Evaluation of Publicly Available Scientific Evidence Regarding Certain Nutrient-Disease Relationships:

8C. Vitamin E and Cancer

December 1991

By

Ching K. Chow, Ph.D.

Prepared for

CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
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Life Sciences Research Office
Federation of American Societies
For Experimental Biology
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Bethesda, Maryland 20814
FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon literature reviews and the scientific analyses by knowledgeable investigators engaged in work in specific areas of biology and medicine.

This report was developed for the Center for Food Safety and Applied Nutrition, Food and Drug Administration (FDA), in accordance with the provisions of Task Order #9 of Contract No. 223–88–2124. Potential authors and reviewing consultants were identified by the LSRO based on their qualifications, experience, and freedom from conflict of interest, with due consideration for balance and breadth in appropriate disciplines. The author and reviewing consultants were selected with the concurrence of the LSRO Advisory Committee (which consists of representatives of each constituent Society of FASEB).

On March 14, 1991, the FDA requested submission of scientific data and information on the ten specific topics for which health claims might be made (Federal Register 56:12932–12933). The scientific data and information provided in response to this request were considered by LSRO in preparing this report. Copies of the submitted materials are available for public inspection at the Dockets Management Branch, FDA (Docket No. 91N–0101). Copies of documents cited in this report are available for public inspection at LSRO, FASEB.

Ching K. Chow, Ph.D., Professor, Department of Nutrition and Food Science, University of Kentucky, Lexington, Kentucky should be cited as the author of this report. The LSRO acknowledges the efforts of Ching K. Chow, Ph.D. and also the critical assistance of Philip Farrell, M.D., Ph.D., Professor and Chair, Department of Pediatrics, University of Wisconsin, Madison, Wisconsin, and Kimberly Kline, Ph.D., Associate Professor, Division of Nutrition, Department of Human Ecology, University of Texas, Austin, Texas, who reviewed several drafts of the manuscript. The appendix tables were prepared by the LSRO staff and author and were critically reviewed by the author and reviewers. Subsequently the draft report and tables were revised by the author, edited by the LSRO scientific staff, and received final concurrence from the author and reviewing consultants.

The evaluation of scientific literature, data, and information submitted to the LSRO was made by the author, reviewers, and the LSRO independently of FDA or any other group, governmental or non-governmental. The author and LSRO accept responsibility for the accuracy of the report conclusions and its appendix table(s). This final report was reviewed and approved by members of the LSRO Advisory Committee under authority delegated by the Federation Board. The LSRO Advisory Committee members who reviewed this report were free of conflicts of interest in regard to the subject matter under policies established by the Federation. Upon completion of these review procedures, the report was approved by the Executive Director, FASEB, and transmitted to FDA.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of each individual member of the FASEB constituent Societies.

December 31, 1991

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office
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I. INTRODUCTION

Cancer is the second leading cause of death in many Western countries. More than 800,000 new cases of cancer are diagnosed each year in the United States, and over 400,000 die of cancer annually (National Cancer Institute, 1990; National Center for Health Statistics, 1988; National Research Council, 1989a; U.S. Department of Health and Human Services, 1988). The cause and possible control of cancer has been intensely investigated. It has been recognized that environmental factors have strong influences on the incidence of human cancer. Many chemical and physical agents in the environment have been identified or are suspected as human carcinogens. These include many direct-acting carcinogens, pro-carcinogens, co-carcinogens, and promoters.

Cancer is not a single disease entity. A large number of chemical and physical agents can affect the development of cancer in many different ways. In spite of the multiplicity of causative factors, most cancers seem to arise by a stepwise evolution involving progressive genetic changes, cell proliferation, and clonal expansion (Mergens and Bhagavan, 1989; National Research Council, 1989a; Weinstein, 1988). The initial stage, initiation, occurs at the DNA level and involves an irreversible genetic alteration such as oncogene activation, tumor suppressor gene inactivation, DNA amplification, gene transposition, or chromosome translocation. This initiation event does not necessarily result in tumor development, rather, it is the first step in a multistage/multifactorial process involving initiation, promotion, and progression. The transition between stages is complex and is thought to involve environmental (exogenous) and autocrine (endogenous) factors. There is generally a long latent period between carcinogen exposure and tumor development. In humans, this period could be as long as 30 years. Thus, it is possible that the process of carcinogenesis could be reversed or slowed if the progressive stages of cancer development were understood.

