Dietary and Plasma Lycopene and the Risk of Breast Cancer

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Abstract

Lycopene is potentially effective in the prevention of breast cancer from laboratory and observational studies. Among 39,876 women initially free of cardiovascular disease and cancer, we first conducted a prospective cohort study of dietary lycopene and its food sources. Participants completed a baseline food frequency questionnaire and provided self-reports of breast cancer risk factors. Dietary lycopene levels were divided into quintiles, and lycopene food sources were categorized. During 9.9 years of follow-up, 1,076 breast cancer cases were confirmed by medical record review. In a nested case-control study, we then identified 508 breast cancer cases and 508 controls matched by age, smoking, and follow-up time. Plasma lycopene and other carotenoids were measured. In the prospective cohort study, women with increasing quintiles of dietary lycopene had multivariate relative risks (RR) of breast cancer of 1.00 (ref), 0.95, 1.00, 1.10, and 1.00 (P, linear trend = 0.71). Women consuming <1.5, 1.5 to <4, 4 to <7, 7 to <10, and ≥10 servings/week of tomato-based products had RRs of 1.00 (ref), 1.00, 1.20, 1.18, and 1.16 (P, linear trend = 0.11). No individual lycopene food sources were associated with breast cancer. In the nested case-control study, women in increasing quartiles of plasma lycopene had multivariate RRs of breast cancer of 1.00 (ref), 0.95, 1.15, and 0.93 (P, linear trend = 0.86). The stepwise addition of individual plasma carotenoids did not impact the RRs for plasma lycopene, nor were other carotenoids associated with breast cancer. In conclusion, neither higher dietary nor plasma lycopene levels were associated with a reduced risk of breast cancer in middle-aged and older women. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1074–81)

Introduction

There is considerable epidemiologic evidence supporting an association between higher levels of fruit and vegetable intake and a reduced risk of total cancer (1). With regard to breast cancer, the evidence is not consistent based on two meta-analyses (2, 3). Specific nutrients rich in fruits and vegetables, including carotenoids, may play a role in breast cancer prevention. Recently, lycopene has garnered specific attention over other carotenoids for its potential role in cancer prevention. Lycopene is a carotenoid without provitamin A activity and is found in a limited number of plant foods (tomato, watermelon, pink grapefruit, papaya, apricot). More than 80% of lycopene intake in the U.S. is obtained from the consumption of tomatoes and tomato products (4), making it more amenable to potential public health strategies to increase intake.

Various studies have also explored promising mechanisms through which lycopene, independent of other carotenoids, may have a role in reducing the risk of breast cancer. Lycopene has been shown to have strong antioxidant properties relative to other carotenoids (5). Lycopene may inhibit the proliferation of mammary human cancer cells (6). Alternatively, lycopene may suppress insulin-like growth factor-I (7), which has been linked to increased risk of premenopausal breast cancer (8). No study has examined lycopene's role for breast cancers positive for steroid hormone receptors (6). Observational evidence from several case-control and cohort studies that examined the association between dietary, blood, or adipose tissue levels of lycopene and the risk of breast cancer has remained inconsistent, along with the findings for other carotenoids (9-21). However, there are no published data specifically examining the association between the consumption of lycopene-containing foods and the risk of breast cancer.

With the availability of both dietary and plasma assessments of lycopene from the Women's Health Study, we simultaneously focused on the roles of dietary consumption of lycopene-containing foods and plasma lycopene in breast cancer risk using corresponding prospective cohort and nested case-control study designs.

Materials and Methods

Study Population. The Women's Health Study is a 2 × 2 factorial trial of low-dose aspirin and vitamin E in the primary prevention of cardiovascular disease and cancer (22). The β-carotene component of the trial was terminated in 1996 due in part to the lack of an effect of β-carotene on cancer incidence in the Physicians' Health Study (23, 24). A total of 39,876 female U.S. health professionals, aged ≥45 years in 1992 and free from prior myocardial infarction, stroke, transient ischemic attack, and cancer (except nonmelanoma skin cancer) were enrolled and randomized into the study. The Institutional Review Board at Brigham and Women's Hospital approved the study protocol and informed consent was obtained for each subject.

Prospective Cohort Study of Dietary Lycopene and its Food Sources. Among those randomized into the Women's Health Study, a 131-item validated Willett semiquantitative food frequency questionnaire was completed by 39,310 women

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(25), of whom 829 were excluded due to insufficient completion of the questionnaire, or because their daily energy intake was <2,510 or ≥14,644 kJ/day (<600 or ≥3,500 kcal/day). After an additional exclusion of those with a pre-randomization revascularization procedure, 38,447 women remained for analyses of dietary lycopene.

Baseline Covariates. On the Women's Health Study baseline questionnaire, women provided self-reported data on age, weight, and height (converted to body mass index, in kg/m^2), smoking status, alcohol intake, frequency of exercise, family history of breast cancer in first-degree relatives, age at menarche, history of oral contraceptive use, age at first pregnancy, number of pregnancies, postmenopausal status, and postmenopausal hormone use.

