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Does lack of tocopherols and tocotrienols put women at increased risk of breast cancer?

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Abstract

Breast cancer is the leading site of new cancers in women and the second leading cause (after lung cancer) of cancer mortality in women. Observational studies that have collected data for dietary exposure to α-tocopherol with or without the other related tocopherols and tocotrienols have suggested that vitamin E from dietary sources may provide women with modest protection from breast cancer. However, there is no evidence that vitamin E supplements confer any protection whatever against breast cancer. Observational studies that have assessed exposure to vitamin E by plasma or adipose tissue concentrations of α-tocopherol have failed to provide consistent support for the idea that α-tocopherol provides any protection against breast cancer. In addition, evidence from studies in experimental animals suggest that α-tocopherol supplementation alone has little effect on mammary tumors. In contrast, studies in breast cancer cells indicate that α- γ-, and δ-tocotrienol, and to a lesser extent δ-tocopherol, have potent antiproliferative and proapoptotic effects that would be expected to reduce risk of breast cancer. Many vegetable sources of α-tocopherol also contain other tocopherols or tocotrienols. Thus, it seems plausible that the modest protection from breast cancer associated with dietary vitamin E may be due to the effects of the other tocopherols and the tocotrienols in the diet. Additional studies will be required to determine whether this may be the case, and to identify the most active tocopherol/tocotrienol.

Keywords: Tocotrienol; Tocopherol; Vitamin E; Breast cancer; Cell proliferation; Diet; Nutrition

1. Food sources of tocopherols, tocotrienols, and vitamin E

Previously, eight dietary components, α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol [1,2,3] were all considered forms of vitamin E (Fig. 1). Alpha-tocopherol is thought to be the most biologically important form of vitamin E [3,4]. Recent guidelines have equated α-tocopherol with vitamin E, discounting other tocopherols and the tocotrienols [3]. Tocopherols and tocotrienols are present in the oil fraction of cereal grains, seeds, and nuts [1]. In most food sources, tocopherols are more prevalent than tocotrienols [1]. The primary tocopherol in a number of nuts and oilseeds is γ-tocopherol [1]. Soybean oil and rice germ oil are particularly good sources of δ-tocopherol while wheat germ is one of the few good sources of β-tocopherol [1]. In comparison, olive oil contains almost exclusively α-tocopherol [1]. In many cereal grains, including rice and flours and meals made from barley, corn, oats, rye, and wheat (except the isolated germ) and in palm oil, tocotrienols are more prevalent than tocopherols [1]. Palm oil is a particularly rich source of α-, γ-, and δ-tocotrienol [1]. Thus, emphasizing the consumption of monounsaturated fat sources, such as olive oil, at the expense of oils rich in saturated fatty acids and polyunsaturated fatty acids, such as palm and soybean oils, respectively, to reduce intermediate markers that may be relevant to cardiovascular disease [5,6,7] or decreased consumption of carbohydrate in the form of cereal grain products, could significantly alter the composition of dietary tocopherols and tocotrienols.

2. Relationship between vitamin E exposure assessed by dietary intake and breast cancer

Data for a number of case-control studies of vitamin E and breast cancer are summarized in Table 1, arranged in ascending order for the lowest (referent) quantile for vita-

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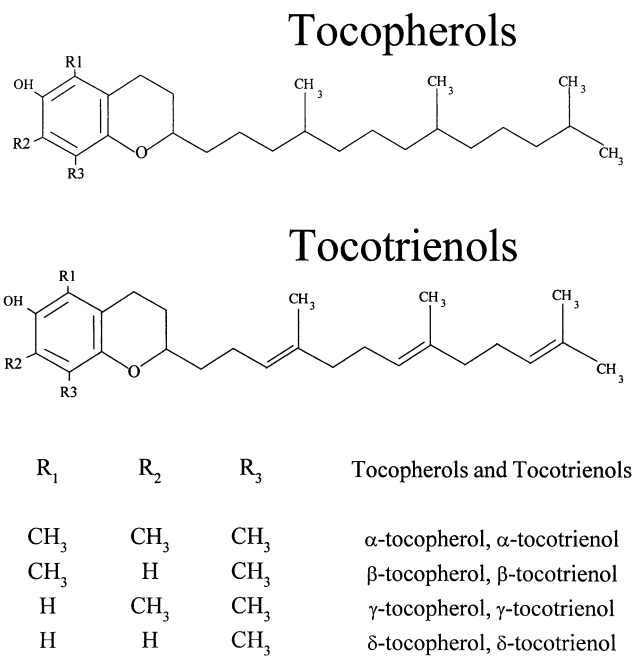

R_1	R_2	R_3	Tocopherols and Tocotrienols
CH_3	CH_3	CH_3	α-tocopherol, α-tocotrienol
CH_3	H	CH_3	β-tocopherol, β-tocotrienol
H	CH_3	CH_3	γ-tocopherol, γ-tocotrienol
H	H	CH_3	δ-tocopherol, δ-tocotrienol

Fig. 1. Illustration of the chemical structures of the four tocopherols and four tocotrienols that were previously considered forms of dietary vitamin E [1,2,3]. Recent guidelines have equated α-tocopherol with vitamin E [3].

min E intake, equating 1 mg of d-α-tocopherol with 1.49 IU vitamin E [3]. One of the difficulties in interpreting case-control studies relating dietary intake of vitamin E with breast cancer concerns the extent to which having the diagnosis of breast cancer might bias reporting of previous dietary intake, or whether a change in diet as a result of the disease, should it occur, might be reflected in the reported dietary intake. Two studies have directly addressed the issue of recall, or reporting bias, in studies relating diet to risk of breast cancer. One nested case-control study in a large population of Canadian women compared prospective reports of dietary intake with retrospective reports for the same period collected 3–6 years later after some of the women had been diagnosed with breast cancer [8]. That study found that retrospective estimates of micronutrient intake from foods, including vitamin E, were very similar to the prospective values for both women who developed breast cancer (cases) and the matched controls selected from among the women who remained free of cancer during the period of follow up (controls). Errors in reporting previous use of supplementary vitamin E and other micronutrients were similar for cases and controls [8]. Consistent with those observations, the relative risks for the association of vitamin E intake with breast cancer determined from the prospective and retrospective dietary data were similar in magnitude with overlapping 95% confidence intervals [8]. Those data suggest that for that population, the reporting of past vitamin E intake was not biased in women with breast cancer compared with controls [8].

In comparison, a study in Finnish women used another approach to consider the extent to which reporting bias confounded the association of dietary intake with risk of breast cancer [9]. In this study, dietary intake was assessed in women referred to the hospital for evaluation of a suspect breast lump, and before the result of the evaluation had been received. By this design, reporting of dietary intake could not be biased by knowledge of breast cancer diagnosis, since it was not known at the time, but could be influenced by worry that the breast lump was indeed malignant [9]. From among the women referred for evaluation, women with diagnosed breast cancer were considered cases while those determined, after a similar evaluation, to be healthy were considered referral controls. A second control group, a random sample of population controls, was obtained by drawing a random sample from the Finnish National Population Register covering the same catchment area, and individually matching these women to the cases by age and long-term residence (urban versus rural). By comparing the dietary information obtained from the referral controls and the population controls, it was possible for the investigators to estimate the extent to which the threat of breast cancer altered dietary reporting, and with that information, to correct the dietary information from women with diagnosed breast cancer to estimate the association of dietary intake with breast cancer risk, independent of the reporting bias associated with the threat of the breast cancer diagnosis. That study found that, compared with population controls, dietary intake of vitamin E, as assessed by a validated food frequency questionnaire, was biased in premenopausal women referred for further evaluation of a breast lump who were later found to be free of breast cancer [9]. Such women over-reported vitamin E intake compared with the population controls. When this reporting bias was accounted for, the odds ratio of breast cancer for the highest quintile of vitamin E intake was 0.5 (0.2–1.0, 95% confidence interval, see also Table 1). Interestingly, such reporting bias was not observed in postmenopausal women from the same population with a suspicious breast lump [9].

Other observational designs that avoid recall bias that threatens case-control studies are prospective studies, including cohort studies and nested case-control studies, in which the exposure is assessed before disease develops. Furthermore, comparability of controls is less of an issue, as the controls are either the remaining cohort free of breast cancer or, for nested case-control studies, controls drawn from the cohort from which cases develop. Table 2 presents summary data for a number of prospective cohort studies and a few nested case-control studies of vitamin E and breast cancer. As for Table 1, these reports are arranged in ascending order for the lowest (referent) quantile for vitamin E intake, again equating 1 mg of d-α-tocopherol with 1.49 IU vitamin E [3]. Among case-control studies, odds ratios for higher compared with lower vitamin E intake are more favorable for those studies in which the lowest quantile of vitamin E intake is ≤7 mg/day [9,10,11,12,13,14]. This level of vitamin E intake represents less than half of the

Table 1
Breast cancer and exposure to vitamin E assessed by dietary intake: case-control studies

Population (Reference)	Age or menopausal status[1]	Number cases/control[2]	Source of control[3]	Instrument (item no.)[4]	Vit E form[5]	Comparison[6]	Odds ratio (95%% C.I.)[7]
Greek	Pre	270/505	Hospital/visitor	FFQ (115)	IU	≥8.6 vs <5.2*	0.50[8] (0.25,1.02)
women [10]	Post	550/1041					0.85[8] (0.53,1.36)
New York	Pre-FH+	35/19	Community	FFQ (171)	IU α-T	>10.4 vs ≤6.3*	0.1[9] (0,1.2)
women [11]	Pre-FH−	224/251					**0.5[9] (0.2,1.0)**
	Post-FH+	61/35					0.7[9] (0.1,4.0)
	Post-FH−	303/401					**0.5[9] (0.3,1.0)**
Uruguay	20–89	400/405	Hospital	FFQ (64)	mg	9.1 versus 4.7*	**0.40[10] (0.26,0.62)**
women [12]	years						
Boston	Post	313/349	Referral	FFQ (116)	mg	Median 11.0 vs. 5.4*	**0.4[12] (0.2,0.9)**
women [13]						Median 368 vs. 5.8*[11]	0.7[12] (0.4,1.3)
White N.Y.	Pre	297/311	Community	FFQ (172)	mg α-T	≥11 vs <6*	**0.55[13] (0.34,0.88)**
women [14]					mg α-T	≥31 vs 0[11]	0.95[13] (0.58,1.55)
Finnish	Pre	119/324	Community	FFQ (110)	mg	≥13 versus ≤7*	1.1[15] (0.5,2.6)
women [9]	Pre[14]	119/178	Referral				**0.5[15] (0.2,1.0)**
Italian	Pre	989/841	Hospital	FFQ (78)	mg	>11.7 vs <8.5*	1.27[16] (0.90,1.78)
women [15]	Post	1577/1745					1.16[16] (0.92,1.46)
Finnish	Pre	14/21	Benign	FFQ (110)	mg	8.2 vs 9.0**	—
women [26]	Post	18/13	Breast			6.9 vs 9.3**	—
French	Pre	138/294	Hospital	FFQ (55)	mg	24.3 versus <14*	1.2[17] (0.7,2.2)
women [16]	Post	246/289					1.3[17] (0.8,2.0)
French	Pre	57/45	Hospital	FFQ (55)	mg	29 versus 47**	—
women [31]	Post	63/64				46 versus 33**	—
Italian	Pre	988/843	Hospital	FFQ (78)	NS	Highest vs lowest*	**0.80[18] (0.7,1.0)**
women [18]	Post	1572/1742				quintile	**0.75[18] (0.6,0.9)**

[1] Pre indicates premenopausal women, Post indicates postmenopausal women, age in years is given if the publication provides that information but no information about menopausal status. FH and FH− indicate women with and without a family history of breast cancer, respectively.

[2] Numbers of case followed by the numbers of controls.

[3] Hospital/visitor refers to a combined group of controls drawn in part from a hospitalized population and in part from visitors to the hospital. Referral indicates women who underwent the same or similar diagnostic procedure as the cases but were found to be healthy. Benign Breast indicates benign breast disease.

[4] FFQ indicates food frequency questionnaire, the number of items are given parenthetically.

[5] Vitamin E form as specified in the publication where IU denotes international units while mg indicates milligrams, α-T indicates α-tocopherol. All vitamin E is derived from food, except as noted. NS denotes not specified. Entries have been arranged in ascending order of vitamin E in referent category (indicated by an asterisk; mean values for cases versus controls are indicated by two asterisks), equating each IU vitamin E reported in the various publications with 0.67 mg d-α-tocopherol [3], and for the purpose of ordering the reports, assuming that mg vitamin E reported reflects α-tocopherol.

[6] Unless indicated otherwise, comparisons are for the highest quantile (quintile, quartile, tertile) compared with the lowest quantile.

[7] Odds ratio is given with the 95% confidence interval in parenthesis. Odds ratios that are significantly different from unity (at $p \leq 0.05$) are indicated in bold typeface.

[8] Adjusted for age, birthplace, body mass index, parity, age at first birth, age at menarche, and total energy intake; relatively little change for additional adjustment for beta-carotene and vitamin C.

[9] Adjusted for age, education, age at menarche, age at first pregnancy, body mass index, and total energy intake.

[10] Adjusted for age, residence, urban/rural, family history of breast cancer, body mass index, age at menarche, parity, menopausal status, and total energy intake.

[11] From supplements.

[12] Adjusted for total energy intake, age, alcohol, age at first birth, parity, family history of breast cancer, age at menopause, age at menarche, history of benign breast disease, body weight.

[13] Adjusted for age, education, body mass index, parity, age at first birth, age at menarche, first degree relative with breast cancer, benign breast disease, total energy intake.

[14] This study also investigated postmenopausal women in whom no association of nutrients with risk of breast cancer was found.

[15] Adjusted for age, geographic location (urban/rural), age at menarche, age at first full term pregnancy, use of oral contraceptives, use of estrogen replacement therapy, family history of breast cancer, history of benign breast disease, education, alcohol, smoking, leisure activity, waist-to-hip ratio, and energy intake.