Increasing evidence indicates that diet and or nutritional status may play an important role in the etiology of human cancer. Diet is a complex mixture of chemical entities that contains factors that may cause or promote cancer (enhancing factors) as well as factors that are antimutagenic or anticarcinogenic (inhibitory factors). Dietary components may directly interact with mutagens or carcinogens and influence metabolic activation and detoxification, as well as regulate DNA repair and expression. Many dietary components have also been shown to be capable of enhancing or diminishing the mutagenic and carcinogenic activity of other substances.
II. VITAMIN E AND CARCINOGENESIS

A. NOMENCLATURE

Vitamin E is the term suggested for all tocol and tocotrienol derivatives qualitatively exhibiting the biological activity of $\alpha$-tocopherol. "Tocopherols" is the generic description for all mono-, di-, and trimethyl tocols and tocotrienols and is not synonymous with the term "vitamin E." All 8 naturally occurring tocopherol compounds isolated from plant sources have a 6-chromanol ring and a phytol side chain (Chow, 1985; Machlin, 1991). There are four each of tocols and tocotrienols that occur naturally, differing in the number and position of methyl groups on the chromanol ring. While vitamin E refers to at least eight tocopherol structures, $\alpha$-tocopherol predominates in many species and, based on animal studies, is significantly more biologically active than any other form of tocopherol.

Tocopherols exist mainly in the free alcohol form and are widely distributed in a variety of plant life. In addition to naturally occurring isomers, several types of synthetic vitamin E are available commercially. Most synthetic tocopherols are esters, particularly the acetate form. The ester form is less susceptible to oxidation and more suitable for food and pharmaceutical applications than the free form. To distinguish it from the synthetic one, the naturally occurring stereoisomer of $\alpha$-tocopherol, formerly known as d-$\alpha$-tocopherol or $\alpha$-tocopherol, has been designated as RRR-$\alpha$-tocopherol. The totally synthetic $\alpha$-tocopherol, previously known as dl-$\alpha$-tocopherol, has been designated as all-rac-$\alpha$-tocopherol. The natural form of tocopherol (RRR) is more active than the synthetic one (all racemic).

B. HUMAN REQUIREMENTS

The biological activity of various forms is expressed as units of activity, and the relative values in international units (IU/mg) are: dl-$\alpha$-tocopheryl acetate (all racemic), 1.00; dl-$\alpha$-tocopherol (all racemic), 1.10; d-$\alpha$-tocopheryl acetate (RRR), 1.36; d-$\alpha$-tocopherol (RRR), 1.49; dl-$\alpha$-tocopheryl succinate (all racemic), 0.89; and d-$\alpha$-tocopheryl succinate (RRR), 1.21. In the United States, the current recommended dietary allowance for vitamin E ranges from 4.5 IU (3 $\alpha$-tocopherol equivalents or 3 mg d-$\alpha$-tocopherol) in infants, 9.0 - 10.5 in children (10 years or under), 15.0 in males (>11 years), 12.0 in females (>11 years), 15.0 in pregnant females, to 18.0 in lactating females (National Research Council, 1989b). The majority of tocopherols consumed in the United States is not the $\alpha$ form (Chow, 1985). Gamma-tocopherol, with 10-35 percent of biological activity of $\alpha$-tocopherol, accounts for over half of the estimated total tocopherol intake.

Dietary deficiency of vitamin E is rare in the United States as normal American diets provide adequate amounts of vitamin E. The intake of vitamin E generally parallels the intake of polyunsaturated fatty acids. Foods with a high polyunsaturated fatty acid content generally are rich sources of tocopherols (Chow, 1985; Machlin, 1991); however, the human requirement for vitamin E can change due to the consumption of fats and oils. Consequently, the dietary requirement for vitamin E is related to the degree of unsaturation of the fatty acids in tissue lipids, which can be altered by dietary lipids (Horwitt, 1974, 1991).

