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Vitamin C intake and breast cancer mortality in a cohort of Swedish women

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Background: Vitamin C may influence cancer progression through its antioxidant properties. However, the evidence from observational epidemiologic studies on vitamin C intake and survival following breast cancer diagnosis is not consistent, and the safety of vitamin C supplements following breast cancer diagnosis has not been extensively studied.

Methods: Using a food-frequency questionnaire we investigated whether vitamin C intake was associated with survival among 3405 women diagnosed with invasive breast cancer in the Swedish Mammography Cohort.

Results: From 1987–2010, there were 1055 total deaths with 416 deaths from breast cancer. Women in the highest quartile of pre-diagnosis vitamin C intake had an adjusted HR (95% CI) of breast cancer death of 0.75 (0.57–0.99) compared with those in the lowest quartile ($P_{trend} = 0.03$). There was a borderline significant association between vitamin C intake and total mortality (HR = 0.84; 95% CI = 0.71–1.00; $P_{trend} = 0.08$). Among 717 breast cancer cases for whom post-diagnosis supplement use was available, there was no association between vitamin C supplement use (≈1000 mg) and breast cancer-specific mortality (HR = 1.06; 95% CI = 0.52–2.17).

Conclusion: Our findings suggest that dietary vitamin C intake before breast cancer diagnosis may be associated with breast cancer survival. In addition, post-diagnosis vitamin C supplementation at the level observed in our population was not associated with survival.

Vitamin C is a water-soluble nutrient that has been hypothesised to influence cancer initiation and promotion through its antioxidant properties including the neutralization of free radicals (Frei, 1994; Willcox *et al*, 2004). In addition, *in vitro* experiments have shown cytotoxic action of vitamin C against cancer cells without subsequent toxicity to normal cells (Chen *et al*, 2008; Ullah *et al*, 2011). However, the evidence from observational epidemiologic studies on vitamin C intake and survival following breast cancer diagnosis is not consistent, with dietary vitamin C intake reported to reduce the risk of mortality in some studies (Rohan *et al*, 1993; Ingram, 1994; Jain *et al*, 1994; Fleischauer *et al*, 2003; McEligot *et al*, 2006) and no association in other studies (Zhang *et al*, 1995; Hebert *et al*, 1998; Holmes *et al*, 1999; Saxe *et al*, 1999; Saquib *et al*, 2011). In addition, the safety of oral vitamin C supplements following cancer diagnosis is not clear (Lawenda *et al*, 2008) and few studies have examined vitamin C supplements in relation to breast cancer survival (Greenlee *et al*, 2009; Nechuta *et al*, 2011; Greenlee *et al*, 2012).

The aim of this study was to investigate whether pre-diagnosis dietary vitamin C intake was associated with total and breast cancer-specific mortality among women diagnosed with invasive breast cancer in the Swedish Mammography Cohort (SMC). We also examined whether the association between vitamin C and survival differed by hormone receptor status, disease stage at diagnosis, age, body mass index (BMI) and smoking. In a subset of women, we examined whether vitamin C supplement intake following breast cancer diagnosis was associated with survival.

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MATERIALS AND METHODS

Study population. This study included 3405 participants in the SMC with invasive breast cancer diagnosed from 1987–2010. Recruitment and characteristics of this cohort have been previously described (Wolk *et al*, 2006). In brief, the SMC is a population-based cohort of 66 651 women born between 1914 and 1948 that were recruited between 1987 and 1990 in Västmanland and Uppsala counties in central Sweden. Participants completed a baseline questionnaire with questions regarding diet, reproductive and other factors. In 1997, a second questionnaire was extended to include dietary supplements, physical activity and smoking status, and was sent to participants who were still alive and residing in the study area; 39 227 (70%) women returned this questionnaire. Those with an incorrect or missing national registration number, previous cancer diagnosis (except non-melanoma skin cancer) and implausible total energy intake (3 standard deviations (s.d) from the mean value for $\log_e$-transformed energy intake) were excluded from the baseline cohort. Completion and return of the self-administered questionnaire was treated as informed consent of study participants. The study was approved by the ethics committee at the Karolinska Institutet.

Histologically confirmed incident invasive breast cancer cases were ascertained by linkage of the cohort with the Swedish Cancer Registry. (Mattsson and Wallgren, 1984). Oestrogen receptor (ER) and progesterone receptor (PR) status, menopausal status at diagnosis, tumour size, grade, lymph node involvement and type of treatment were available for ~77% of the cases. More detailed information on the evaluation of hormone receptor status in this cohort has been described previously (Larsson *et al*, 2009).

