), the active form of vitamin B6 (3), serves as a cofactor in more than one hundred enzymatic reactions (4). Vitamin B6 is involved in one-carbon metabolism, a sequence of biochemical processes that enrich the cellular supply of methyl groups for DNA synthesis, repair, and methylation (5). Apart from its role as a coenzyme, vitamin B6 might affect carcinogenesis directly through inhibition of cell proliferation, oxidative stress, angiogenesis, and enhanced immune function [reviewed in (6)].

Results from studies of the association of dietary vitamin B6 intake (7–17) or circulating PLP levels (18–20) with breast cancer risk are inconsistent. A randomized trial of combined folic acid, vitamin B6, and vitamin B12 reported significantly reduced breast cancer risk among women in the treatment arm of older (≥ 65 years old), but not younger women (21). We investigated prediagnostic plasma levels of PLP in relation to invasive breast cancer risk among postmenopausal women in the Multiethnic Cohort (MEC) study.

Methods

Study design and population

This case-control study was nested within the biospecimen subcohort of the MEC, a large prospective study of residents of Hawaii and Southern California, which included 118,441 women (22). The population-based sampling frames for the MEC included rolls from drivers' licenses, voters' registration, and the Health Care Financing Administration and was targeted to five ethnic groups: African American, Hawaiian, Japanese, Latino, and whites. At cohort entry, participants completed a self-administered, 26-page baseline questionnaire that included queries on demographic characteristics, anthropometry, medical history, family history of cancer, reproductive and menstrual history, cancer risk factors, and detailed questions on diet. The MEC biospecimen subcohort was established from1996 through 2006, with the majority of recruitment occurring after 2001. All surviving cohort members still residing in the catchment area were re-contacted and asked to provide biologic specimens, including blood samples. Blood samples were drawn at a clinical laboratory or at the participants' homes and were kept refrigerated and protected from light until processed on average within 3 hours of collection. After centrifugation, blood components were aliquoted into 0.5-cc cryovials and stored in the vapor phase of liquid nitrogen (−150°C). A total of 36,458 female cohort members contributed to the biorepository from which the cases and controls were selected for the present study. Although the participants in the biospecimen subcohort were slightly more educated (mean 13.6 versus 12.7 years) and were more likely to be nonsmokers (13% versus 17%) and have a family history of cancer (46% versus 42%) than nonparticipants, they were similar in relation to other exposures and were generally representative of all cohort members.

The study protocol was approved by the institutional review boards of the University of Hawaii and the University of Southern California, and all participants signed informed consent.

Case ascertainment and control selection

Linkages of the cohort to the Surveillance, Epidemiology, and End Results cancer registries in Hawaii and California are conducted annually to identify incident breast cancer cases diagnosed during the follow-up period. Linkages to state death files and the National Death Index are conducted to identify deaths. Postmenopausal women participating in the

biospecimen repository who were diagnosed with primary invasive breast carcinoma prior to the most recent tumor registry linkage (December 31, 2010) and provided blood sample before breast cancer diagnosis were included in this analysis. Breast cancer cases were identified using the International Classification of Diseases for Oncology, third edition, (codes C50.0-C50.9). Controls were randomly selected from the pool of postmenopausal women who were alive and free of a breast cancer diagnosis and individually matched to cases in a 1:1 ratio on study site, ethnicity, date of birth (± 1 year), date of blood draw (± 6 months), time of blood draw (morning hours), hours fasting prior to blood draw (<6, 6 to < 8, 8 to < 10, ≥ 10), and use of menopausal hormones at blood draw (none; current use of estrogen, progesterone, or combination of both). A total of 706 postmenopausal breast cancer cases and 706 matched controls were available for analysis. Tumor estrogen receptor (ER) and progesterone receptor (PR) status was available for 617 (87%) and 597 (85%) of the cases, respectively.

