

# Plasma Retinol and Prognosis of Postmenopausal Breast Cancer Patients

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## Abstract

**Background:** The role of retinol (vitamin A) in breast cancer prognosis has never been investigated in postmenopausal women. We prospectively assessed the long-term prognostic role of retinol plasma levels in a cohort of postmenopausal breast cancer patients.

**Patients and Methods:** We investigated 208 women self-reported as postmenopausal operated on for T<sub>1-2</sub>N<sub>0</sub>M<sub>0</sub> breast cancer who participated in a chemoprevention trial as controls and never received chemotherapy or hormone therapy. Plasma samples were collected 3 months (median) after surgery and assayed within 3 weeks for retinol. Minimum and median potential follow-up were 12 and 15 years, respectively. The main analyses were on all women and on a subgroup ages  $\geq 55$  years, assumed too old to be in perimenopause. The main end point was breast cancer death. Breast cancer survival was estimated by the Kaplan-Meier

method. The hazard ratios of breast cancer death by retinol level were estimated by Cox models stratified for age, where relevant, and recruitment period, and adjusted for tumor size and histology.

**Results:** At 12 years, patients with low retinol ( $< 2.08 \mu\text{mol/L}$ , median of distribution) had lower breast cancer survival than those with high retinol (log-rank  $P = 0.052$ ); the difference was significant for women  $\geq 55$  years (log-rank  $P = 0.006$ ). The adjusted hazard ratios for low versus high retinol were 2.11 (95% confidence interval, 1.08-4.14) for all women and 3.58 (95% confidence interval, 1.50-8.57) for those  $\geq 55$  years.

**Conclusions:** Low plasma retinol strongly predicts poorer prognosis in postmenopausal breast cancer patients. Retinol levels should be determined as part of the prognostic workup. (Cancer Epidemiol Biomarkers Prev 2009;18(1):42-8)

## Introduction

Retinol (vitamin A) is involved in various physiologic processes, including vision, embryogenesis, reproduction, inflammation, cell growth, and cell differentiation (1).

The role of retinol in normalizing cell differentiation and reducing inflammation suggests it might influence the risk of developing cancer, and the relationship between retinol and cancer has been studied extensively (2, 3).

The relation of retinol to the risk of breast cancer has been investigated in case-control (4-7) and prospective studies (8-15). In the case-control studies, retinol levels in newly diagnosed women were compared with those in matched-control women. One study found that high retinol was significantly associated with reduced breast cancer risk (7), another found a significant trend of reduced retinol levels with more advanced disease stage at diagnosis (6), and the two other studies (4, 5) found no relation between breast cancer and retinol. Four of the prospective studies (10, 13-15) found an inverse associ-

ation between retinol levels and breast cancer risk, but the association was significant in one study only (10); in another of these studies (15), high retinol was associated with significantly decreased risk of breast cancer with lymph node metastases at diagnosis.

Although, taken together, these investigations do not present a consistent picture, they do suggest the possibility of a role of retinol in breast cancer etiology. Furthermore, it is known that retinol plasma levels vary with age (9) and the phase of the menstrual cycle (16, 17), yet no studies have considered menopausal status as a possible factor confounding the relation between retinol and breast cancer.

We found only one study that looked at the influence of retinol on breast cancer prognosis (18). The study found that in premenopausal women with node-positive breast cancer receiving adjuvant chemotherapy, significantly low levels of retinol-binding protein (RBP; also known as RBP4; ref. 1) were associated with early disease recurrence; however, only 39 women were studied.

An association of vitamin A with breast cancer is also supported by evidence that retinoids, natural and synthetic analogues of vitamin A, protect against mammary carcinogenesis in animal models (19). Fenretinide seemed the most active and least toxic of these compounds (19) and, for this reason, was tested in a randomized trial for its ability to prevent breast cancer recurrence after surgery for early stage (T<sub>1-2</sub>N<sub>0</sub>) breast cancer (20, 21). In the trial, fenretinide was associated

Received 5/30/08; revised 9/2/08; accepted 10/17/08.

