Antioxidants as Chemopreventive Agents for Breast Cancer

MICHAEL J. GONZALEZ, DSC, PHD, FACN, NEIL H. RIORDAN, RPA, HUGH D. RIORDAN, MD

Summary: In the last decade the simplistic, stepwise concept of initiation, promotion, and progression has matured to a better understanding of the genotypic changes that ultimately lead to cancer. The hypothesis has been proposed that it may be possible to intervene pharmacologically during this process and that carcinogenesis may be arrested or reversed by synthetic or naturally occurring agents.

Evidence supports the potential role of antioxidant agents in cancer prevention. Basic research has demonstrated that antioxidants can trap organic free radicals and prevent tissue damage. Although observational epidemiologic studies are not entirely consistent, many show an inverse association between dietary intake or blood levels of antioxidants contained in foods and subsequent cancer risk. The Breast Cancer Prevention Trial (BCPI) recently concluded that the antioxidant drug tamoxifen (Nolvadex) which was being evaluated for its potential ability to prevent primary breast cancer among high-risk candidates reduces breast cancer risk by 45% among women younger than 50. Long term side effects have not been evaluated.

A current interest has arisen in utilizing a strategy known as chemoprevention in breast cancer prevention. Chemoprevention is defined as the inhibition or reversal of carcinogenesis by the use of non-cytotoxic nutrients or pharmacologic compounds that protect against the development and progression of mutant clones of malignant cells [1]. The goal of any cancer chemoprevention strategy is to reduce cancer incidence while producing minimal to no side effects.

Cancer Prevention Agents: From Drugs to Vitamins

A range of compounds, from synthetic drugs to naturally occurring micronutrients in the diet, have been proposed as cancer prevention agents [2-5]. Epidemiologic studies have suggested that some antioxidants agents as well dietary constituents with antioxidant properties may be acting as naturally occurring cancer prevention agents and may explain some of the differences in cancer incidence seen in populations with varying dietary intake [6-9]. In vitro and in vivo laboratory studies have supported this concept [10]. These areas of research have led to human trials testing the effect of potential cancer prevention agents on cancer incidence and/or potential biomarkers of carcinogenesis. Although the field of clinical cancer chemoprevention is in its infancy and still evolving, there has been a general consensus among investigators that potential chemoprevention agents identified in epidemiologic and laboratory studies should proceed through clinical evaluation [11-13]. The clinical development of cancer chemoprevention agents is similar to that of cytotoxic agents. On the basis of promising preclinical studies, agents with potential efficacy are introduced to the clinic as a phase I trial to study the dose-toxicity relationship of the agent and its human pharmacology.

In 1992 the National Cancer Institute (NCI) funded the Breast Cancer Prevention Trial (BCPI). In this large-scale, multi-center trial the drug tamoxifen (Nolvadex) is being evaluated for its potential ability to prevent primary breast cancer among high-risk candidates [14]. Of 16,000 women that will be randomized to receive tamoxifen or placebo for a period of 5 years, over 11,000 women have entered the trial to date [15]. A recent press release indicates that tamoxifen reduces breast cancer risk by 45% among women younger than 50. Long term side effects have not been evaluated.

Tamoxifen and Breast Cancer

The nonsteroidal agent tamoxifen has been studied and used as an adjuvant agent in the treatment of primary breast cancer since the 1970's [16]. Tamoxifen was selected as a potential chemopreventive agent in breast cancer for several reasons: first, because it exhibits antiestrogenic properties and increases the production of steroid-binding globulin. It inhibits protein kinase C, which is considered to be one of its extra-estrogens receptor site of action. Tamoxifen also decreases insulin-like growth factor-1, a stimulator of breast cancer growth; down-regulates transforming growth factor-a, an enhancer of tumor growth; and induces transforming growth factor-b; [17-21]. As an antioxidant, tamoxifen inhibits the chain reactions of lipid peroxidation and acts a scavenger of

Address correspondence to Michael J, Gonzalez, DSc, PhD
University of Puerto Rico , Medical Sciences Campus , School of public Health,PO Box 365067, San Juan Puerto Rico 00936-5067

free radicals in vitro. Tamoxifen was found to act as an efficient membrane antioxidant with an IC50-value (26 microM). Treatment of female Sprague-Dawley rats with tamoxifen (12 or 48 mg/kg) was found to decrease serum levels of lipid peroxides.[22]

When used clinically as an adjuvant treatment in primary breast cancer and taken for longer tan 2 years, tamoxifen reduces the risk of development of contralateral breast malignancy, with few known side effects as compared to chemotherapeutic agents [17]. In addition to its anti-cancer activities, protective antioxidant action of tamoxifen on low-density lipoprotein has been shown to reduce mortality risks from myocardial infarction, reduce serum cholesterol and fibrinogen, and protect against post-menopausal bone loss of the lumbar spine [23-25].

