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# Clinical Evidence Supporting Cancer Risk Reduction with Antioxidants and Implications for Diet and Supplementation

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## BACKGROUND OF THIS SYNOPSIS

The role of antioxidants in cancer risk reduction continues to be debated in the scientific literature as well as the lay press. This article will summarize recent, pertinent supporting scientific and clinical literature as well as clarify why the debate is not easily resolved, if indeed it can be resolved at all. From this pertinent evidence, we will also suggest some implications for diet and supplementation policy. Central to the debate on the efficacy of antioxidants in cancer risk reduction is the question of what studies constitute definitive evidence. The argument of appropriate evidence came to a head in the case of *Pearson v Shalala* (1999) before the U.S. Court of Appeals1 wherein the FDA refused to authorize four health claims, including one stating that "consumption of antioxidant vitamins may reduce the risk of certain kinds of cancer." The FDA's opinion is an excellent example of the thinking of those who contend that data validity to a near-certain degree are requisite to truth in such health claims. This view was rejected by the court in its decision against the FDA, and the court's decision allowing the claims, although not further appealed by the FDA, remains under FDA review. A recent review of the decision and the controversy surrounding proper evidence summarizes the debate:

*Almost all nutrition science exists along a continuum of relative acceptance in the relevant scientific community. Few single nutrient-single-disease propositions, for example, command universal acceptance among those who study the relationship. Instead truths in nutrition science exist along a continuum that runs from views held by a small majority of scientists to views held by a substantial majority. A true claim may be made about all such views along the continuum, provided that appropriate qualifications are used to indicate the degree, quality, and quantity of scientific evidence present to support it. It would be difficult to identify any single nutrient-disease proposition that commands universal acceptance by nutrition scientists.*[2]

The discrepancy in expectations for the scientific evidence explains much of the lack of concurrence in the literature about the possible efficacious role of antioxidants to reduce cancer risk. Within this noted continuum of acceptance, we present pertinent supporting clinical scientific

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published evidence for a role of certain antioxidants and related compounds in reducing selected cancers and cancer risk. The cancers with sufficient published evidence on this relationship will be emphasized. These are prostate, gastric and esophageal, oral and oropharyngeal, skin, lung, cervical, colorectal, bladder, and hepatocellular carcinomas. We focus here specifically only on vitamin A and certain retinoids, certain carotenoids, vitamin C, vitamin E (i.e., alpha tocopherol), and selenium, and will reference folic acid only when particularly pertinent, although folic acid is not typically considered as an antioxidant. In a subsequent article, we will consider certain herbs, spices, and functional foods as they may pertain to cancer risk reduction.

The "gold standard" for clinical scientific evidence today that risk of cancer is reduced is the randomized, placebo-controlled, double-blinded trial where the end-point is reduction of cancer incidence and where large numbers of subjects are enrolled with long follow-up periods. Such trials are relatively few in today's literature pertaining to cancer prevention by vitamins, antioxidants, and other micronutrients. However even when such well-designed larger trials are performed, results may differ, and final definitive proof of an effect on cancer prevention may prove elusive. The ATBC (alpha-tocopherol beta carotene) and CARET (beta caroptene and retinol efficacy) trials, both large trials that were randomized in Finland, demonstrated enhanced lung carcinogenesis with beta carotene in smokers[3,4,5] the U.S. Physicians Health Study, of similar design but differing in that it recruited predominantly non-smokers in contrast to the ATBC and CARET trials, found no such association and continued to accrue study subjects after the results of the Finnish studies were published.[6] Other sources of cancer prevention data are (a) observational with data more commonly reflecting cross-sectional analysis, migration patterns, and longitudinal cohort studies that are both retrospective and prospective, and (b) experimental studies using case study methods, consecutive case series, non-randomized intervention trials, and non-blinded clinical studies of a variety of design structures. Surrogate Endpoint Biomarker (SEBM) studies are of importance to generate early and reliable data relevant to cancer incidence reduction, given the high cost and long period of observation which are the major limitations of cancer incidence reduction trials.[7] Such studies and data all contribute to a judgement as to whether a substance may or may not be termed a chemopreventative (ie, an agent that reduces the risk of cancer in one or more of its aspects).

In the review by Lippman et al,[8] the "definitive" evidence for an agent (antioxidant or other substance) having demonstrated cancer prevention, that is, unequivocal proven risk reduction for a cancer, included the stipulation that invasive cancer was prevented and that clinical trials in excess of 1,000 patients were necessary to support this evidence. However such a limited definition has been challenged,[9] and modified[10] recognizing that both intermediate biomarkers of cancer risk reduction, including effects on precancerous or non-invasive lesions are valid, useful, and reliable data sources as indicators of an agent's cancer risk reduction effectiveness and clinical magnitude. It should be recognized that the transition from normal tissue through the premalignant state and then to overt clinical cancer has been estimated[11] as follows: prostate– 40 years; breast – 30 years; lung – 5 to 20 years for 20-40 pack-years of smoking; colon –10 to 35 years; cervix–10 to 20 years; (Barrett's) esophagus–5 to 25 years; and (HBV+) liver – 20 to 40 years. These data emphasize the utility of intermediate markers and high risk premalignant lesions in clinical studies for cancer prevention, offering reasonable turnaround times for valid data generation. Studies of premalignant lesions and biomarker studies may, however, not yield key data on mortality incidence, survival, or quality of life issues relative to cost; deriving these data may indeed require the "gold standard" approach to ascertain cancer prevention.

## LITERATURE SOURCES

Using the MEDLINE/HEALTH STAR guides and databases which contain data from 8,400 journals, citations, and abstracts, relevant literature on antioxidants was reviewed. We will use the term "antioxidant" as in biochemistry and medicine, where antioxidants are enzymes or other substances such as vitamin E or beta carotene that are capable of counteracting the damaging effect of oxidation in animal and/or human tissue. We recognize however that as more research into antioxidants proceeds, unanticipated anticarcinogenic mechanisms in them will become newly recognized which are not "antioxidant" in nature[12-15] but which will still accrue to the use of these antioxidant compounds. Cancer causation is linked experimentally and clinically to cellular and DNA damage by oxidants, and therefore antioxidants may properly be viewed as potentially reducing the risk of cancer. This report emphasizes data published since 1993, supplementing previous reports in the literature, and is limited to those clinical scientific papers published only in English and referenced in the respected *Index Medicus*.