[16] Adjusted for age, geographic location, body mass index, total energy intake, beta carotene, alcohol, physical activity, and education.

[17] Adjusted for age, history of benign breast disease, family history of breast cancer, age at menarche, age at first pregnancy, and education (no adjustment for energy intake).

[18] Adjusted for age, geographic location, education, parity/age at first birth, and energy intake.

currently recommended daily intake of 15 mg α-tocopherol per day [3]. For studies reporting vitamin E intake in the lowest quantile to be ≤ 7 mg/day [9,10,11,12,14], the odds ratio for breast cancer for premenopausal women in the highest quantile of vitamin E intake is typically about 0.5, indicating 50% protection. On the other hand, among the studies for which vitamin E intake in the lowest quantile was greater than 8 mg/day [15,16], all odds ratios for breast

Table 2
Breast cancer and exposure to vitamin E assessed by dietary intake: Prospective studies

Population (Reference)	Age or menopausal status[1]	Number total/cases[2]	Follow up time in years[3]	Instrument (item no.)[4]	Vit E form[5]	Comparison[6]	Relative risk (95% C.I.)[7]
Females nurses [21]	34–59 years	89,494/1439	Up to 8	FFQ (16/121)[8]	IU	≥24.1 versus <3.9*[9]	0.90[10] (0.77,1.06)
						≥600[10] versus no use*	1.01[10] (0.69,1.49)
Women in Iowa [22}	Post	21,782/570	Up to 7	FFQ (127)	IU	≥9.6 versus <4.9*	1.08[12] (0.74,1.58)
		34,387/879				>250 versus 0*[11]	0.96[12] (0.76,1.23)
Female nurses [17]	Pre	53,938/784	Up to 14	FFQ (61/126)[8]	IU	Median 10 versus 5*	0.81[13] (0.64,1.02)
	Pre	53,938/784				Median 251 versus 5*[9]	1.22[13] (0.98,1.52)
	Post	29,296/1913				Median 10 versus 5*	0.96[13] (0.83,1.11)
	Post	29,296/1913				Median 251 versus 5*[9]	**0.84**[13] **(0.72,0.96)**
Swedish women [24]	40–76 years	59,036/1271	Mean 8.6	FFQ (67)	mg	Median 9.3 versus Median 3.8*	0.83[14] (0.60,1.14)
N.Y. State Cohort [118]	Post	18,586/359	Up to 7	FFQ (45)	mg	≥9.3 versus <4.3*	0.86[15] (0.61,1.21)
Netherlands Cohort study [32]	Post	62,573/650	4.3	FFQ (150)	mg	Median 19.8 versus median 6.9*	1.25[16] (0.85,1.85)
Canadian women [20]	40–59 years	1182/519	Up to 5	FFQ (86)	mg α-T	>7 versus <3*	1.05[17] (0.65,1.70)
					mg	>25 versus <12*	0.96[17] (0.63,1.45)
					mg α-T	>3 versus 0*[11]	1.20[17] (0.83,1.75)
					mg	>4 versus 0*[11]	1.00[17] (0.65,154)
Canadian women [8]	Not provided	628/325	Prospective	FFQ (86)	IU	18.56 versus 18.19**	1.32[18] (0.85,2.05)
		628/325	Retrospective			19.10 versus 18.78**	1.02[18] (0.57,1.82)
Finnish women [25]	>15 years	4697/88	Up to 25	Dietary interview	NS	Highest versus lowest* tertile	1.08[19]

[1] Pre indicates premenopausal women, Post indicates postmenopausal women. Age in years is given if the publication provided that information but no information concerning menopausal status.

[2] Numbers in the entire cohort for prospective cohort studies followed by number of incident cases, or for nested case-control studies, number of controls followed by the number of cases.

[3] Length of follow up in years is given for prospective cohort studies. Prospective and retrospective indicates results for dietary intake assessed prospectively and retrospectively for a nest case-control study.

[4] FFQ indicates food frequency questionnaire, the number of items are given parenthetically, Dietary interview indicates diet ascertained by interview.

[5] Vitamin E form as specified in the publication where IU denotes international units while mg indicates milligrams, α-T indicates α-tocopherol. All vitamin E is derived from food, except as noted. NS denotes not specified. Entries have been arranged in ascending order of vitamin E in referent category (indicated by an asterisk; mean values for cases versus controls are indicated by two asterisks), equating each IU vitamin E reported in the various publications with 0.67 mg d-α-tocopherol [3], and for the purpose of ordering the reports, assuming that mg vitamin E reported reflects α-tocopherol.

[6] Unless indicated otherwise, comparisons are for the highest quantile (quintile, quartile, tertile) compared with the lowest quantile.

[7] Relative risk is given with the 95% confidence interval in parenthesis. Relative risks that are significantly different from unity (at $p \leq 0.05$) are indicated in bold typeface.

[8] A baseline 61 item food frequency questionnaire was expanded to the larger number of items for serial collection of dietary data for later years of the study.

[9] From food and supplements.

[10] Adjusted for age, length of follow up, energy intake, body mass index, parity, age at first birth, age at menarche, family history of breast cancer, menopausal status, alcohol, history of benign breast disease.

[11] From supplements.

[12] Adjusted for age, age at menarche, age at menopause, age at first birth, parity, body mass index at baseline, body mass index at age 18, family history of breast cancer, history of benign breast disease, alcohol, and education. Dietary vitamin E was adjusted for energy intake while supplemental vitamin E was not adjusted for energy intake.

[13] Adjusted for age, length of follow up, total energy intake, body mass index at age 18, weight change from 18 years, parity, age at first birth, age at menarche, family history of breast cancer, alcohol, history of benign breast disease, and height. For postmenopausal women in addition by age at menopause and postmenopausal hormone use.

[14] Adjusted for age, family history of breast cancer, height, body mass index, education, parity, age at first birth, total energy intake, alcohol, fiber, and monounsaturated fatty acids.

[15] Adjusted for age and education.

[16] Adjusted for age, energy intake, alcohol, history of benign breast disease, family history of breast cancer, age at menarche, age at menopause, age at first live birth, parity.

[17] Adjusted for age, energy intake, age at menarche, surgical menopause, age at first live birth, education, family history of breast cancer, history of benign breast disease.

[18] Adjusted for total energy intake.

[19] Adjusted for age, body mass index, parity, region, occupation, smoking.

cancer for higher versus lower vitamin E intake were greater than 1, although not significantly so. This suggests that no additional reduction in risk of breast cancer is conferred by dietary vitamin E greater than about 8 mg/day. Thus, in comparison to current guidelines [3], only women whose dietary intake of vitamin E is less than 53% of the recommended value appear to experience increased risk of breast cancer. Generally, relative risks for the highest compared with the lowest quantile of vitamin E intake are closer to 1 for the prospective studies reported in Table 2 than for the case-control studies reported in Table 1. Potentially this could be due in part to bias in reporting of dietary intake in the case-control studies. In addition, it is possible that the differences in the populations that were described may have contributed to these differences.

Most of the case-control studies reported odds ratios separately for premenopausal and postmenopausal women, and, for populations with lower levels of vitamin E intake, odds ratios for highest compared to lowest quantile of vitamin E intake were typically lower for premenopausal than postmenopausal women (Table 1). In comparison, most of the prospective studies either reported on a combined population of premenopausal and postmenopausal women, or only reported on postmenopausal women. One large prospective study in female nurses has reported values for the relative risk of breast cancer associated with the highest quintile of vitamin E separately for premenopausal and postmenopausal women [17]. After adjustment for age, length of follow up, total energy intake, body mass index at age 18, weight change from 18 years, parity, age at first birth, age at menarche, family history of breast cancer, alcohol, history of benign breast disease, and height, and additionally in postmenopausal women for age at menopause and postmenopausal hormone use, relative risks were 0.81 (0.64–1.02, 95% confidence interval) and 0.96 (0.83–1.11) for premenopausal and postmenopausal women, respectively. In comparison, a case-control study in Greek women with similar low level of vitamin E intake reported odds ratios of breast cancer for the highest compared with the lowest quintile of vitamin E intake to be 0.50 (0.25–1.02, 95% confidence interval) and 0.85 (0.53–1.36, 95% confidence interval) for premenopausal and postmenopausal women, respectively, after adjustment for age, birthplace, body mass index, parity, age at first birth, age at menarche, and total energy intake [10]. It is not clear what might account for the more favorable estimates of the protective effect of dietary vitamin E in the case-control [10] than the prospective [17] study. However, the prospective study adjusted for several additional risk factors for breast cancer that were not considered in the case-control study, including previous benign breast disease [14,16], family history of breast cancer [14,16], alcohol consumption [16], and for postmenopausal women, age at menopause [16] and postmenopausal hormone use [17]. Two other case-control studies in populations with higher vitamin E intakes [16] or unspecified vitamin E intake [18] reported odds ratios for breast cancer in quantiles with highest compared with the lowest vitamin E intake to be not different from unity and not different between premenopausal and postmenopausal women. One of these studies also observed that odds ratios for risk of breast cancer (adjusted for age, education, parity/age at first birth, energy intake, and geographic location) for the highest quintile of vitamin E intake compared with the lowest quintile differed little among women <45, 45–54, 55–64, or ≥ 65 years [18]. Considering the available data, it seems probable that the reduction in risk of breast cancer associated with higher dietary vitamin E may be greater for premenopausal than postmenopausal women [9,10,17].

The etiology of breast cancer in women with a family history and those without a family history may differ, and it is plausible that dietary factors, such as vitamin E, may differentially influence risk of breast cancer in women with and without this risk factor. Several studies have presented data relevant to this point. A case-control study in women from western New York compared women with primary histologically confirmed breast cancer with controls drawn from the community and matched to the cases by age and county of residence [11]. Dietary intake was assessed by an extensive food frequency questionnaire and food composition data that captured α-tocopherol intake from 252 foods. Odds ratios were calculated for risk of breast cancer in the highest compared with the lowest quartile of α-tocopherol intake for women with and without a family history of breast cancer. After adjustment for age, education, age at menarche, age at first pregnancy, and body mass index, and total energy intake, odds ratios were 0.2 (0.1–1.1, 95% confidence interval) for women with a family history of breast cancer and 0.4 (0.3–0.7, 95% confidence interval) for women without a family history of breast cancer [11]. For women without a family history of breast cancer, there was a significant linear trend for reduced risk with higher intake of α-tocopherol [11]. In a subsequent analysis, the authors determined the odds ratio for breast cancer for the highest compared with the lowest quartile of dietary α-tocopherol in women stratified both by menopausal status and by family history of breast cancer. This analysis indicated odds ratios of 0.1 (0.0–1.2), 0.5 (0.2–1.0), 0.7 (0.1–4.0), and 0.5 (0.3–1.0) for premenopausal women with a family history of breast cancer, premenopausal women without a family history of breast cancer, postmenopausal women with a family history of breast cancer, and postmenopausal women without a family history of breast cancer, respectively [11].

More recently, a prospective study of 83,234 women, ages 33–60 years at baseline, participants in the Nurses' Health Study, reported on the role of family history of breast cancer on development of breast cancer during up to 14 years of follow up [17]. Among the 53,938 women who were premenopausal at baseline, 90 incident cases of breast cancer developed in women with a family history of breast cancer while 689 cases of incident breast cancer developed in women without a family history of breast cancer. Within this cohort, the relative risk for breast cancer during the

follow up period for the highest quintile of vitamin E intake was 0.57 (0.28–1.15, 95% confidence interval) for premenopausal women with a family history of breast cancer compared with 0.84 (0.66–1.07) for women without a family history of breast cancer, after adjustment for age, length of follow up, total energy intake, parity, age at first birth, age at menarche, history of benign breast disease, alcohol, body mass index at age 18, weight change from age 18 years, and height [17]. These relative risks are substantially attenuated compared with odds ratios calculated from the much smaller case-control study [11], which considered about a third as many breast cancer cases. However, this larger prospective study adjusted for several risk factors for breast cancer that were not considered in the very small case-control study, including parity [10,14,19], history of benign breast disease [14,16], and alcohol consumption [16]. Other differences between these studies, in addition to the design (retrospective case-control versus prospective study) were the adjustment of the odds ratios for educational attainment, a risk factor for breast cancer in some populations [15,16,19], only in the case-control study [11], and the assessment of different measures of vitamin E (total vitamin E from food in the prospective study and dietary α-tocopherol in the case-control study). Further investigation will be needed to determine whether dietary vitamin E confers differential protection from breast cancer in women with and without a family history of breast cancer.

It is of interest to consider whether it is α-tocopherol or perhaps α-tocopherol together with other tocopherols and tocotrienols that might have been included in total dietary vitamin E, that may protect against breast cancer. Among the studies summarized in Tables 1 and 2, only three reported data for dietary α-tocopherol [11,14,20]. Only one of these studies, a nested case-control study in Canadian women, reported data for both dietary α-tocopherol and total dietary vitamin E [20]. In that study, dietary intake of α-tocopherol, which was assessed by a food frequency questionnaire containing 86 food items, accounted for only about one third of total dietary vitamin E [20]. For that population, the odds ratio for breast cancer in the highest quantile of dietary α-tocopherol and total dietary vitamin E, respectively, compared with the respective lowest quantile were 1.05 (0.65–1.70) and 0.96 (0.63–1.45), very similar to one another. Further studies, in which dietary intake of the different tocopherols and tocotrienols are quantitated separately, will be needed to sort out the relative roles of the compounds in prevention of breast cancer.