On the semiquantitative food frequency questionnaire, a common unit or portion size for each food was specified, and the participants selected from nine responses ranging from ''never or less than once per month'' to ''6 + per day.'' Dietary lycopene intake, adjusted for total energy intake using the residual method (25), was calculated in micrograms per day and based on food tables maintained by the Harvard School of Public Health, Boston, MA. Other studies have reported moderate-to-high correlations between the semiquantitative food frequency questionnaire and dietary records for lycopene food sources (26, 27). Four major lycopene food sources were also considered, including tomatoes, tomato juice, tomato sauce, and pizza. Other nutrients and foods considered for analyses included total fiber, folate, and saturated fat intake, which were also adjusted for total energy intake, plus total fruit and vegetable intake.

Ascertainment of Breast Cancer. Women completed follow-up questionnaires every 6 months during the first year, then annually thereafter during the course of the study. Women reported a diagnosis of breast cancer either through these follow-up questionnaires, by letter, or by telephone. Family members or postal authorities usually reported deaths. Medical records were obtained for all reported cases of breast cancer, with an Endpoints Committee of physicians to confirm or refute the occurrence of breast cancer. Additional details on breast cancer (e.g., hormone receptor status) were also abstracted. Only confirmed cases of invasive and *in situ* breast cancer were included.

Nested Case-Control Study of Plasma Lycopene. Baseline blood samples were collected from 28,345 (71%) participants and stored in liquid nitrogen freezers until analysis. A nested case-control study was conducted, identifying 508 case-control pairs with baseline blood samples. Cases included women developing breast cancer confirmed by an Endpoints Committee during a mean of 7 years of follow-up. Each case was then matched with a control subject according to age (± 1 year), smoking status (never, former, and current smoker), and follow-up time (± 6 months), while remaining free of breast cancer throughout follow-up.

All investigators and laboratory personnel were blinded to the subject's case-control status, and blood samples were handled identically. Baseline plasma blood samples were thawed and assayed for lycopene and other carotenoids at Our Lady of Mercy Medical Center, Bronx, NY. All analytes were quantitated by reversed-phase high-performance liquid chromatography following extraction and concentration by conventional methodology (28). Plasma total cholesterol was assayed by enzymatic, end point spectroscopy using commercially available diagnostic kits (Sigma-Aldrich Chemical, Co., St. Louis, MO) and conventional methodology (29, 30). Because plasma lipoproteins are nonspecific carriers for all plasma carotenoids, the measurement of total cholesterol represents the best way to control for the confounding effects due to differences in lipoprotein levels between subjects (31). Although confounding by total cholesterol is largely unknown for breast cancer, we took a conservative data analysis approach and controlled for plasma cholesterol.

Laboratory performance was based on blind quality control samples provided from both the Women's Health Study and the Department of Pathology Quality Assurance program at Our Lady of Mercy Medical Center, and on repeated assays of laboratory-prepared sample pools. Several blind quality control samples and laboratory pools were assayed with each analysis run to monitor laboratory performance. The laboratory accuracy was within 7% for each measured carotenoid, whereas the day-to-day and within-day precision (coefficient of variation) for these analytes was 5% (32).

Data Analyses. For the prospective cohort study, lycopene intake was grouped by quintiles based on its overall distribution of intake. Participants were first compared by quintile of lycopene intake, using mean values or proportions of baseline coronary risk factors. Cox proportional hazards modeled the relative risk (RR) and 95% confidence interval (CI) of breast cancer, with the lowest quintile as the reference. The ≥95th percentile versus lowest quintile of lycopene intake was also compared. Models were first adjusted for age, total energy intake, and randomized treatment assignments, then life-style and clinical factors, and finally by dietary factors. Linear trends across quintiles of lycopene intake were tested using the median level for each quintile as an ordinal variable.

Categories of major lycopene food sources paralleled a previous study from the Women's Health Study (33): tomatoes (one serving = one tomato), none, 1 to 3 servings/month, 1 to 4 servings/week, ≥5 servings/week; tomato juice (small glass), none, 1 to 3 servings/month, 1 serving/week, and ≥2 servings/week; tomato sauce (1/2 cup or 118 mL), none, 1 to 3 servings/month, 1 serving/week, and ≥2 servings/week; and pizza (2 slices), none, 1 to 3 servings/month, 1 serving/week, and ≥2 servings/week. Serving sizes reflect what is mentioned on the semiquantitative food frequency questionnaire, and the categories were determined a priori based on the distribution of intake for each lycopene food source. We summed the total number of tomato-based products as <1.5, 1.5 to <4, 4 to <7, 7 to <10, and ≥10 servings/week (34). These analyses included total energy intake in each model. The proportional hazards assumption was satisfied using a Wald test for the interaction of time with each lycopene indicator variable.