**Grant support:** The Italian Association for Cancer Research and Italian Ministry of Health. The US National Cancer Institute, U.S. Department of Health and Human Services, NIH, and the Italian National Research Council also supported the study.

**Note:** This work has not been published previously in any form.

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doi:10.1158/1055-9965.EPI-08-0496

with reduced occurrence of second breast cancer in premenopausal women (21), an effect that persisted for 10 years after stopping treatment (22).

In the present study, we prospectively examined the relationship between plasma retinol concentrations and breast cancer prognosis in a large cohort of breast cancer patients with long follow-up and information on menopausal status. The cohort consisted of the untreated postmenopausal patients who had participated in the trial to test the efficacy of fenretinide in preventing breast cancer recurrence (19).

## Patients and Methods

**Study Patients.** We analyzed the data of 208 postmenopausal control patients prospectively recruited at the Istituto Nazionale Tumori, Milan, Italy, to the fenretinide breast cancer prevention trial (21). The trial began on March 1, 1987, and accrual closed on July 31, 1993. The trial design, protocol, and results have been published elsewhere (20-22). Eligible patients were operated on for T<sub>1-2</sub>N<sub>0</sub>M<sub>0</sub> breast cancer and did not receive adjuvant chemotherapy or hormone therapy. At baseline, the patients were assessed for metastases by chest X-ray, bone scan, blood tests for liver function, and liver ultrasonography, and for contralateral breast cancer by mammography. Those recruited also had normal metabolic function and normal erythrocyte, leukocyte, and platelet counts.

Because fenretinide treatment is associated with lowered plasma retinol (23, 24), retinol and fenretinide were determined at baseline and during treatment to assess compliance. Written informed consent was obtained to use the blood samples for retinol and fenretinide determinations and for future ancillary studies. Blood samples were available for 208 of the 235 enrolled postmenopausal control patients. The reason for missing samples was difficulty of venous access or refusal (21). Samples from the 208 women, mean age 58.6 ± 5.1 y (range, 48-70 y) at recruitment, were taken a median of 2.8 mo after surgery (38% within 1 mo; 89% within 12 mo, and 11% 12-55 mo after surgery). Postmenopausal women were those who self-reported amenorrhea for >1 y before recruitment. In a study on all control patients recruited to the fenretinide breast cancer prevention trial, it was found that among women recruited ages 46 y to 55 y, retinol levels increased significantly in the 5 y after recruitment but were stable if they were already in menopause at recruitment (25). We therefore also analyzed the subgroup ages ≥55 y at recruitment using this age cutoff to identify a subgroup of women too old to be in perimenopause. Patients who had undergone hysterectomy without ovariectomy were considered postmenopausal if they were ≥51 y old at enrollment.

On December 31, 2004 (end of follow-up), the minimum potential follow-up was 11.7 y and the median follow-up was 15.1 y (interquartile range, 13.4-15.8 y). No participant received adjuvant chemotherapy or hormone therapy. Life status was checked annually. No cases were lost to life status follow-up and all causes of death were recorded. To our knowledge, no patients were covered by cancer registry or other archives. During the first 5 y, follow-up consisted of checkup, including

clinical examination and blood tests every 6 mo, annual mammography and chest X-ray, and bone scan every 18 mo. New breast cancer events (local relapse, regional relapse, distant metastasis, contralateral primary breast cancer, and ipsilateral primary breast cancer unrelated to the first) were recorded; however, 26 (12.5%) women were lost to clinical follow-up at 10 y, 38 (18.3%) were lost at 12 y (minimum potential follow-up), and 62 (29.8%) at 15 y (median follow-up).