Laboratory analyses

Plasma samples were analyzed in the Analytical Biochemistry laboratory of the University of Hawaii Cancer Center (AAF, director) by laboratory personnel blinded to the case-control status of the samples. The PLP assay was based on the method of Rybak et al. (23). Briefly, 150 μL freshly thawed plasma was mixed for 5 minutes with 10 μL aqueous $^{2}H_{2}$-pyridoxal-5'-phosphate (internal standard, donated by Stephen P. Coburn, Purdue University, Fort Wayne, IN) and 50 μL 10% aqueous metaphosphoric acid (Sigma, St. Louis, MO). After shaking with 200 μl CH_2Cl_2, the supernatant aqueous layer was transferred into 350 μl round bottom plates (MicroLiter Analytical Supplies, Georgia 07-1111N) and 20 μL of the aqueous layer were injected into the LCMSMS system (model Accela and TSQ-Ultra from Thermo Scientific Inc., Waltham, MA). PLP was separated on an Ascentis Express C18 column (150 × 3.0 mm; 2.7 μm; Supelco Inc, Bellefonte, PA) and a 0.2 μm pre-filter cartridge (2.1 mm ID, ThermoFisher) at a flow rate of 0.6 mL/min with the following linear mobile phase gradient consisting of 5% aqueous acetic acid (A) and 0.1% formic acid in acetonitrile (B): %B= 4% for 1 minute then to 95% in 2 minutes, held at 95% for 1 minute and changed to 4% in 0.1 minute followed by equilibration for 4 minutes. Quantitation was performed by tandem mass spectrometry after positive electrospray ionization in selected reaction monitoring mode by scanning the transitions m/z 248.0 to m/z 150.1 (at 18 eV) and m/z 229.9 (at 14 eV) for PLP and m/z 250.0 to m/z 152.0 (at 18 eV) for deuterated PLP (internal standard). Instrument settings were as follows: spray voltage, 4000 V; sheath gas, nitrogen; sheath gas pressure, 45 arbitrary units; ion transfer capillary temperature, 350 °C. External calibration was performed with aqueous solutions of authentic standards (Sigma, St. Louis, MO) in the range 5–1000 nM and stock concentrations were determined by absorbance readings (ε= 5070 at 388 nm in water). Based on a total of 47 duplicate and 23 triplet samples, the intra-batch and inter-batch coefficients of variation for PLP were 4.4% and 3.8%, respectively.

Statistical analysis

Statistical analysis was performed using SAS 9.2 (Cary, NC, USA). Conditional logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (CI) for the association of prediagnostic plasma PLP levels with postmenopausal breast cancer risk. Quartiles of the log-transformed PLP levels were based on the distribution among controls and entered into the models as indicator variables with the lowest quartile as a reference category. Trend was assessed based on a variable assigned the median of the appropriate quartile for the transformed variable. To identify potential confounders, covariates were tested individually and jointly and were included in the models if they produced a >10% change in regression coefficients for PLP or were associated with breast cancer risk or PLP levels among controls. All final models were uniformly adjusted for the

following baseline variables: (BMI kg/m^2; continuous, log-transformed), education (continuous), family history of breast cancer among first degree female relatives, age at menarche (≤12; 13–14; ≥ 15 years), parity (0; 1; 2–3; ≥4) and age at first live birth (nulliparous; ≤ 20; 21–25; ≥ 26 years), years of contraceptive hormone use (none;< 5 years; ≥ 5 years), age at natural menopause (≤ 50; 50–55; >55 years), oophorectomy, hysterectomy, smoking status (never; past; current), average ethanol consumption (grams/day; continuous, log-transformed), and hours of daily moderate and vigorous physical activity (continuous, log-transformed). We also examined the inclusion of one-carbon compounds, such as folic acid, 5-methyltetrahydrofolate, methylmalonic acid (indicator for vitamin B12), cysteine, homocysteine, methionine, choline, and betaine, but none of them individually or jointly influenced the association of PLP with breast cancer risk. A sensitivity analysis to examine whether the association of PLP with breast cancer risk was influenced by the time between blood collection and diagnosis was conducted. A Wald test was used to assess heterogeneity across subtypes of cancer by hormone receptor status and time from blood collection to diagnosis (≤1 year; >1 year), using polytomous conditional logistic regression modeled via proportional hazards regression (24). Interactions between PLP and all covariates included in the models as well as other one-carbon compounds on the risk of postmenopausal breast cancer were examined using the Wald test of cross-product terms of PLP (as a trend variable) and each of the risk factors, in turn. All P-values were two-sided. P values < 0.05 were considered statistically significant.