In this review, we do not include data of antioxidant effects in animal cancer models or *in vitro* systems, nor do we examine diet-related epidemiologic data. Such studies are of course critical and valid precursor investigations often necessary for construction of clinical trials of specific micronutrient or supplement effects on preventing

cancer; also, with the introduction of transgenic and specific gene knockout mouse models, the value of experimental animal data in the field of cancer chemoprevention is increasing. Yet even prospective, blinded, controlled trials yield data that confound such precursor investigations. The striking positive effects of beta carotene in pre-clinical and diet-related epidemiologic systems foundered importantly in several large-scaled, well-designed clinical trials for lung cancer prevention[3,4,16,17] albeit there was little experimental animal data supporting a role for beta-carotene in cancer chemoprotection. The reasons for this dichotomy of research results have been extensively explored and serve to defend the focus of this review. Further we examine only those reports where clinical outcomes overall address noted useful supplement effects on clinical carcinogenesis; many of the cited references however do include conflicting or indeterminant results of other studies, and interested readers are referred to our primary sources.

## CANCER PROCESS

The cancer process incorporates (a) the abnormal tissue in certain organs which when left unchecked will result in invasive or widespread clinical cancer {Type I - or recognized pre-malignancies}, (b) the disease which may be *in situ* when first detected but which, when unchecked, is known to then become invasive, and then widespread and lethal {Type II - or early *in situ* carcinoma}, and (c) invasive malignancy that is first detected in a local, regional, or a widespread state, the more common clinical form {Type III - invasive carcinoma}. To a lesser extent, we will address reduction by antioxidants of risk of certain cancers recurring at a different site in the same organ or elsewhere {Type IV - or recurrent carcinoma or new primaries}. The foundation of such Type I and II cancer categories (and to some extent Type IV) is the recognition that for some cancers, there is evidence of a tissue "field cancerization"[18,19] by which the authors[18] meant that for a local tissue area, pre-malignant and malignant tissue changes recurred repeatedly. Examples of field cancerization are found in bladder cancer, head and neck cancer, skin cancer, and to some extent in lung cancer. Premalignant tissue changes include atypical hyperplasia of the breast, non-invasive cancer of the breast (ductal carcinoma *in situ*), lobular carcinoma *in situ*, prostate (high-grade prostate intraepithelial neoplasia), cervix (cervix intraepithelial neoplasia), skin (actinic keratoses), oral mucous membranes (leukoplakia and erythroplakia), lung (atypical hyperplasia and respiratory papillomatoses), esophagus (Barrett's esophagus with dysplasia), and gastrointestinal tract (adenomatous or villous polyps). All cancer definitions are appropriate to the clinical situation and parallel modern medical concepts of "cancer prevention," a term intrinsically related to reduction in cancer risk.

## PROSTATE CANCER

Clinical cancer of the prostate {Type III} has an impressively variable geographic and world-wide incidence, while latent (microscopic, non-clinical) prostate cancer incidence is similar[20,21] throughout the world. Dietary factors are among the risk modulating influences in this clinical cancer.[22] The alpha tocopherol beta carotene cancer (ATBC) prevention study[23] focused on antioxidant supplement risk-reducing effects on lung cancer, was primarily addressed for beta carotene, but the effects of supplements on other cancers were also examined. In this study from Finland,[24] subjects receiving 50 mg/day of alpha-tocopherol had a 32% reduced incidence of prostate clinical cancer {Type III}. These data strikingly support a cancer risk reduction role for the antioxidant supplement alpha-tocopherol in prostate cancer, and the authors of the study recommended the use of alpha-tocopherol for prostate cancer prevention. The results with alpha-tocopherol were seen starting within two years of taking the supplement. In analyses of the Physician Health Study[6] using beta carotene and alpha-tocopherol in healthy males, those subjects who at the onset of the study were in the lowest tertile of serum alpha-tocopherol also had evidence for a reduction in prostate cancer incidence.

Paralleling these results, several studies reflecting the pioneering work of Clark et al[25,26] show impressive reductions of cancer in controlled, placebo trials with selenium (200 mcg/day), reducing overall cancer mortality by 21% and prostate cancer by 65% in selenium-treated subjects from geographic areas in the U.S. with low selenium soil content. These selenium effects were statistically significant for the subjects in both the lowest tertile and middle tertile; in subjects from the highest tertile of baseline serum selenium levels, a non-significant reduction of cancers was noted with selenium supplementation. The studies by Clark et al used a yeast-derived selenium compound. Other forms of selenium commonly used include sodium selenite ($Na_2SeO_3$) which is highly toxic at five to ten times the normally tolerated nutrition level for other selenium derivatives.[27] Selenomethionine, selenite, and seleno-cysteine also are found in many supplement preparations. Most studies showing positive effects of selenium in cancer prevention indicate the yeast-derived selenium as the more efficacious compound.[28] Studies demonstrating differences between tissue repletion[29] and anticarcinogenic effects have been noted in animal systems also;[30] selenite and selenomethionine compounds appear to have less anticarcinogenic activity.

Beta carotene has shown cancer prevention efficacy in men with low pre-morbid baseline serum carotene levels[30,31] prevention with beta carotene supplementation is most pronounced with respect to limiting metastatic cancer progression and aggressiveness of established cancer and may not apply to primary prevention.

## GASTRIC AND ESOPHAGEAL CANCER

Gastric cancer, because of its relationship to precancerous atrophic gastritis and gastric metaplasia lends itself favorably to cancer risk reduction studies. This relationship may be influenced by the presence of *Helicobacter pylori*, an organism responsible for fostering mutagenic intermediates necessary for frank carcinogenesis. The interactions between gastric juice vitamin C levels, serum levels of vitamin C, other antioxidant blood levels such as beta carotene and retinol, have all helped to elucidate this complex interaction of carcinogenesis. Studies[32] have shown that serum and gastric levels of ascorbic acid are lower in cancer patients than controls, and that these levels correlate inversely with lipid peroxides in the serum and gastric mucosa. Oxidative damage to DNA is related to both *H pylori* infection, atrophic gastritis, and intestinal metaplasia – all precursors to invasive cancer.[33,34] Dietary vitamin C may be protective of gastric cancer by scavenging mucosal oxygen radicals,[35] believed important in gastric carcinogenesis. Dietary studies of ingestion of carotenoids[36,37-44] indicate gastric cancer protection, and a prospective cohort study[45] of antioxidant vitamins and retinol in post-menopausal women suggests benefit for cancer of the upper digestive tract. Secondary analyses in a few reports[39,40] involving controlled trial data, drawn from the ATBC Finnish studies, support a role for these antioxidant vitamins.