**Blood Sample Storage and Retinol Assay.** Blood samples were collected between 9 a.m. and 3 p.m. into heparin-containing containers. Plasma was separated and divided into samples: one stored at -20°C pending retinol and fenretinide assay (21, 24) and the others stored at -80°C for future studies (26, 27). Retinol and fenretinide were determined within 3 wk of collection by high-performance liquid chromatography, the method of choice for quantitating retinoids (28). Details of the high-performance liquid chromatography assay and quality control methods are reported elsewhere (24, 29). In all procedures, the samples were protected from the light. Briefly, 0.2 mL aliquots were added to 0.4 mL acetonitrile containing butylated hydroxytoluene (125 µg/mL; Sigma) as antioxidant, vortexed and centrifuged. Retinol and fenretinide were determined in the recovered supernatants with the use of a liquid chromatograph (Perkin-Elmer) fitted with a C18 (5 µm) reverse-phase column (150 × 4.6 mm) and C18 precolumn (Perkin-Elmer). The mobile phase was CH<sub>3</sub>CN:H<sub>2</sub>O:CH<sub>3</sub>COOH (75:23:2, volume for volume for volume), flow rate 2 mL/min. The detector was a Perkin-Elmer LC95 absorbance detector set at 340 nm. Samples from pooled plasma, stored at -80°C, were included in each analytic batch. *N*-(4-ethoxyphenyl)-retinamide (kindly provided by the Johnson Pharmaceutical Research Institute) was used as internal standard by adding it to the CH<sub>3</sub>CN used to precipitate proteins. The retinol reference standard was from Sigma. The retinol calibration curve was linear in the concentration range 0.01 to 6 µmol/L. Intra-assay and inter-assay coefficients of variation never exceeded 20%.

**Statistical Analysis.** For the main analyses, the 208 women were divided into low-retinol (<2.08 µmol/L) and high-retinol (≥2.08 µmol/L) groups, with the median (2.08 µmol/L) as cutoff. The significance of differences in clinical and pathologic variables between the two groups was evaluated by  $\chi^2$  test. For ancillary analyses, the retinol levels were divided into tertiles. Differences in mean retinol levels were assessed by the nonparametric Kruskal-Wallis test; trends across age classes and recruitment periods were analyzed by the Cuzick test.

The main end point was breast cancer survival, defined from date of blood sampling to date of breast cancer death, with deaths due to other causes censored at date of death. The second end point was distant metastasis-free survival, defined from date of blood sampling to date of diagnosis of distant metastases; women with another first event (local or regional relapse, contralateral or ipsilateral primary unrelated to the first cancer) were censored at the date of event. Women lost to clinical follow-up were censored at the date of latest clinical checkup. Data were analyzed at 12 and 15 y,

**Table 1. Clinical and pathologic characteristics at recruitment of 208 postmenopausal women operated on for early breast cancer according to baseline plasma retinol levels**

Characteristic	Retinol concentration ( $\mu\text{mol/L}$ )		$P$ ( $\chi^2$ test)
	<2.08*	$\geq 2.08^*$	
	$n$ (%)	$n$ (%)	
Age, y			
48-54	27 (26.2)	31 (29.5)	0.595
55-70 <sup>†</sup>	76 (73.8)	74 (70.5)	
Tumor size <sup>‡</sup>			
pT <sub>1</sub>	80 (77.7)	87 (82.9)	0.347
pT <sub>2</sub>	23 (22.3)	18 (17.1)	
Histology			
IDC	53 (51.5)	55 (52.4)	0.721
IDC with intraductal component	10 (9.7)	12 (11.4)	
ILC	24 (23.3)	20 (19.1)	
IDC plus ILC	4 (3.9)	8 (7.6)	
Other infiltrating histotype	12 (11.6)	10 (9.5)	
Treatment			
Breast-conserving surgery	71 (68.9)	75 (71.4)	0.920
Mastectomy	27 (26.2)	25 (23.8)	
Missing data	5 (4.9)	5 (4.8)	
Total	103 (100.0)	105 (100.0)	

Abbreviations: IDC, pure infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma.

\* Median retinol concentrations.

<sup>†</sup> Assumed too old to be in perimenopause.

<sup>‡</sup> International Union Against Cancer TNM classification (1987): pT<sub>1</sub>,  $\leq 2$  cm; pT<sub>2</sub>, 2 to 5 cm.

corresponding to the rounded values of minimum potential follow-up and median follow-up, respectively. Survival was represented by Kaplan-Meier curves. The significance of breast cancer survival and distant metastasis-free survival differences in relation to retinol levels were investigated by the log-rank test. Hazard ratios (HR) for breast cancer death or distant metastasis were estimated by Cox multivariable modeling.