Although the side effects of tamoxifen were deemed to be minimal prior to the start of BCPT, one of the objectives of the study is to evaluate the long term toxicity and side effects and to assess the risk to benefit ratio of its long term use. To date, the relatively mild side effects of tamoxifen use that have been include: reduced libido; menopausal symptoms, menstrual irregularities (amenorrhea, irregular menses, and vaginal bleeding), visual disturbances (corneal changes, retinopathy and cataracts), inflammatory polyarthritis, ovarian cysts , endometriosis and endometrial proliferation, depression, and asthma. [26-28]

Additional side effects with life-threatening potential reported in women using tamoxifen include: blood clotting disorders; agranulocytosis, pancytopenia, thrombocytopenia, hemolytic anemia, and leukemia, increased risk of endometrial carcinoma, hepatocellular damage and fatal hepatic failure [29-30]. A Swedish trial reported 3 cases of liver cancer in women using 40mg/day of tamoxifen for more than 2 years versus 1 case in the control group [31]. Another Swedish trial found a nearly six-fold increase in endometrial cancers, and a threefold increase in gastrointestinal cancers in postmenopausal patients undergoing tamoxifen therapy for early stage breast cancer. It has been pointed out that patients receiving tamoxifen for less tan seven years may not have had sufficient exposure time for the drug to induce liver cancer [32]. Because of the reported side effects of tamoxifen, and long-term risk to benefit ratio, other chemopreventive agents and/or strategies for breast cancer are being considered.

Dietary intake of several macronutrients with antioxidant properties such as selenium, carotenoids, and vitamins A, C, and the tocopherols and tocotrienols (vitamin E), have been postulated to decrease the risk of breast cancer in certain case-control and follow-up investigations [33]. Epidemiological studies have not confirmed this hypothesis for breast cancer, possibly because of methodological limitations, although in a number of studies, inverse associations have been observed between antioxidants and the risk of various cancers most notably squamous epithelial cell cancers of the upper aerodigestive tract, lung, breast, intestinal tract, and cervix. In contrast, several recent clinical trials designed lo test the effectiveness of antioxidants in preventing certain of these cancers have not shown protective effects [34].

Oxidative Damage

Molecular oxygen and its reaction products, including the superoxide radical and hydrogen peroxide, can cause injury to biological organisms through a variety of mechanisms.

Oxidative damage to DNA may result in protooncogene activation and/or tumor suppressor gene inactivation [35], and these events are likely to accumulate with age.

Free radical alterations of unsaturated lipids in cell membranes may result in loss of membrane fluidity, and can lead to cell lysis. In addition, the oxidative degradation of polyunsaturated fatty acids has been shown to create aldehydes such as malondialdehyde, which can cause cross-linking in nucleic acids, proteins, and lipids [36]. Oxidative damage to enzymes and proteins could lead to enzyme inactivation, cross-linking, and denaturation. Oxidative damage to carbohydrates can result in alteration of cellular receptor function. Molecular oxygen reaction products appear to activate the enzyme protein kinase C which participates in the modulation of growth-related genes [37].

Molecular defense mechanisms which protect against oxidation include enzymes such as glutathione peroxidase, a selenium-dependent free radical scavenger, and smaller molecules such as the carotenoids, ascorbic acid and the tocopherols and tocotrienols with vitamin E activity [35-37]. These anti-oxidants are proposed to protect cells and their molecular components from oxidative stress by scavenging free radicals and quenching lipid peroxidation chain reactions [38]. Recent laboratory research has indicated, that some carotenoids and tocopherols may have peroxidant activity under certain conditions, such as an oxygen-rich environment.

Carotenoids

Between five and ten carotenoids with antioxidant activity have been analyzed in the blood of human subjects, including lycopene, lutein, cryptoxanthin, alpha-carotene. and beta-carotene [39]. Basic research has demonstrated that b -carotene can trap organic free radicals and/or deactivate excited oxygen molecules.