Results

The mean age of the study participants at blood draw was 67.9 years (SE: 0.3; range: 58–87 years) (Table 1). All matching variables were similar between cases and controls. Cases and controls were also similar with respect to years of education, ages at menarche, first live birth, and menopause, and hours of daily moderate or vigorous physical activity. Cases were more likely than were controls to have had a family history of breast cancer and to be overweight or obese. Cases also had lower parity than controls, reported higher daily alcohol consumption, and were more likely to be current tobacco smokers. Cases had lower mean prediagnostic PLP levels than did controls (83.3 nM versus 92.7 nM). The percentage of cases with positive hormone receptor status tumors was higher among Japanese (86%), white (86%), and Hawaiian (85%) women than among Latino (78%) or black women (75%).

Among controls, mean PLP levels were significantly lower among obese (BMI ≥ 30 kg/m^2: 78.4 ± 7.1 nM), and overweight (BMI 25–29.9 kg/m^2: 92.6 ± 5.2 nM) women compared to lean women (BMI<25 kg/m^2: 102.9 ± 4.5 nM; p<0.0004). Lower PLP levels were also observed among current smokers (75.2 ± 10.1 nM) compared to nonsmokers (97.7 ± 3.4 nM; p=0.01). Although no statistically significant relation between ethnicity and PLP levels were observed (p=0.30), PLP levels among Latina controls were significantly (p= 0.02) lower than among Japanese controls (76.8 ±7.4 nM vs. 102.8±5.3 nM). No statistically significant associations of PLP levels with other covariates were observed (data not shown).

Plasma PLP levels were inversely associated with postmenopausal breast cancer risk (Table 2). Women with PLP levels in the highest quartile had a 30% reduced risk of invasive breast cancer (95% CI: 0.50–0.98) compared to the women with PLP levels in the lowest quartile (P trend=0.02). This association remained unchanged after excluding cases (and their matched controls) whose blood was collected within one year of their breast cancer diagnosis. In the analysis stratified by tumor hormone receptor status, strong inverse associations of plasma PLP levels with breast cancer risk among women with ER-positive, PR-positive, and ER-positive/PR-positive tumors were observed. No significant associations of PLP with postmenopausal breast cancer risk were observed among women diagnosed with hormone receptor negative tumors (P for heterogeneity=0.04 for the difference in breast

cancer risk by hormone receptor status). No significant modification of the association of PLP levels with breast cancer risk was found by any of the covariates, including family history of breast cancer education, BMI, age at menarche, parity, age at first parturition, use of contraceptive and menopausal hormones, age at menopause, alcohol consumption, tobacco smoking and physical activity, age at blood collection, hours of fasting before blood collection as well as other one one-carbon compounds (P>0.16) (data not shown), Although there was some statistically significant (P=0.04) heterogeneity by ethnicity. Among Latino women, no association of PLP with breast cancer risk was observed (Table 2).

Discussion

In this large prospective study of postmenopausal women, prediagnostic plasma PLP levels were inversely associated with invasive breast cancer risk. This association was strongest in women with hormone-receptor positive tumors and remained unchanged in the analysis restricted to women whose blood was collected more than one year prior to cancer diagnosis. Inverse trends in the risk of postmenopausal breast cancer associated with higher PLP levels were generally consistent in all ethnic groups, with the exception of Latinas among whom PLP was (non-significantly) positively related to risk. We also found comparatively low PLP levels among Latina controls and a higher percentage of hormone-receptor negative tumors among Latina cases than among cases from other ethnicities. However, we were limited by our sample size to further explore these population subgroups.