The following were evaluated as possible covariates: (a) period of recruitment (women were recruited over 2,103 d; we divided this into periods 1, 2, and 3, each of 701 d), (b) size of primary, as pT<sub>1</sub> versus pT<sub>2</sub>, according to the 1987 tumor-node-metastasis classification (ref. 30; two women classified as pT<sub>1</sub>-pT<sub>2</sub> were considered pT<sub>2</sub>), (c) histology (pure infiltrating ductal carcinoma versus other), (d) age at recruitment as continuous variable, and (e) treatment (breast-conserving surgery versus mastectomy). Hormone receptor status and body mass index were determined in 78% and 38%, respectively, of the cohort; these subgroups were not representative of the whole cohort and were therefore not considered in the HR estimates.

The variables retained in the final models were period of recruitment as stratification variable, and tumor size and histology as covariates. Period of recruitment was retained because breast cancer survival increased (nonsignificant) with this variable (at 12 y of follow-up, survival was 77%, 81%, and 87% in recruitment periods 1, 2, and 3, respectively); furthermore, period of recruitment was significantly related to retinol levels (means  $\pm$  SD: 2.19  $\pm$  0.52, 2.15  $\pm$  0.50, and 1.92  $\pm$  0.38  $\mu\text{mol/L}$  for periods 1, 2, and 3, respectively;  $P_{\text{trend}} = 0.004$ ). Tumor size and histology were retained because both influenced HR estimates. Age at recruitment (continuous variable) and type of treatment did not influence HRs and were excluded from the final models. However, models on the entire cohort were

stratified into two groups: <55 y versus  $\geq 55$  y, to have a group of women too old to be in perimenopausal stage. In the final models, HRs for women  $\geq 55$  y were stratified for period of recruitment and adjusted for tumor size and histology covariates.

For estimating HR, the proportional hazards assumption (checked by scaled Schoenfeld residuals) was satisfied up to the minimum potential follow-up (12 y) but not up to the median follow-up (15 y). At 12 y of follow-up, 38 breast cancer deaths had occurred, resulting in a minimum detectable HR of 2.30, assuming a power of 80% and an  $\alpha$ -level of 0.05 (one-sided test; ref. 31).

Ninety-five percent confidence intervals (95% CI) were estimated for all HRs. All  $P$  values were obtained from two-sided statistical tests; differences with  $P \leq 0.05$  were considered significant. The analyses were done with the STATA statistical software package, release 9.0 (2005; Stata Corporation).