The intake of total tocopherols is, in general, proportional to the amount of vegetable oil consumed as vegetable oils are among the best sources of tocopherols. The normal intake of vitamin E in American diets ranges between 4 and 33 $\alpha$-tocopherol equivalents ($\alpha$-TE) daily in adults not taking...
vitamin E supplements, with average values between 11 and 13 \( \alpha\)-TE (Machlin, 1989). This level of intake results in average plasma/serum levels in adults of approximately 9.5 \( \mu g/ml \) (Machlin, 1991).

C. FUNCTIONS

Vitamin E was discovered approximately 70 years ago as a lipid-soluble dietary factor necessary for the prevention of fetal death and resorption in rats (Evans and Bishop, 1922). Subsequently a number of species-dependent manifestations of vitamin E deficiency, such as liver necrosis in rats and pigs, erythrocyte hemolysis in rats and chickens, and white muscle disease in calves, sheep, mice and mink, have been documented (Machlin, 1991; Scott, 1970). Because of the lack of a definite clinical deficiency syndrome attributable to vitamin E, the need or use of this vitamin in humans had been questioned.

The essentiality of vitamin E for humans was recognized in the late 1960s in connection with studies on premature infants in which hemolytic anemia was associated with vitamin E deficiency (Bieri and Farrell, 1976). While it is difficult to produce vitamin E deficiency under experimental conditions in adult humans, recent studies have shown that deficiencies or subclinical deficiencies such as neurologic abnormalities do occur in association with malabsorption syndromes of various etiologies (Bieri and Farrell, 1982; Machlin, 1991; Sokol, 1988, 1989). Although many biochemical abnormalities have been found to be associated with vitamin E deficiency, the mechanism by which vitamin E prevents various metabolic and pathological lesions has not yet been elucidated.

Vitamin E is the most important lipid-soluble antioxidant and free radical scavenger. The suggestion that the function of vitamin E may be related to its membrane stabilization property (Lucy, 1972) does not conflict with the free radical scavenging mechanism or antioxidant function of vitamin E (Tappel, 1972). Several non-antioxidant functions of vitamin E have also been suggested. Vitamin E, for example, has been reported to regulate the de novo synthesis of xanthine oxidase (Catignani et al., 1974), modulate the activities of protein kinase C (Packer, 1991) and microsomal enzymes (Chen et al., 1982; Chow and Gairola, 1984), and inhibit tumor cell proliferation (Prasad and Edward-Prasad, 1982; Boscoboinik et al., 1991). Also, vitamin E may regulate immune response or cell-mediated immunity by modulating generation of prostaglandins and other lipid peroxidation products (Meydani et al., 1990).

The functional interrelationships among vitamin E and other micronutrients, notably selenium and vitamin C, have long been recognized (Chow, 1979, 1988). Selenium has been shown to prevent or reduce the severity of several symptoms of vitamin E deficiency (Chow, 1985; Machlin, 1991; Scott, 1970). As an integral part of the enzymes glutathione (GSH) peroxidase (Rotruck et al., 1972) and phospholipid hydroperoxide GSH peroxidase (Ursini et al., 1985), selenium may complement the antioxidant function of vitamin E via the hydroperoxide reduction function and thus reduce the requirement for the vitamin.