Several studies have considered the influence of vitamin E supplements, much of which would be in the form of α-tocopherol, on risk of breast cancer. Two reports of the association of vitamin E intake with risk of breast cancer in the same cohort of more than 80,000 female nurses, one after up to 8 [21] and the second after up to 14 [17] years of follow up have investigated the role of vitamin E supplementation. After 8 years of follow up, the multivariate relative risk (adjusted for age, length of follow up, energy intake, parity, age at first birth, age at menarche, family history of breast cancer, menopausal status, body mass index, alcohol intake, and history of benign breast disease) for the highest compared with the lowest quintile of vitamin E from food and supplements was 0.90 (0.77–1.06, 95% confidence interval), and when vitamin E from supplements only was considered, the relative risk was numerically increased to 1.01 (0.69–1.49), providing no evidence for any benefit from vitamin E supplements. After 14 years of follow up, multivariate relative risks for premenopausal women were 0.81 (0.64–1.02, 95% CI), 1.22 (0.98–1.52, 95% CI) for vitamin E from foods and from foods and supplements, respectively. Corresponding relative risks for postmenopausal women were 0.96 (0.83–1.11, 95% CI) and 0.84 (0.72–0.96, 95% CI), respectively [17]. Also in this study, the investigators reported on the association of breast cancer with both dose and duration of use of vitamin E supplements. Compared with women who never used a vitamin E supplement, the multivariate relative risk of breast cancer for women (premenopausal and postmenopausal combined) in the highest quintile of vitamin E supplement use ($\geq$600 IU/day) was 0.92 (0.70–1.21). Also, the relative risk for women using vitamin E supplements for $\geq$ 10 years compared with those who never used a vitamin E supplement was 1.11 (0.92–1.35). Similarly, the Iowa Women's Health Study, a cohort of 34,387 postmenopausal women, reported that multivariate relative risks for breast cancer (adjusted for age, energy intake, age at menarche, age at menopause, age at first live birth, parity, body mass index at baseline, body mass index at age 18, family history of breast cancer, history of benign breast disease, alcohol intake, and educational attainment) in postmenopausal women in the highest quintile of vitamin E intake from food only was 1.08 (0.74–1.58), compared with 1.05 (0.83–1.33) when vitamin E from food and supplements was considered, and 0.96 (0.76–1.23) for the highest quintile of vitamin E supplement compared with no supplemental vitamin E [22]. Importantly, these relative risks were not altered when women who developed breast cancer during the first two years of follow up, who may have had early stage (not detectable) cancer at baseline, were excluded [22]. One other prospective study reported no association of use of vitamin E supplement with risk of breast cancer in an elderly cohort of women [23]. Two other studies, a nested case-control study [20] and a case-control study [14] also provide evidence that both α-tocopherol [14,20] and total vitamin E [20] supplements are not associated with reduced (multivariate) odds of breast cancer. Thus, together these studies suggest that α-tocopherol has no influence on risk of breast cancer in women.

One study in the Swedish Mammography Screening Cohort, a large population-based cohort study [24] is of interest. This study not only considered the association of dietary vitamin E with risk of breast cancer but also considered several subgroups within the cohort with the intent to gain insight into to the mechanism(s) by which vitamin E could

influence risk of breast cancer. Overall, after adjustment for age, family history of breast cancer, height, body mass index, education, parity, age at first birth, total calorie intake, and intake of alcohol, fiber, and monounsaturated fatty acids, the hazard ratio for breast cancer was 0.83 (0.60–1.14, 95% confidence interval) for women in the highest compared with the lowest quintile of vitamin E intake [24]. Hazard ratios for the highest compared with the lowest quintile for vitamin E intake for subgroups stratified by body mass index (<25 versus ≥ 25 kg/m^2), high versus low intake of linoleic acid (<4 versus >6 g/day), and the subgroup with both higher body mass index and higher linoleic acid intake all were similar and not significantly different from unity [24], suggesting that higher vitamin E intake did not confer protection from breast cancer in any of these subgroups.

One very abbreviated report for Finnish women is worthy of note because of its prolonged length of follow up [25]. In this study, data for baseline diet was collected by a dietary history interview method in women 15 years of age and older and initially free of cancer. During 25 years of follow up, 88 of the original 4697 women developed breast cancer, resulting in a relative risk of breast cancer for the highest tertile of vitamin E intake compared with the lowest of about 1.0, after adjustment for age, body mass index, parity, geographic location, occupation, and smoking [25]. Unfortunately, no information was provided for the range of vitamin E intake or for premenopausal compared with postmenopausal women.

Overall, observational studies of the association of vitamin E with breast cancer risk suggest the possibility that increased dietary exposure to vitamin E may slightly reduce breast cancer risk. It is possible that certain subgroups, such as premenopausal women and those with a family history of breast cancer, may derive more protection from breast cancer with higher levels of dietary vitamin E. However, there is no evidence that supplemental vitamin E, most, if not all of which is in the form of α-tocopherol, confers any protection from breast cancer.

3. Concerns regarding investigation of the relationship between vitamin E, other tocopherols, and the tocotrienols and breast cancer by dietary intake

Assessment of dietary intake in large populations is only feasible with simple instruments such as food frequency questionnaires. However, even the best of such instruments for assessing dietary intake are relatively crude and show only modest correlations with dietary intake as assessed by more precise and valid measures. For example, a large prospective study of diet and breast cancer incidence reported a correlation of 0.55 between vitamin E assessed by five 24 hr dietary recalls and by a 127 item food frequency questionnaire [22]. A small case control study reported that the correlation between vitamin E intake assessed by 14 day diet records and by a 110 item food frequency questionnaire was 0.68 [26]. However, another large prospective study of breast cancer incidence in Swedish women reported that the correlation between vitamin E intake as assessed by a more qualitative food frequency questionnaire containing only 67 items and as assessed by records of the types and weights of all foods consumed was only 0.30 [24].

A further difficulty in the interpretation of studies of dietary intake is the extent to which dietary tocopherols and tocotrienols are included in the analysis, and whether and in what way data for tocopherols and tocotrienols are combined or reported. For example, one accepted practice has been to combine the different dietary tocopherols and tocotrienols using weighting factors that are thought to reflect biological activity [4,27,28,29]. Among naturally occurring sterioisomers (RRR tocopherols and R tocotrienols), with α-tocopherol as 100%, weighting factors for β-, γ-, and δ-tocopherol are reported to be 12–50%, 1–35%, and 0.3–3%, while weighting factors for α-, β-, γ-, and δ-tocotrienol are reported to be 17–30%, 1–5%, 10%, and 0%, respectively [4,27,28,29]. These broad ranges of activity reflect the different bioassays that were used. Often, for a given report, the specific weighting factors used for these dietary tocopherols and tocotrienols are not clear. Until recently [3], it was recommended that vitamin E activity derived from natural sources in the diet be expressed as α-tocopherol equivalents (α-TE) [27], using weighting factors of 0.5, 0.1, and 0.3 for the milligrams of β- and γ-tocopherol and α-tocotrienol, respectively. However, it often is not clear how values for vitamin E that are reported have been computed; specifically, it is not clear which weighting factors were used and the extent to which dietary tocotrienols might have been included in computations. In other cases, dietary tocopherol is reported, and it is not clear whether this reflects the summation of all dietary tocopherols directly, or whether weighting factors such as those indicated above have been applied. Thus, the available literature on vitamin E or tocopherol and risk of breast cancer can provide little if any insight into the role that different tocopherols or the tocotrienols play in influencing risk of breast cancer.

A further concern relates to the complication of studies of dietary intake by the food matrix from which tocopherols and tocotrienols are obtained. As discussed above, tocopherols and tocotrienols are present in the oil fraction of cereals, seeds, and nuts [1], food sources that are rich sources of fatty acids. Evidence suggests that ω–6 polyunsaturated fatty acids may increase risk of breast cancer while monounsaturated fatty acids may be protective [30]. It is well known that nutrient intake is correlated with total energy intake; thus studies of dietary intake of vitamin E or tocopherol commonly adjust intake of these nutrients for total energy intake [8,9,10,11,12,13,14,15,17,18,20,21,22,24,26,31,32]. However, the importance of adjusting intake of fat soluble nutrients such as vitamin E, other tocopherols,

Table 3
Breast cancer and exposure to vitamin E assessed by serum levels: Retrospective and propective case-control studies

Population (Reference)	Age or menopausal status[1]	Number cases/control[2]	Source of control[3]	Form Vit E[4]	Comparison[5]	Statistical test[6]
Retrospective case-control studies						
Boston women [13]	Post	377/403	Referral	α-tocopherol	Median 48 vs median 22	OR: 0.8[7] (0.5,1.2)
Korean women [47]	Mean 38–44 years	44/46	Benign Breast	α-tocopherol γ-tocopherol	18.2 ± 2.0 vs 19.8 ± 2.1[8] 3.8 ± 0.4 vs 3.8 ± 0.4[8]	— —
Indian women [49]	Pre post post	28/23 29/19	Hospital	Not specified	38.4 ± 1.1 vs 24.9 ± 0.9[8] 29.9 ± 1.4 vs 24.6 ± 1.0[8]	t-test: $p < 0.05$ t-test: $p < 0.02$
Detroit women [48]	Mean years	27/28	Mammo.	α-tocopherol γ-tocopherol	≤20.5 versus ≥35 ≤2.12 versus ≥7.57	OR: 0.76[9] (0.10,5.75) OR: 0.31[9] (0.04,1.93)
Prospective nested case-control studies						
Wash. Co. Maryland [50]	Post	30/59	Cohort (14)	α-tocopherol	Cases = 0.25[10] controls = 0.24[10]	—
Columbia Missouri [51]	Mean 58 years	105/203	Cohort (up to 9.5)	α-tocopherol	≥31.3 vs ≤21.6	OR:1.2[11] (0.5,2.8)

[1] Post indicates postmenopausal women, Pre indicates premenopausal women. Mean age or age range is given if the publication provided that information but no information concerning menopausal status.

[2] Number of cases followed by the number of controls.

[3] Referral indicates women who underwent the same or similar diagnostic procedure as the cases but were found to be healthy, Benign Breast indicates benign breast disease, Hospital indicates patients admitted to the hospital with minor surgical problems other than breast disease or malignancy, Mammo. indicates normal women recruited at mammography screening. For nested case-control studies, the control is indicated as Cohort with years of follow up in parenthesis.

[4] Indicates whether the serum measurement is for α-tocopherol, or another tocopherol, or for undetermined tocopherols termed vitamin E. Values are given in units of μmol/L.

[5] Unless indicated otherwise, comparisons are for the highest quantile (quintile, quartile, tertile) compared with the lowest quantile.

[6] OR indicates odds ratio which is given with 95% confidence interval in parenthesis. t-test indicates Students t-test.

[7] Adjusted for age, alcohol intake, age at first birth, parity, family history of breast cancer, age at menopause, age at menarche, body weight, history of benign breast disease, and plasma concentrations of cholesterol, HDL cholesterol, and triglycerides.

[8] Value for control compared with case ± SEM.

[9] This study included approximately equal numbers of African American and Caucasian women. The odds ratio reported in the publication is for the lowest quartile compared with the highest quartile. The odds ratio was adjusted for race.

[10] As given in the publication. It is likely that an error of a factor of 100 was made.

[11] Matched on age, benign breast disease, time of blood collection; adjusted for serum cholesterol, smoking, and body mass index.

and tocotrienols for individual fatty acids is less well appreciated. Among the studies summarized in Tables 1 and 2, only one adjusted dietary vitamin E for intake of specific fatty acid classes, and this study only did so for monounsaturated fatty acids [24]. In future studies directed toward understanding the role that tocopherols and tocotrienols play in breast cancer, it will be necessary to consider the level and composition of dietary fatty acids.

4. Relationship between vitamin E exposure assessed by blood levels and breast cancer risk

A number of case control studies [19,31,33,34,35,36] or nested case-control studies [37,38,39,40] have reported on blood levels of vitamin E in samples stored at or above -20°C for several to many years [31,37,38,39,40] or for an unspecified time [19,33,35,36], or did not report the conditions under which plasma or serum was stored before analysis [34]. However, in the early to middle 1990's it became clear that significant losses of vitamin E occur in plasma and whole blood stored at -20°C even for as little as 24 months [41,42]. Importantly, when values for 55 plasma samples, measured after 24 months of storage at -20°C were compared with those measured in fresh samples, only 1/3 of samples remained in the same tertile as in the original analysis and almost 1/3 were classified in the opposite tertile on the repeat analysis [42]. This makes interpretation of results of any epidemiological study relying of measurements of vitamin E in blood, serum or plasma stored at -20°C very tenuous. Thus, the discussion of this review will be limited to those studies that took the precaution of storing serum, plasma or whole blood at -70°C before measurement of α-tocopherol, other tocopherols or the tocotrienols.

After exclusion of the studies that did not store serum samples appropriately before analysis, only a limited number of studies remain. These data are summarized in Table 3. As shown in Table 3, there are data for four case-control studies in which serum samples were stored properly. Among these studies, one large study in women in Boston

reported on α-tocopherol concentrations in serum of postmenopausal women with breast cancer compared with controls who underwent the same or similar diagnostic procedure at the same institution, and who either did not require a breast biopsy or whose breast biopsy revealed normal tissue or nonproliferative benign breast disease [13]. In this population of postmenopausal women, the odds ratio for breast cancer for women with the highest compared with the lowest quartile of α-tocopherol in serum was 0.8 (0.5–1.2) after adjustment for age, alcohol intake, age at first birth, parity, family history of breast cancer, age at menopause, age at menarche, body weight, history of benign breast disease, and serum cholesterol, HDL cholesterol, and triglyceride concentrations [13]. The adjustment for these lipids, in addition to the risk factors for breast cancer, is important because tocopherols correlate with plasma lipids and lipoproteins [43,44,45,46]. The remaining three case-control studies included very small numbers of women and did not adjust concentrations of the measured α- or γ-tocopherol [47,48] or vitamin E [49] for either risk factors for breast cancer or any measure of plasma lipids. Among these smaller studies, one reported higher concentrations of α-tocopherol in plasma of both premenopausal and postmenopausal breast cancer patients, but this study also reported differences in lipids and lipoproteins between these cases and their respective controls [49], potentially confounding the observations for plasma α-tocopherol. The same study reported no difference in plasma vitamin E concentrations among women with differing stage of breast cancer [49], however, lack of adjustment for plasma lipids complicates interpretation of such data.