## Results

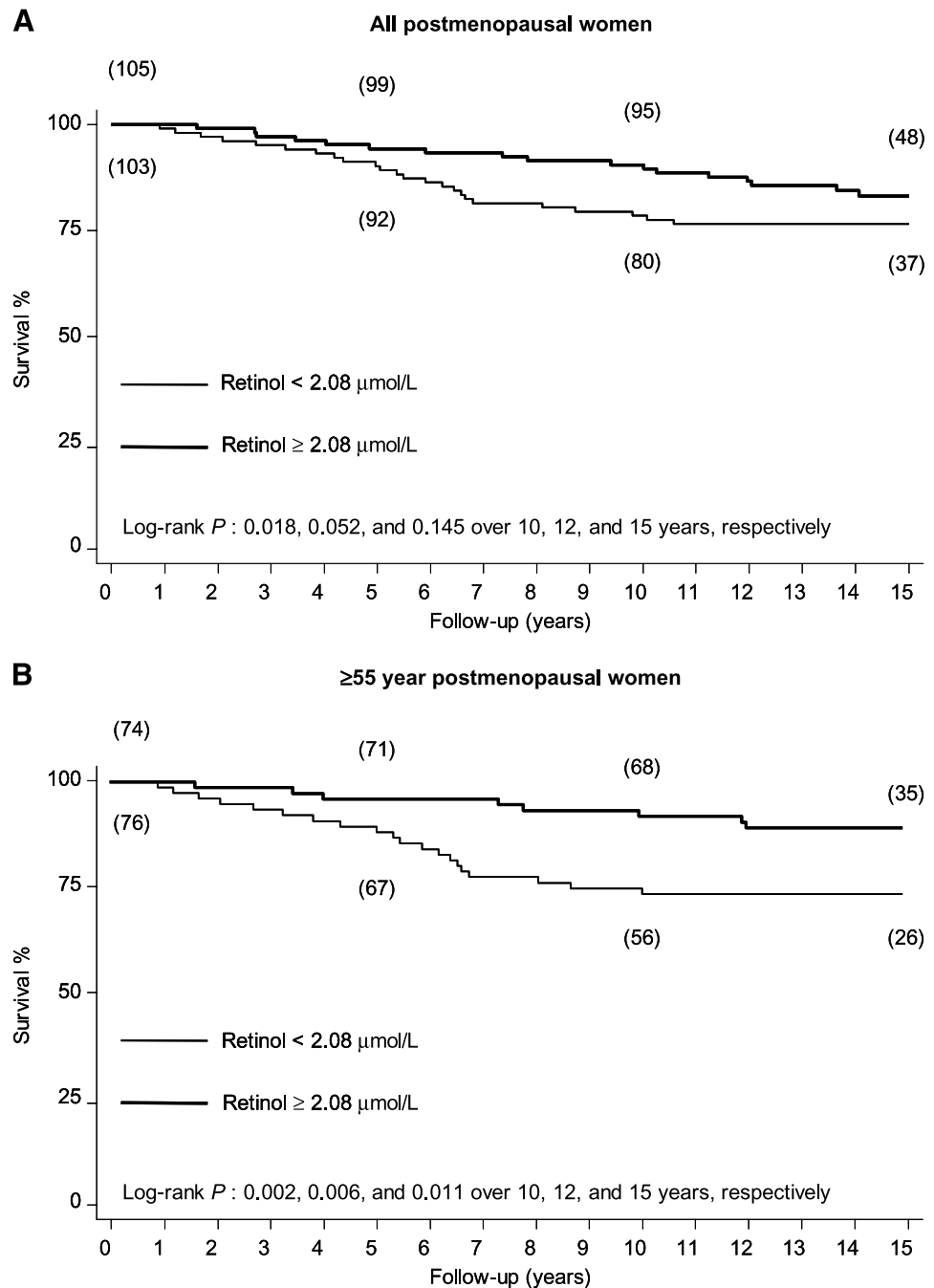
Table 1 shows the baseline characteristics of the 208 postmenopausal women by retinol level. Women  $\geq 55$  years formed 72% of the cohort, 74% of the low-retinol group, and 71% of the high-retinol group. The retinol groups were well balanced for stage (78% of the low-retinol and 83% of the high-retinol group were pT<sub>1</sub>), histology (52% in both groups had pure infiltrating ductal carcinoma), and treatment (69% and 71%, respectively, received breast-conserving surgery).

Women in the low-retinol group had worse breast cancer survival than those in the high-retinol group (Fig. 1). Most breast cancer deaths occurred in the first 10 years in the low-retinol group but were spread over the entire follow-up period in the high-retinol group. For

the entire cohort, differences between the two curves were significant over the first 10 years (log-rank  $P = 0.018$ ) but not over 12 years (log-rank  $P = 0.052$ ) and 15 years (log-rank  $P = 0.145$ ). For women  $\geq 55$  years, the survival disadvantage for the low-retinol group was more marked and significant over the first 15 years of follow-up (log-rank  $P = 0.011$ ). For the entire cohort, 10-year breast cancer survival was 78% in the low-retinol and 90% in the high-retinol group; 12-year breast cancer survival was 76% versus 87%; and 15-year breast cancer survival was 76% versus 83%. For women  $\geq 55$  years,

10-year breast cancer survival was 75% in the low-retinol and 93% in the high-retinol group; 12-year breast cancer survival was 73% versus 90%; and 15-year breast cancer survival was 73% versus 89%.