Dietary assessment. Diet was assessed using a 67-item food-frequency questionnaire (FFQ) at baseline and a 96-item FFQ in 1997. Participants were asked how often, on average, they had consumed each item during the previous 6 months (1987) or year (1997). Vitamin C intake was calculated as the frequency of consumption of each food item multiplied by its vitamin C content per age-specific serving (Bergström *et al*, 1991), Women were asked about dietary supplement use on the 1997 questionnaire including predefined questions about vitamin C. In Swedish populations, 1000 mg has been reported as the most frequently used dose of single nutrient vitamin C supplements (Holmquist *et al*, 2003; Messerer and Wolk, 2004). The FFQ has been previously validated for vitamin C and for foods that were the main sources of vitamin C. The correlation coefficients between the questionnaire and four 1-week diet records were 0.3 for dietary vitamin C, 0.5 for citrus fruits, boiled potatoes and apples/pears, 0.4 for juice, tomatoes and bananas, and 0.3 for fruit drinks (A Wolk, unpublished data, 1992). The sensitivity and specificity of vitamin C supplement use have been estimated to be 67% and 95%, respectively (Messerer and Wolk, 2004). Nutrient intakes were adjusted for energy using the residual method (Willett and Stampfer, 1986).

Outcome assessment. Date of death was identified through linkage to the Swedish National Death Registry at Statistics Sweden. It is estimated that 93% of all deaths in Sweden are reported within 10 days and 100% are reported within 30 days (Ludvigsson *et al*, 2009). Cause of death was determined by International Classification of Diseases (ICD) codes (ICD9 and ICD10) through linkage to the Cause of Death Registry at the National Bureau of Health and Welfare.

Statistical analysis. Cox proportional hazard models were used to calculate hazard ratios (HRs) and 95% confidence intervals (95% CIs) for death from any cause. Participants contributed person-time from the date of breast cancer diagnosis until death from any cause, or end of follow-up on 16 October 2010. We also examined death from breast cancer and non-breast cancer death as the end-points with end of follow-up on 31 December 2008 as information on the cause of death was not available after this time. Baseline diet (1987) was considered the exposure in all analyses except when dietary supplement use and dietary change were examined. Dietary vitamin C intake was categorised in quartiles with the lowest quartile as the reference group. Vitamin C supplement use was categorised as supplement user and non-supplement user. Total caloric intake and age at diagnosis were included in all models.

Education, marital status, menopausal status at diagnosis, BMI, alcohol and year of diagnosis were considered potential confounders in all multivariable models. Parity/age at first birth, oral contraceptive use, postmenopausal hormone use, height and family history of breast cancer were not observed to be confounders in the study population and therefore were not included in the final models. Categories were created for missing data. Multivariable models were adjusted for the following clinical characteristics: stage, grade of tumour, radiation treatment and chemotherapy/hormonal therapy. Additional adjustment for the clinical covariates tumour size and number of positive lymph nodes did not further alter the effect estimates, thus were not included in the final models. Tests for linear trend were performed by assigning the median value of each category to each participant in that group.

We examined whether the association between vitamin C and breast cancer survival differed by hormone receptor status (ER+, ER−, PR+, PR−, ER+/PR+, ER−/PR−), disease stage at diagnosis (I, II, III/IV), age (<65 years, ⩾65 years), BMI (<30 kg m^{-2}, ⩾30 kg m^{-2}) and smoking status (never, ever), with a likelihood ratio test comparing the model with the cross-product term between vitamin C and each potential effect modifier to the model with main effects only. All tests of statistical significance were two-sided, and analyses were performed using SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

During 30 080 person-years of follow-up contributed by 3405 breast cancer cases, there were 1055 deaths with 416 deaths from breast cancer. The mean (±s.d.) age at diagnosis 65 years (±10.3) and the median follow-up time was 7.8 years (range 1 month to 23.5 years). Pre-diagnosis dietary assessment occurred a mean of 11.0 years before breast cancer diagnosis (range 1 month to 23.4 years). Among the subset of women with post-diagnosis dietary information, dietary assessment occurred a mean of 4.6 years after breast cancer diagnosis (range 1 year to 10 years). The mean dietary vitamin C intake was 72.2 mg d^{-1} (±40.1). The main sources of dietary vitamin C in the study population were citrus fruit (28.0%), boiled potatoes (14.0%), juice (13.5%), tomatoes (8.8%) and apples/pears (7.7%). Women in the highest quartile of vitamin C intake were more likely to have used oral contraceptives and postmenopausal hormones, were younger at cohort enrolment and had a lower mean BMI than women in the lowest quartile of vitamin C intake (Table 1).