Two prospective nested case-control studies reported on the prognostic value of α-tocopherol concentrations in serum [50,51]. Of these, the more recent larger study in older women of whom about 80% were postmenopausal [51] reported a relative risk of 1.2 (0.5–2.8) for breast cancer for the highest quartile of serum α-tocopherol compared with the lowest quartile for an analysis that involved matching on age, benign breast disease, time of blood collection and adjustment for serum cholesterol, smoking, and body mass index. The smaller, older study, which did not adjust serum α-tocopherol in any way, reported no differences in concentrations of α-tocopherol among women who did and did not develop breast cancer during up to 14 years of follow up [50]. Thus, if one considers only reports for which data were collected from serum or plasma properly stored to preserve vitamin E and the other tocopherols and tocotrienols, there is no convincing evidence that either higher or lower α- or γ-tocopherol influence risk of breast cancer in women. Potentially, this apparent discrepancy with data for dietary intake of vitamin E may be related to differing transport and metabolism of α-tocopherol and other tocopherols and tocotrienols (see below) that may have been included in computations of dietary vitamin E.

5. Relationship between vitamin E exposure assessed by adipose tissue concentrations and breast cancer risk

Several studies that carefully collected adipose tissue and stored this tissue at -70°C or lower before analysis have investigated the relationship between breast adipose tissue tocopherols and breast cancer risk. One case-control study assessed α-tocopherol concentrations in breast adipose tissue from patients with early invasive breast carcinoma compared with those with non malignant tumors in a mixed population of premenopausal and postmenopausal women [52]. In that study, the concentration of α-tocopherol was 6-fold higher for breast adipose tissue of the women with non-malignant tumors (controls) than for breast adipose tissue of those with invasive breast carcinoma [52]. However, the women with malignant tumors were on average 9 years older than the controls. The investigators did not adjust for this age difference, or for any reproductive risk factors [10,14,16,19] for breast cancer. Other potential confounding factors that were ignored were body mass index, which is not only a risk factor for breast cancer [10,14,16] and also is important because tocopherols and tocotrienols are distributed among the body fat stores, adipose tissue cholesterol and triglyceride concentrations, which are important because of the relationships between adipose tissue vitamin E and these factors [53,54,55], and fatty acid composition, which influences the vitamin E requirement [56]. Another smaller case-control study reported on the comparison of breast adipose tissue concentrations of tocopherol (forms not specified) for women with early breast cancer compared with those with benign breast disease, stratified by menopausal status [26]. Among premenopausal women, and after adjustment for age and body mass index, breast adipose tissue tocopherol concentrations did not differ between cases and controls (100 ± 13 versus 104 ± 12 mg/g, mean $\pm$ SEM). Compared with premenopausal women, breast adipose tissue tocopherol concentrations tended to be lower for postmenopausal women, and were significantly lower for postmenopausal breast cancer cases compared with postmenopausal controls, after adjustment for age and body mass index (69 ± 5 versus 90 ± 8 mg/g) [26]. This study also did not adjust breast adipose tissue concentrations of tocopherol for reproductive and other risk factors for breast cancer. Because neither of these studies controlled for risk factors for breast cancer or used appropriate adjustment for all factors known to influence adipose tissue vitamin E, and because different measures of vitamin E were assessed (α-tocopherol versus unspecified forms(s) of tocopherol), it is difficult to draw any conclusions from these data.

The European Community Multicentre Study on Antioxidants, Myocardial Infarction, and Cancer of the Breast, a large case control study among postmenopausal women from five European countries, reported on the association of breast cancer with α-tocopherol exposure as assessed by

buttocks adipose α-tocopherol concentration [57]. This study included more than three times the numbers of cases and controls as were involved in the two studies discussed above. Cases were women with newly diagnosed breast cancer histologically classified as ductal carcinoma without metastasis. Controls were women drawn from the population in the same catchment area and without a history of breast cancer. In this study, the odds ratio for breast cancer for the highest tertile of adipose tissue α-tocopherol compared with the lowest was 1.15 (0.75–1.77), after adjustment for age, geographic location, parity, age at first birth, and alcohol [57], suggesting that α-tocopherol, as assessed in buttocks adipose tissue, was unrelated to risk of breast cancer. However, again unfortunately no adjustment was made for adipose tissue fatty acid composition, or adipose tissue cholesterol and triglyceride, or body mass index.

One study in women with breast cancer reported on the detection of each of the tocopherols (α, β, γ, δ) and both α- and β-tocotrienol in breast adipose tissue for women with breast cancer [58]. Unfortunately, that study did not include comparative data for women without breast cancer [58]. Information regarding the prognosis of women with breast cancer in relation to breast adipose tissue concentrations of tocopherols and tocotrienols would provide insight into the roles that tocopherols and tocotrienols might play in breast cancer. However, no such information is yet available. Data indicate that concentrations of tocopherols and tocotrienols are similar for adipose tissue adjacent to, and distant from, the breast tumor [58]. Another study also reported that concentrations of α- and γ-tocopherol for cancerous breast did not differ from the corresponding concentrations for noncancerous breast from the same women [59]. Thus, these studies suggest that the tumor did not have any local effect on concentrations of these tocopherols and tocotrienols in breast adipose tissue or the breast overall. Further study will be needed to determine how breast epithelial and/or adipose tissue concentrations of tocopherols (α, β, γ, δ) and tocotrienols (α, β, γ, δ) might influence risk of breast cancer in women.

6. Importance of tocopherol and tocotrienol transport and metabolism to the perceived influence of vitamin E on breast cancer risk

Absorption of tocopherols and tocotrienols requires adequate fat [60]. Fortunately, most of the dietary tocopherol and tocotrienol occurs in foods together with fat [1]. However, supplemental α-tocopherol may or may not be consumed with the necessary fat to promote absorption, and thus may or may not contribute to plasma α-tocopherol concentrations. The same would apply to supplements of the other tocopherols or the tocotrienols. A recent study specifically investigated absorption and metabolism of a 300 mg dose of mixed tocotrienols administered either in the fasted state or with a high fat meal [61]. That study demonstrated that peak plasma concentrations of the individual tocotrienols (α, γ, and δ), and the area under the plasma concentration-time curves for these tocotrienols (a parameter indicative of the average exposure of tissues to these tocotrienols), were 60–72% and 59–65% lower, respectively, after administration of the tocotrienol preparation in the absence of food [61]. While that study did not observe any effect of the fatty meal on the rates of clearance of the tocotrienols from the plasma, tocotrienols were cleared from the plasma more rapidly than reported values for α-tocopherol [61], consistent with the well known differences in metabolism of tocotrienols and α-tocopherol as discussed in more detail below.

All tocopherols and tocotrienols are equally absorbed and appear in the blood in chylomicrons during fat absorption [46,60]. In addition, data suggests that the absorption of the individual tocopherols is unaffected by large amounts of the other tocopherols [3,60]. During the postabsorptive phase, chylomicrons containing tocopherols and tocotrienols are cleared from the blood by uptake by the liver, adipose tissue, and other tissues [46]. The liver tocopherol binding protein selectively recycles α-tocopherol by packaging and secreting α-tocopherol in lipoproteins [60,62]. A small amount of γ-tocopherol is secreted in lipoproteins because γ-tocopherol competes with α-tocopherol for binding to the tocopherol binding protein. However, when α-tocopherol supplements are consumed with sufficient fat to promote absorption, the large influx of α-tocopherol into the liver favors the binding of α-tocopherol to the tocopherol binding protein, and the secretion of only α-tocopherol from the liver into the plasma [60,62]. Even in the absence of supplemental α-tocopherol, β- and δ-tocopherol and the tocotrienols are not secreted from the liver [60]. During α-tocopherol supplementation, plasma concentrations of tocopherols other than α-tocopherol and the tocotrienols are reduced [63].

As a result of these metabolic differences, during the postabsorptive phase blood concentrations of α-tocopherol remain relatively stable while those of other tocopherols and the tocotrienols decline [46,60,62]. Under such circumstances, a single measure of plasma concentrations of individual tocopherols and tocotrienols cannot provide an accurate measure of exposure to these dietary constituents [64]. Furthermore, at higher levels of α-tocopherol intake, plasma concentrations of α-tocopherol are not linearly related to α-tocopherol intake, due to both decreased fractional absorption and regulation of the secretion of α-tocopherol on lipoproteins by the tocopherol binding protein [3,60].

As discussed above, all of the tocopherols and both α- and β-tocotrienol have been identified in human breast [59,65] and breast adipose tissue [26,52,58] while α- and γ-tocopherol have been measured in a number of reproductive tissues [65]. Another study showed that both α- and γ-tocopherol are present in collecting duct of the breast [66]. The ratio of γ-tocopherol/α-tocopherol is the same for

breast adipose tissue and collecting duct, although absolute concentrations are lower in the collecting duct [66]. In addition, each of α-, β-, γ-, and δ-tocopherol have been detected in breast milk of normal healthy women [67]. Importantly, the ratio of γ-tocopherol/α-tocopherol in human breast milk is higher than that in plasma, and is suppressed less by α-tocopherol supplementation than is the plasma γ-tocopherol concentration [66]. Concentrations of the various tocopherols and tocotrienols differ among tissues [53,66]. However, evidence suggests that there is little difference in α-tocopherol concentrations among breast adipose tissue and adipose tissue from the axilla or the subcutaneous abdominal fat depot from the same women [66]. Importantly, in human beings, γ-tocopherol is enriched in adipose tissue and several other tissues compared with what might be expected from the ratio of γ-tocopherol/α-tocopherol in plasma [68]. Similarly, a study in hamsters showed that for adipose tissue, the ratio of tocotrienols:tocopherols was almost as high as in the diet, even as the ratio of tocotrienols:tocopherols in plasma was much lower [46]. Potentially this can be explained by transport of tocotrienols into adipose tissue along with triglycerides in chylomicrons [46]. Other data also indicate that ratios of the tocotrienols to α-tocopherol and β-, γ-, and δ-tocopherol to α-tocopherol in the blood *underestimate* the corresponding ratios in both the diet and adipose tissue [46,54,66,68]. Furthermore, absolute values for concentrations of α-tocopherol are about ten times as high for breast adipose tissue as for plasma [66]. Together, such data suggest that concentrations of β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol in breast adipose tissue may be more than ten-fold the corresponding concentrations in plasma. Importantly, tocopherol concentrations of adipose tissue require more than a year to reach a new steady state when the composition of ingested tocopherols is dramatically altered, compared with only a few weeks for plasma tocopherol concentrations [55]. Thus, adipose tissue provides a better measure of long term tocopherol intake (and most likely also tocotrienol intake) than do plasma concentrations.

Thus, whether or not tocopherols and tocotrienols have similar or different biological effects, given the differences in transport and metabolism among α-tocopherol, the other tocopherols, and the tocotrienols, it is not surprising that studies that assessed different measures of dietary, blood, or adipose tissue vitamin E (or different forms of vitamin E, α-tocopherol only, total tocopherol, or total vitamin E-including the contribution of tocotrienols) found different relationships between vitamin E and breast cancer.

7. Inhibition of breast cancer by tocopherols and tocotrienols in experimental animals

Vitamin E deficiency increases the incidence [69,70] and reduces the latency [69] of mammary tumors in experimental animals. This is consistent with the observational studies in human individuals that suggest that only dietary vitamin E less than about half of the recommended intake [3] appears to be associated with increased risk of breast cancer [9,10,11,12,13,14]. Compared with vitamin E adequate diets, most [70,71,72,73,74] but not all [75,76] studies of α-tocopherol supplementation alone found that intervention to have little effect on chemically-induced mammary tumors in animals. Again, this is consistent with observational studies in women, discussed above, that suggest that supplemental α-tocopherol confers no protection from breast cancer. However, many studies in experimental animals consistently reported that α-tocopherol supplementation potentiated the reduction in mammary tumor incidence and prolongation of mammary tumor latency by supplemental selenium [71,77,78,79,80]. Further studies will be needed to determine whether selenium and α-tocopherol supplementation may interact to reduce risk of breast cancer in women. Several studies have provided inconsistent results for the influence of the tocotrienol-rich fraction of palm oil on chemically induced mammary carcinogenesis in rats [74, 81]. Unfortunately, neither of these studies reported plasma or tissue concentrations of tocopherols or tocotrienols. Thus, it is difficult to know the relevance of such data to breast cancer in women.

8. Effects of tocopherols and tocotrienols on proliferation of breast cancer cells. Role of the estrogen receptor

A number of studies have reported on the ability of γ-tocotrienol [82,83,84], δ-tocotrienol [82,83,84], α-tocotrienol [82,83,84] or the tocotrienol-rich fraction from palm oil (which also contains some α-tocopherol) [83,84,85] to inhibit proliferation of estrogen receptor (ER) positive human MCF-7 [82,83] and ZR-75–1 [84] breast cancer cells and ER negative MDA-MB-231 [82] and MDA-MB-435 [83,85] breast cancer cells. Values for concentrations of these tocotrienols that inhibited growth of breast cancer cells 50% (IC_{50}), either as presented in those reports or as interpolated from the graphs presented, are summarized in Table 4. As shown, IC_{50} for the tocotrienols ranged from 5–18 μmol/L for estrogen-receptor positive breast cancer cells and were 10–20 fold higher for estrogen receptor negative cells. For estrogen receptor positive breast cancer cells cultured in the absence of estrogen, γ- and δ-tocotrienol were equally potent inhibitors of cell proliferation and more effective than α-tocotrienol [83,84]. For estrogen receptor negative MDA-MB-435 cells, α- and δ-tocotrienol were equally potent inhibitors of proliferation and about 1/3 as potent as γ-tocotrienol [83]. For the growth inhibitory tocotrienols, most of the cells were viable even at the IC_{50} for these compounds, suggesting that the antiproliferative effects of these compounds were not due to cytotoxicity [83]. In contrast to the growth inhibitory effects of the tocotrienols, many studies [82,84,85,86] found α-tocoph-

Table 4
Inhibition of proliferation of breast cancer cells by tocopherols and tocotrienols: concentrations required to inhibit growth 50% (IC_{50})[1]

Cell line (reference)	Expose time (days)	α-Tocopherol	Tocotrienols[2]			TRF[3]
			α-T-3	γ-T-3	δ-T-3	
Estrogen receptor negative cells						
MDA-MB-231 [86]	Up to 5	>100[4]	—	—	—	—
MDA-MB-435 [83][5]	2	>2320[4]	212 ± 7	73 ± 5	227 ± 8	439 ± 7
MDA-MB-435 [85][5]	2 days	>2320[4]	—	—	—	439
Estrogen receptor positive cells						
MCF-7 [88]	4–7	>1000[4]	—	—	—	—
MCF-7 [83][5]	5	290 ± 7	14 ± 0.7	4.9 ± 0.2	5.0 ± 0.1	9.8 ± 0.2
MCF-7 [87][6]	3	>1000[4]	—	—	—	—
MCF-7 [87][6]	12	<100	—	—	—	—
ZR-75-1 [84][6]	12	>23[4]	18	10	9	13
ZR-75-1 [84][6,7]	12	>23[4]	5	6	9	13

None of these studies, nor any other published reports, invested the influence of β-, γ-, δ-tocopherol or β-tocotrienol on breast cancer cell proliferation. All cells were cultured in the presence of 5–10% serum. No estrogen was present except as indicated for ZR-75-1 cells.