Table 2 shows disease and vital status according to retinol levels at 12 years of follow-up (all women had at least 12 years of follow-up). One hundred and seven (51%) women had no breast cancer event, 50 in the low-retinol and 57 in the high-retinol group. Thirty-nine women had distant metastasis as first event, 26 in the low-retinol and 13 in the high-retinol group (21 and 8,



**Figure 1.** Kaplan-Meier curves of 15-y breast cancer survival in postmenopausal patients operated on for early breast cancer according to dichotomized plasma retinol levels determined after treatment. (A) entire cohort, (B)  $\geq 55$  y postmenopausal women. Figures in parentheses, numbers of women at risk.

**Table 2. Disease status and vital status at 12 y of follow-up according to plasma retinol levels at baseline in 208 women operated on for early breast cancer**

	Patients	Retinol concentration ( $\mu\text{mol/L}$ )	
		<2.08	$\geq 2.08$
		<i>n</i> (%)	<i>n</i> (%)
All postmenopausal women			
Disease status			
BC free	107 (51.4)	50 (48.5)	57 (54.3)
New BC event: distant metastasis	39 (18.8)	26 (52.2)	13 (12.4)
New BC event: other event <sup>†</sup> *	24 (11.5)	9 (8.7)	15 (14.3)
Lost to clinical follow-up <sup>†</sup>	38 (18.3)	18 (17.5)	20 (19.0)
Vital status			
Alive	166 (79.8)	77 (74.8)	89 (84.8)
Deceased due to BC	38 (18.3)	24 (23.3)	14 (13.3)
Due to other causes <sup>‡</sup>	4 (1.9)	2 (1.9)	2 (1.9)
Total	208 (100.0)	103 (100.0)	105 (100.0)
Women $\geq 55$ y			
Disease status			
BC free	73 (48.7)	34 (44.7)	39 (52.7)
New BC event: distant metastasis	29 (19.3)	21 (27.6)	8 (10.8)
New BC event: other event <sup>†</sup> *	16 (10.7)	6 (7.9)	10 (13.5)
Lost to clinical follow-up <sup>†</sup>	32 (21.3)	15 (19.7)	17 (23.0)
Vital status			
Alive	119 (79.3)	54 (71.1)	65 (87.8)
Deceased due to BC	27 (18.0)	20 (26.3)	7 (9.5)
Due to other causes <sup>‡</sup>	4 (2.7)	2 (2.6)	2 (2.7)
Total	150 (100.0)	76 (100.0)	74 (100.0)

Abbreviation: BC, breast cancer.

\*Thirteen contralateral breast cancers (9 in women  $\geq 55$  y at recruitment), 6 local relapses (4 in women  $\geq 55$  y at recruitment), 3 ipsilateral breast cancers (2 in women  $\geq 55$  y at recruitment), and 2 cases of contralateral plus ipsilateral breast cancer (1 in a woman  $\geq 55$  y at recruitment).

<sup>†</sup>Known to be alive at the end of follow-up.

<sup>‡</sup>One lung cancer, one stroke, one cardiac arrest, and one amyotrophic lateral sclerosis.

respectively, in those  $\geq 55$  years). Twenty-four women (11.5%) had another breast cancer event as first event, 9 in the low-retinol and 15 in the high-retinol group (6 and 10, respectively, in those  $\geq 55$  years). Thirty-eight women died of breast cancer: 24 (23%) in the low-retinol and 14 (13%) in the high-retinol group [20 (26%) and 7 (10%), respectively, in those  $\geq 55$  years].

Table 3 shows the HRs of breast cancer death estimated over 12 years of follow-up. Women with low retinol had a significantly greater hazard for breast cancer death than those with high retinol (reference). The adjusted HR (model 2) was 2.11 (95% CI, 1.08-4.14) for the entire cohort and 3.58 (95% CI, 1.50-8.57) for those  $\geq 55$  years (model 4).