Pre-diagnosis dietary vitamin C intake was associated with a decreased risk of breast cancer death (Table 2). Women in the highest quartile of dietary vitamin C intake had a covariate-adjusted HR (95% CI) of death from breast cancer of 0.74 (0.57–0.98) compared with those in the lowest quartile ($P_{trend} = 0.04$). Further adjustment for clinical characteristics and treatment did not alter the results (HR = 0.75; 95% CI = 0.57–0.99; $P_{trend} = 0.03$). In a sensitivity analysis that excluded women with stage IV breast cancer, results were not materially different from the main analysis (covariate and clinical characteristics-adjusted

Table 1. Characteristics at baseline of 3405 women with invasive breast cancer in the Swedish Mammography Cohort by quartiles of vitamin C intake[a]

	Quartile of vitamin C intake (mg d^{-1})			
	<42.9	**42.9–65.5**	**65.6–92.4**	**⩾92.5**
Median vitamin C intake (mg d^{-1})	32.0	53.7	78.1	113.6
Breast cancer deaths (*n*)	128	103	97	88
Non-breast cancer deaths (*n*)	192	145	153	149
Age at enrolment (years)	54.1	52.5	52.6	52.3
Age at diagnosis (years)	66.5	65.4	65.4	65.4
Post-secondary education (%)	11.3%	15.1%	14.7%	13.9%
Married (%)	67.6%	73.8%	72.9%	69.5%
Body mass index (kg m^{-2})	25.2	24.9	24.9	24.7
Height (cm)	164.6	164.4	164.8	164.9
Age at menarche (years)	13.3	13.1	13.2	13.1
Nulliparous (%)	13.7%	13.9%	11.4%	12.1%
Age at first birth among parous women (years)	24.3	24.7	24.6	24.7
Number of children	2.3	2.3	2.3	2.3
Family history of breast cancer (%)	10.5%	11.7%	10.8%	11.4%
Ever use of oral contraceptives (%)	51.7%	56.2%	56.8%	58.4%
Ever use of postmenopausal hormones (%)	41.6%	48.0%	47.8%	48.6%
Postmenopausal at diagnosis (%)	92.6%	90.9%	90.8%	91.4%
Fruit intake (servings per d)	0.7	1.2	1.7	2.4
Alcohol intake (g d^{-1})	2.4	2.7	3.1	3.0
Total energy intake (kcal d^{-1})	1591	1586	1624	1533
Disease stage (%)[b]				
Stage I	53.9%	53.4%	52.3%	54.6%
Stage II	39.8%	41.1%	41.9%	40.7%
Stage III/IV	6.3%	5.5%	5.9%	4.8%
Treatment (%)[c]				
Radiation	49.4%	56.0%	50.5%	52.4%
Chemotherapy	13.7%	15.2%	14.2%	13.2%
Hormonal	32.7%	33.6%	28.8%	34.2%
Oestrogen receptor-positive (%)	80.0%	83.1%	81.8%	82.4%
Progesterone receptor-positive (%)	68.7%	64.4%	68.5%	67.1%
Oestrogen receptor-/progesterone receptor-positive (%)	62.3%	60.2%	63.3%	63.6%

[a]Data represent mean unless otherwise indicated.
[b]Percents may not equal 100 due to missing values.
[c]>100% because some breast cancer patients receive more than one treatment.

HR for top to bottom quartile = 0.75; 95% CI = 0.56–1.00; P_{trend} = 0.03). There was a borderline significant association between vitamin C intake and total mortality (HR = 0.84; 95% CI = 0.71–1.00; P_{trend} = 0.08); however, there was no association between vitamin C intake and non-breast cancer deaths (HR = 0.91; 95% CI = 0.73–1.13; P_{trend} = 0.65) (Table 2).

When the association between pre-diagnosis dietary vitamin C and breast cancer death was stratified by hormone receptor status of the tumour, the protective association appeared strongest among those with ER-negative/PR-negative tumours (Table 3). Among women with ER-negative/PR-negative tumours, those in the highest quartile of dietary vitamin C intake had a covariate and clinical characteristics-adjusted HR of 0.46 (95% CI = 0.22–0.96; P_{trend} = 0.008) compared with women in the lowest quartile. The corresponding HR for ER-positive/PR-positive breast cancer was 0.80 (95% CI = 0.47–1.35; P_{trend} = 0.52); however, the interaction was not significant ($P_{interaction}$ = 0.73). The stronger association observed among women with ER-negative/PR-negative tumours appeared to be driven by the ER-negative receptor status as a significant inverse association was observed among women with ER-negative tumours (HR = 0.50, 95% CI = 0.28–0.89; P_{trend} = 0.004), but not among those with PR-negative tumours (HR = 0.76, 95% CI 0.45–1.27; P_{trend} = 0.15). A similar association was observed when total mortality was the outcome (data not shown).