Results from three other prospective studies of the association of circulating PLP levels with postmenopausal breast cancer risk have been reported (18–20). In accordance with our findings, an inverse relation of PLP to postmenopausal breast cancer risk was observed in the Women's Health Study among 521 postmenopausal cases and 520 controls (RR=0.64; 95% CI: 0.42–0.99, comparing the highest and lowest quintiles) (20). Although not statistically significant, an inverse association of plasma PLP to risk among 487 postmenopausal breast cancer cases and matched controls in the Nurses' Health Study was suggested (RR=0.66; CI: 0.43–1.01, comparing the highest and lowest quintiles) (19). No association of serum PLP with risk was found In a smaller study of 109 postmenopausal breast cancer cases and matched controls nested within the Washington County Cohort (18). Although vitamin B6 intake was inversely associated with breast cancer in two studies (8, 17), most studies have not found its association with breast cancer risk (7, 9–16), A significant reduction of breast cancer risk was found among older women (age ≥ 65 years), but not younger women, in the randomized trial of folic acid, vitamin B6, and vitamin B12 (21). Although, it is not possible to distinguish the affects attributable to vitamin B6, folic acid, or vitamin B12.

Our finding of an inverse association of plasma PLP with the risk of hormone receptor positive breast cancer, but not ER-negative or PR-negative tumors, is novel as no heterogeneity was observed (p>0.84) in the association of circulating PLP with breast cancer risk by hormone receptor status in the Women's Health Study (8). The differential association of PLP with breast cancer risk by hormone receptor status may have biological relevance as the etiology of these tumors is thought to be distinct (25). Vitamin B6 is a coenzyme in one-carbon metabolism reactions, facilitating the transfer of methyl groups to tetrahydrofolate to yield 5,10-methylenetetrahydrofolate and the downstream generation of thymidylate and purine for DNA synthesis. Disruption of these reactions may create an imbalance in the generation of methyl groups required for DNA methylation and other biochemical processes. Altered DNA methylation has been observed in tumors at several sites (26), and ER and PR expression in breast tumors has found to be associated with methylation of ER and PR gene CpG islands (27, 28).

Experimental studies suggest that supraphysiological doses of vitamin B6 can reduce tumor cell growth in culture (29–36), including the growth of mammary tumor lines (34–36). The exact mechanism through which vitamin B6 exerts anti-tumorigenic effects is presently unknown (6). Decreased growth of tumor cells by vitamin B6 treatment has been attributed to modulation of steroid hormone receptor-mediated gene expression (34, 37–39). Because steroid hormones are involved in the regulation of cellular growth, differentiation, and maintenance of physiological function, the potential for PLP to modulate these hormones could represent a novel therapeutic approach to the inhibition of breast cancer growth. Aside from its role in growth regulation, vitamin B6 possesses anti-inflammatory properties that might reduce the risk of breast malignancy (40, 41). *In vitro* studies indicate that vitamin B6 may act as an antioxidant (33, 42); thus, the suppressive effects of vitamin B6 on cellular proliferation might be mediated through reduced oxidative stress.

Major strengths of this study include its prospective design and the evaluation of prediagnostic levels of plasma PLP in a large sample of postmenopausal women and controls matched on several factors associated with breast cancer risk. A wide range of potential confounders of the PLP-breast cancer association was available. Furthermore, the measurement of vitamin B6 was performed by a sensitive tandem mass spectrometry-based method with low inter-assay variation. Our analysis was limited by a single measurement of PLP which may not be representative of exposure during periods in a woman's life critical to breast cancer susceptibility and reduces the precision of the measurement. Although the study sample was relatively large, statistical power to conduct subgroup analyses of hormone-negative tumors was modest.

This nested case-control study provides new evidence that circulating levels of vitamin B6 may be inversely related to the risk of invasive postmenopausal breast cancer. Although the exact mechanism by which vitamin B6 may reduce the risk of breast cancer is presently unknown, laboratory data are overwhelming that this compound is critical to multiple pathways that might inhibit breast carcinogenesis. In conclusion, these results, in combination with information from two other prospective studies, suggest a role for vitamin B6 in the prevention of postmenopausal breast cancer.