The possible protective effects of multiple vitamin/mineral supplementation in the Linxian, China studies in esophageal/gastric dysplasia and cancer[40-43] does indicate a role for antioxidant vitamins to reduce risk of cancer of the stomach in comparatively undernourished populations. Therefore these supplements, including vitamin E, selenium, and beta carotene, may be of important relevance to reduce cancer of the stomach in the understudied populations in the United States which comprise both economically-challenged and under-nourished groups. Risk reduction studies with antioxidants for esophageal and stomach cancer are continuing and *en toto* support the view that antioxidant vitamin supplements, perhaps given in combinations including vitamins C and alpha-tocopherol, may reduce gastric cancer risk. The important interplay of carcinogenesis with infection may be reflected in other cancers as well.

## ORAL AND OROPHARYNGEAL CARCINOMA

Precancerous lesions for oral and oropharyngeal cancers include leukoplakia, particularly when this lesion includes severe cellular atypia, and erythroplakia. Both smoking and alcohol intake are thought to impact on cancer development in most cases as well as diet. Further, because of the high incidence of second primary cancers of the head and neck region following the development of an initial primary cancer, chemoprevention and/or cancer risk reduction studies have been particularly targeted to this clinical setting generally in subjects at risk for new cancer development, second cancers of the region, or recurrent cancers. Recent published results have focused on several vitamin A derivatives, both naturally occurring and synthesized. In some studies retinoids have been combined with beta carotene and other antioxidants in an effort to assess effects on precancerous lesions. Studies showed a pronounced reduction in cancer risk with the use of 13-cis-retinoic acid, the provitamin A antioxidant.[46] Antioxidant supplements (30 mg beta carotene, 1000 mg ascorbic acid, and 800 IU of alpha tocopherol) were given to 79 patients with oral leukoplakia with improvement noted in 55.7% of patients, particularly those who reduced their intake of alcohol and tobacco.[47] The supplement increased tissue levels of beta carotene, ascorbic acid, and alpha-tocopherol.

In another risk reduction trial with an antioxidant supplement of beta carotene, vitamin C, and E in dysplastic oral mucosal lesions,[47,48] biomarkers of cancer risk (number of nuclear organizer regions in the cell and micronuclei) were reduced. Short term responses were seen in the precancerous lesions where alcohol and tobacco cessation complemented supplement use. Other cancer-related biomarkers studied included such growth factors as epidermal growth factor receptor (EGFR), transforming growth factor alpha (TGF-alpha) and her-2/neu expression. In other biomarker studies using micronuclei expression in mucosal scrapings,[49] both isotretinoin and beta carotene suppressed micronuclei counts in 57 patients.

13-cis-retinoic acid at a dose of 1 mg/kg/day produced complete resolution of leukoplakia. Pretreatment expression of EGF, TBF-alpha and her-2/neu were elevated in leukoplakia compared to normal mucosa; TGF-alpha decreased in leukoplakia lesions but not in normal mucosa.[50] A double-blind placebo study with vitamin A (retinyl acetate 300,000 IU/week for 12 months or beta carotene 360 mg/week for 12 months) among 160 subjects (in Kerala, India) with leukoplakia lesions showed a 10% complete regression rate on placebo, 52% with vitamin A, and 33% with beta carotene. Relapses after therapy cessation were common. Vitamin A

effects on disease regression were significant and strongly supported vitamin A's cancer risk reduction role.

In addition, the protective effects of vitamin A derivatives when applied topically were consistent with the beneficial effects when consumed orally. For example, in other studies of oropharyngeal or laryngeal lesions, the topical application of the vitamin A derivative fenretinide (4-HPR) produced total complete remissions of oral lichen planus and leukoplakia; remissions seemed greater than with use of the supplement only by oral administration.[51] Other controlled studies[52] confirmed the effectiveness of fenretinide in preventing relapses of leukoplakia, new localizations of the lesion, and new carcinoma development during the period of treatment. Laryngeal leukoplakia treated with high dose retinyl palmitate (300,000 IU/day for response, then 150,000 IU/day for maintenance) showed a complete remission rate of 75% with follow-up to 18 months; the results were viewed as demonstrating the antioxidant supplement to have substantial activity and represents an excellent candidate as a preventative agent for laryngeal cancer.[53] The controlled placebo studies with antioxidant micronutrient supplements including vitamin A given subjects who "reverse-smoke tobacco leaf"[54] produced regression of palatal precancerous lesions in 52% of subjects (8% regression in the placebo group), and progression of lesions in 10% of the treatment group and 47% of the placebo group; new lesions developed in 12% of those on supplement (which included also riboflavin, zinc, and selenium taken twice a week for a year), while 38% developed significantly more new lesions while on placebo. In an intervention study from Uzbekistan in an area of high oral cancer,[55,56] using a double-blind two-by-two factorial design study, a decrease in precursor lesions of the mouth was seen after six months treatment with a supplement of retinol (100,000 IU), beta carotene (40 mg/day), and riboflavin (80 mg/day) with an odds ratio of 0.62 for the supplement. Where low dose isotretinoin (0.5 mg/kg/day) was compared to beta carotene (30 mg/day) in the prevention of oral carcinogenesis in a randomized study of 59 patients, low dose supplement therapy with isotretinoin was more active against leukoplakia than beta carotene when these supplements were used following high dose (1.5 mg/kg/day) isotretinoin therapy.[57] Beta carotene supplementation (20 mg/day) or alpha tocopherol (50 mg/day) given in the ATBC cancer prevention study[58] increased beta-carotene mucosal concentration which correlated with serum levels; mucosal changes in beta carotene concentration was limited to leukoplakia lesions and was seven times greater than mucosal levels without supplementation.

In summary, these recent studies demonstrate that antioxidant supplements such as beta carotene and alpha-tocopherol can (a) raise serum and mucosal antioxidant levels in appropriate target tissue at well-tolerated doses, (b) can favorably influence morphologic, genetic, and chemical biomarkers indicative of cancer risk, and (c) support the view that antioxidant vitamin A retinoids may reduce cancer risk in oral, laryngeal, and head and neck cancers. Other studies[59] of combination antioxidant supplementation showed 13-cis-retinoic acid had a 10% complete response rate in oral premalignancy and that alpha-tocopherol supplements may increase this rate to 78%. These supplement interventional trials in oral cancer risk reduction using pre-cancerous lesions as indicator surrogates for invasive neoplasia complement directly other recent nutrient studies[60] and dietary evaluation efforts[61] where diets rich in carotenoids and vitamin A/retinoids reduce the risk of cancer and precancerous lesion development. Further tissue response to the retinoids and to beta carotene in head and neck leukoplakia may vary importantly, with laryngeal lesions noted to respond most favorably,[62] perhaps on the basis of tissue differences in response to the pharmacology of these agents.