We examined whether the association between vitamin C and breast cancer mortality varied by the reactive oxygen species (ROS)-related factors of age, obesity and smoking. Vitamin C intake had a stronger inverse association with breast cancer mortality among women who were aged ⩾65 (HR = 0.48;

Table 2. Hazard ratios (HR) and 95% confidence intervals (95% CI) of breast cancer death by vitamin C intake among 3405 invasive breast cancer cases in the Swedish Mammography Cohort

	Quartile of vitamin C intake (mg d^{-1})				
	<42.9	42.9–65.5	65.6–92.4	⩾92.5	P_{trend}[a]
Person-years	7458	7523	7584	7516	—
Breast cancer deaths	128	103	97	88	—
Age-adjusted model	1.00	0.80 (0.62–1.04)	0.75 (0.58–0.98)	0.69 (0.52–0.90)	0.007
Covariate-adjusted model[b]	1.00	0.84 (0.65–1.09)	0.81 (0.62–1.06)	0.74 (0.57–0.98)	0.04
Covariate-adjusted model + clinical characteristics[c]	1.00	0.89 (0.69–1.16)	0.77 (0.59–1.01)	0.75 (0.57–0.99)	0.03
Non-breast cancer deaths	192	145	153	149	
Age-adjusted model	1.00	0.81 (0.65–1.00)	0.93 (0.75–1.15)	0.90 (0.72–1.11)	0.58
Covariate-adjusted model[b]	1.00	0.83 (0.67–1.03)	0.96 (0.77–1.19)	0.92 (0.74–1.14)	0.70
Covariate-adjusted model + clinical characteristics[c]	1.00	0.84 (0.68–1.05)	0.97 (0.78–1.20)	0.91 (0.73–1.13)	0.65
Total deaths	320	248	250	237	
Age-adjusted model	1.00	0.80 (0.68–0.95)	0.85 (0.72–1.00)	0.80 (0.68–0.95)	0.03
Covariate-adjusted model[b]	1.00	0.84 (0.71–0.99)	0.90 (0.76–1.06)	0.85 (0.71–1.00)	0.12
Covariate-adjusted model + clinical characteristics[c]	1.00	0.86 (0.72–1.01)	0.89 (0.75–1.05)	0.84 (0.71–1.00)	0.08

[a]Determined using category medians.
[b]Cox proportional hazard model adjusted for age (continuous), energy intake (continuous), education level (primary, high school, university), marital status (single, married, divorced, widowed, living with partner), menopausal status at diagnosis, (premenopausal, postmenopausal, unknown), body mass index (<20, 20–24.9, 25–29.9, ⩾30 kg m^{-2}), alcohol intake (non-drinker, <3.4, 3.4–9.9, ⩾10 g d^{-1}) and calendar year of diagnosis (continuous).
[c]Cox proportional hazard model adjusted for the variables above plus disease stage (I, II, III/IV), grade (I, II, III), radiation treatment (yes/no), and chemotherapy and/or hormonal treatment (no chemotherapy or hormonal treatment, hormonal therapy and no chemotherapy, chemotherapy and no hormonal therapy, and hormonal therapy and chemotherapy).

95% CI = 0.31–0.74; $P_{trend} = 0.007$) and obese (HR = 0.54; 95% CI = 0.22–1.35; $P_{trend} = 0.04$), although the interaction was only significant for age ($P_{interaction} = 0.02$) (Table 4). A similar association was also seen for age when total mortality was the outcome (data not shown). In the subset of 1917 women who reported their smoking habits in 1997, there was the suggestion of a stronger inverse association among those who had never smoked (HR = 0.53; 95% CI = 0.28–0.99; $P_{trend} = 0.03$; $P_{interaction} = 0.10$); however, these results are based on small numbers (Table 4).

We evaluated the association of foods that were the main sources of dietary vitamin C in our population to explore if any of these foods could explain the association between vitamin C and breast cancer-specific mortality. None of the foods that were the main sources of dietary vitamin C in the study population were significantly associated with mortality and vitamin C was still significantly associated with mortality following adjustment for these foods (data not shown). In addition, we adjusted for other micronutrients including vitamin E and carotenoids as well as fibre and vegetable intake, and did not observe any materially changes in the effect estimates (data not shown).