When retinol was categorized into tertiles, the adjusted HRs showed a linear trend: 2.40 (95% CI, 1.01-5.71) for low retinol, 2.01 (95% CI, 0.82-4.94) for medium retinol, and 1.0 (reference) for high retinol ( $P$  for trend = 0.050) for all women, and 2.69 (95% CI, 0.99-7.32), 2.26 (95% CI, 0.71-5.44), and 1.0 ( $P$  for trend = 0.054) in those  $\geq 55$  years (data not shown in the tables). Retinol was strongly associated with distant metastasis: 12-year metastasis-free survival was 72% versus 85% in the low-retinol versus high-retinol groups in all women and 71% versus 87% in those  $\geq 55$  years. The differences between curves over the first 12 years were significant: log-rank  $P = 0.018$  for the entire cohort and log-rank  $P = 0.009$  for those  $\geq 55$  years. The adjusted HRs of metastasis were 2.46 (95% CI, 1.25-4.84) and 3.16 (95% CI, 1.39-7.24) for low versus high retinol in all women and those  $\geq 55$  years, respectively (data not shown in the tables).

## Discussion

To date, this is the only study to prospectively evaluate the association of plasma retinol levels with breast cancer outcomes. We found that in postmenopausal women (ages 48-70 years at diagnosis) with a minimum potential of follow-up of 12 years, low retinol levels after breast cancer surgery were significantly associated with poorer outcomes.

Previous studies have suggested that retinol may be associated with a reduced risk of developing breast cancer. However, the results of prospective (8-15) and case-control studies (4-7) have been inconsistent, and only a few studies (6, 7, 10) have shown a significant inverse association between retinol levels and breast cancer risk. Little is known about the influence of hormones on retinol levels. However, cyclic changes in levels of retinol (16, 32) and its transport protein RBP (17) with the menstrual cycle suggest an association between changes in sex hormones and changes in retinol. Use of estrogen-containing oral contraceptives is also known to provoke a significant increase in plasma retinol levels (17, 33, 34). It is for this reason that we included only postmenopausal women in our study. However, a previous study on participants of the fenretinide breast cancer prevention trial showed that retinol levels increased significantly in the 5 years after recruitment in nonmenopausal women ages 46 to 55 years but were stable in same-aged women recruited after menopause (25). Another study found that retinol levels increase significantly (10%) in the first 2 years of menopause (35).

We therefore decided to stratify the entire cohort by age and also to analyze the subgroup  $\geq 55$  years separately. However, analyses done classifying women according to other cut points (52 and 60 years) produced similar results (data not shown). The hormonal situation was not taken into account in previous studies on retinol and breast cancer and may be one reason for their inconsistent results.

RBP4, the specific transport protein for retinol in the blood, has recently been shown to be secreted by adipocytes (36). High serum RBP4 levels have been found in subjects with obesity, metabolic syndrome, and type 2 diabetes (37)—all conditions whose increasing prevalence in the Western world has been linked to breast cancer (38). Notwithstanding high RBP4 levels, retinol levels in obese and diabetic patients have been reported as similar to those in controls (39-41). These findings suggest that, in these cases, retinol levels may not be associated with breast cancer. Note, however, that in these studies too, the age and the hormonal situation in the few women investigated were not taken into account.

For our entire cohort, we found that low retinol was associated with significantly lower 12-year survival than high retinol and that, for those  $\geq 55$  years, the association was even stronger. Similarly, the HRs of breast cancer death increased from 2.11 (95% CI, 1.08-4.14) for all women to 3.58 (95% CI, 1.50-8.57) for those  $\geq 55$  years. The weaker association of adverse breast cancer outcomes with low retinol in all women is therefore likely to be attributable to the misclassification of premenopausal or perimenopausal women.

Our findings on breast cancer survival were mirrored by those on distant metastases. Low-retinol women had a 2-fold (3-fold in those  $\geq 55$  years) greater risk of distant metastasis than high-retinol women. In a prospective study on breast cancer risk, retinol was unrelated to the risk of breast cancer in general, but high retinol was associated with a significantly decreased risk of breast cancer with nodal metastases (15), suggesting that low retinol levels predict metastasis risk (i.e., they seem to be

associated with disease progression). Retinol levels, which are tightly controlled by physiologic mechanisms (42), decrease during inflammation (43, 44). Previous studies have established that systemic inflammatory status influences prognosis in patients with various solid tumors, including breast cancer (45-47). Thus, low retinol levels could indicate an inflammatory state that, in turn, may adversely affect breast cancer prognosis—a hypothesis worth investigating in future studies.