In addition to being an important water-soluble free radical scavenger and reducing agent, vitamin C seems to have a synergistic effect on vitamin E. This effect has been attributed to the involvement of vitamin C in the regeneration or restoration of vitamin E after it exerts its antioxidant function (Niki et al., 1982; Packer et al., 1979; Wefers and Sies, 1988). Similarly, GSH, in association with an enzyme or enzyme system(s), may also be involved in the regeneration of vitamin E (Reddy et al., 1982; Wefers and Sies, 1988). Furthermore, a GSH-dependent dehydro-ascorbate reductase and a NADH-semidehydro-ascorbate reductase appear to be involved in the regeneration or restoration of vitamin C (Chow, 1988; Diliberto et al., 1982).
Experimental evidence for the vitamin E regeneration or restoration in vivo, however, remains to be provided (Chow, 1991). While the nature of the vitamin E regenerative process in vivo, if any, and the agent(s) responsible are not yet clear, it does provide a rational explanation for the fact that it is very difficult to deplete adult animals or human subjects of the vitamin (Horwitt, 1962). Also, the possible involvement of vitamin C, GSH and other reducing agents in the regeneration of vitamin E and its functional interaction with selenium indicate an interdependence among various antioxidant defense systems (Chow, 1988, 1991).

D. POSSIBLE ROLE FOR VITAMIN E IN CARCINOGENESIS

Free radical-initiated peroxidative damage to the cell has been implicated in the pathogenesis of many cell injury and disease states including cancer, cardiovascular disease, and autoimmune diseases (Esterbauer and Cheeseman, 1987; Freeman and Crapo, 1982; Halliwell, 1987). Whether vitamin E, the most important biological antioxidant and free radical scavenger, can prevent or slow down carcinogenesis has received considerable research interest. Investigation into the influence of dietary vitamin E on the induction and development of cancer has produced a large amount of data. Cell culture and certain animal studies, although not conclusive, suggest that vitamin E may possess antitumor properties (Chen et al., 1988; Mergens and Bhagavan, 1989).

Vitamin E has been suggested to play a role in several stages of carcinogenesis. These include a) inhibition or blockage of mutagen or carcinogen formation from precursors via direct chemical interaction, b) prevention of mutagens or carcinogens from reaching or reacting with DNA by scavenging mutagens or by enhancing detoxification processes, and c) prevention of cancer progression by the enhancement of normal immune responses.
III. ASSESSMENT OF VITAMIN E STATUS

In an epidemiological study, the intake of vitamin E is estimated based on information obtained from subject interview and questionnaire, and the nutritional status of vitamin E is assessed based on the concentration of tocopherols in body stores. Many sources of potential errors are associated with each type of nutritional epidemiological study. Until they are properly addressed, meaningful assessment of the relationship between nutrient and cancer risk can not be achieved. It should be noted that, due to the absence of vitamin E deficiency in the general population, any relationship between this nutrient and human cancer may be difficult to demonstrate. Also, most clinical intervention trials are actually pharmacologic interventions that are not entirely relevant to dietary considerations.

The major problems associated with the assessment of vitamin E intake and status in epidemiological studies of human cancer are briefly summarized below.

A. BIOCHEMICAL ASSESSMENT

At present there is no suitable index which accurately reflects dietary intake or body stores of vitamin E. Several indices, such as tocopherol concentrations in plasma/serum, erythrocytes, platelets or tissues, degree of erythrocyte hemolysis, and amounts of lipid peroxidation products (e.g., ethane, pentane, and malonaldehyde) generated, have been used to assess the nutritional status of vitamin E. Direct measurement of tocopherol concentrations, especially in tissues, is a logical choice over the indirect methods. Analysis of adipose tissue and liver biopsy samples for tocopherols, for example, is conceivably a reliable index of body stores of vitamin E and thus long-term vitamin E status (Rautalahti et al., 1990). However, in addition to its invasive nature, ethical and other considerations, such as whether representative samples are used, render it impractical for large population studies.

Measurement of tocopherol concentrations in erythrocytes is a better indicator for vitamin E than that of plasma/serum. However, it has not been widely employed since it is technically more cumbersome and difficult to determine tocopherol in the erythrocytes than in serum/plasma. Platelets have been shown to be more sensitive for measuring dose response to dietary vitamin E when compared to plasma, erythrocytes, or lymphocytes (Lehmann, 1981). Also, platelet tocopherol concentrations are independent of serum lipid levels (Vatassery et al., 1983), an important advantage relative to serum or plasma tocopherol concentrations. However, it is cumbersome to isolate platelets, and the procedure requires a larger blood sample. Therefore, measurement of tocopherol concentrations in blood plasma/serum remains the most practical method for the assessment of the nutritional status of vitamin E by most investigators. The discussion here is limited to serum/plasma since all the studies reviewed here employed only these samples for assessing vitamin E status.