[1] Concentrations are given in μmol/L with SEM (if provided in the publication); — indicates not tested.

[2] α-T-3, γ-T-3, δ-T-3; α-tocotrienol, γ-tocortrienol, and δ-tocotrienol, respectively.

[3] Tocotrienol rich fraction from palm oil.

[4] <50% inhibition of cell growth at the indicated concentration, which was the maximum concentration that was tested.

[5] Concentrations shown in this report as μg/ml have been converted to μmol/L for presentation here.

[6] Values interpolated from data presented in figures.

[7] In the presence of 10^{-8} mol/L estradiol.

erol not to influence proliferation of ZR-75–1 [84], MDA-MB-435 cells [83,85], or MDA-MB-231 cells [82,86] at the highest concentrations tested, which were often as high as 1000 μmol/L. For MCF-7 cells, the literature is inconsistent [83,87,88]. However, even those studies that found α-tocopherol to inhibit growth of MCF-7 cells found that concentrations of α-tocopherol required for 50% growth inhibition were more than 20-fold higher than the growth inhibitory concentrations of the tocotrienols [83].

Comparison of data from the multiple reports shown in Table 4, and data presented in reference [87] suggest that the low growth inhibitory effect of α-tocopherol is enhanced as cells are exposed to α-tocopherol for longer periods of time. However, even after exposure to cells for as long as 12 days, the IC_{50} for growth inhibition by α-tocopherol in MCF-7 cells was about 7–20 fold as high as those for the tocotrienols in the same cell line exposed to tocotrienols for only 5 days (Table 4). Limited data suggest that growth inhibitory effect of the tocotrienols on breast cancer cells is maintained or increases with duration of exposure to the tocotrienols during up 10 days exposure [85,89]. Thus, it seems unlikely that, after prolonged exposure, the growth inhibitory potency of α-tocopherol would approach that of the tocotrienols. However, the limited data (Table 4) and different durations of exposure used in the various studies make it difficult to draw conclusions concerning the relative susceptibility of estrogen receptor positive and estrogen receptor negative breast cancer cells to the growth inhibitory effects of the tocotrienols.

Several studies have investigated potential mechanism(s) by which tocotrienols inhibit breast cancer cell proliferation [82,90]. As yet, the mechanism(s) by which tocotrienols inhibit breast cancer cell proliferation remain to be determined. However, it appears that the growth regulatory effects of the tocotrienols are independent of mutated ras and p53 functions [90] and do not involve increased expression of growth inhibitory insulin-like binding proteins [82] or altered expression of the estrogen-regulated gene pS2 (suggesting that tocotrienols do not inhibit breast cancer cell growth by estrogen-receptor mediated pathways) [82].

9. Effects of tocopherols and tocotrienols on proliferation of breast cancer cells. Role of estrogen and estrogen agonists/antagonists

Importantly, α-, γ-, and δ-tocotrienol [82,83,84] and the tocotrienol-rich fraction from palm oil [82,83,84] inhibited proliferation of MCF-7 [82,83] and ZR-75–1 [84] cells both in the absence and the presence of estradiol. In the presence of pharmacological (10^{-8} mol/L) concentrations of estradiol, both cell lines demonstrated consistently declining growth with exposure to increasing concentrations of the tocotrienol-rich fraction of palm oil [82,84]. A similar response was also observed for MCF-7 cells cultured in the absence of estradiol [82]. In the absence of estrogen, growth of the ZR-75–1 cells was inhibited for all concentrations of the tocotrienol-rich fraction >2 μmol/L, and strongly inhibited for α-, γ-, and δ-tocotrienol individually at >7–10 μmol/L [84]. However, low concentrations (<2 μmol/L) of the tocotrienol-rich fraction of palm oil and slightly higher concentrations (up to 4–5 μmol/L) of α-, γ-, and δ-tocotrienol promoted growth of ZR-75–1 cells [84]. This is of potential concern. However other experiments in the ab-

sence of estrogen, or when estrogen might be expected to be functionally absent, are more reassuring. Specifically, in the presence of both10^{-8} mol/L estradiol and 10^{-8} mol/L of the antiestrogen ICI 161 384, low concentrations of the tocotrienols did not stimulate growth of ZR-75–1 cells, and the individual tocotrienols were potent inhibitors of growth of ZR-75–1 cells at higher concentrations (IC_{50} 7–18 μmol/L) [84]. In addition, ZR-75–1 cells grown in the absence of estradiol and in the presence of 10^{-7} or 10^{-8}mol/L tamoxifen could be further growth inhibited by 1–13 μmol/L concentrations of α-, γ-, and δ-tocotrienol [84].

In a particularly comprehensive study, Guthrie et al. investigated the influence of the tocotrienol-rich fraction of palm oil, α-tocopherol, α-, γ-, and δ-tocotrienol, and tamoxifen on proliferation of MCF-7 and MDA-MB-435 cells [83]. In addition to considering the individual effects of these compounds, they considered the growth inhibitory effects of equal mixtures (w/w) of tamoxifen with the tocotrienol-rich fraction or its three constituent tocotrienols. For MCF-7 cells, concentrations of tamoxifen required for 50% inhibition of cell proliferation were reduced from 110 ± 3 (mean ± SEM) nmol/L for tamoxifen alone to 27 ± 0.5 nmol/L and 8.1 ± 0.3 nmol/L, respectively, for equal mixtures (w/w) of tamoxifen and each of γ-, and δ-tocotrienol, respectively [83]. For this cell line, combination of α-tocotrienol with tamoxifen slightly increased the concentration of tamoxifen needed for 50% inhibition of cell proliferation (110 ± 3 to 269 ± 13 nmol/L) [83]. Interestingly, these investigators also found that tamoxifen inhibited proliferation of estrogen receptor negative MDA-MB-435 cells, although at a much higher concentration than was required for the estrogen receptor positive MCF-7 cells (IC_{50} 242 ± 11 versus 0.11 ± 0.003 μmol/L) [83]. Those investigators also found that concentrations of tamoxifen required for 50% inhibition of MDA-MB-435 cell proliferation were reduced from 242 ± 11 μmol/L for tamoxifen alone to 10 ± 0.5 μmol/L, 4.0 ± 0.1 μmol/L, 5.1 ± 0.05 μmol/L, and 16 ± 0.3 μmol/L, respectively, for equal (w/w) mixtures of tamoxifen and each of the tocotrienol-rich fraction of palm oil, α-, γ-, and δ-tocotrienol, respectively [83]. In comparison to the substantial reduction of the concentration of tamoxifen required for 50% inhibition of growth by combination with γ- or δ-tocotrienol for MCF-7 cells and α-,γ-, and δ-tocotrienol for MDA-MB-435 cells, α-tocopherol combined with tamoxifen increased the IC_{50} for tamoxifen in MCF-7 cells more than 1000-fold to 126 ± 5 μmol/L [83]. Other studies have similarly shown α-, γ-, and δ-tocotrienol to enhance the growth inhibitory effect of tamoxifen on estrogen receptor positive ZR-75–1 cells [84], while 100 μmol/L α-tocopherol completely blocked the potent growth inhibitory effect of 5 μmol/L tamoxifen on estrogen receptor negative MDA-MB-231 cells [86]. These limited data suggest that tocotrienols in combination with tamoxifen may be worthy of investigation as potential therapy for women with both estrogen receptor positive and estrogen receptor negative breast tumors. The tissue culture data relating to the combined effects of α-tocopherol and tamoxifen on breast cancer cell proliferation raise the concern that supplemental α-tocopherol may reduce the effectiveness of tamoxifen therapy in women receiving such therapy for treatment of breast cancer.

For the MDA-MB-231 cells, this antagonism of the growth inhibitory effect of tamoxifen by α-tocopherol was associated with the blockage of tamoxifen-mediated decrease in protein kinase C activity in the intact cells [86]. In comparison, for several other cells types, similar concentrations of α-tocopherol (100 μmol/L) both reduce protein kinase C activity and inhibit cellular proliferation, while other tocopherols have variable effects on cell proliferation that do not correlate with effects on protein kinase C activity [91,92,93].

10. Effects of tocopherols and tocotrienols on apoptosis of breast cancer cells

Several studies have investigated the ability of tocopherols and tocotrienols to induce breast cancer cells to undergo apoptosis. Data indicate that exposure to α-, γ-, and δ-tocotrienol or δ-tocopherol during 3 days induced MCF-7 and MDA-MB-435 breast cancer cells to undergo apoptosis as determined by nuclear morphology after staining with 4,6-diamidino-2-phenylindole (DAPI) ([94], Table 5). In comparison, the same duration of exposure to α- β-, and γ-tocopherol did not induce apoptosis in either of these cell lines exposed to up to 464−480 μmol/L concentrations of these tocopherols [94]. However, another study reported that a higher concentration of α-tocopherol, 1000 μmol/L, induced apoptosis as indicated by DNA fragmentation in 23% of MCF-7 cells [87]. None of these studies investigated the effect of β-tocotrienol on apoptosis. For MCF-7 cells, rank order of concentrations required to induce 50% of cells to undergo apoptosis (EC_{50}) was δ-tocopherol > γ-tocotrienol ≥ α-tocotrienol > δ-tocotrienol whereas for MDA-MB-435 cells rank order for EC_{50} was α-tocotrienol ≥ δ-tocopherol > γ-tocotrienol > δ-tocotrienol [94]. For the tocotrienols, EC_{50} for promotion of apoptosis in each of MCF-7 and MDA-MB-435 cells (Table 5) were several fold the IC_{50} for inhibition of proliferation by the corresponding tocotrienols in these cell lines (Table 4), suggesting that the tocotrienols were not cytotoxic.

11. Relative growth inhibitory and proapoptotic effects of tocopherols and tocotrienols in malignant and preneoplastic breast cells

One comprehensive study compared the influence of three tocopherols (α, γ, and δ) and the corresponding tocotrienols on both proliferation and apoptosis in three breast cell types, preneoplastic, neoplastic, and malignant mouse mammary epithelial cells [89]. The preneoplastic line, de-

Table 5
Promotion of apoptosis in breast cancer cells by tocopherols and tocotrienols concentrations: required to promote apoptosis in 50% of cells (EC_{50})[1]

Cell line (reference)	Exposure time (days)	Tocopherols[2]				Tocotrienols[3]		
		α-T	β-T	γ-T	δ-T	α-T-3	γ-T-3	δ-T-3
MCF-7 [94][4]	3	>464[5]	>480[5]	>480[5]	241 ± 12	33 ± 5	36 ± 5	18 ± 2
MCF-7 [87]	3	>1000[5]	—	—	—	—	—	—
MDA-MB-435 [94][4]	3	>464[5]	>480[5]	>480[5]	360 ± 82	414 ± 54	68 ± 6	33 ± 9

All cells were cultured in the presence of 5–10% serum in the absence of estrogen.
[1] Concentrations are given in μmol/L with SEM (if provided in the publication); — indicates not tested.
None of these studies, nor any other published reports, investigated the influence of β-tocotrienol on apoptosis in breast cancer cells.
[2] α-T, β-T, γ-T, δ-T; α-tocopherol, β-tocopherol, γ-tocopherol, respectively.
[3] α-T-3, γ-T-3, δ-T-3; α-tocotrienol, γ-tocotrienol, and δ-tocotrienol, respectively.
[4] Concentrations shown in this report as μg/ml have been converted to μmol/L for presentation here.
[5] This concentration, which was the maximum concentration that was tested, failed to promote apoptosis in 50% of cells.

noted CL-S1, is immortal in culture but will not form solid tumors when transplanted back into the mammary gland [89]. The neoplastic and malignant cell lines, denoted -SA and +SA, respectively, were derived from adenocarcinomas that developed spontaneously from the original D1 cell line [89]. When injected into the mammary gland fat pad of syngeneic female mice, cells of the -SA line form well differentiated tumors while those of the +SA line form anaplastic adenocarcinomas [89]. The growth and metastatic characteristics of +SA tumors are more aggressive than the -SA tumors [89]. In agreement with other results shown in Table 4, this study found the tocotrienols to be growth inhibitory in each of the breast cell lines tested, with the same rank order of potency, δ-tocotrienol ≥ γ-tocotrienol > α-tocotrienol. In comparison, δ-tocopherol was found to be growth inhibitory in each cell line at higher concentrations than the tocotrienols, whereas neither α- nor γ-tocopherol altered cell growth even at the highest concentration tested (120 μmol/L, Table 6) [89]. While mechanism(s) accounting for these effects remain to be determined, these preneoplastic, neoplastic, and malignant mouse mammary epithelial cells exposed to 5 μmol/L α-, γ-, and δ-tocotrienol accumulated amounts of these tocotrienols comparable to similar cells exposed to 120 μmol/L of the corresponding tocopherols, suggesting that preferential cellular accumulation of the tocotrienols may have contributed to their greater growth inhibitory activity [89]. However, treatments that induced similar cellular concentrations of tocopherols and tocotrienols did not result in similar antiproliferative and cytotoxic effects in the preneoplastic and neoplastic cell lines [89].