It should be noted that oropharyngeal cancers are recognized as clinically diverse; the undifferentiated nasopharyngeal carcinoma (NPC) is not related to usually pre-morbid risk factors of tobacco and alcohol. A relationship between EBV (Epstein-Barr Virus) infection and HPV-16 (human papilloma virus-16) infection has recently been amplified.[63] The above observation on head and neck cancer prevention, or on supplement effects on leukoplakia are not necessarily applicable to undifferentiated NPC.

## SKIN CANCER

Skin cancer carcinogenesis is frequently thought to represent a transition from a variety of keratotic benign lesions (actinic keratosis, now termed keratocytic intraepithelial neoplasia), often reflecting age and/or sun exposure, to clinical invasive basal cell or squamous cell carcinoma, or in the case of malignant melanoma, a transition from dysplastic or atypical cutaneous moles to frank invasive melanoma. In recent randomized, double-blind controlled trials with retinol supplementation for moderate risk non-melanoma skin cancer,[45] 25,000 IU of retinol effectively prevented squamous cell skin cancer. Another similarly controlled and designed trial[64,65] in renal transplant patients (who develop skin cancer commonly associated with their immune-suppressive therapy) with at least 10 precancerous keratotic skin lesions at the onset of the study, 30 mg/day of acetretin for six months had a significant effect in preventing squamous cell carcinoma of the skin in 44 patients studied (47% incidence in placebo group versus 10% in the acetretin group of new skin cancer incidents); results

also demonstrated a decrease in the number of keratotic skin lesions (acetretin:13.4% decrease versus placebo:28.2% increase). The report noted that the acetretin effect was most pronounced in patients with prior squamous cell skin cancers. Occlusive application of retinoids also reduced the pre-melanoma lesions of benign atypical moles.[66] In summary, several of the vitamin A derivatives have demonstrated their ability to reduce skin cancer risk in certain identified populations at high risk of cancer development. A similar study of a xeroderma pigmentosum variant (xy-v) showed dramatic reduction in non-melanoma skin cancer with synthetic retinoids.[67] The clinical implications of these studies are important, given their derivation from vigorously-controlled trials albeit in relatively small numbers of patients; the noted review by Lippman et al[8] is even stronger, holding that definitive evidence shows that antioxidant vitamin A retinoids prevent certain skin cancers.

## LUNG CANCER

The use of antioxidant supplements to reduce clinical lung cancer emanated from a wide variety of observational dietary studies indicating that diets rich in antioxidant nutrients were associated with reduced lung cancer risk.[68-70] Alpha-tocopherol (50 mg, used alone) did reduce the incidence of mesothelioma in asbestos workers at a supplement dose of 50 mg; risk reduction rates were significant.[71] Alpha-tocopherol serum and tissue levels were also raised by supplement administration[72-75] in the ATBC trial, and highest quintile of serum alpha tocopherol levels were associated with a 19% reduction in lung cancer risk with a particularly strong inverse relationship between alpha-tocopherol supplementation and reduced cancer risk in younger men with more limited duration of smoking.[76] Further, in the important secondary analyses of the ATBC data by Woodson et al,[76] subjects having longer exposure to alpha tocopherol "may have accrued some marginal benefit (a 10%-15% reduction) in cancer incidence".[76] In analyses of food and dietary supplements of middle-aged women in the Nurses Health Study,[77] two antioxidants yielded a reduction in lung cancer: for more than 10 years of vitamin E supplementation, relative risk was 0.7 (i.e., there was 30% fewer occurrences of lung cancer among this group taking vitamin E supplementation); for more than 15 years of vitamin C, relative risk was 0.7.

In summary, the data in lung cancer and mesothelioma offer evidence that (a) alpha-tocopherol supplementation and long term use of vitamin C with other vitamins may indeed reduce the risk of cancer of the lung {Type III} and mesothelioma {Type III} in certain populations and (b) the synthetic retinoids (and folate)[78,79] will induce regression of premalignant lesions as reflected in a few, limited, but promising, studies which may lead to new approaches in retinoid delivery.[80,81]

## CERVICAL CANCER

A role for beta carotene in cervical cancer risk reduction is supported by studies which are concerned primarily in disease prevention and the progression of cervical intraepithelial neoplasm {CIN I-III} in which CIN II-III is considered to have the potential for progression to invasive squamous cell cancer.[82] CIN is intimately related to human papilloma virus (HPV) infection,[83-85] particularly HPV-16, with persistent infection increasing the risk of persistent CIN and therefore invasive cervical carcinoma. A recent study[86] demonstrated that beta carotene is being useful in treating precancerous lesions. Another study of the 30 mg dose of beta carotene[87] compared with placebo showed enhanced serum carotene levels in non-smokers with beta carotene supplementation. Biochemical studies showed[87] that doses of beta carotene will raise serum levels in women with CIN, increase vaginal epithelial concentrations of beta carotene,[49] and up-regulate favorably transforming growth factor beta-1, which is thought to favor CIN development and progression through loss of TGF-beta-1 itself or lack of responsiveness to the growth factor. Furthermore CIN and HPV infection is associated with lower intake of foods containing vitamin C, beta-carotene and folate.[88] Serum levels of vitamin C in patients with cervical premalignant lesions associated with HPV infection are reported low,[89,90] with parallel observations for H. pylori infection and gastric cancer development.