For the nested case-control study, women were first compared according to case-control status. Plasma lycopene levels were then divided into quartiles based on its distribution among 508 controls, for which baseline characteristics were compared. We computed the RRs and 95% CIs of breast cancer using conditional logistic regression for increasing quartiles of plasma lycopene levels, with the lowest quartile as the reference. Linear trends across quartiles of plasma lycopene levels were tested using the median level for each quartile as an ordinal variable. Models were first adjusted for randomized treatment assignments and plasma total cholesterol level, then adjusted sequentially for life-style, clinical, and dietary factors as done for the cohort study. We also considered whether simultaneous adjustment for other plasma carotenoids attenuated the association between plasma lycopene and breast cancer. Other analyses were limited to cases that were positive for estrogen and progesterone receptors (344 case-control pairs). Finally, multivariate models were then compared for plasma lycopene with other carotenoids categorized into quartiles among the controls and entered into a separate model.

Results

Prospective Cohort Study of Dietary Lycopene and its Food Sources. Overall, the mean (SD) lycopene intake was 9,199 (6,443) μg/day in 38,447 women aged 53.9 (7.0) years. Both tomato sauce (40.5%) and tomatoes (39.8%) were the major food sources of dietary lycopene intake, followed by tomato juice (12.3%), pizza (4.7%), grapefruit (2.7%), and salsa (0.1%). Table 1 compares selected baseline characteristics of women according to their quintiles of lycopene intake. Higher dietary lycopene intake was associated with slightly lower body mass index, smoking rates, and family history of breast cancer, and was only weakly associated with reproductive risk factors. Women consuming greater amounts of lycopene also exercised more and consumed greater amounts of fruits and vegetables, fiber, and folate, as well as less saturated fat.

Over 9.9 years of follow-up for the 38,447 women comprising the baseline population, 1,076 women developed breast cancer, of whom 719 had breast cancers that were positive for estrogen and progesterone receptors. Table 2 presents results on the association between increasing quintiles of lycopene intake and risk of breast cancer. Increasing quintiles of dietary lycopene were not associated with the risk of breast cancer in age- and treatment-adjusted models (P, linear trend = 0.89). Women consuming the greatest amount of lycopene, at or above the 95th percentile of intake (≥20,519 μg/day), also had no association with the risk of breast cancer. Adjustment for life-style, reproductive, and dietary risk factors only marginally impacted the RRs. Analyses limited to breast cancers that were positive for estrogen and progesterone receptors also revealed no association with dietary lycopene intake in full multivariate models (P, linear trend = 0.17). In addition, we found no meaningful differences in the RRs comparing pre- versus postmenopausal women (data not shown).

Next, we considered whether higher intake of tomato-based food products, including tomatoes, tomato juice, tomato sauce, and pizza, were associated with the risk of breast cancer (Table 3). For tomato-based food products, 17.1% of women consumed <1.5 servings/week, 37.0% consumed 1.5 to <4 servings/week, 29.6% consumed 4 to <7 servings/week, 11.9% consumed 7 to <10 servings/week, and 4.5% of women consumed ≥10 servings/week. The multivariate RRs of breast cancer for increasing consumption of tomato-based food products were 1.00 (ref), 1.00, 1.20, 1.18, and 1.16 (P, linear trend = 0.11). Results were also equivocal for individual tomato-based food products, with modest nonsignificant increases in breast cancer risk for tomato intake. Analyses confined to women developing breast cancer positive for estrogen and progesterone receptors had comparable RRs to total breast cancer, including results for tomato intake.

Table 1. Selected baseline characteristics of 38,447 women according to quintiles of dietary lycopene intake