The antioxidant activities of these carotenoids appears to derive from their ability to form a carboncentered carotenoid radical which is resonance stabilized by the carotenoid's conjugated double bond system. The provitamin A carotenoids (alpha- beta-, and gamma-carotenes and cryptoxanthin) also may function in cancer prevention through conversion to retinol and retinyl esters in the intestine and possibly in the liver [40]. Retinol deficiency causes dietary ß-carotene to convert to preformed vitamin A, or retinol. If very low retinol levels are related to carcinogenesis, ß-carotene could indirectly play a role in cancer prevention. In well-nourished populations, however, most dietary carotene is absorbed directly from the intestine without undergoing transformation to retinol. Dietary sources of carotenoids are dark green leafy and yellow vegetables and fruits.

Animal Studies: Carotenoids have decreased the incidence of skin and mammary tumors in rodents as well as the number of tumors. Also in mice, dietary

carotenoids delayed the appearance and decreased the incidence of virally induced tumors [41]. Orally supplemented carotenoid prevented photochemically-induced breast cancer in mice [42].

Dietary Intake Studies of ß-carotene

Analytic epidemiologic studies using both case-control and prospective cohort designs have demonstrated inverse associations between either dietary intake or blood levels of ß-carotene and cancer risk. In addition, data are now available from two large- scale randomized trials testing ß-carotene in cancer prevention, one in a well-nourished and another in a poorly nourished population.

The largest observational study of dietary ß-carotene and cancer was conducted by Hirayama and colleagues [43] in Japan. Statistically significant inverse associations were seen between ß-carotene intake and cancer risk, with relative risks in the highest intake category of 0.76 for men and 0.87 for women. Other dietary studies have also reported significant inverse associations between ß-carotene intake and cancer risk [44-46].

But interpreting observational dietary intake studies can be problematic and lead to erroneous conclusions, since it is not possible to control for potential effects of confounding variables. For example the protection afforded by consumption of a particular food may be multifactorial, with several components of the food having chemopreventive effects. Six prospective cohort studies found no significant association between dietary ß-carotene intake and cancer risk [47-48] But a recent report on breast cancer from the Nurses' Health Study, a prospective cohort investigation of more than 120,000 US women, found a statistically significant decreased cancer risk among woman with the highest intake levels of total vitamin A [49].

While the prospective observational evidence concerning ß-carotene and cancer point to possible benefits of this agent in cancer risk, the available data are not all consistent. Only one large-scale randomized trial has tested ß-carotene in cancer prevention among a well nourished population [50]. The recently published alpha-Tocopherol/ ß-carotene (ATBC) Cancer Prevention Study involved six years of randomized treatment with 20 mg of ß-carotene and/or 50 mg of vitamin E daily in 29,133 Finnish male smokers, aged 50 to 69. No protective effect on lung cancer was observed for either of the two vitamins. In fact, those assigned to ß-carotene had a statistically significant 18% higher risk of lung cancer. The chief limitation of the Finnish trial is the relatively short six-year duration of treatment and follow-up which may have been inadequate to detect an anti-cancer effect [51].

Another large-scale randomized trial that assessed antioxidant vitamins in cancer prevention, the Chinese Cancer Prevention Trial demonstrated a modest reduction in cancer mortality from a combined regimen of ß-carotene, vitamin E, and selenium. The effect of the individual agents could not be assessed, and because the trial was carried out among 29,584 residents of rural a county in north-central China [52] a nutritionally deficient population, its results may not have direct relevance to well-nourished individuals.

Thus, conclusive evidence on the balance of benefits and risks of ß-carotene in cancer prevention will emerge from several ongoing large-scale randomized trials among well-nourished populations.

Ongoing trials of antioxidants include the 'CARET" study, testing ß-carotene and retinol among 18,000 individuals at high risk for lung cancer, and the SU.VI. MAX study, testing ß-carotene, vitamin E, and vitamin C, as well as zinc and selenium, in healthy French man and women.