The protective effects of retinoids when applied topically to the cervix are consistent with the beneficial effects when consumed orally. Intravaginal direct topical application retinoids (Retinamide II) for six months in 27 women (10 mg daily) showed an overall CIN response rate of 96.2% and a complete response rate of 88.9% with an overall effective rate of 74.3% after two courses of treatment, a treatment effect comparable to laser beam radiation or electrocautery of the lesions.[91] Further local delivery of all trans-retinoic acid in a phase II trial produced a complete response rate of 50%. Moderately severe CIN (II-III) treated with cervical caps with 0.372% of beta-trans RA or placebo showed a 43% retinoid complete regression rate compared to 27% in the placebo group.[92]

The recent data, in summary, do support the effect of oral dietary antioxidant supplements on modulating favorably a variety of biochemical parameters in women with cervical dysplasia and reducing the risk of cervical cancer by causing regression of *in situ* cancer {Type II} lesions. Studies also showed biochemical parameters were

improved. Local (topical) application of other vitamin A antioxidant retinoids in well-designed studies present convincing data that the progression of cervical precancerous lesions and their persistence is favorably influenced by such therapy with vitamin A derivatives, demonstrating that the protective effects of vitamin A derivatives are consistent with the beneficial effects when consumed orally. These data support the view that vitamin A derivatives may reduce the risk of cervical cancer.[89,90]

## COLORECTAL CANCER

A study[93] with the vitamins A, C, and E showed a decrease (5.7%) in recurrence rate of colon polyps {Type II cancer} as compared to a 14.7% recurrence rate of patients given lactulose and a 35.9% polyp recurrence rate in untreated controls. Studies with selenium[26,94,95] also yield indication of a decreased cancer incidence and a decrease in new adenoma formation as do studies with folic acid.[96,97]

## BLADDER AND HEPATOCELLULAR TUMORS

Studies indicate a role for antioxidant vitamins in cancer risk reduction of bladder cancer,[98] and the acyclic retinoid polyprenoic acid[99] reduced the occurrence of secondary primary hepatocellular carcinoma development after surgical resection of the first primary tumor. Evaluation at 62 months mean follow-up showed a reoccurrence rate for placebo of 74% versu 46% for the retinoid. Selenium is reported as reducing hepatocellular cancer rates after HBV infection.[100]

## OVERALL CANCER RISK REDUCTION IMPACT

The discussions above document the perspective that some antioxidant vitamins and other related supplements have a substantial peer-reviewed literature research base with respect to their reduction of cancer risk. Their mechanisms of action range from free radical scavenging and reducing nitrosamine formation, to influencing immune status favorably and thereby modulating infectious causes participating in carcinogenic processes and reducing oxidized DNA damage and genetic injury and repair.[101-103] Other studies demonstrate effects on proliferation initiating receptors,[104] growth factor modulation,[105] and oncogene activation.[12,106] The "classic" concept of antioxidant effects on tissues as modulating cellular reactive oxygen spaces (ROS) is increasingly being extended to new biologic modifying systems. How combinations of such supplements impact these systems remains a fruitful arena for further research in chemoprevention.

Thus, in this report, the considered and documented view has been offered that there is now new (since 1993) compelling evidence that supplements including antioxidant vitamins, namely alpha-tocopherol natural and synthetic derivatives of vitamin A and retinol, vitamin C, selenium, and folate, may reduce the risk of certain cancers. Data in support of this view come from human clinical trials, oftentimes conducted in higher risk populations.

## CANCER RISK REDUCTION AND DIET

Cancer risk-reducing effects are more commonly seen in subjects with impaired, low, or deficient antioxidant serum levels, usually reflective of poor dietary intake of certain foods or of a malnourished state. The link between dietary habits, malnourishment, and antioxidant supplement effect is blurred in much of the U.S. and in some international trials. Participants in such trials are often not reflective of poverty-level populations. Other special groups where economic or cultural factors do not facilitate either trial participation, or concomitant "healthy" diets, rarely participate in trials of cancer prevention.[107] These latter populations are deficient characteristically in plasma antioxidant and specific vitamin and mineral levels,[108] fruit and vegetable intake, and may more likely have long-standing smoking habits. It is recognized as more difficult to promote "healthy" dietary intake and educational efforts in diet-deficient groups including groups in the United States[109,110,49,111] with notable disparities in health services delivery. Such groups may also be less susceptible to public education regarding the benefits of healthy dietary intake. The role for antioxidant and other nutritional supplements would appear to have potential for the most profound benefit in reducing the risk of certain identified diseases, such as cancer, in these populations where adequate antioxidant plasma levels and intake are not achieved through appropriate dietary sources.

Perhaps related, African-Americans who have the lowest serum alpha-tocopherol levels1[112-114] have the highest incidence of prostate cancer among U.S. populations. In young African-American males below the age of 50, the incidence of high-grade prostate intraepithelial neoplasia (HPIN) is 7%; 50% of these will have invasive prostate cancer at the time of the next prostate biopsy.[115,116] Needed data on dietary cancer prevention in such a group must be considered in the light of noted cancer prevention results with nutritional supplements. As the U.S. increasingly addresses health disparities in its diverse populations where nutritional information may be predominantly limited, the role of diet and health supplements should be expected to receive new and more relevant focus.

Studies cited in this report are predominantly directed at adult populations. Adult populations because of prolonged "exposure" to adverse diets, environmental pollutants, and adverse lifestyle and social habits will have necessarily the more developed and advanced carcinogenic potential and an intrinsic health analogue for clinical cancer development. In this regard, the report by Woodson, et al[76]