	Quintile of lycopene intake				
	1st (<4,443 μg/d)	2nd (4,443 to <6,471 μg/d)	3rd (6,471 to <9,083 μg/d)	4th (9,083 to <13,044 μg/d)	5th (≥13,044 μg/d)
Number of women	7,690	7,689	7,690	7,689	7,689
Age (years)	54.3 ± 7.2*	53.7 ± 6.9	54.1 ± 7.0	54.0 ± 7.0	53.6 ± 6.9
Smoking status (%)					
Never	52.6	51.8	51.0	51.3	48.4
Former	33.0	34.9	36.2	36.4	39.4
Current	14.4	13.3	12.9	12.3	12.2
Body mass index (kg/m^2)	26.2 ± 5.2	25.9 ± 4.9	26.1 ± 5.0	25.9 ± 5.0	25.9 ± 5.0
History of breast cancer in first-degree relative (%)	6.6	6.5	6.2	5.9	5.8
Exercise (%)					
Rarely/never	44.3	39.4	37.2	36.2	34.1
<One/wk	19.2	20.7	20.1	20.1	19.4
One to three times/wk	27.8	30.0	32.3	31.9	34.2
≥Four times/wk	8.8	9.9	10.5	11.8	12.4
Alcohol consumption (%)					
Rarely/never	52.2	45.7	42.1	42.6	41.2
One to three drinks/mo	12.8	13.6	13.7	13.6	12.3
One to six drinks/wk	26.6	31.7	33.9	32.8	33.7
≥1 drink/d	8.4	9.0	10.3	11.0	12.9
Menarche ≥14 years (%)	19.5	18.0	17.9	18.7	17.4
Ever used oral contraceptives (%)	66.4	71.3	68.8	69.9	71.4
Age at first pregnancy (%)					
<20 years	11.4	10.5	10.3	9.9	10.0
20-34 years	83.4	84.9	84.7	85.6	84.8
≥35 years	3.3	2.6	3.1	2.8	3.1
Nulliparous (%)	3.3	3.4	3.1	3.1	3.3
Postmenopausal (%)	56.4	53.6	55.5	54.6	53.6
Postmenopausal hormone use (%)					
Never	48.8	49.2	47.8	46.8	46.8
Former	10.8	10.0	9.7	10.7	10.1
Current	40.4	40.9	42.5	42.5	43.1
Fruit and vegetable intake (servings/d)	4.8 ± 2.8	5.2 ± 2.8	5.9 ± 3.0	6.7 ± 3.1	7.6 ± 3.7
Other energy-adjusted dietary factors (25)					
Total fiber intake (g/d)	17.1 ± 6.0	17.8 ± 5.4	18.8 ± 5.4	19.8 ± 5.6	21.5 ± 6.2
Folate intake (μg/d)	400 ± 222	412 ± 220	428 ± 224	437 ± 218	465 ± 231
Saturated fat intake (g/d)	20.5 ± 5.3	20.2 ± 4.8	19.8 ± 4.6	19.3 ± 4.6	18.5 ± 4.7

*Mean ± SD.

Table 2. RR of breast cancer according to quintiles of dietary lycopene intake among 38,447 women

	Quintile of lycopene intake*					≥95th Percentile	*P*, linear trend
	1st (*n* = 7,690)	2nd (*n* = 7,689)	3rd (*n* = 7,690)	4th (*n* = 7,689)	5th (*n* = 7,689)		
Median lycopene intake (μg/d)*	3,326	5,427	7,683	10,813	16,741	≥20,519	
Total breast cancer	(208)†	(213)	(215)	(237)	(203)	(51)	
Age-, treatment-adjusted RR‡	1.00 (reference)	1.04 (0.86-1.26)	1.04 (0.86-1.26)	1.15 (0.96-1.39)	1.00 (0.82-1.21)	0.99 (0.73-1.33)	0.89
Multivariate-adjusted RR§	1.00 (reference)	0.95 (0.76-1.17)	0.98 (0.79-1.21)	1.07 (0.87-1.32)	0.96 (0.77-1.19)	1.03 (0.74-1.42)	0.98
Multivariate-adjusted RR‖	1.00 (reference)	0.95 (0.77-1.18)	1.00 (0.80-1.24)	1.10 (0.89-1.36)	1.00 (0.80-1.25)	1.09 (0.78-1.51)	0.71
Positive for estrogen and progesterone receptors	(133)	(139)	(136)	(167)	(144)	(38)	
Age-, treatment-adjusted RR‡	1.00 (reference)	1.07 (0.84-1.36)	1.03 (0.81-1.31)	1.27 (1.01-1.59)	1.11 (0.88-1.41)	1.18 (0.83-1.68)	0.23
Multivariate-adjusted RR§	1.00 (reference)	0.97 (0.75-1.27)	0.94 (0.72-1.23)	1.17 (0.91-1.51)	1.06 (0.81-1.37)	1.19 (0.81-1.74)	0.35
Multivariate-adjusted RR‖	1.00 (reference)	0.98 (0.75-1.28)	0.96 (0.73-1.25)	1.21 (0.94-1.57)	1.12 (0.85-1.47)	1.28 (0.86-1.90)	0.16

*Energy-adjusted using the residual method (25).
†Number of events in brackets.
‡Adjusted for age, randomized aspirin, randomized vitamin E, and randomized β-carotene.
§Adjusted for the covariates above plus body mass index, family history of breast cancer, physical activity, age at menarche, ever use of oral contraceptives, age at first pregnancy, number of pregnancies, postmenopausal status, and postmenopausal hormone use.
‖Adjusted for the covariates above plus alcohol intake, fiber intake, folate intake, saturated fat intake, and fruit and vegetable intake.

Nested Case-Control Study of Plasma Lycopene. We then examined the association of plasma lycopene with the risk of breast cancer in a subset of 508 cases and an equal number of controls remaining free of breast cancer and matched on age and smoking status. There were no significant differences comparing baseline characteristics of breast cancer cases and controls except for age at first pregnancy (Table 4). Cases were more likely to have a later age of first pregnancy. Women who developed breast cancer also tended to consume more alcohol, reached menarche at a younger age, and were less likely to use oral contraceptives, although these differences were not significant. There were no appreciable differences in dietary factors among cases and controls, including dietary lycopene intake ($P = 0.44$).