All of the tocotrienols and the tocotrienol rich fraction from palm oil had cytotoxic effects on all cells lines, whereas δ-tocopherol was cytotoxic only to -SA and +SA cells [89]. Concentrations inducing 50% cell death were 2–4 fold those required for 50% growth inhibition. However, apoptosis was observed in all cell lines exposed to IC_{50} concentrations of tocotrienols, and in -SA and +SA

Table 6
Growth inhibitory and cytotoxic effects of tocopherols and tocotrienols in preneoplastic and malignant mammary epithelial cells[1]

Cell line	Exposure time (days)	Tocopherols[2]			Tocotrienols[3]			TRF[4]
		α-T	γ-T	δ-T	α-T-3	γ-T-3	δ-T-3	
Concentrations required to inhibit growth 50% (IC_{50})[5,6]								
CL-S1 preneoplastic	5	>120[7]	>120[7]	55	12	8	7	13
−SA neoplastic	5	>120[7]	>120[7]	47	7	5	4	7
+SA malignant	5	>120[7]	>120[7]	23	5	4	3	6
Concentrations required to reduce numbes of viable cells by 50% (LD_{50})[5]								
CL-S1 preneoplastic	1	>250[8]	>250[8]	>250[8]	27	19	16	50
−SA neoplastic	1	>250[8]	>250[8]	166	28	17	15	43
+SA malignant	1	>250[8]	>250[8]	126	23	14	12	38

[1] Data from reference [89]. This study did not investigate the influence of β-tocopherol or β-tocotrienol on proliferation or apoptosis in these cell lines.
[2] α-T, γ-T, δ-T; α-tocopherol, γ-tocopherol, δ-tocopherol, respectively.
[3] α-T-3, γ-T-3, δ-T-3; α-tocotrienol, γ-tocotrienol, and δ-tocotrienol, respectively.
[4] Tocotrienol rich fraction from palm oil.
[5] Concentrations are given in μmol/L.
[6] Treatment with IC_{50} concentrations of δ-tocopherol, or α-, γ-, δ-tocotrienol for 24 hours induced significant apoptosis as indicated by DNA fragmentation.
[7] <50% inhibition of cell growth at the indicated concentration, which was the maximum concentration that was tested.
[8] This concentration, which was the maximum concentration that was tested, failed to reduce cell viability by ≥50%.

cells exposed to the IC_{50} concentration of δ-tocopherol [89]. Rank order of susceptibility to both growth inhibitory and proapoptotic effects of the tocotrienols/δ-tocopherol were +SA > -SA > CL-S1 cells, with the most striking differences apparent between the preneoplastic CL-S1 cells and the neoplastic or malignant cells [89]. If such differential susceptibility were to also occur in vivo, this would suggest that, with tocotrienol supplementation, growth inhibition and promotion of apoptosis would occur preferentially in cancerous compared with noncancerous cells within the breast.

12. Inhibition of breast cancer cell proliferation and promotion of apoptosis by synthetic analogs of vitamin E

As discussed above, many studies have shown that α-tocopherol has little if any effect on proliferation [82,84,85, 86,89] or apoptosis [87,89,94] of breast cancer cells. In contrast, a number of studies have shown potent inhibition of proliferation [88,95,96] and promotion of apoptosis [94, 97,98,99] in several breast cancer cell lines by α-tocopheryl succinate and other vitamin E analogs. These effects are evident at concentrations as low as 20 mol/L [94,95,97,98, 99]. However, several lines of evidence suggest that the mechanism(s) accounting for these effects of α-tocopheryl succinate are quite different from any growth regulatory and proapoptotic effects of free α-tocopherol. First, hydrolysis and release of free α-tocopherol is not required for the growth inhibitory effect of α-tocopheryl succinate [88]. Second, cholesteryl succinate also inhibits growth of breast cancer cells, and does so at the same concentrations at which α-tocopheryl succinate is effective [88]. Third, another ester of α-tocopherol, α-tocopheryl acetate, does not influence apoptosis in breast cancer cells [94].

13. Is it feasible and safe for human beings to achieve plasma and tissue concentrations of tocotrienols that might regulate growth and apoptosis of breast cancer cells?

It is of interest to consider whether it would be feasible and safe for women (and men, who also can develop breast cancer, albeit with a lower incidence than women) to attempt to achieve plasma and tissue concentrations of tocotrienols that are similar to those that have been shown to inhibit proliferation of breast cancer cells and promote apoptosis in these cells in vitro. In humans not consuming supplements containing tocotrienols, plasma concentrations of total tocotrienols are less than 1 μmol/L [100,101,102]. However, after supplementation with a palm oil concentrate providing 200 mg of tocotrienol+tocopherol (15–20% α-tocopherol, 12–15% α-tocotrienol, 35–40% γ-tocotrienol, 25–30% δ-tocotrienol) for 4 weeks, plasma α- γ-, and δ-tocotrienol concentrations were increased to 8.14 ± 0.68, 7.46 ± 0.73, and 5.91 ± 0.67 μmol/L (mean ± SD) [100]. These plasma tocotrienol concentrations are similar to those that inhibit proliferation of estrogen receptor positive breast cancer cells in vitro by 50% and about one third those shown to promote apoptosis of 50% of estrogen receptor positive breast cancer cells (above). Importantly, these plasma concentrations of tocotrienols were achieved without any apparent adverse effect [100]. Other studies of supplementation of human individuals with palm oil tocotrienol concentrate have also reported no significant adverse effects of supplementation with up to 240 mg tocotrienol for up to 16 months duration [102,103,104]. Furthermore, as discussed above, it is likely that breast adipose tissue concentrations of tocotrienols will be five to ten-fold those in the plasma [46,54,66], so that lower levels of tocotrienol supplementation might be adequate to reach breast adipose tissue tocotrienol concentrations similar to those that inhibit proliferation and promote apoptosis in breast cancer cells in vitro. Thus, it appears to be both feasible and safe to increase plasma and breast adipose tissue concentrations of tocotrienols to levels that regulate growth and viability of breast cancer cells in vitro. Further studies will be needed to determine whether individuals that achieve such plasma and tissue tocotrienol concentrations are protected from development of breast cancer.

14. Conclusions

The modest apparent protection from breast cancer associated with dietary vitamin E inferred from studies that collected information on dietary intake is consistent with the idea that either vitamin E, or some dietary constituent that is correlated with vitamin E, may protect against breast cancer. The fact that no observational study has found supplemental vitamin E to be associated with any protection from breast cancer, and the lack of convincing evidence that higher plasma or tissue α-tocopherol concentrations protect against breast cancer, suggest that the active dietary constituent is not α-tocopherol, but instead some other dietary component that is correlated with α-tocopherol. Experiments in animals also provide little support for the notion that α-tocopherol protects against breast cancer. In contrast, studies in breast cancer cells in vitro indicate that the α- γ-, and δ- tocotrienol, and to a lesser extent δ-tocopherol, have potent antiproliferative and proapoptotic effects. Breast epithelial cell proliferation is a cardinal feature of mammary tumors and increased indices of proliferation of breast neoplasms are correlated with poor prognosis [105,106,107]. While plasma concentrations of tocotrienols are low, as discussed above, data indicate that for fatty tissues such as the breast, tissue concentrations of tocotrienols (and tocopherols) are higher than what might be expected based on plasma concentrations. Many vegetable sources of α-tocopherol also contain other tocopherols or tocotrienols.

Thus, it seems plausible that the modest protection from breast cancer associated with dietary vitamin E, but not supported by blood or tissue concentrations of α-tocopherol or duplicated by vitamin E supplements, may be due to the effects of other tocopherols and the tocotrienols in the diet.

15. Future directions

It seems clear that, for protection from breast cancer, focus on α-tocopherol may have been misguided. Data suggest that is it now time to reconsider the role of tocopherols and tocotrienols in prevention of breast cancer. The individual tocopherols and tocotrienols may well have different biological activities due in part to intrinsic effects on cellular function, as suggested above by their effects on breast cancer cells. Thus, it will be necessary to quantitate separately each of the four tocopherols and the four tocotrienols. Because past dietary studies have not aimed at separate assessment of these dietary constituents, it seems likely that this will require development of new instruments for dietary assessment.

Additionally, because the ability of tocopherols and tocotrienols to reduce risk of breast cancer is likely to be determined by their delivery to the breast, it will be important to determine concentrations of these dietary constituents in breast, and to relate these concentrations to dietary intake. Obviously, it is not feasible to propose to determine concentrations of tocopherols and tocotrienols in breast or breast adipose tissue of large numbers of women, particularly not in women who are free of disease. However, as discussed above, evidence suggests that concentrations of corresponding tocopherols and tocotrienols are similar for adipose tissue collected from different anatomic sites. Thus, it seems plausible that measures of adipose tissue tocopherol and tocotrienol concentration in peripheral fat depots may provide a useful surrogate that might provide a relatively accurate proxy for tocopherol and tocotrienol concentrations for breast adipose tissue, and possibly suggest the concentrations of these dietary components to which breast epithelial cells are exposed. Previous studies have sampled adipose tissue from the buttocks by fine needle aspiration [53,54,57,108,109] from conscious women. Evidence indicates that collecting adipose tissue in such a way is well tolerated and perceived as causing less discomfort than standard venipuncture [53].

In addition to relating dietary intake and tissue levels of tocopherols and tocotrienols to risk of breast cancer, it will be important to investigate the mechanism(s) by which these dietary constituents might be protective. Oxidative stress has been implicated in breast cancer [110,111,112, 113], and may influence breast cancer by altering gene expression [114] or by promoting oxidative DNA damage [112,113]. Evidence indicates that all tocopherols (α, β, γ, and δ) and tocotrienols (α, β, γ, and δ) have antioxidant activity [28,115]. However, relative antioxidant activity of these compounds in vivo remains to be determined. It will be relevant for future studies to investigate whether tissue tocopherol and tocotrienol status alters oxidative stress in breast tissue in vivo, and how such changes in oxidative stress are related to measures of tumor aggression, such as cellular proliferation. As discussed above, studies have shown the tocotrienols and δ-tocopherol to have potent growth regulatory and proapoptotic effects on breast cancer cells in vitro. With respect to cellular proliferation, previous studies have shown that in mammalian cells in vitro, tocotrienols inhibit the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase [116], the rate limiting enzyme of cholesterol synthesis, while other data suggest that this inhibitory effect would be likely to reduce cellular proliferation [117]. In addition, supplementation of human individuals with a tocotrienol rich palm oil fraction reduced plasma cholesterol concentrations [100], suggesting that 3-hydroxy-3-methylglutaryl-coenzyme A reductase might have been inhibited in these individuals. It will be appropriate for future studies to consider whether proliferative activity of breast epithelial cells can be regulated in vivo by the tocotrienols or δ-tocopherol, and the mechanism(s), perhaps including inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity, by which such effects occur. The data discussed above concerning the synergistic inhibition of breast cancer cell proliferation in vitro by the tocotrienols in combination with tamoxifen is particularly intriguing and potentially important and worthy of further investigation.

References

[1] P.J. Mclaughlin, J.L. Weihrauch, Vitamin E content of foods, J. Am. Diet. Assoc. 75 (1979) 647–665.
[2] A.M. Papas, Oil-soluble antioxidants in foods, Toxicol. Ind. Health. 9 (1993) 123–150.
[3] Subcommittee on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Vitamin E. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*, Panel on Dietary Antioxidants and Related Compounds, Institute of Medicine, National Academy Press, Washington, DC, 2000, pp. 186–283.
[4] S. Kijima, Chemistry of vitamin-E, In: M. Mino et al. (Eds.), Vitamin E—Its Usefulness in Health and in Curing Diseases, Japan Sci. Soc. Press, Tokyo/S. Karger, Basel, 1993, pp. 3–11.
[5] P. Khosla, K. Sundram, Effects of dietary fatty acid composition on plasma cholesterol, Prog. Lipid Res. 35 (1996) 93–132.
[6] P. Mata, O. Varela, R. Alonso, C. Lahoz, M. Deoya, L. Badimon, Monounsaturated and polyunsaturated n-6 fatty acid- enriched diets modify LDL oxidation and decrease human coronary smooth muscle cell DNA synthesis, Arterioscler. Thromb. Vasc. Biol. 17 (1997) 2088–2095.
[7] S. Tsimikas, A. Philistsimikas, S. Alexopoulos, F. Sigari, C. Lee, P.D. Reaven, LDL isolated from Greek subjects on a typical diet or from American subjects on an oleate-supplemented diet induces less monocyte chemotaxis and adhesion when exposed to oxidative stress, Arterioscler. Thromb. Vasc. Biol. 19 (1999) 122–130.
[8] C.M. Friedenreich, G.R. Howe, A.B. Miller, Recall bias in the association of micronutrient intake and breast cancer, J. Clin. Epidemiol. 46 (1993) 1009–1017.