Vitamin C

Ascorbic acid (vitamin C) is a water-soluble compound which is distributed throughout the body, with especially high concentrations found in a number of tissues including the adrenal, thymus, and pituitary glands and the retina. Ascorbic acid acts as an antioxidant by functioning as a free radical scavenger. In addition to its antioxidant activity, ascorbic acid has a number of biologic functions which may affect carcinogenesis, including enhancement and stimulation of immune response, involvement in collagen synthesis, and inhibition of in vitro formation of N-nitroso compounds. The major dietary sources of ascorbic acid include citrus fruits, dark-green leafy vegetables, tomatoes, and potatoes.

Animal Studies Animal studies indicate that vitamin C supplementation delays the appearance and decreases the incidence of skin tumors in mice exposed to ultraviolet light [53]. Another study found that it prevented 1 ,2-dimethylhydrazine (DMH)-induced colon tumors and reduced DMH-induced kidney tumors [54]. The administration of ascorbic acid was found to inhibit the development of metaplastic and neoplastic lesions of the respiratory tract of mice exposed to fiberglass dust, plutonium dioxide and asbestos [55-57], and to protect hamster lung cell cultures subjected to repeated cigarette smoke against abnormal growth and malignant transformation. Vitamin C decreased the incidence of kidney tumors in hamsters exposed to estrogen. In vitro, vitamin C has demonstrated a preferential toxicity against malignant melanoma cells. This potential has also been observed in vivo, in B-16 melanoma bearing mice, and transplantable mouse melanoma tumors [58-61].

In relation to breast cancer, vitamin C has been shown to decrease the rate of tumor appearance and growth of spontaneous mammary tumors in mice [62]. Vitamin C has also been shown to block the formation of nitrosamine, a carcinogen, from dietary sources [63], and to inhibit the incidence of DMBA induced breast carcinoma in rats. In addition, vitamin C has demonstrated antineoplastic action on human mammary tumor xenografts in immune deficient mice [64], most notably in the presence of copper sulfate.

Human Studies Of 46 epidemiological studies in which a dietary vitamin C index was calculated, 33 found a statistically significant protection, with a high intake producing a two-fold increase in protection from cancer development compared a low intake [65]. A study by Colditz et al [66] found that people who ingested the highest amount of strawberries and tomatoes had

significantly lower relative risks (RR=O.3 and RR=O.5, respectively) for development of cancer at several sites. But again, since the fundamental problem in nutritional epidemiology is that certain foods contain a number of potential beneficial elements and that confounding factors may not be entirely controlled, accurate conclusions require randomized clinical trials. While three recent studies showed a reduced risk of breast cancer associated with higher intakes of vitamin C [67,68], and another study found a favorable prognostic index in patients with breast cancer consuming a diet high in vitamin C [69], a cohort study in the Netherlands found no strong role for any of these dietary compounds in the etiology of breast cancer [70]. Table 1 shows the relative rates for breast cancer according to quintiles of intakes of vegetable, fruits, potatoes, provitamins and dietary fiber according to use of vitamin C supplements, as described in the Netherlands study. Prolongation of survival of cancer patients taking high doses of vitamin C has also been reported, discussed, and contradicted [71,72].

Vitamin E

The tocopherols and tocotrienols are lipid soluble antioxidant compounds of plant origin which have vitamin E activity. These fat-soluble antioxidants are thought to have a primary role in protecting low-density lipoproteins and the polyunsaturated fatty acids in cell membranes from oxidation. Compounds with vitamin E activity appear to scavenge oxygen radicals attacking cell membranes, and also to terminate free radical chain reactions within cell membranes. Alpha-tocopherol appears to be must important in terms of vitamin E activity, with betatocopherol, gammatocopherol, and alpha tocotrienol having less activity. Vegetable oils are the richest source of dietary vitamin E, although whole grains, wheat germ, nuts, and seeds also contribute to vitamin E intake in humans [73].

Animal and cell culture studies suggest that vitamin E possesses chemopreventive potential [74]. As vitamin C, vitamin E inhibits nitrosamine and nitrosamide formation [75]. It may also play a role in immune-mediated cancer prevention [76]. The possible role of vitamin E in cancer prevention was reported for the first time in 1934, when supplementation with wheat germ oil was found to decrease tar-induced carcinomas in mice [75]. Later, in a study using methylcholanthrene induced tumor in animals were reduced when animals were fed with supplemented with wheat germ oil diet [77]. A direct role of vitamin E in cancer prevention was demonstrated by Harbar et al [78]. In this study, a diet supplemented with alpha tocopherol reduced cancer incidence in mediylcholanthrene-treated female mice. Alpha tocopherol has also been reported to reduce dimethylbenzanthrazene (DMBA) induced mammary tumors in female Sprague-Dawley rats [79]. There is evidence that vitamin E enhances the ability of selenium to suppress DMBA-induced mammary carcinogenesis in rats fed high fat diets, although vitamin E alone was without effect [80]. Oral administration of alpha tocopherol (7 IU 2 times/wk) reduced DMBA-induced buccal pouch tumors in adult Syrian Golden hamsters [81]. In vitro, Vitamin E has inhibit growth of rat glioma cells and enhanced the differentiation of rat neuroblastoma cells [82].