Table 3. RR of breast cancer according to total and individual intake of tomato-based products (tomatoes, tomato juice, tomato sauce, and pizza)

	Categories of intake					*P*, linear trend
	1st	2nd	3rd	4th	5th	
Tomato-based food products	<1.5 servings/wk	1.5 to <4 servings/wk	4 to <7 servings/wk	7 to <10 servings/wk	≥10 servings/wk	
Number of women (breast cancer cases)	6,568 (175)*	14,206 (375)	11,368 (352)	4,587 (130)	1,717 (44)	
Age-, energy-, treatment-adjusted RR†	1.00 (ref)	1.01 (0.85-1.21)	1.19 (0.98-1.43)	1.09 (0.86-1.38)	0.98 (0.70-1.39)	0.44
Multivariate-adjusted RR‡	1.00 (ref)	1.00 (0.81-1.23)	1.20 (0.96-1.49)	1.18 (0.89-1.55)	1.16 (0.78-1.72)	0.11
Tomatoes	None*	1-3 servings/mo	1-4 servings/wk	≥5 servings/wk		
Number of women (breast cancer cases)	2,080 (40)	7,772 (222)	23,406 (680)	4,963 (133)		
Age-, energy-, treatment-adjusted RR†	1.00 (ref)	1.47 (1.05-2.06)	1.44 (1.05-1.98)	1.29 (0.91-1.85)		0.94
Multivariate-adjusted RR‡	1.00 (ref)	1.54 (1.04-2.29)	1.54 (1.05-2.24)	1.45 (0.94-2.22)		0.66
Tomato juice	None	1-3 servings/mo	1 serving/wk	≥2 servings/wk		
Number of women (breast cancer cases)	22,548 (620)	9,245 (260)	3,709 (123)	2,325 (54)		
Age-, energy-, treatment-adjusted RR†	1.00 (ref)	1.00 (0.87-1.16)	1.17 (0.96-1.42)	0.81 (0.61-1.07)		0.36
Multivariate-adjusted RR‡	1.00 (ref)	0.97 (0.83-1.15)	1.16 (0.93-1.44)	0.81 (0.59-1.11)		0.37
Tomato Sauce	None	1-3 servings/mo	1 serving/wk	2-4 servings/wk		
Number of women (breast cancer cases)	3,654 (101)	13,115 (388)	14,092 (364)	6,857 (205)		
Age-, energy-, treatment-adjusted RR†	1.00 (ref)	1.12 (0.90-1.39)	1.01 (0.81-1.26)	1.20 (0.93-1.53)		0.20
Multivariate-adjusted RR‡	1.00 (ref)	1.03 (0.80-1.33)	0.98 (0.76-1.27)	1.23 (0.93-1.64)		0.046
Pizza	None	1-3 servings/mo	1 serving/wk	≥2 servings/wk		
Number of women (breast cancer cases)	9,768 (259)	18,054 (542)	9,056 (250)	1,305 (20)		
Age-, energy-, treatment-adjusted RR†	1.00 (ref)	1.23 (1.06-1.43)	1.19 (0.99-1.43)	0.66 (0.41-1.04)		0.35
Multivariate-adjusted RR‡	1.00 (ref)	1.25 (1.05-1.48)	1.17 (0.95-1.44)	0.78 (0.48-1.26)		0.54

*Number of events in parentheses.
†Adjusted for age, total caloric intake, randomized aspirin, randomized vitamin E, and randomized β-carotene.
‡Adjusted for the covariates above plus body mass index, family history of breast cancer, physical activity, age at menarche, ever use of oral contraceptives, age at first pregnancy, number of pregnancies, postmenopausal status, postmenopausal hormone use, alcohol intake, fiber intake, folate intake, saturated fat intake, and fruit and vegetable intake.

Table 4. Baseline characteristics among 508 women who developed breast cancer (cases) and an equal number of women who remained free of breast cancer (controls)