We examined vitamin C supplement use following breast cancer diagnosis among the 717 breast cancer cases who were diagnosed with breast cancer from 1987–1996 and completed a FFQ in 1997 after their breast cancer diagnosis. Among these women, 14.1% reported post-diagnosis use of a supplement containing vitamin C in 1997 compared with 16.7% of women who completed the 1997 FFQ pre-diagnosis. There was no association between any post-diagnosis vitamin C supplement use and breast cancer-specific (HR = 1.06; 95% CI = 0.52–2.17) or total mortality (HR = 0.81; 95% CI = 0.53–1.26); however, these results are based on only 66 breast cancer deaths and 228 total deaths. In addition, 25% of these women reported regular use of a multivitamin and regular multivitamin use was not associated with mortality (data not shown). Finally, we compared dietary vitamin C intake among women who completed both the 1987 and 1997 FFQs ($N = 2176$). Among the subset of women who were diagnosed with breast cancer from 1987–1996 and completed a FFQ in 1997 following breast cancer diagnosis ($N = 717$), up to 82.2% of these women remained in the same or adjacent quartile of dietary vitamin C intake following breast cancer diagnosis compared with 81.4% of all women who completed the 1997 FFQ. Those in the highest quartile of post-diagnosis dietary vitamin C intake had a covariate- and clinical-adjusted HR (95% CI) of 0.71 (0.35–1.43) for breast cancer-specific death and 0.75 (0.52–1.09) for total death compared with those in the lowest quartile ($P_{trend} = 0.53$ and 0.14, respectively).

DISCUSSION

In this prospective cohort study among 3405 women with breast cancer, dietary vitamin C intake was inversely associated with breast cancer-specific mortality. In addition, the association appeared to differ by age with a stronger inverse association observed among women aged ⩾65 years. There was no association between post-diagnosis vitamin C supplement use and mortality, however these results were based on small numbers.

Results from observational studies on dietary vitamin C intake and survival following breast cancer diagnosis have not been consistent, with dietary vitamin C intake reported to reduce the risk of mortality in some studies (Rohan *et al*, 1993; Ingram, 1994; Jain *et al*, 1994; Fleischauer *et al*, 2003; McEligot *et al*, 2006) and no association reported in other studies (Zhang *et al*, 1995; Hebert *et al*, 1998; Holmes *et al*, 1999; Saxe *et al*, 1999; Saquib *et al*, 2011). In addition, two additional studies have only examined post-diagnosis supplement use reporting inverse associations between vitamin C supplement use and all-cause mortality or recurrence (Nechuta *et al*, 2011; Greenlee *et al*, 2012). The varied results may be in part because the measurement of dietary vitamin C has occurred both pre- and post-diagnosis as well as among populations with varying intakes of vitamin C, with not all studies capturing supplement use. Consistent with our results, four (Rohan *et al*, 1993; Ingram, 1994; Jain *et al*, 1994; McEligot *et al*, 2006) of

Table 3. Hazard ratios (HR) and 95% confidence intervals (95% CI) of breast cancer death across hormone receptor subtypes by quartile of vitamin C intake among 3405 invasive breast cancer cases in the Swedish Mammography Cohort