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Table 1

Characteristics of participants of the breast cancer case-control study nested in the Multiethnic Cohort in Hawaii and Southern California

Participant characteristics	Cases n=706	Controls n=706	*p*[a]
Mean age (SD) at blood draw (years)	67.9 (7.5)	67.9 (7.5)	Matching variable
Hours of fasting (SD) prior to blood draw	12.7 (2.9)	12.6 (2.9)	Matching variable
Ethnicity, N (%)			Matching variable
African American	106 (15)	106 (15)	
Hawaiian	66 (9)	66 (9)	
Japanese	254 (36)	254 (36)	
Latino	132 (19)	132 (19)	
White	148 (21)	148 (21)	
Study site			Matching variable
Hawaii	419 (59)	419 (59)	
California	287 (41)	287 (41)	
Years of education, mean (SD)	13.6 (3.1)	13.6 (3.1)	*0.89*
History of breast cancer among first degree relatives, N (%)	109 (15)	82 (12)	*0.04*
Body mass index, mean (SD)	26.1 (5.4)	26.7 (5.2)	*0.01*
< 25	290 (41)	357 (51)	
25–29.9	260 (37)	206 (29)	
≥ 30	156 (22)	143 (20)	*0.001*
Age at menarche (years), mean (SD)	13.0 (1.6)	13.1 (1.6)	*0.42*
Age at natural menopause, mean (SD)	49.6 (4.8)	49.2 (4.6)	*0.28*
Parity			
Nulliparous	94 (13)	73 (10)	
1	80 (11)	78 (11)	
2–3	334 (47)	317 (45)	
≥ 4	198 (28)	238 (34)	*0.08*
Age at first live birth, mean (SD)	23.7 (4.6)	23.5 (4.5)	*0.50*
Current use of menopausal hormones	190 (27)	190 (27)	Matching variable
Alcohol consumption (ethanol, grams/day), mean (SD)	4.8 (15.5)	3.6 (11.0)	*0.09*
Current smoking status *n* (%)			
Nonsmoker	607 (87)	626 (90)	
Smoker	92 (13)	72 (10)	*0.10*
Moderate or vigorous physical activity (hours/day), mean (SD)	1.2 (1.3)	1.2 (1.3)	*0.66*
Pyridoxal-5 phosphate (nM), mean (SD)	83.3 (78.0)	92.7 (85.3)	*0.02*

Abbreviations: SD, standard deviation.

Note: Cases and controls were matched on age of birth (± 1 year), study site (Hawaii or California), ethnicity, date of blood draw (± 6 months), hours fasting prior to blood draw (<6, 6 to < 8, 8 to < 10, ≥ 10), and use of menopausal hormones at blood draw (none; estrogen alone; progestin alone, combined estrogen and progestin).

[a] *P* values from chi-square test (for categorical variables) and from t-test (for continuous variables).

Table 2

Association of pyridoxal 5'-phosphate prediagnostic plasma levels with invasive breast cancer risk