[9] S. Mannisto, P. Pietinen, M. Virtanen, V. Kataja, M. Uusitupa, Diet and risk of breast cancer in a case-control study: does the threat of disease have on influence on recall bias?, J. Clin. Epidemiol. 52 (1999) 429–439.
[10] K. Bohlke, D. Spiegelman, A. Trichopoulou, K. Katsouyanni, D. Trichopoulos, Vitamins A, C, and E and the risk of breast cancer: results from a case-control study in Greece, Br. J. Cancer 79 (1999) 23–29.
[11] C.B. Ambrosone, J.R. Marshall, J.E. Vena, R. Laughlin, S. Graham, T. Nemoto, J.L. Freudenheim, Interaction of family history of breast cancer and dietary antioxidants with breast cancer risk (New York, United States), Cancer Causes & Control 6 (1995) 407–415.
[12] A. Ronco, E. Destefani, P. Boffetta, H. Deneopellegrini, M. Mendilaharsu, F. Leborgne, Vegetables, fruits, and related nutrients and risk of breast cancer: a case-control study in Uruguay, Nutrition & Metabolism 35 (1999) 111–119.
[13] S.J. London, E.A. Stein, I.C. Henderson, M.J. Stampfer, W.C. Wood, S. Remine, J.R. Dmochowski, N.J. Robert, W.C. Willet, Carotenoids, retinol, and vitamin E and risk of proliferative benign breast disease and breast cancer, Cancer Causes & Control 3 (1992) 503–512.
[14] J.L. Freudenheim, J.R. Marshall, J.E. Vena, R. Laughlin, J.R. Brasure, M.K. Swanson, T. Nemoto, S. Graham, Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients, J. Natl. Cancer Inst. 88 (1996) 340–348.
[15] M. Mezzetti, C. Lavecchia, A. Decarli, P. Boyle, R. Talamini, S. Franceschi, Population attributable risk for breast cancer: diet, nutrition, and physical exercise, J. Natl. Cancer Inst. 90 (1998) 389–394.
[16] S. Richardson, M. Gerber, S. Cenee, The role of fat, animal protein and some vitamin consumption in breast cancer: a case control study in southern France, Int. J. Cancer 48 (1991) 1–9.
[17] S. Zhang, D.J. Hunter, M.R. Forman, B.A. Rosner, F.E. Speizer, G.A. Colditz, J.E. Manson, S.E. Hankinson, W.C. Willet, Dietary carotentoids and vitamins A, C, and E and risk of breast cancer, J. Natl. Cancer Inst. 91 (1999) 547–556.
[18] C. Braga, C. Lavecchia, E. Negri, S. Franceschi, M. Parpinel, Intake of selected foods and nutrients and breast cancer risk: an age- and menopause-specific analysis, Nutr. Cancer 28 (1997) 258–263.
[19] M. Gerber, F. Cavallo, E. Marubini, S. Richardson, A. Barbieri, E. Capitelli, A. Costa, A. Crastes de Paulet, P. Crastes de Paulet, A. Decarli, U. Pastorino, H. Pujol, Liposoluble vitamins and lipid parameters in breast cancer. A joint study in northern Italy and southern France, Int. J. Cancer 42 (1988) 489–494.
[20] T.E. Rohan, G.R. Howe, C.M. Friedenreich, M. Jain, A.B. Miller, Dietary fiber, vitamins A, C, and E, and risk of breast cancer: a cohort study, Cancer Causes & Control 4 (1993) 29–37.
[21] D.J. Hunter, J.E. Manson, G.A. Colditz, M.J. Stampfer, B. Rosner, C.H. Hennekens, F.E. Speizer, W.C. Willett, W.C., A prospective study of the intake of vitamin-C, vitamin-E, and vitamin-A and the risk of breast cancer, New Engl. J. Med. 329 (1993) 234–240.
[22] L.H. Kushi, R.M. Fee, T.A. Sellers, W. Zheng, A.R. Folsom, Intake of vitamins A, C, and E and postmenopausal breast cancer—The Iowa Women's Health Study, Am. J. Epidemiol. 144 (1996) 165–174.
[23] A. Shibata, A. Paganini-Hill, R.K. Ross, B.E. Henderson, Intake of vegetables, fruits, beta-carotene, vitamin-c and vitamin supplements and cancer incidence among the elderly—a prospective study, Br. J. Cancer. 66 (1992) 673–679.
[24] K.B. Michels, L. Holmberg, L. Bergkvist, H. Ljung, A. Bruce, A. Wolk, Dietary antioxidant vitamins, retinol, and breast cancer incidence in a cohort of Swedish women, Int. J. Cancer 91 (2001) 563–567.
[25] R. Jarvinen, P. Knekt, R. Seppanen, L. Teppo, Diet and breast cancer risk in a cohort of Finnish women, Cancer Lett. 114 (1997) 251–253.
[26] Z. Zhu, M. Parviainen, S. Mannisto, P. Pietinen, M. Eskelinen, K. Syrjanen, M. Uusitupa, Vitamin E concentration in breast adipose tissue of breast cancer patients (Kuopio, Finland), Cancer Causes & Control 7 (1996) 591–595.
[27] Subcommittee on the Tenth Edition of the RDAs, Food and Nutrition Board Commission on Life Sciences, National Research Council, Vitamin E, in Recommended Dietary Allowances, National Academy Press, Washington, DC, 1989, pp. 99–107.
[28] A. Kamaleldin, L.A. Appelqvist, The chemistry and antioxidant properties of tocopherols and tocotrienols, Lipids 31 (1996) 671–701.
[29] B.J. Weimann, H. Weiser, Functions of vitamin E in reproduction and in prostacyclin and immunoglobulin synthesis in rats, Am. J. Clin. Nutr. 53 (1991) 1056S–1060S.
[30] H. Bartsch, J. Nair, R.W. Owen, Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: emerging evidence for their role as risk modifiers, Carcinogenesis 20 (1999) 2209–2218.
[31] M. Gerber, S. Richardson, P. Crastes de Paulet, H. Pujol, A. Crastes de Paulet, Relationship between vitamin E and polyunsaturated fatty acids in breast cancer. Nutritional and metabolic aspects, Cancer 64 (1989) 2347–2353.
[32] D.T.H. Verhoeven, N. Assen, R.A. Goldbohm, E. Dorant, P. van't Veer, F. Sturmans, R.J.J. Hermus, P.A. Vandenbrandt, Vitamins C and E, retinol, beta carotene and dietary fibre in relation to breast cancer risk: a prospective cohort study, Br. J. Cancer 75 (1997) 149–155.
[33] M. Gerber, S. Richardson, F. Cavallo, E. Marubini, P. Crastes de Paulet, A. Crastes de Paulet, H. Pujol, H., The role of diet history and biologic assays in the study of "diet and breast cancer", Tumori 76 (1990) 321–330.
[34] T.K. Basu, G.B. Hill, D. Ng, E. Abdi, N. Temple, Serum vitamins A and E, beta-carotene, and selenium in patients with breast cancer, J. Am. Coll. Nutr. 8 (1989) 524–528.
[35] M. Gerber, S. Richardson, R. Salkeld, P. Chapuis, Antioxidants in female breast cancer patients, Cancer Invest. 9 (1991) 421–428.
[36] M. Torun, S. Akgul, H. Sargin, Serum vitamin E in patients with breast cancer, J. Clin. Pharm. Therapeutics 20 (1995) 173–178.
[37] P. Knekt, Serum vitamin E level and risk of female cancers, Int. J. Epidemiol. 17 (1988) 281–286.
[38] P. Knekt, A. Aromaa, J. Maatela, R.K. Aaran, T. Nikkari, M. Hakama, T. Hakulinen, R. Peto, L. Teppo, Vitamin-E and cancer prevention, Am. J. Clin. Nutr. 53 (1991) S283–S286.
[39] N.J. Wald, J. Boreham, J.L. Hayward, R.D. Bulbrook, R.D., Plasma retinol, beta-carotene and vitamin E levels in relation to the future risk of breast cancer, Br. J. Cancer 49 (1984) 321–324.
[40] M.J. Russell, B.S. Thomas, R.D. Bulbrook, A prospective study of the relationship between serum vitamins A and E and risk of breast cancer, Br. J. Cancer 57 (1988) 213–215.
[41] G.W. Comstock, A.J. Alberg, K.J. Helzlsouer, Reported effects of long-term freezer storage on concentrations of retinol, beta-carotene, and alpha- tocopherol in serum or plasma summarized, Clin. Chem. 39 (1993) 1075–1078.
[42] M.C. Ocke, J. Schrijver, G.L. Obermanndeboer, B.P.M. Bloemberg, G.R.M.M. Haenen, D. Kromhout, Stability of blood (pro)vitamins during four years of storage at -20 degrees C: consequences for epidemiologic research, J. Clin. Epidemiol. 48 (1995) 1077–1085.
[43] T. Ogihara, M. Miki, M. Kitagawa, M. Mino, Distribution of tocopherol among human plasma lipoproteins, Clin. Chim. Acta 174 (1988) 299–305.
[44] W. Behrens, R. Madere, Alpha and gamma tocopherol concentrations in human serum, J. Am. Coll. Nutr. 5 (1986) 91–96.
[45] J. Lehmann, M.W. Marshall, H.T. Slover, J.M. Iacono, Influence of dietary fat level and dietary tocopherols on plasma tocopherols of human subjects, J. Nutr. 107 (1977) 1006–1015.
[46] K.C. Hayes, A. Pronczuk, J.S. Liang, Differences in the plasma transport and tissue concentrations of tocopherols and tocotrienols—observations in humans and hamsters, Proc. Soc. Exp. Biol. Med. 202 (1993) 353–359.

[47] K.J. Yeum, S.H. Ahn, S.A. Rupp de Paiva, Y.C. Lee-Kimm, N.I. Krinsky, R.M. Russell, Correlation between carotenoid concentrations in serum and normal breast adipose tissue of women with benign breast tumor or breast cancer, J. Nutr. 128 (1998) 1920–1926.
[48] M.S. Simon, Z. Djuric, B. Dunn, D. Stephens, S. Lababidi, L.K. Heilbrun, An evaluation of plasma antioxidant levels and the risk of breast cancer: a pilot case control study, The Breast Journal 6 (2000) 388–395.
[49] G. Ray, S.A. Husain, Role of lipids, lipoproteins and vitamins in women with breast cancer, Clin. Biochem. 34 (2000) 71–76.
[50] G.W. Comstock, K.J. Helzlsouer, T.L. Bush, Prediagnostic serum levels of carotenoids and vitamin-E as related to subsequent cancer in Washington County, Maryland, Am. J. Clin. Nutr. 53 (1991) S260–S264.
[51] J.F. Dorgan, A. Sowell, C.A. Swanson, N. Potischman, R. Miller, N. Schussler, H.E. Stephenson, Jr., Relationship of serum carotenoids, retinol, alpha-tocopherol, and selenium with breast cancer risk: results from a prospective study in Columbia, Missouri (United States), Cancer Causes & Control 9 (1998) 89–97.
[52] V. Chajes, C. Lhuillery, W. Sattler, G.M. Kostner, P. Bougnoux, Alpha-tocopherol and hydroperoxide content in breast adipose tissue from patients with breast tumors, Int. J. Cancer 67 (1996) 170–175.
[53] L. Kohlmeier, M. Kohlmeier, Adipose tissue as a medium for epidemiologic exposure assessment, Environ. Health Perspect. 103 (Suppl 3) (1995) 99–106.
[54] M. Ohrvall, S. Tengblad, B. Vessby, Tocopherol concentrations in adipose tissue. Relationships of tocopherol concentrations and fatty acid composition in serum in a reference population of Swedish men and women, Eur. J. Clin. Nutr. 48 (1994) 212–218.
[55] G.J. Handelman, W.L. Epstein, J. Peerson, D. Spiegelman, L.J. Machlin, E.A. Dratz, Human Adipose alpha-tocopherol and gamma-tocopherol kinetics during and after 1 y of alpha-tocopherol supplementation, Am. J. Clin. Nutr. 59 (1994) 1025–1032.
[56] R. Muggli, Physiological requirements of vitamin E as a function of the amount and type of polyunsaturated fatty acid, World Rev. Nutr. Diet. 75 (1994) 166–168.
[57] P. van't Veer, J.J. Strain, J. Fernandez-Crehuet, B.C. Martin, M. Thamm, A.F. Kardinaal, L. Kohlmeier, J.K. Huttunen, J.M. Martin-Moreno, F.J. Kok, Tissue antioxidants and postmenopausal breast cancer: the European Community Multicentre Study on Antioxidants, Myocardial Infarction, and Cancer of the Breast (EURAMIC), Cancer Epidem. Biomarkers Prevent. 5 (1996) 441–447.
[58] M. Rautalahti, D. Albanes, L. Hyvonen, V. Piironen, M. Heinonen, Effect of sampling site on retinol, carotenoid, tocopherol, and tocotrienol concentration of adipose tissue of human breast with cancer, Ann. Nutr. Metab. 34 (1990) 37–41.
[59] H. Langemann, J. Torhorst, A. Kabiersch, W. Krenger, C.G. Honegger, Quantitative determination of water- and lipid-soluble antioxidants in neoplastic and non-neoplastic human breast tissue, Int. J. Cancer 43 (1989) 1169–1173.
[60] M.G. Traber, Regulation of human plasma vitamin E, Adv. Pharmacol. 38 (1997) 49–63.
[61] S.P. Yap, K.H. Yuen, J.W. Wong, Pharmacokinetics and bioavailability of alpha-, gamma- and delta-tocotrienols under different food status, J. Pharm. Pharmacol. 53 (2001) 67–71.
[62] M.G. Traber, Determinants of plasma vitamin E concentrations, Free Radic. Biol. Med. 16 (1994) 229–239.
[63] G.J. Handelman, L.J. Machlin, K. Fitch, J.J. Weiter, E.A. Dratz, Oral alpha-tocopherol supplements decrease plasma beta and gamma tocopherol levels in humans, J. Nutr. 115 (1985) 807–813.
[64] R.A. Shipley, R.E. Clark, Tracer methods for in vivo kinetics, Academic Press, New York, 1972, pp. 1–233.
[65] P.R. Palan, G.L. Goldberg, J. Basu, C.D. Runowicz, S.L. Romney, Lipid-soluble antioxidants: beta-carotene and alpha-tocopherol levels in breast and gynecological cancers, Gynecol, Oncol, 55 (1994) 72–77.
[66] N.V. Dimitrov, R.Q. Pan, J. Bauer, T.I. Jones, Some aspects of vitamin E related to humans and breast cancer prevention, Adv. Exp. Med. Biol. 364 (1994) 119–127.
[67] E.L. Syvaoja, V. Piironen, P. Varo, P. Koivistoinen, K. Salminen, Tocopherols and tocotrienols in Finnish foods: human milk and infant formulas, Internat. J. Vit. Nutr. 55 (1985) 159–166.
[68] G.W. Burton, M.G. Traber, R.V. Acuff, D.N. Walters, H. Kayden, L. Hughes, K.U. Ingold, Human plasma and tissue alpha-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitamin E, Am. J. Clin. Nutr. 67 (1998) 669–684.
[69] K. Ishii, L.X. Zhen, D.H. Wang, Y. Funamori, K. Ogawa, K. Taketa, Prevention of mammary tumorigenesis in acatalasemic mice by vitamin E supplementation, Jpn. J. Cancer Res. 87 (1996) 680–684.
[70] C. Ip, Dietary vitamin E intake and mammary carcinogenesis in rats, Carcinogenesis 3 (1982) 1453–1456.
[71] P.M. Horvath, C. Ip, Synergistic effect of vitamin E and selenium in the chemoprevention of mammary carciongenesis in rats, Cancer Res. 43 (1983) 5335–5341.
[72] M. Beth, M.R. Berger, M. Aksoy, D. Schmahl, Effects of vitamin A and E supplementation to diets containing two different fat levels on methylnitrosourea-induced mammary carcinogenesis in female SD-rats, Br. J. Cancer 56 (1987) 445–449.
[73] M. Hirose, A. Masuda, T. Inoue, S. Fukushima, N. Ito, Modification by antioxidants and p,p′-diaminodiphenylmethane of 7,12-dimethylbenz[a]anthracene-induced carcinogenesis of the mammary gland and ear duct in CD rats, Carcinogenesis 7 (1986) 1155–1159.
[74] M.N. Gould, J.D. Haag, W.S. Kennan, M.A. Tanner, C.E. Elson, A comparison of tocopherol and tocotrienol for the chemoprevention of chemically induced rat mammary tumors, Am. J. Clin. Nutr. 53 (1991) 1068S–1070S.
[75] S. Cognault, M.L. Jourdan, E. Germain, R. Pitavy, E. Morel, G. Durand, P. Bougnoux, C. Lhuillery, Effect of an alpha-linolenic acid-rich diet on rat mammary tumor growth depends on the dietary oxidative status, Nutr. Cancer 36 (2000) 33–41.
[76] A. Hagiwara, P. Boonyaphiphat, H. Tanaka, M. Kawabe, S. Tamano, H. Kaneko, M. Matsui, M. Hirose, N. Ito, T. Shirai, Organ-specific modifying effects of caffeine, and two naturally occurring antioxidants alpha-tocopherol and n-tritriacontane-16,18-dione, on 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced mammary and colonic carciongenesis in female F344 rats, Jpn. J. Cancer Res. 90 (1999) 399–405.
[77] C. Ip, G. White, Mammary cancer chemoprevention by inorganic and organic selenium: single agent treatment or in combination with vitamin E and their effects on in vitro immune functions, Carcinogenesis 8 (1987) 1763–1766.
[78] C. Ip, Feasibility of using lower doses of chemopreventive agents in a combination regimen for cancer protection, Cancer Lett. 39 (1988) 239–246.
[79] H. Takada, T. Hirooka, T. Hatano, Y. Hamada, M. Yamamoto, Inhibition of 7,12-Dimethylbenz⟨a⟩anthracene-induced lipid peroxidation and mammary tumor development in rats by vitamin-E in conjunction with selenium, Nutr. Cancer. 17 (1992) 115–122.
[80] C. Ip, Attenuation of the anticarciongenic action of selenium by vitamin E deficiency, Cancer Lett. 25 (1985) 325–331.
[81] K. Nesaretnam, H.T. Khor, J. Ganeson, Y.H. Chong, K. Sundram, A. Gapor, The effect of vitamin E tocotrienols from palm oil on chemically induced mammary carciongeneis in female rats, Nutr. Res. 12 (1992) 879–892.
[82] K. Nesaretnam, R. Stephen, R. Dils, P. Darbre, Tocotrienols inhibit the growth of human breast cancer cells irrespective of estrogen receptor status, Lipids 33 (1998) 461–469.
[83] N. Guthrie, A. Gapor, A.F. Chambers, K.K. Carroll, Inhibition of proliferation of estrogen receptor-negative MDA-MB- 435 and -positive MCF-7 human breast cancer cells by palm oil tocotrienols and tamoxifen, alone and in combination, J. Nutr. 127 (1997) S544–S548.