	Quartile of vitamin C intake (mg d^{-1})					
	<42.9	42.9–65.5	65.6–92.4	⩾92.5	P_{trend}[a]	$P_{heterogeneity}$[b]
ER-positive/PR-positive						
Breast cancer deaths	35	25	29	25	—	—
Person-years	3113	3426	3287	3410	—	0.73
Covariate-adjusted model[c]	1.00	0.66 (0.39–1.11)	0.84 (0.51–1.38)	0.70 (0.42–1.17)	0.32	—
Covariate-adjusted model + clinical characteristics[d]	1.00	0.75 (0.44–1.27)	0.85 (0.51–1.42)	0.80 (0.47–1.35)	0.52	—
ER-negative/PR-negative						
Breast cancer deaths	18	23	16	15	—	—
Person-years	749	573	641	802	—	—
Covariate-adjusted model[c]	1.00	1.36 (0.71–2.59)	0.96 (0.48–1.93)	0.67 (0.33–1.36)	0.14	—
Covariate-adjusted model + clinical characteristics[d]	1.00	1.17 (0.60–2.29)	0.49 (0.23–1.06)	0.46 (0.22–0.96)	0.008	—
ER-positive						
Breast cancer deaths	52	36	40	39	—	—
Person-years	3961	4527	4239	4286		0.65
Covariate-adjusted model[c]	1.00	0.62 (0.41–0.96)	0.77 (0.51–1.17)	0.73 (0.48–1.12)	0.32	—
Covariate-adjusted model + clinical characteristics[d]	1.00	0.73 (0.47–1.13)	0.82 (0.54–1.26)	0.88 (0.57–1.35)	0.72	—
ER-negative						
Breast cancer deaths	32	29	20	21	—	—
Person-years	1113	866	1059	1039	—	—
Covariate-adjusted model[c]	1.00	1.08 (0.65–1.82)	0.74 (0.42–1.32)	0.66 (0.37–1.15)	0.07	—
Covariate-adjusted model + clinical characteristics[d]	1.00	0.97 (0.57–1.64)	0.42 (0.23–0.78)	0.50 (0.28–0.89)	0.004	—
PR-positive						
Breast cancer deaths	49	31	33	31	—	—
Person-years	3491	3735	3715	3647	—	0.61
Covariate-adjusted model[c]	1.00	0.61 (0.39–0.97)	0.70 (0.45–1.09)	0.64 (0.41–1.02)	0.11	—
Covariate-adjusted model + clinical characteristics[d]	1.00	0.69 (0.43–1.10)	0.65 (0.41–1.03)	0.72 (0.45–1.15)	0.18	—
PR-negative						
Breast cancer deaths	35	34	27	29	—	—
Person-years	1588	1659	1559	1652	—	—
Covariate-adjusted model[c]	1.00	0.91 (0.56–1.48)	0.78 (0.47–1.31)	0.79 (0.48–1.31)	0.32	—
Covariate-adjusted model + clinical characteristics[d]	1.00	0.99 (0.60–1.63)	0.64 (0.38–1.09)	0.76 (0.45–1.27)	0.15	—

[a]Determined using category medians.
[b]*P*-value from likelihood ratio test comparing a model with the cross-product term between vitamin C and hormone receptor status to the model with main effects only.
[c]Cox proportional hazard model adjusted for age (continuous), energy intake (continuous), education level (primary, high school, university), marital status (single, married, divorced, widowed, living with partner), menopausal status at diagnosis, (premenopausal, postmenopausal, unknown), body mass index (<20, 20–24.9, 25–29.9, ⩾30 kg m^{-2}), alcohol intake (non-drinker, <3.4, 3.4–9.9, ⩾10 g d^{-1}) and calendar year of diagnosis (continuous).
[d]Cox proportional hazard model adjusted for the variables above plus disease stage (I, II, III/IV), grade (I, II, III), radiation treatment (yes/no), and chemotherapy and/or hormonal treatment (no chemotherapy or hormonal treatment, hormonal therapy and no chemotherapy, chemotherapy and no hormonal therapy, and hormonal therapy and chemotherapy).

the seven studies (Rohan *et al*, 1993; Ingram, 1994; Jain *et al*, 1994; Zhang *et al*, 1995; Saxe *et al*, 1999; Fleischauer *et al*, 2003; McEligot *et al*, 2006) examining pre-diagnosis dietary intake reported a significant inverse association with mortality while only one (Fleischauer *et al*, 2003) of the four studies (Hebert *et al*, 1998; Holmes *et al*, 1999; Fleischauer *et al*, 2003; Saquib *et al*, 2011) examining post-diagnosis dietary intake reported a similar association. We had longer follow-up than previous studies (median = 7.8 years) as well as more than twice as many deaths providing us with ample power to examine these associations. In addition, the differences in results between pre- and post-diagnosis intake may indicate that the timing and duration of vitamin C intake may be important. In the FASTCAB study, vitamin C supplement use, including pre- and post-diagnosis intake for >4 years, was associated with statistically significant decreased risk of breast cancer-related mortality and recurrence, while supplement use for 0–3 years had a non-significant inverse association with breast cancer-related mortality and recurrence (Fleischauer *et al*, 2003). In addition, Nechuta *et al* (2011) reported an inverse association between >3 months of post-diagnosis vitamin C supplement use and total mortality, but no association with ⩽3 months of use.

Cancer cell proliferation is hypothesised to be stimulated by hydrogen peroxide (H_2O_2) through the transformation of H_2O_2 into hydroxyl radicals as well as through the involvement of H_2O_2 in cell signalling events (Loo, 2003). Vitamin C may inhibit cancer cell proliferation through the suppression of H_2O_2 and its ROS products (Frei, 1994; Willcox *et al*, 2004). In addition, at high doses vitamin C may also function as a pro-oxidant causing cytotoxicity to cancer cells without similar effects on normal cells (Chen *et al*,

Table 4. Hazard ratios (HR) and 95% confidence intervals (95% CI) of breast cancer death stratified by selected characteristics by quartile of vitamin C intake among 3405 invasive breast cancer cases in the Swedish Mammography Cohort