	Quartile 1 (≤41.1 nM)	Quartile 2 (41.2–66.0 nM)	Quartile 3 (66.1–116.6 nM)	Quartile 4 (>116.6 nM)	P_{trend}	p[b]
ALL PARTICIPANTS (706 cases/706 controls)						
N cases/controls	198/177	197/176	165/176	146/177		
OR (95% CI)[a]	1.00 (reference)	0.91 (0.66–1.25)	0.77 (0.55–1.08)	0.70 (0.50–0.98)	*0.02*	
EXCLUDING CASES (AND MATCHED CONTROLS) WITH BLOOD COLLECTION WITHIN ONE YEAR OF DIAGNOSIS						
N cases/controls	165/146	165/149	137/146	122/148		
OR (95% CI)[a]	1.00 (reference)	0.88 (0.63–1.24)	0.74 (0.51–1.07)	0.69 (0.48–0.99)	*0.03*	*0.99*
STRATIFIED ACCORDING TO THE TUMOR ER/PR STATUS						
ER+						
N cases/controls	154/125	140/125	115/129	92/122		
OR (95% CI)[a]	1.00 (reference)	0.80 (0.54–1.18)	0.58 (0.38–0.90)	0.55 (0.36–0.84)	*0.002*	
ER−						
N cases/controls	28/34	32/30	30/23	26/29		
OR (95% CI)[a]	1.00 (reference)	1.31 (0.47–3.69)	2.35 (0.79–6.98)	0.94 (0.34–2.62)	*0.86*	*0.09*
PR+						
N cases/controls	128/95	119/105	90/113	77/101		
OR (95% CI)[a]	1.00 (reference)	0.73 (0.48–1.11)	0.52 (0.32–0.83)	0.56 (0.35–0.89)	*0.006*	
PR−						
N cases/controls	45/56	51/46	48/35	39/46		
OR (95% CI)[a]	1.00 (reference)	1.24 (0.61–2.52)	1.70 (0.80–3.61)	0.88 (0.41–1.89)	*0.88*	*0.06*
ER+/PR+						
N cases/controls	125/92	117/105	88/110	76/99		
OR (95% CI)[a]	1.00 (reference)	0.71 (0.47–1.09)	0.53 (0.33–0.85)	0.58 (0.37–0.92)	*0.01*	
ER−/PR−						
N cases/controls	24/31	30/29	28/20	25/27		
OR (95% CI)[a]	1.00 (reference)	1.63 (0.50–5.36)	3.46 (1.02–11.77)	1.09 (0.35–3.35)	*0.70*	*0.04*

	Quartile 1 (≤41.1 nM)	Quartile 2 (41.2–66.0 nM)	Quartile 3 (66.1–116.6 nM)	Quartile 4 (>116.6 nM)	P_{trend}	P[b]
STRATIFIED ACCORDING TO RACE/ETHNICITY						
Black (106 cases/106 controls)						
N cases/controls	40/30	39/21	15/21	12/30		
OR (95% CI)[a]	1.00 (reference)	1.17 (0.46–3.00)	0.27 (0.07–1.03)	0.12 (0.03–0.46)	*0.002*	
Hawaiian (66 cases/66 controls)						
N cases/controls	23/15	15/22	15/10	13/19		
OR (95% CI)[a]	1.00 (reference)	0.15 (0.03–0.86)	0.31 (0.04–2.38)	0.15 (0.03–0.78)	*0.06*	
Japanese (254 cases/254 controls)						
N cases/controls	62/55	58/55	65/72	69/72		
OR (95% CI)[a]	1.00 (reference)	0.82 (0.44–1.52)	0.69 (0.38–1.26)	0.72 (0.40–1.30)	*0.25*	
Latino (132 cases/132 controls)						
N cases/controls	38/41	39/37	31/32	24/22		
OR (95% CI)[a]	1.00 (reference)	1.32 (0.54–3.27)	1.03 (0.42–2.54)	2.02 (0.79–5.18)	*0.25*	
White (148 cases/148 controls)						
N cases/controls	35/32	46/41	39/41	28/34		
OR (95% CI)[a]	1.00 (reference)	0.54 (0.23–1.27)	0.65 (0.27–1.60)	0.57 (0.24–1.33)	*0.32*	*0.04*

[a]Odds ratios (OR) and 95% confidence intervals (CI) from conditional logistic regression models including cases and controls matched on date of birth (± 1 year), study site, ethnicity, date of blood draw (± 6 months), hours fasting prior to blood draw (<6, 6 to < 8, 8 to < 10, ≥ 10), and hormone replacement therapy use at blood draw (none; estrogen alone, progestin alone, estrogen and progestin) and adjusted for education, family history of breast cancer, BMI (continuous, kg/m^2), age at menarche, parity, age at first parity, use of contraceptive hormones (none, <5 years ≥ 5 years), oophorectomy, hysterectomy, age at natural menopause, current smoking status, ethanol consumption (continuous, grams/day), hours of daily moderate/vigorous physical activity.

[b]P for heterogeneity of PLP association with breast cancer risk by ethnicity, time between blood collection and diagnosis, and tumor hormone receptor status.