[84] K. Nesaretnam, S. Dorasamy, P.D. Darbre, Tocotrienols inhibit growth of ZR-75–1 breast cancer cells, Int. J. Food Sci. Nutr. 51 (Suppl) (2000) S95–S103.
[85] K. Nesaretnam, N. Guthrie, A.F. Chambers, K.K. Carroll, Effect of tocotrienols on the growth of a human breast cancer cell line in culture, Lipids 30 (1995) 1139–1143.
[86] U. Gundimeda, Z.H. Chen, R. Gopalakrishna, Tamoxifen modulates protein kinase C via oxidative stress in estrogen receptor-negative breast cancer cells, J. Biol. Chem. 271 (1996) 13504–13514.
[87] G. Sigounas, A. Anagnostou, M, Steiner, dl-alpha-tocopherol induces apoptosis in erythroleukemia, prostate, and breast cancer cells, Nutr. Cancer 28 (1997) 30–35.
[88] Z. Djuric, L.K. Heilbrun, S. Lababidi, C.K. Evertt-Bauer, M.W. Fariss, Growth inhibition of MCF-7 and MCF-10A human breast cancer cells by alpha-tocophyeryl hemisuccinate, cholesterol hemisuccinate, cholesteryl hemisuccinate and their ether analogs, Cancer Lett. 111 (1997) 133–139.
[89] B.S. McIntyre, K.P. Briski, A. Gapor, P.W. Sylvester, Antiproliferative and apoptotic effects of tocopherols and tocotrienols on preneoplastic and neoplastic mouse mammary epithelial cells, Exp. Biol. Med. 224 (2000) 292–301.
[90] H. Mo, C.E. Elson, Apoptosis and cell-cycle arrest in human and murine tumor cells are initiated by isoprenoids, J. Nutr. 129 (1999) 804–813.
[91] A. Azzi, I. Breyer, M. Feher, R. Ricciarelli, A. Stocker, S. Zimmer, J. Zingg, Nonantioxidant functions of alpha-tocopherol in smooth muscle cells, J. Nutr. 131 (2001) 378S–381S.
[92] A. Azzi, I. Breyer, M. Feher, M. Pastori, R. Ricciarelli, S. Spycher, M. Staffieri, A. Stocker, S. Zimmer, J.M. Zingg, Specific cellular responses to alpha-tocopherol, J. Nutr. 130 (2000) 1649–1652.
[93] E. Chatelain, D.O. Boscoboinik, G.M. Bartoli, V.E. Kagan, F.K. Gey, L. Packer, A. Azzi, Inhibition of smooth muscle cell proliferation and protein kinase-C activity by tocopherols and tocotrienols, Biochim. Biophys. Acta. 1176 (1993) 83–89.
[94] W. Yu, M. Simmons-Menchaca, A. Gapor, B.G. Sanders, K. Kline, Induction of apoptosis in human breast cancer cells by tocopherols and tocotrienols, Nutr. Cancer 33 (1999) 26–32.
[95] J.M. Turley, F.W. Ruscetti, S.J. Kim, T. Fu, F.V. Gou, M.C. Birchenall-Roberts, Vitamin E succinate inhibits proliferation of BT-20 human breast cancer cells: increased binding of cyclin a negatively regulates e2f transactivation activity, Cancer Res. 57 (1997) 2668–2675.
[96] A. Charpentier, S. Groves, M. Simmons-Menchaca, J. Turley, B. Zhao, B.G. Sanders, K. Kline, RRR-alpha-tocopheryl succinate inhibits proliferation and enhances secretion of transforming growth factor-beta (TGF-beta) by human breast cancer cells, Nutr. Cancer 19 (1993) 225–239.
[97] J.M. Turley, T. Fu, F.W. Ruscetti, J.A. Mikovits, D.C. Bertolette, M.C. Birchenall-Roberts, Vitamin E succinate induces fas-mediated apoptosis in estrogen receptor-negative human breast cancer cells, Cancer Res. 57 (1997) 881–890.
[98] B.H. Zhao, W.P. Yu, M. Qian, M. Simmonsmenchaca, P. Brown, M.J. Birrer, B.G. Sanders, K. Kline, Involvement of activator protein-1 (AP-1) in induction of apoptosis by vitamin e succinate in human breast cancer cells, Mol. Carcinogen. 19 (1997) 180–190.
[99] W. Yu, M. Simmons-Menchaca, H.H. You, P. Brown, M.J. Birrer, B.G. Sanders, K. Kline, RRR-alpha-tocopheryl sucinate induction of prolonged activation of c-jun amino-termonal kinase and c-jun during induction of apoptosis in human MDA-MB-435 breast cancer cells, Mol Carcinogenesis 22 (1998) 247–257.
[100] A.A. Qureshi, N. Qureshi, J.J.K. Wright, Z. Shen, G. Kramer, A. Gapor, Y.H. Chong, G. DeWitt, A.S.H. Ong, D.M. Peterson, B.A. Bradlow, Lowering of serum cholesterol in hypercholesterolemic humans by tocotrienols (Palmvitee), Am. J. Clin. Nutr. 53 (1991) S1021–S1026.
[101] C.K. Chow, Distribution of tocopherols in human plasma and red blood cells, Am. J. Clin. Nutr. 28 (1975) 756–760.
[102] M.L. Wahlqvist, Z. Krivokuca-Bogetic, C.S. Lo, H. Hage, R. Smith, W. Lukito, Differential serum responses of tocopherols and tocotrienols during vitamin supplementation in hypercholesterolaemic individuals without changes in coronary risk factors, Nutr. Res. 12 (Suppl 1) (1992) S181–S201.
[103] A.A. Qureshi, B.A. Bradlow, L. Brace, J. Manganello, D.M. Peterson, B.C. Pearce, J.J.K. Wright, A. Gapor, C.E. Elson, Response of hypercholesterolemic subjects to administration of tocotrienols, Lipids 30 (1995) 1171–1177.
[104] A.C. Tomeo, M. Geller, T.R. Watkins, A. Gapor, M.L. Bierenbaum, Antioxidant effects of tocotrienols in patients with hyperlipidemia and carotid stenosis, Lipids 30 (1995) 1179–1183.
[105] C.A. Lockwood, C. Ricciardelli, W.A. Raymond, R. Seshadri, K. McCaul, D.J. Horsfall, A simple index using video image analysis to predict disease outcome in primary breast cancer, Int. J. Cancer 84 (1999) 203–208.
[106] S. Masood, M.M. Bui, L. Lu, Comparison of proliferative activity in breast carcinoma by flow cytometry analysis of S-phase and quantative analysis of MIB-1, Ann. Clin. Lab. Sci. 28 (1998) 315–323.
[107] J.S. Ross, J.A. Fletcher, HER-2/neu (c-erb-B2) gene expression and protein in breast cancer, Am. J. Clin. Pathol. 112 (1 Suppl 1) (1999) S53–S67.
[108] L. Kohlmeier, N. Simonsen, P. van't Veer, J.J. Strain, J.M. Martin-Moreno, B. Margolin, J.K. Huttunen, J.F.-C. Navajas, B.C. Martin, M. Thamm, A.F.M. Kardinaal, F.J. Kok, Adipose tissue trans fatty acids and breast cancer in the European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer, Cancer Epidem. Biomarkers Prevent. 6 (1997) 705–710.
[109] S.M. Virtanen, P. Vantveer, F. Kok, A.F.M. Kardinaal, A. Aro, Predictors of adipose tissue carotenoid and retinol levels in nine countries: the EURAMIC study, Am. J. Epidemiol. 144 (1996) 968–979.
[110] N.F. Boyd, V. McGuire, The possible role of lipid peroxidation in breast cancer risk, Free Radic. Biol. Med. 10 (1991) 185–190.
[111] Y.L. Huang, J.Y. Sheu, T.H. Lin, Association between oxidative stress and changes of trace elements in patients with breast cancer, Clin. Biochem. 32 (1999) 131–136.
[112] D. Li, W. Zhang, A.A. Sahin, W.N. Hittelman, DNA adducts in normal tissue adjacent to breast cancer: a review, Cancer Detect. Prev. 23 (1999) 454–462.
[113] A. Matsui, T. Ikeda, H. Enomoto, K. Hosoda, H. Nakashima, K. Omae, M. Watanabe, T. Hibi, M. Kitajima, Increased formation of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, in human breast cancer tissue and its relationship to GSTP1 and COMT genotypes, Cancer Lett. 151 (2000) 87–95.
[114] J.Y. Lee, S.S. Galoforo, C.M. Berns, J.C. Chen, B.H. Davis, J.E. Sim, P.M. Corry, D.R. Spitz, Glucose deprivation-induced cytotoxicity and alterations in mitogen-activated protein kinase activation are mediated by oxidative stress in multidrug-resistant human breast carcinoma cells, J. Biol. Chem. 273 (1998) 5294–5299.
[115] C. Suarna, R.T. Dean, R. Stocker, The reactivity of tocotrienols and other lipid-soluble antioxidants towards peroxyl radicals. In: A.S.H. Ong, L. Packer (Eds.), Lipid-Soluble Antioxidants: Biochemistry and Clinical Applications, Birkhauser Verlag, Basel, Switzerland, 1992, pp. 17–26.
[116] R.A. Parker, B.C. Pearce, R.W. Clark, D.A. Gordon, J.J.K. Wright, Tocotrienols regulate cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, J. Biol. Chem. 268 (1993) 11230–11238.
[117] O. Larsson, H. Blegen, Regulatory role of mevalonate in the growth of normal and neoplastic human mammary epithelial cells, Anticancer Res. 13 (1993) 1075–1079.
[118] S. Graham, M. Zielezny, J. Marshall, R. Priore, J. Freudenheim, J. Brasure, B. Haughey, P. Nasca, M. Zdeb, M., Diet in the epidemiology of postmenopausal breast cancer in the New York State Cohort, Am. J. Epidemiol. 136 (1992) 1327–1337.