	Cases (n = 508)	Controls (n = 508)	P
Body mass index (kg/m^2)	25.5 ± 4.4	25.9 ± 4.8	0.27
History of breast cancer in first-degree relative (%)	7.2	7.7	0.76
Exercise (%)			0.78
Rarely/never	36.6	39.8	
<Once/wk	20.1	18.7	
One to three times/wk	32.9	31.7	
≥Four times/wk	10.4	9.8	
Alcohol consumption (%)			0.17
Rarely/never	41.7	45.3	
One to three drinks/mo	12.2	15.2	
One to six drinks/wk	34.5	30.1	
≥One drink/d	11.6	9.5	
Menarche ≥14 years (%)	16.9	18.9	0.40
Ever used oral contraceptives (%)	62.1	65.7	0.50
Age at first pregnancy (%)			0.028
<20 years	5.9	10.1	
20-34 years	86.8	85.3	
≥35 years	5.9	2.6	
Nulliparous (%)	2.3	2.6	0.44
Postmenopausal (%)	63.2	61.0	0.23
Postmenopausal hormone use (%)			0.30
Never	43.8	40.6	
Former	9.5	12.2	
Current	46.8	47.1	
Fruit and vegetable intake (servings/d)	6.2 ± 3.2	6.0 ± 3.1	0.16
Energy-adjusted dietary factors			
Lycopene intake (μg/d)	9,359 ± 6,780	9,038 ± 6,409	0.44
Total fiber intake (g/d)	19.1 ± 5.6	19.2 ± 6.2	0.84
Folate intake (μg/d)	434 ± 228	441 ± 239	0.62
Saturated fat intake (g/d)	19.5 ± 5.0	19.7 ± 4.7	0.52
Plasma lycopene (μg/dL)	10.8 ± 5.7	10.3 ± 4.7	0.20
Plasma total cholesterol (mg/dL)	220 ± 37	221 ± 39	0.61
Plasma β-cryptoxanthin (μg/dL)	11.4 ± 9.7	11.1 ± 8.7	0.52
Plasma lutein/zeaxanthin (μg/dL)	19.4 ± 10.5	18.5 ± 8.8	0.16
Plasma α-carotene (μg/dL)	8.9 ± 8.5	8.2 ± 7.5	0.19
Plasma β-carotene (μg/dL)	27.1 ± 23.3	25.4 ± 22.0	0.24

NOTE: Cases and controls were matched on age and smoking status.

Among 508 controls, there was a moderate and significant Spearman correlation of 0.25 between plasma and dietary lycopene ($P < 0.0001$). Plasma lycopene was also significantly correlated (all $P < 0.05$) with the intake of tomato-based food products ($r = 0.20$), tomatoes ($r = 0.14$), tomato juice ($r = 0.11$), and tomato sauce ($r = 0.25$), but not pizza (Spearman $r = 0.09$; $P = 0.06$). Baseline characteristics were largely similar across quartiles of plasma lycopene among the 508 controls free of breast cancer (data not shown), defined as ≤7.2, 7.2 to 9.55, 9.56 to 13.0, and >13.0 μg/dL. Only a few risk factors from Table 4 differed. For example, controls with higher levels of plasma lycopene tended to be leaner, have a lower prevalence of family history of breast cancer, and have their first pregnancy at an earlier age. Among dietary factors, women with higher levels of plasma lycopene consumed greater amounts of fruits, vegetables and fiber, along with smaller amounts of saturated fat.

We next examined the association between increasing quartiles of plasma lycopene and the risk of breast cancer (Table 5). Crude models matched for age and smoking status, as well as multivariate models each showed no association between plasma lycopene and the risk of breast cancer (all P, linear trend >0.05). Controlling for potential confounders of the association between plasma lycopene and breast cancer only attenuated the RRs in the highest quartile. We also examined plasma lycopene and the risk of breast cancers that were positive for estrogen and progesterone receptors in 344 case-control pairs of women. In fully adjusted models, there was still no association between higher levels of plasma lycopene and a lower risk of breast cancer with a positive receptor status (P trend = 0.80).

Of note, among 508 controls free from breast cancer during follow-up, plasma lycopene was significantly correlated with other plasma carotenoids such as β-cryptoxanthin, lutein/zeaxanthin, α-carotene, and β-carotene to a similar magnitude, with Spearman correlations ranging from 0.32 to 0.38. In Table 5, further adjustment for other plasma carotenoids such as β-cryptoxanthin, lutein/zeaxanthin, α-carotene, and β-carotene in individual models had virtually no impact on the RRs of breast cancer. In addition, we found no meaningful differences in the RRs comparing pre- versus postmenopausal women (data not shown).

Quartiles of plasma carotenoids aside from plasma lycopene were also considered for their independent multivariate associations with the risk of breast cancer in Table 6. As with models for plasma lycopene, there was very little confounding of the association between each plasma carotenoid and risk of breast cancer. Higher quartiles of neither α- nor β-carotene were associated with the risk of breast cancer in multivariate models (both P trend >0.05). Women with elevated levels of plasma β-cryptoxanthin and lutein/zeaxanthin had slight nonsignificant reductions in the risk of breast cancer compared with those in the lowest quartile.