Human Studies Case control experimental designs have yielded inconsistent results on the relationship between plasma vitamin E levels and cancer risk.

In a large prospective study in which alpha tocopherol levels were assessed, the risk of developing breast cancer in women with vitamin E levels in the lowest quintile was about five times higher that the risk for women with vitamin E levels in the highest quintile [83]. These authors believe that a differential degradation of vitamin E occurred during the plasma storage and concluded that their earlier results were inaccurate, but another similar study found no relationship between serum vitamin E levels and breast cancer risk [84]. In contrast a 10 year Finnish longitudinal study established a strong inverse relationship between serum alpha-tocopherol and risk of cancer [85]. This relationship persisted even after adjustments for serum cholesterol, vitamin A, selenium and various other confounding factors.

The epidemiologic evidence for a relationship between vitamin E consumption and breast cancer was studied in seven case-control and three prospective studies Table 2. An association of dietary intake of vitamin E and breast cancer in an inverse direction was found in seven of these studies (3 of which were statistically significant) [86-88]. In three of the case-control studies, a significant inverse association between breast cancer and dietary intake of vitamin E was observed [86-88], whereas in a fourth, an inverse association was suggested, but the findings were not statistically significant [87]. Grahani and coworkers (36) studied 439 postmenopausal breast cancer cases identified from a hospital base in Western New York. When the risk of breast cancer was examined adjusting for other nutrients (and for age and education), however, the trend was significant only when adjusted for calories and not when adjusted for carotene, vitamin C, or folic acid. This suggests that the vitamin E-breast cancer association may have been confounded by other nutrients.

London and co-workers [88] derived their study population from postmenopausal women who presented for evaluation of breast abnormalities. Women who had breast biopsies showing nonproliferative breast disease, proliferative disease without atypia, and atypical hyperplasia were also studied to evaluate the association of vitamin E intake with degree of atypia on breast biopsy. No significant trends were noted. The only OR that was significant was the odds of low vitamin E intake from food sources only [OR = 0.4, 95% confidence interval (CI) = 0.2-0.9, p (trend)=0.002]

In the remaining studies that have explored the association between breast cancer and vitamin E intake, a null relationship or an increased risk of breast cancer with higher vitamin E intake [85-90] was found. Toniolo and co-workers [89] performed a population-based study of Italian women who were less than 75 years old. When quartiles of vitamin E intake were analyzed, the trend adjusted for age and total caloric were not significant..

In a French hospital-based study by Richardson and colleagues [91] in which the

Table 1. Relative rates of breast cancer according to antioxidant level stratified by category of intake of energy adjusted PUFAs (polyunsaturated fatty acids) The Netherland Cohort Study

	Quintile of antioxidant level[b]					X^2 for trend
Antioxidant/PUFA	1[c]	2	3	4	5	(*P*-value)
β-Carotene						
Low PUFA[a]						
No. of cases per subcohort	34/112	40/119	45/120	39/114	50/108	
Relative rate	1.00	1.14	1.22	1.02	1.52	2.05 (0.15)
95% Confidence interval	–	0.66–1.97	0.71–2.10	0.58–1.81	0.88–2.62	
High PUFA[a]						
No. of cases per subcohort	47/96	45/101	38/121	46/108	37/112	
Relative rate	1.00	0.94	0.60	0.94	0.70	2.04 (0.15)
95% Confidence interval	–	0.56–1.59	0.35–1.02	0.55–1.59	0.40–1.21	
Vitamin C						
Low PUFA[a]						
No. of cases per subcohort	44/98	32/131	41/110	36/111	55/123	
Relative rate	1.00	0.50	0.81	0.64	0.85	0.00 (0.97)
95% Confidence interval	–	0.29–0.87	0.48–1.38	0.37–1.12	0.51–1.43	
High PUFA[a]						
No. of cases per subcohort	47/107	44/101	42/113	42/118	38/99	
Relative rate	1.00	0.92	0.78	0.75	0.77	2.00 (0.16)
95% Confidence interval	–	0.54–1.55	0.47–1.32	0.44–1.28	0.44–1.33	