## REFERENCES


1. Pearson v Shalala (1999), 164 F3d 650 (DC Cir 1999) reh'g denied 172 F3d 72 (DC Cir. 1999).
2. Emord JW. The beginning of the end for FDA speech suppression. J Pub Pol and Market.Spring 2000;19(1):139-143.
3. Alpha-Tocopherol and Beta Carotene Cancer Prevention Study Group (ATBCPSG). The effect of vitamin E and beta carotene on the incidence of lung cancer in male smokers. NEJM. 1994;330: 1029-1035.
4. Omenn G S, Goodman G, Thornquist M. et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. NEJM. 1996;334: 1150-1155.
5. Omenn GS, Goodman G, Thornquist M, et al. Chemoprevention of lung cancer: the beta caroteneand retinol efficacy trial (CARET) in high-risk smokers and asbestos-exposed workers. IARC Sci. Publ. 1996;136: 67-85.
6. Christen WG, Graziano JM., Hennekens C H, et al. Design of Physicians' Health Study II. A randomized trial of beta carotene, vitamin E and C, and multivitamins in preventing cancer, cardiovascular disease, and eye disorders, and review of results of completed trials. Ann Epidemiol. 2000;10: 125-134.
7. Montironi R, Mazzucchelli R, Marshall JR, et al. Prostate cancer prevention: review of target populations, pathologic biomarkers, and chemopreventative agents. J Clin Pathol. 1999;52:793-803.
8. Lippman SM, et al. Cancer chemoprevention: progress and promise. J Natl Cancer Inst. 1998;90:1514-1528.
9. Meyskens FL Jr. Re: Cancer prevention and promise. J Natl Cancer Inst. 1999;91:563-564.
10. Lippman S M, et al. Response. J Natl Cancer Inst. 1999;91: 564.
11. Kelloff G J. Perspectives in cancer prevention. In: Vadnerwoude GF, Klein, G, eds. Research and Drug Development Advances in Cancer Research. London: Academic Press; 2000;199-334.
12. Bertram J S. Carotenoids and gene regulation. Nutr Reviews. 1999;57:182-191.
13. Ripple M O, Henry F, Schwartz SR, et al. Effects of antioxidants on androgen-induced AP-1 and NF-K8 DNA binding activity in prostate cancer cells. J Natl Cancer Inst. 1999;91:1227-1232.
14. Wang XD, Russell RM. Procarcinogenic and anticarcinogenic effects of beta carotene. Nutr Rev. 1999;57(9pt1):263-272.
15. Perocco P, Paolini M, Mazzullo M, et al. Beta carotene as an enhancer of cell transforming activity of powerful carcinogens and cigarette smoke condensate in Balb/c 373 cells in vitro. Mut Res. 1999;440:83-90.
16. Lotan R. Lung cancer promotion by beta carotene and tobacco smokers: relationship to suppression of retinoic acid and receptor-beta and increased activator protein-1? I Natl Cancer Inst. 1999;91: 7-9.
17. Halliwell B. The antioxidant paradox. Lancet. 2000;355: 1179-1180.
18. Slaughter D P, Southwick HW, Smejkal W. "Field cancerization" in oral stratified squamous Epithelium: clinical implications of multicentric origin. Cancer. 1953;6:963-68.
19. Soloway MS, Perito PE. Superficial bladder cancer: diagnosis, surveillance, and therapy. J Cell Biochem. 1992;16(suppl):120-1127.
20. Muir CS, et al. The epidemiology of prostate cancer: geographic distribution and time trends. Acta Oncol. 1991;30:133-140.
21. Gittes RF. Carcinoma of the prostate. NEJM. 1991;324: 236-245.
22. Nomura AM, Kolenel LN. Prostate cancer: a current perspective. Epidemiol Rev. 1991;13:200-227.
23. Alpha-Tocopherol and Beta Carotene Cancer Prevention Study Group (ATBCPSG). The effect of vitamin E and beta carotene on the incidence of lung cancer in male smokers. NEJM. 1994;330:1029-1035.
24. Heinonen OP, et al. Prostate cancer and supplementation with alpha-tocopherol and B carotene: Incidence and mortality in a controlled trial. J Natl Cancer Inst. 1998;90:440-446.
25. Clark LC, Krongrad A, Slate E, et al. Decreased incidence of prostate cancer with selenium (Se) supplement: results of a randomized control trial. FASEB Proc. 1998;Abstract 781.
26. Clark LC, Combs GF Jr, Turnbull BW, et al. Effect of selenium supplement for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. JAMA. 1996;276:1957-1963.
27. Levander OA. Selenium. In: Mertz W, ed. Trace Elements in Human and Animal Nutrition. Orlando, Fla: Academic Press; 1986:209-280.
28. Ip C, Ganther H. Activity of methylated forms of selenium in cancer prevention. Cancer Res. 1996;50:1206-1251.
29. Whanger P, Butler J. Effects of various dietary levels of selenium as selenite or methylated selenium compound administered at cancer prevention levels in rats. J Nutr. 1998;118: 846-852.
30. Cook NR, Stampfer MJ, Ma J, et al. Beta carotene supplements for patients with low baseline levels and decreased risk of fatal prostate carcinoma. Cancer. 1999;86:1783-1792.
31. Heinonen O, Albanes D, Virtano J, et al. Prostate cancer and supplementation with alpha tocopherol and beta carotene: incidence and mortality in a controlled trial. J Natl Cancer Insti. 1998;90:440-446.
32. Webb PM, et al. Gastric cancer, gastritis, plasma vitamin C: results from an international correlation and cross sectional study. Intl J Cancer. 1997;73:684-689.
33. Choi MA, et al. Serum antioxidant levels and lipid peroxidation in gastric carcinoma patients. Cancer Lett. 1999;136: 89-93.
34. Farinati F, et al. Oxidative DNA damage accumulation in gastric carcinogenesis. Gut.1998;42:351-356.
35. Drake IM, et al. Ascorbic acid may protect against human gastric cancer by scavenging mucosal oxygen radicals. Carcinogenesis. 1996;17:559-562.
36. Hansson LE, et al. Nutrients and the risk of gastric cancer: a population-based case control study in Sweden. Int J Cancer. 1994;57:638-644.
37. Gail MH, et al. Factorial trial of three interventions to reduce the progression of precancerous gastric lesions in Shandong, China: design issues and initial data. Controlled Clin Trials. 1998;19: 352-369.
38. Ramon JM, et al. Nutrient intake and gastric cancer risk: a case control study in Spain. Int J Epidemiol. 1993;22:983-988.
39. Hu J, et al. Risk factors for oesophageal cancer in Northeast China. Int J Cancer. 1994;57:38-46.
40. Dawsey SM, et al. Effects of vitamin/mineral supplementation on the prevalence of histologic dysplasia and early cancer of the esophagus and stomach from the results of the dysplasia trial in Linxian, China. Cancer Epidemiol. Biomarkers and Prevention. 1994;3:167-172.
41. Wang GQ, et al. Effects of vitamin/mineral supplementation in the prevalence of histologic dysplasia and early cancer of the esophagus and stomach: results from the general population trial in Linxian, China.