Discussion

In this large-scale prospective study of middle-aged and older women, neither dietary intake of lycopene nor plasma lycopene levels were significantly associated with the risk of breast cancer. Whereas the benefits of dietary lycopene intake

Table 5. RR and 95% CIs of breast cancer according to quartiles of plasma lycopene

	Quartile of plasma lycopene				*P*, linear trend
	1st (≤7.2 g/dL)	2nd (7.2-9.55 g/dL)	3rd (9.56-13.0 g/dL)	4th (>13.0 g/dL)	
Median plasma lycopene (μg/dL)	5.6	8.5	11.2	15.9	
Total breast cancer (508 case-control pairs)					
Crude*	1.00 (ref)	0.97 (0.69-1.36)	1.09 (0.76-1.57)	1.06 (0.74-1.51)	0.64
Multivariate-adjusted RR†	1.00 (ref)	0.96 (0.60-1.52)	1.12 (0.68-1.83)	0.89 (0.56-1.43)	0.72
Multivariate-adjusted RR‡,§	1.00 (ref)	0.95 (0.59-1.55)	1.15 (0.69-1.90)	0.93 (0.56-1.52)	0.86
+Plasma β-cryptoxanthin	1.00 (ref)	0.96 (0.59-1.56)	1.15 (0.69-1.91)	0.94 (0.56-1.57)	0.91
+Plasma lutein/zeaxanthin	1.00 (ref)	0.95 (0.59-1.55)	1.14 (0.69-1.89)	0.91 (0.55-1.51)	0.81
+Plasma α-carotene	1.00 (ref)	0.95 (0.59-1.55)	1.15 (0.69-1.90)	0.92 (0.55-1.54)	0.87
+Plasma β-carotene	1.00 (ref)	0.95 (0.59-1.55)	1.15 (0.69-1.90)	0.92 (0.55-1.54)	0.87
Positive for estrogen and progesterone receptors (344 case-control pairs)					
Crude*	1.00 (ref)	0.96 (0.63-1.45)	0.99 (0.63-1.56)	0.95 (0.61-1.47)	0.85
Multivariate-adjusted RR†	1.00 (ref)	0.96 (0.52-1.79)	1.20 (0.62-2.30)	0.85 (0.46-1.56)	0.61
Multivariate-adjusted RR‡	1.00 (ref)	0.96 (0.50-1.84)	1.27 (0.65-2.49)	0.90 (0.47-1.71)	0.80

*Matched on age and smoking status, and adjusted for randomized aspirin treatment, randomized vitamin E treatment, randomized β-carotene treatment, and plasma cholesterol level.
†Adjusted for the covariates above plus body mass index, family history of breast cancer, physical activity, age at menarche, ever use of oral contraceptives, age at first pregnancy, number of pregnancies, postmenopausal status, and postmenopausal hormone use.
‡Adjusted for the covariates above plus alcohol intake, fiber intake, folate intake, saturated fat intake, and fruit and vegetable intake.
§Other carotenoids were added separately into the model.

and plasma lycopene levels have been shown for prostate, lung, and stomach cancer (35) as well as cardiovascular disease (32, 33, 36), this study does not suggest a role for either dietary lycopene intake or plasma lycopene in the development of breast cancer. Increasing the consumption of either dietary lycopene or tomato-based food products did not exhibit a dose-response relation with the risk of breast cancer, including breast cancers that were positive for estrogen and progesterone receptors. Other plasma carotenoids besides lycopene—including β-cryptoxanthin, lutein/zeaxanthin, α-carotene, and β-carotene—were also not associated with the risk of breast cancer.

Epidemiologic studies assessing whether dietary (9-13) or blood (14-17) lycopene are associated with the risk of breast cancer have provided no clear answer. Numerous other studies have examined not only lycopene but also other dietary and blood carotenoids, with equally inconsistent results (37). Among studies of dietary lycopene, only a Swiss case-control study (289 cases) reported a strong inverse association with breast cancer (38). On the other hand, previous long-term prospective studies of dietary lycopene consumption among Canadian (10) and U.S. (11, 13, 39) women have found no association with pre- and postmenopausal breast cancer risk. The present study is the first to additionally examine the role of tomato-based food products with the risk of breast cancer, and no association was apparent.

Among studies of serum and plasma lycopene, a nested case-control study of U.S. women (295 cases) found a strong association between plasma lycopene and breast cancer risk (15). In another nested case-control study of Swedish women (201 cases) selected from multiple cohorts, an inverse association (*P* trend = 0.01) between plasma lycopene and breast cancer was limited to a subcohort of only 67 postmenopausal cases (16). In contrast, two case-control studies of serum lycopene found no association with breast cancer (14, 17).