	Quartile of vitamin C intake (mg d^{-1})				
	<42.9	42.9–65.5	65.6–92.4	⩾92.5	P_{trend}[a]
Age					
<65					
Breast cancer deaths	56	53	54	58	
Covariate-adjusted model + clinical characteristics[b]	1.00	0.91 (0.62–1.34)	0.81 (0.55–1.19)	1.09 (0.75–1.59)	0.69
⩾65					
Breast cancer deaths	72	50	43	30	
Covariate-adjusted model + clinical characteristics[b]	1.00	0.88 (0.61–1.28)	0.76 (0.52–1.12)	0.48 (0.31–0.74)	0.0007
$P_{interaction}$[c] = 0.03					
BMI					
<30					
Breast cancer deaths	102	84	90	74	
Covariate-adjusted model + clinical characteristics[b]	1.00	0.86 (0.64–1.15)	0.84 (0.63–1.12)	0.80 (0.59–1.09)	0.17
⩾30					
Breast cancer deaths	21	18	4	8	
Covariate-adjusted model + clinical characteristics[b]	1.00	1.33 (0.64–2.77)	0.36 (0.12–1.10)	0.54 (0.22–1.35)	0.04
$P_{interaction}$[c] = 0.30					
Smoking					
Never smoker					
Breast cancer deaths	25	22	17	19	
Covariate-adjusted model + clinical characteristics[b]	1.00	0.80 (0.44–1.45)	0.55 (0.29–1.04)	0.53 (0.28–0.99)	0.03
Ever smoker					
Breast cancer deaths	18	12	12	20	
Covariate-adjusted model + clinical characteristics[b]	1.00	0.93 (0.42–2.02)	0.70 (0.33–1.49)	1.82 (0.90–3.67)	0.14
$P_{interaction}$[c] = 0.10					

[a]Determined using category medians.
[b]Cox proportional hazard model adjusted for age (continuous), energy intake (continuous), education level (primary, high school, university), marital status (single, married, divorced, widowed, living with partner), menopausal status at diagnosis, (premenopausal, postmenopausal, unknown), body mass index (<20, 20–24.9, 25–29.9, ⩾30 kg m^{-2}), alcohol intake (non-drinker, <3.4, 3.4–9.9, ⩾10 g d^{-1}), calendar year of diagnosis (continuous), disease stage (I, II, III/IV), grade (I, II, III), radiation treatment (yes/no) and chemotherapy and/or hormonal treatment (no chemotherapy or hormonal treatment, hormonal therapy and no chemotherapy, chemotherapy and no hormonal therapy, and hormonal therapy and chemotherapy). BMI is not adjusted for in models stratified by BMI and alcohol intake is not adjusted for in models stratified by alcohol.
[c]*P*-value from likelihood ratio test comparing a model with the cross-product term between vitamin C and potential effect modifier to the model with main effects only.

2008; Ullah *et al*, 2011). While *in vitro* studies support a role for vitamin C in cancer outcomes, the literature is not clear on the safety of oral supplements containing vitamin C following cancer diagnosis (Lawenda *et al*, 2008) and few studies have specifically examined breast cancer survival (Greenlee *et al*, 2009). In addition, it has been hypothesised that use of antioxidant supplements, including vitamin C, during cancer treatment may actually protect cancer cells from treatment agents (D'Andrea, 2005; Lawenda *et al*, 2008). Two recent observational studies examined post-diagnosis supplement use in women with breast cancer and both observed that vitamin C supplement use was associated with a decreased risk of breast cancer mortality and/or recurrence (Nechuta *et al*, 2011; Greenlee *et al*, 2012), while in one study frequent use of carotenoids was associated with increased mortality risk (Greenlee *et al*, 2012). In contrast, early randomized trials of oral vitamin C supplements in cancer patients demonstrated no benefit of high-dose vitamin C on cancer survival (Creagan *et al*, 1979; Moertel *et al*, 1985); however, there have been no randomized trials evaluating oral vitamin C supplementation specifically among breast cancer patients. In the subset of women with information on post-diagnosis diet, we did not observe an association between vitamin C supplement use and survival; however, we had limited power for this analysis. The most frequently reported dose of vitamin C supplement in two Swedish populations has been reported to be 1000 mg (Holmquist *et al*, 2003; Messerer and Wolk, 2004), which is higher than in the Shanghai Breast Cancer Survival Study, where ~85% of women with dosage information used ⩽400 mg per day (Nechuta *et al*, 2011), but lower than the mega-doses (>1 g) used in other studies of supplement use and cancer outcomes (Lawenda *et al*, 2008; Greenlee *et al*, 2009). Levine *et al* (1996) have reported that plasma concentrations of vitamin C reach near saturation at doses of 400 mg d^{-1} and that bioavailability declines at doses of 500 mg d^{-1} and higher. The amount of vitamin C obtained from dietary sources is considerably lower than what can be obtained through oral supplementation. Thus adequate dietary intake of vitamin C may influence cancer