Cancer Epidemiol. Biomarkers and Prevention. 1994;3:161-166.
42. Blot WJ, et al. Nutritional intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general populations. J Natl Cancer Inst. 1993;85:1483-1492.
43. Li JY, et al. Nutrition intervention trials in Linxian, China: multiple vitamin/mineral supplementation, cancer incidence, and disease specific mortality among adults with esophageal dysplasia. J Natl Cancer Inst.1993; 85:1492-1498.
44. Varis K, et al. Gastric cancer and premalignant lesions in atrophic gastritis: a controlled trial on the effects of supplementation with alpha-tocopherol and beta carotene. Scand J Gastroenterol. 1998;33:294-300.
45. Zeng W, et al. Retinol, antioxidant vitamins, and cancers of the upper digestion tract in a prospective cohort study of post-menopausal women. Am J Epidemiol. 1995;142:955-960.
46. Hong WK, et al. Prevention of second primary tumors with isotrefinoin in squamous-cell carcinoma of the head and neck. N Engl J Med. 1990;323:795-801.
47. Barth TJ, et al. Redifferentiation of oral dysplastic mucosa by the application of the antioxidants beta carotene, alpha-tocopherol and vitamin C. Int J Vit and Nutr Res. 1997;67:368-376.
48. Kaugars GE, et al. A clinical trial of antioxidant supplements in the treatment of oral leukoplakia. Oral Surg, Oral Med, and Oral Path. 1994;78:462-468.
49. Comerci JT Jr, et al. Induction of transforming growth factor-beta 1 in cervical intraepithelial neoplasia in vivo after treatment with beta-carotene. Clin Cancer Res. 1997;3:157-160.
50. Beenken SW, et al. Retinoid modulation of biomarkers in oral leukoplakia/dysplasia. J Cell Biochem. 1994;19:270-277.
51. Tradati N, et al. Successful treatment of oral lichen planus and leukoplakia with fenretinide (4-HPR). Cancer Lett. 1994;76:109-111.
52. Chiesa F, et al. Fenretinide (4-HPR) in chemoprevention of oral leukoplakia. J Cell Biochem. 1993;(suppl17F): 255-261.
53. Issing WJ, et al. Long term follow-up of larynx leukoplakia under treatment with retonyl palmitate. Head and Neck. 1996;18: 560-565.
54. Krishnaswamy K, et al. A case study of nutrient intervention of oral precancerous lesions in India. Europ J Cancer. 1995;31(ptB):B41-48.
55. Zaridze D, et al. Chemoprevention of oral leukoplakia and chronic esophagitis in an area of high incidence of oral and esophageal cancer. Ann Epidemiol. 1993;3:225-234.
56. Benner SE, et al. Micronuclei, a biomarker for chemoprevention trials: results of a randomized study in oral pre-malignancy. Int J Cancer. 1994;59:457-459.
57. Lippman SM, et al. Comparison of low dose isotretinoin with beta carotene to prevent oral carcinogenesis. NEJM. 1993;328:15-20.
58. Liede KE, et al. Long term supplementation with alpha tocopherol and beta carotene and prevalence of oral mucosal lesions in smokers. Oral Dis. 1998;4:78-83.
59. Dimery IW, et al. Phase I trial of alpha tocopherol effects on 13-cis-retinoic acid. Ann Oncol. 1997;8: 85-89.
60. Ramaswany G, et al. Serum vitamin's status in oral leukoplakias-a preliminary study. Oral Oncol Eur J Cancer. 1996;32B:120-122.
61. Lippman SM, et al. Cancer chemoprevention. J Clin Oncol. 1994;12:851-873.
62. Davies P, Lippman SM. Biologic basis of retinoid pharmacology. Adv Oncol. 1996;12:2-10.
63. Gillison ML, Koch WM, Capone RB, et al. Evidence for a casual association between human papillomavirus and a subset of head and neck cancer. J Natl Cancer Inst. 2000;92:709-720.
64. Baverick JN, et al. Prevention of skin cancer and reduction of keratotic skin lesions during acetretin therapy in renal transplant recipients: a double blind, placebo controlled study. J Clin Oncol. 1995;13:1933-1938.
65. Gibson GE. Low dose retinoid therapy for prophylaxis of skin cancer in renal cell transplant patients. J Europ Acad Derm and Venereol. 1998;10:42-47.
66. Stam-Posthuma JJ, Virik J, le Cessie S, et al. Effect of topical tretinoin under occlusion in atypical naevi. Melanoma Res. 1998;8:539-548.
67. Kraemer KH, Di Giovanna JJ, Moshell AN, et al. Prevention of skin cancer with oral 13-cis retinoic acid in xeroderma pigmentosum variant (xy-v). NEJM. 1988;318:33-38.
68. Bjelke E. Dietary vitamin A and humans lung cancer. Int J Cancer. 1975;15:561-565.
69. Kvale G, et al. Dietary habits and lung cancer. Int J Cancer. 1983;15:397-405.
70. Fontham ETH. Protective dietary factors and lung cancer. Int J Epidemiol. 1990;19(51):532-542.
71. de Klerk NH, et al. Vitamin A and cancer prevention II: comparison of the effects of retinol and beta carotene. Int J Cancer. 1998;75:362-367.
72. Knekt P, et al. Role of various carotenoids in lung cancer prevention. J Natl Cancer Inst. 1999;91:182-184.
73. Christen WG, et al. Beta-carotene supplementation: a good thing, a bad thing, or nothing? Curr Opin Lipid. 1999;10: 29-33.
74. Omenn GS, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. NEJM. 1996;334:1150-1155.
75. Huttunen JK. Why did antioxidants not protect against lung cancer in the ATBC Cancer Prevention Study? IARC Scient Publ. 1996;136:63-65.
76. Woodson K, et al. Serum alpha-tocopherol and subsequent risk of lung cancer among male smokers. J Natl Cancer Inst. 1999;91:1738-1743.
77. Speizer FE, Colditz GA, Hunter DJ, et al. Prospective study of smokers, antioxidant intake and lung cancer in middle aged women (U.S.A.). Cancer Causes and Control. 1999;10:475-482.
78. Heimburger DC, Alexander CB, Nirch R, et al. Improvement in bronchial squamous metaplasia in smokers treated with folate and B12. Report of a preliminary randomized double-blind intervention trial. JAMA. 1988;259:1525-1530.
79. Pastorino V, Infante M, Maioli M, et al. Adjuvant treatment of stage I lung cancer with high dose of vitamin A. J Clin Oncol. 1993;11:1216-1222.
80. Mulshine JL. Reducing lung cancer risk: early detection. Chest. 1999;116:4935-4965.
81. Mulshine JL, De Luca LM, Dedrick RL. Regional delivery of retinoids: a new approach to early lung detection intervention. In: Martinet Y, Vignaud JM, et al., eds. Clinical and Biological Bases of Lung Cancer Prevention. Basel, Switzerland:1999.
82. Richart RM, Barron BA. A follow up study of patients with cervical dysplasia. Am J Obst Gynecol. 1969;105:386-393.
83. Koutsky LA, et al. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papilloma virus infection. NEJM. 1992;327:1272-1278.