[a]Relative rate after adjustment for age, energy intake, alcohol intake (0, 0.1–14, 15–29, 30+ g day^{-1}), history of benign breast disease, maternal breast cancer, breast cancer in sister(s), age at menarche, age at menopause, age at first birth, parity. [b]Cut-points: β-carotene (mg eq vitamin A day^{-1}), 0.252, 0.337, 0.428, 0.567; vitamin C (mg day^{-1}), 70.90, 93.66, 113.40, 141.82. [c]Reference category. [d]Low and high PUFA are defined as the two lowest quintiles and the two highest quintiles of intake of PUFAs, i.e. an intake of <12.85 g day^{-1} and ≥ 15.89 g day^{-1} respectively.

OR was adjusted for age, family history of breast cancer, history of benign breast disease, age at menarche, parity, educational level, and Quetelet index, a positive association was suggested, although it was not statistically significant.

Rohan and co-workers [92] analyzed the association between dietary fiber, vitamins A, C, and E, and various food groups and breast cancer risk in The Canadian National Breast Cancer Screening Study. In this population, dietary fiber was inversely associated with breast cancer risk, even after adjustment for other dietary factors. An inverse association was also found for intake of pastas, cereals, and vitamin A and C and breast cancer risk.

Hunter and colleagues [93] examined the relationship between vitamins A, C, and E and breast cancer in the Nurses' Health Study cohort. After multivariate adjustment for known risk factors and vitamin A intake, the RR for breast cancer was 0.99 (95% CI = 0.83-1.19) in women in the highest (>24.1 IU/day) vs. lowest quintile (<3.9 IU/day) of vitamin E intake. In summary, epidemiologic analysis of the relationship of dietary vitamin E intake and breast cancer risk is inconclusive.

Selenium

Selenium is an essential constituent of the enzyme glutathione peroxidase, which reduces peroxides before they damage cell membranes. Evidence suggests that selenium can inhibit DNA synthesis and cell proliferation.

Animal Studies Selenium compounds have been found to inhibit and/or retard tumorigenesis in a variety of experimental animal models. Of 78 studies on animal models, 49 presented results which suggest reduction of tumor incidence and/or numbers of tumors per animal. In half of these studies, a dramatic reduction in tumorigenesis was attained, while in 20% of the cases selenium did not show no effect on tumorigenesis or inhibition of tumor growth [94].

Dietary selenium supplementation in mice has decreased the incidence of skin tumors [95], and decreased the incidence and multiplicity of chemically-induced colon tumors, liver tumors, lung tumors, and mammary tumors [96-98].

Human Studies Dietary sources of selenium are seafood and organ meats, followed by grains. The selenium content of grains is heavily dependent on the concentration of selenium in the soil. Because there is much diversity in soil selenium levels, the selenium content of food varies considerably

Most of the evidence for a cancer protective effect of selenium in humans comes from studies that have correlated dietary and blood levels of selenium with cancer incidence in populations. [99,100]. Shamberger and Willis [100] found that mortality due to lymphomas, gastrointestinal tract, peritoneum, lung, and breast cancers were lower for both males and females residing in areas of the United States which have moderate (0.06 to 0.10 ppm selenium) to high (>0.10 ppm selenium) selenium concentrations in local forage crops. Shrauzer documented overall cancer mortality rates in 27 developed countries and found that leukemia, colon, rectum, breast, ovarian, and lung cancer correlated inversely to the estimates of the average per capita ingestion rates of

Table 2. Serum Vitamin E Levels and Cancer Risk [a]

Reference	Population	No. of Cases	No. of Controls	Comparison	OR/RR	95% CI
			Case-control studies			
Gerber et al. (44)	Italy and France	314	344	Highest vs. lowest quintile	4.2	1.9–9.0
London et al. (38)	Boston	377		Highest vs. lowest quintile	0.8	0.5–1.2
Basu et al. (50)	Canada	30	30	Mean case-control differences		NS[b]