progression and survival through different mechanisms than supplementation and a potential U-shaped relation may exist between total vitamin C intake (from diet and supplements) and survival. In addition, the route of administration, oral *vs* intravenous, of vitamin C intake may also have a role in the efficacy and safety of vitamin C use in cancer patients as these different routes of administration have differing effects on plasma concentrations (Padayatty *et al*, 2004).

To our knowledge, only one study has examined whether the association between vitamin C intake and mortality among women with breast cancer differs by hormone receptor status. In addition, cohort studies examining dietary vitamin C intake and breast cancer risk have not supported an association that varies by hormone receptor status (Cui *et al*, 2008). Jain *et al* (1994) reported marginally more inverse hazard ratios for mortality for hormone receptor-positive tumours compared with receptor-negative tumours, but the direction and magnitude of the effects were similar between hormone receptor-positive and -negative tumours, no *P*-values for interaction were reported, and the total number of deaths was only 88. The protective association between dietary vitamin C and breast cancer survival in our population was most evident among women with ER-negative/PR-negative tumours, although the interaction did not reach statistical significance. The potential mechanism behind the stronger association between dietary vitamin C intake and mortality among those with ER-negative/PR-negative tumours is unclear. However, the stronger association observed with these tumours may simply be more apparent, as they are not as susceptible to other factors that are mediated through oestrogen exposure (Huang *et al*, 2000; Colditz *et al*, 2004).

We also investigated whether the association between dietary vitamin C intake and breast cancer mortality differed by ROS-related factors. We observed suggestions of stronger inverse associations between dietary vitamin C intake and breast cancer mortality among women aged $\geqslant 65$ and obese; however, the interaction was only significant for age. The mechanism underlying the interaction between age and dietary vitamin C intake is unclear; however, age is associated with increased ROS production coupled with a decrease in the clearance of ROS that increases oxidative stress (Cannizzo Elvira *et al*, 2012). Consequently vitamin C may have a greater impact as a scavenger of free radicals with increasing age. Obesity is also associated with oxidative stress (Olusi, 2002; Keaney *et al*, 2003; Furukawa *et al*, 2004), and may interact with vitamin C intake in a similar manner.

The limitations of our study need to be considered. Seventy-nine per cent of our participants had only a pre-diagnosis assessment of diet and thus we had limited power to examine post-diagnosis diet. Studies among women with breast cancer have reported dietary changes following diagnosis in 30–40% of women (Salminen and Lagstrom, 2000; Maunsell *et al*, 2002; Salminen *et al*, 2004), with increased consumption of fruits and vegetables reported in most (Salminen and Lagstrom, 2000; Maunsell *et al*, 2002; Salminen *et al*, 2004; Velentzis *et al*, 2011) but not all (Wayne *et al*, 2004) studies. One study examined vitamin C intake and reported a significant increase in vitamin C intake following breast cancer diagnosis (Velentzis *et al*, 2011). However, younger women were most likely to report these changes and the average age at breast cancer diagnosis in our cohort was 65.1 years. In our study, up to 82% of women who completed a FFQ post-diagnosis remained in the same or adjacent quartile of vitamin C intake following diagnosis. In addition, supplement use was not assessed at baseline and we had limited power to examine the association between post-diagnosis supplement use and mortality. Residual or unmeasured confounding by lifestyle or other dietary factors is also a possibility. However, we adjusted for foods that contributed to vitamin C intake as well as for physical activity in the subset of cases and the associations did not materially change.

To our knowledge, this is the largest study to examine the relation between dietary vitamin C and mortality among women with breast cancer, giving us the power to examine whether the association varied by hormone receptor status or ROS-generating factors. We also have complete follow-up of all cases, a long follow-up period and data on many important covariates, including clinical and lifestyle characteristics.

In conclusion, we observed that dietary vitamin C intake before breast cancer diagnosis was associated with breast cancer-specific survival. This association was strongest among women aged $\geqslant 65$. In addition, we did not observe a harmful effect of post-diagnosis vitamin C supplementation with the dosages of ~ 1000 mg. Future studies examining vitamin C intake from food and supplements are needed to further our understanding of the impact of the timing and dose of vitamin C intake on outcomes in women with breast cancer.

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