The finding of only a modest correlation between dietary intake of lycopene and plasma lycopene levels (Spearman r = 0.25) suggests that our attempt to directly associate diets that are simply rich in lycopene with higher plasma lycopene levels may be too simplistic. In support of this suggestion, we also reported a weak but significant correlation of 0.14 in another nested case-control study using data from the Women's Health Study (32). Lycopene in foods occurs mainly in the thermodynamically stable all-*trans* form (40), whereas the more unstable *cis*-isomer accounts for 50% to 70% of total lycopene in human plasma (40, 41). To further confound the issue, others have reported the correlation between dietary

Table 6. Multivariate RR and 95% CIs of breast cancer comparing quartiles of plasma lycopene with other plasma carotenoids in separate models

	Quartile of plasma biomarker				*P*, linear trend
	1st	2nd	3rd	4th	
Plasma lycopene (μg/dL)	5.6*	8.5	11.2	15.9	
Multivariate-adjusted RR	1.00 (ref)	0.95 (0.59-1.55)	1.15 (0.69-1.90)	0.93 (0.56-1.52)	0.86
Plasma β-cryptoxanthin (μg/dL)	3.5	6.7	11.2	20.6	
Multivariate-adjusted RR	1.00 (ref)	1.31 (0.79-2.17)	0.83 (0.49-1.43)	0.82 (0.46-1.44)	0.21
Plasma lutein/zeaxanthin (μg/dL)	9.7	14.2	19.8	28.8	
Multivariate-adjusted RR	1.00 (ref)	0.88 (0.52-1.49)	0.72 (0.42-1.25)	0.78 (0.45-1.38)	0.40
Plasma α-carotene (μg/dL)	2.2	4.3	7.9	15.9	
Multivariate-adjusted RR	1.00 (ref)	1.04 (0.60-1.81)	0.89 (0.52-1.53)	1.06 (0.61-1.84)	0.85
Plasma β-carotene (μg/dL)	8.3	15.0	24.3	45.6	
Multivariate-adjusted RR	1.00 (ref)	1.24 (0.75-2.05)	1.20 (0.71-2.02)	1.36 (0.79-2.33)	0.36

NOTE: Matched on age and smoking status, and adjusted for randomized aspirin treatment, randomized vitamin E treatment, randomized β-carotene treatment, plasma cholesterol level, body mass index, family history of breast cancer, physical activity, age at menarche, ever use of oral contraceptives, age at first pregnancy, number of pregnancies, postmenopausal status, postmenopausal hormone use, alcohol intake, fiber intake, folate intake, saturated fat intake, and fruit and vegetable intake.
*Median value for each plasma biomarker for that quartile.

lycopene intake and plasma lycopene to be stronger in men than in women (42, 43). For example, among 162 nonsmoking women in the Nurses' Health Study, the adjusted correlation was 0.21 for lycopene, whereas in 110 nonsmoking men from the Health Professionals Follow-up Study the correlation was 0.47 (42). Reasons for this gender disparity remain unclear. One possible explanation for the lower correlations between dietary lycopene intake and plasma lycopene in studies may include differences in actual dietary patterns, which include lower intakes of tomato sauce that contain lycopene in a more bioavailable food matrix (34, 44).

Despite our lack of association for lycopene and breast cancer, lycopene may still have chemopreventive properties. Research suggests that lycopene strongly inhibits the proliferation and cell cycle proliferation of mammary human cancer cells (6) and suppresses insulin-like growth factor-I (7), which has been strongly linked with an increased risk of breast cancer in premenopausal women in some studies.

We considered the association between dietary lycopene intake and plasma lycopene with the risk of breast cancers that were positive for steroid hormone receptors based on previous findings to suggest that estrogen receptor status is an important factor for breast cancer cell response to carotenoids, including lycopene (6). We hypothesized that if lycopene or other carotenoids had a role in the prevention of breast cancer through an endogenous hormonal mechanism, a stronger inverse association would be observed for women positive for estrogen and progesterone receptors. However, based on our findings for both dietary lycopene intake and plasma lycopene, we found no evidence to support this mechanism.

A number of potential limitations of the present study also warrant discussion. First, we relied on a single baseline measurement of dietary or plasma lycopene, raising the possibility of regression to the mean that could bias our RRs toward the null hypothesis and underestimate the observed risk reductions. However, total plasma lycopene has been shown to be reasonably stable in samples taken 3 to 4 years apart with a correlation of 0.63 (45). Furthermore, even with the use of repeated dietary lycopene measurements, no association remained with breast cancer among U.S. nurses (11). Second, we have not directly assessed the long-term stability of plasma lycopene stored at −140°C in liquid nitrogen–chilled freezers since 1993. However, studies comparing measurements of plasma lycopene and other carotenoids support the long-term stability of these biochemical markers (46, 47). Third, the women in our study did not consume large amounts of specific tomato-based food products, resulting in narrow distributions of intake and lower levels of plasma lycopene. This fact may have limited our ability to discern possible dose-response or threshold effects that might have been present in our data. Finally, residual confounding may also be of concern, but seems unlikely. We comprehensively controlled or matched for major breast cancer risk factors, which had a trivial impact on the reported RRs.

In summary, neither higher dietary nor plasma lycopene levels were associated with a reduced risk of breast cancer in this population of middle-aged and older female health professionals. Despite promising mechanistic studies, this large-scale study of dietary lycopene, tomato-based food products, and plasma lycopene does not support a role for lycopene in breast cancer carcinogenesis.

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