Reference	Population	Population No.	No. of Cases/ Matched Controls	Comparison	OR/RR	95% CI
			Prospective studies			
Wald et al. (47)	Guernsey (followed 7–14 yrs)	5,004	39/78	Highest vs. lowest quintile	0.5	
Knekt (48)	Finland (followed 10 yrs)	15,093	67/578	4 highest vs. lowest quintile	0.88	0.32–2.39[c]
Russell et al. (51)	Guernsey (followed 8 yrs)	5,086	30/288	Mean case-control difference		NS[b]
Comstock et al. (49)	Maryland (followed 15 yrs)	25,802	30/59	Highest vs. lowest quartile	1.7	
Willett et al. (52)[d]	Boston (followed 5 yrs)	10,940	14/31	Mean case-control difference		NS[b]

a: See Table 2 footnote for definition of abbreviations.
b: Not significant (mean serum levels in cases and controls not significantly different at $p < 0.05$).
c: RR adjusted for smoking status and serum cholesterol after exclusion of breast cancer cases diagnosed during 1st 2 yrs of follow-up.
d: Nested in case-control studies.

selenium in those countries [101]. In a more detailed and in-depth analysis which involved an extensive review of the methodology used the studies and interpretations of results, Clark [102] concluded that mortality rates were significantly lower in countries with an intermediate and high selenium soil content when compared to countries with low soil selenium content for cancers of all sites in both sexes. Inverse associations of selenium exposure were also found for females with cancer of the breast, ovarian and cervix, although other cancers such as liver, stomach, Hodgkin's disease and leukemia correlated positively with selenium exposure in both sexes [102].

Case control studies have also been employed to examine the hypothesis that selenium status is related to cancer risk in human populations. Most of these cross-sectional studies support the hypothesis that low selenium status can increase cancer risk. They indicate that cancer patients have generally lower selenium levels than do healthy, matched controls [103].

In another study, Willet et al [104] demonstrated that subjects in the lowest quintile of serum selenium had a relative cancer risk of 20 when compared to the highest quintile group. The relative risk associated with low selenium appeared to be greatest in those subjects who were also the lowest in plasma retinol and alpha tocopherol levels.

There are conflicting reports as to the role of selenium in preventing breast cancer. On the negative side, Garland et al were unable to associate an effect of selenium intake on breast cancer risk, at least within the range of human diets [105]. And a recent study showed a tendency for slightly higher selenium levels among future breast cancer cases by analyzing nail clippings for selenium. [106-107]

The majority of the well designed prospective and retrospective case control studies have indicated an association of relatively low selenium status with increased cancer risk. In principle, inverse associations between cancer risk and selenium status will only be detectable if the selenium gradient in the study population is sufficiently large. Thus, the determination of the potential role of selenium in preventing human carcinogenesis still needs to be addressed in well-planned intervention trials.

The European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Cancer of the Breast (EURAMIC)

Time-integrated exposure of tocopherols and ß-carotene in adipose tissue, and selenium in toenails was investigated in a case-control study among postmenopausal women, ages 50-74 years, from five European countries. The study group comprised 347 incident breast cancer cases and 374 controls.

Mean antioxidant levels, adjusted for age and center, did not significantly differ for a-tocopherol (cases were 4.5% higher than controls), ß-carotene (3.0% lower). or selenium (1.8% lower). Odds ratios for highest *versus* lowest tersiles of exposure, adjusted for potential confounders, were 1.15 *(95%* confidence interval, *0.75-* 1.77), 0.74 (0.45-1.23), and 0.96 (0.63-1.47), respectively, without evidence for a decreasing trend. No statistically significant interactions were observed, and the results do not support the hypothesis that dietary antioxidants are important determinants of this hormone-related malignancy among postmenopausal women. [108]

Conclusion

There is a definite role of antioxidants in the regulation of certain molecular risk factors These agents appear to affect the activation of protocogenes, loss of antioncogenes, suppressor genes, and stimulation of cell signaling systems, which can result in abnormal cell proliferation and differentiation .

But, from an epidemiological perspective, the antioxidant defense system is more than just the sum of the individual parts. Future research will elucidate the concurrent application of safe, appropriate doses of antioxidants that could exert a potent synergistic chemopreventive effect. This preventive course of action continued for an extended period of time may reduce cancer risk without toxicity.

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