Our study has a number of limitations. Twenty years ago, information on human epidermal growth factor receptor 2 and p53 expression was not collected routinely; neither was hormone receptor status determined in all women. The 162 (77% of the low-retinol and 80% of the high-retinol group) women in whom receptor status was determined are not a representative sample of the whole cohort. However, when hormone receptor status was included in a multivariable analysis, the adjusted HRs did not change substantially (data not shown). Similarly, body mass index could only be calculated in 38% of the cohort (weight was available in 187 women, but in only 80 of these was height available). Body mass index did not change with increasing retinol tertiles: means were  $25.5 \pm 4.7$ ,  $26.2 \pm 5.3$ , and  $25.3 \pm 5.1$  kg/m<sup>2</sup> ( $P$  trend = 0.663). However, weight slightly decreased (not significantly) with retinol levels: mean weights were  $64.2 \pm 11.3$ ,  $63.3 \pm 11.5$ , and  $61.9 \pm 11.2$  kg ( $P$  trend = 0.228). From this, the possibility emerges that the effect of high retinol on breast cancer survival may be partially explained by its association with low body weight. However, when weight was included in a multivariable Cox analysis, the adjusted HRs did not change substantially (data not shown). It is also important to note that the study participants did not receive any adjuvant therapy. Today, hormone receptor-positive patients would receive hormonal therapy, and selected patients would be offered chemotherapy; thus, the generality of our findings may be limited for this reason. Finally, the participants were recruited over a long period (March 1987 to July 1993) and, when divided into three equal periods, it was found that the intervals were inversely associated with hazard of breast cancer death and significantly inversely associated with retinol levels. However, we took this into account by stratifying the final Cox model by recruitment period.

An important strength of our study is that, by confining itself to postmenopausal women, it is the first to take account of the fact that retinol levels are influenced by hormonal pattern. To conclude, we have found that in women operated on for early breast cancer, lower levels of retinol were associated with poorer outcomes, and our findings suggest that retinol levels should be determined as part of the prognostic workup of postmenopausal women with breast cancer. Although further studies on the relation of retinol in breast cancer prognosis are necessary, particularly for women receiving adjuvant systemic antihormonal and/or chemotherapy, the observation that low retinol levels affect distant metastases and survival in women without systemic therapy is an important one with future therapeutic potential.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

**Table 3. HRs for breast cancer death over 12 y of follow-up in low-retinol (<2.08  $\mu\text{mol/L}$ ) and high-retinol ( $\geq 2.08$   $\mu\text{mol/L}$ ) subgroups of postmenopausal patients operated on for early breast cancer**

	Retinol level	
	Low	High*
All postmenopausal women		
No. events/no. patients	24/103	14/105
Stratified HR (95% CI), model 1 <sup>†</sup>	1.89 (0.98-3.65)	1
Adjusted HR (95% CI), model 2 <sup>‡</sup>	2.11 (1.08-4.14)	1
Women $\geq 55$ y		
No. events/no. patients	20/76	8/74
Stratified HR (95% CI), model 3 <sup>§</sup>	3.68 (1.54-8.78)	1
Adjusted HR (95% CI), model 4 <sup>  </sup>	3.58 (1.50-8.57)	1

NOTE: HRs estimated by Cox regression model.

\* Reference category.

<sup>†</sup> Model 1: stratified by age (<55 and  $\geq 55$  y).

<sup>‡</sup> Model 2: stratified by age (<55 and  $\geq 55$  y) and by period of recruitment, and adjusted for size and histology of primary.

<sup>§</sup> Model 3: stratified by period of recruitment.

<sup>||</sup> Model 4: stratified by period of recruitment and adjusted for size and histology of primary.

## Acknowledgments

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We thank Don Ward for help with the English and the patients of the Fenretinide Breast Cancer Prevention Trial for their continuing cooperation.

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*Cancer Epidemiol Biomarkers Prev* 2009;18:42-48.

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