84. Ho GYF, et al. Persistent genital human papilloma virus infection as a risk factor for persistent cervical dysplasia. J Natl Cancer Inst. 1995;87:1365-1371.
85. Morrison EAB, et al. Human papilloma virus infection and other risk factors for cervical neoplasia: a case-control study. Int J Cancer. 1991;49:6-13.
86. Mackerras D, et al. Randomized double blind trial of beta carotene and vitamin C in women with minor cervical abnormalities. Br J Cancer. 1999;79:1448-1453.
87. Palau PR, et al. Plasma concentrations of micronutrients during a nine-month clinical trial of beta carotene in women with precursor cervical cancer lesions. Nutrition and Cancer. 1998;30:46-52.
88. Poteschman N, Brinton KA. Nutrition and cervical cancer. Cancer Causes Control. 1996;7:113-126.
89. Nogata C, Shimizu H, Yoshikawa H, et al. Serum carotinoids and vitamins and risk of cervical dysplasia from a case control study in japan. Br J Cancer. 1999;81:1234-1237.
90. Lehtinen M, Luostarinen T, Youngman LD. Low levels of vitamins A and E in blood and subsequent risk for cervical cancer: interaction with HPV seropositivity. Nutr and Cancer. 1999;34:229-234.
91. Ruidi C, et al. Chemoprevention of cancer of uterine cervix: a study of chemoprevention of retinamide II from cervical precancerous lesions. J Cell Biochem. 1997;(suppl28-29):140-143.
92. Meyskens FL Jr, et al. Enhancement of regression of cervical intraepithelial neoplasia II with topically applied all-trans-retinoic acid: a randomized trial. J Natl Cancer Inst. 1994;86:539-543.
93. Ponz de Leon M, Roncucci L. Chemoprevention of colorectal tumors; the role of lactulose and of other chemoprevention agents. Scand J Gastroenterol. 1997;(suppl222):72-75.
94. Knekt P, Aromao A, Maatela J, et al. Serum vitamin E, serum selenium and the risk of gastrointestinal cancer. Int J Cancer. 1988;42:846-850.
95. Bonnelli L, et al. Chemoprevention of metachronous adenomas of large bowel by means of antioxidants.: a double blind randomized study trial. The International Selenium Tellurium Development Association. Scottsdale, Arizona. 1998.
96. Kim, Y-I. Folate and carcinogenesis: evidence, mechanisms, and implications. J Nutri Biochem.. 199910:66-88.
97. Choi S-W, Mason JB. Folate and carcinogenesis: an integrated scheme. J Nutri. 2000;131:129-32.
98. Krinsky NI. Carotenoids and cancer. Basic research studies. In: Frei B, ed. Natural Antioxidants in Health and Disease. San Diego: Academic Press Inc;1994:239-261.
99. Muto Y, et al. Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. NEJM. 1996;334:1561-1567.
100. Yu SY, Chu Y J, Gong XL, et al. Regional variation of cancer mortality and incidence and its relation to selenium levels in China. Biol Trace Elements Res. 1985;7:21.
101. Kelley DS. Essential nutrients and immunologic functions. Am J Clin Nutr. 1996;63:9945-9965.
102. De Flora S, et al. Modulation of genotoxic and related effects by carotenoids and vitamin A in experimental models: mechanistic issues. Mutagenesis. 1999;14(2):153-172.
103. Collins AR. Oxidative DNA damage, antioxidants, and cancer. Bioessays. 1999;21(3):238-246.
104. Kurie JM.The biologic basis of retinoids in cancer prevention. Curr Opin Oncol. 1999;11:497-502.
105. Kim DG, Lee DY, Cho S, et al. Down regulation of interleukin growth factor building proteins and growth modulation by retinoic acid. Hepatology. 1999;29:1091-1098.
106. Hampton MB, Orrenius S. Redux regulation of apoptotic cell death. Biofactors. 1998;18:1-5.
107. Mathers JC, et al. Nutrition and cancer prevention. Curr Opin Oncol. 1999;11:402-407.
108. Lee IM. Antioxidant vitamins in the prevention of cancer. Proc Assoc Am Physicians. 1999;111:10-15.
109. Glanz K, et al. The health impact of worksite nutrition and cholesterol programs. Am J Health Promot. 1996;10:453-470.
110. Sorensen G, et al. Working well: results from a worksite-based cancer prevention trial. Am J Publ Health. 1996;86:939-947.
111. National Cancer Institute SEER cancer statistics review, 1973-94. Bethesda, Md: National Cancer Institute, 1997 (NIH publication No. 97-2789).
112. Ford ES, Sowell A. Serum tocopherol status in the United States population: Findings of the Third National Health and Examination Survey. *Am J Epidemoil*. 1999; 150:290-300.
113. Mayne ST. et al. Effects of supplemental B-carotene on plasma concentrations of carotenoids, retinol, and alpha tocopherol in humans. *Am J Clin Nutr*. 1998; 68:642-677.
114. Nierenberg DW, et al. Effects of 4 years of oral supplemental with beta. carotene on serum concentrations of retinol tocopherol, and 5 carotenoids. *Am J Clin Nutr*. 1997; 66:315-319.
115. Sakr WA, Grignon DJ, Haas GP, et al. Age and racial distribution of prostate intraepithelial neoplasia. Eur Urol. 1996;30:138-144.
116. Will ML, Hamper NM, Partini AW, et al. Incidence of HPIN in sextant needle biopsy specimens. Urology. 1997;49:367-373.
117. Subar A, et al. Fruit and vegetable intake in the United States: the baseline survey of the Five a Day for Better Health Program. Am J Health Promotion. 1992;9:352-360.
118. Buller DR. Randomized trial testing the effect of peer education in increasing fruit and vegetable intake. J Natl Cancer Inst. 1992;91:491-500.
119. Carroquino MJ, et al. The U.S. EPA Conference on Preventable Causes of Cancer in Children: a research agenda. Environ Health Persp. 1998;106(suppl3):867-873.
120. Preston-Martin S, et al. Results from an International case-control study of childhood brain tumors: the role of prenatal vitamin supplementation. Environ Health Persp. 1998;106(suppl 3): 887-892.
121. Vanhet Hof KH, West CE, Westrate V, et al. Dietary factors that affect bioavailability of carotenoids. J Nutri. 2000;130:503-506.
122. Li R, Serdula M, Bland S, et al. Trends in fruits and vegetables consumption among adults in 16 U.S. states: behavioral risk factor surveillance system. Am J Public Health. 2000;90:771-781.
123. Veggie Nutrients Dip in Tests. Newhouse News Service, Washington, DC. Omaha World-Herald. January 29, 2000:6.
124. Finley J, Matthys SL, Shuler T, et al. Selenium content of foods purchased in North Dakota. Nutr Res.1996;16:723-728