1. Sample storage

Major problems associated with the measurement of serum/plasma levels of vitamin E in epidemiological studies are the length and conditions of sample storage, as well as the frequency and manner of sample handling. Tocopherol oxidation (destruction) is accelerated by exposure to light, heat, alkali, and the presence of iron and copper salts. As exemplified in the articles cited in this review, serum/plasma samples had been stored at temperature ranging from -18°C to -70°C for as long as 13 years. Wald et al. (1988) re-analyzed the vitamin E levels in those original serum samples still available and compared these values to those obtained in 1981 (Wald et al., 1984) to determine the effect of sample storage and handling on serum vitamin E level. They found the mean level of
vitamin E declined from 6.45 mg/L to 3.1 mg/L during a 5-year storage period (from 1981 to 1986) at
-20°C and suggested that the values reported in their 1984 publication might have been artifacts of
freezing and thawing. This stresses the importance of ensuring that samples used for prospective
follow-up study be stored properly and that records, especially freezing and thawing, are kept to
insure the comparability of cases and controls.

2. Methodology

Methods employed for quantifying plasma/serum vitamin E can have significant effects on the values
obtained. High performance liquid chromatography with either UV–visible or fluorescence detection
is a far more specific and sensitive method than fluorometric or spectrophotometric determination.
While the majority of recent studies used a high performance liquid chromatographic technique to
measure serum or plasma tocopherols, usually only the levels of α–tocopherol were reported to
represent the status of vitamin E. However, the contribution of non-α tocopherols to the total vitamin
E activity can be significant (Chow, 1985; Machlin, 1991).

3. Clinical considerations

The ability to absorb nutrients, including vitamin E, may be impaired in subjects with gastrointestinal
cancer, especially for those with pancreatic or intestinal cancers. Consequently, it should not be
 surprising that the levels of plasma/serum vitamin E are lower in patients with gastrointestinal cancer.
Both disease symptoms and drug treatments are likely to affect digestion and absorption of nutrients,
including vitamin E, in other types of cancer. Also, certain types of chemotherapies and drugs may
interact with and alter the nutritional status of vitamin E, independent of dietary intake.

4. Role of blood lipid levels

Vitamin E is transported in blood via lipoproteins. In humans, α–tocopherol is found in all lipoprotein
fractions. Since vitamin E is found in chylomicrons, fasted serum samples eliminate this source of
potential variation based on the time blood is drawn after the subject's last meal. The ratio of plasma
tocopherol to total lipid is usually a more reliable criterion of vitamin E status than tocopherol alone
(Machlin, 1991). Often the ratio of tocopherol to cholesterol or tocopherol to the sum of cholesterol
and triglycerides is used as a more convenient index than total lipids. Stähelin et al. (1989) reported
that the adjustment of fasted plasma values to the sum of cholesterol and triglycerides allowed a
lipid–independent estimation of vitamin E status. Since vitamin E is carried in the lipid fraction of
the blood and shows significant correlations with lipid levels, blood lipid levels should be taken into
consideration.

5. Biological variability

A great deal of genetic and environmentally derived diversity is found in serum vitamin E levels.
Helzlsouer et al. (1989), for example, have shown that the serum levels in controls averaged 11.1 μg/ml
with a range of 5.3 to 25.7 μg/ml (n = 70). Also, Tangney et al. (1987) reported that day–to–day
intraindividual variations in serum vitamin E levels can constitute an important source of error if
single samples of blood are used to categorize individuals. Knekt et al. (1988a) studied the relationship
between serum α–tocopherol levels and many of its possible determinants in 301 adult Finnish men
and women, ages 40–79 years. They found that the mean α–tocopherol level among men was 8.6 μg/ml
and among women was 10.5 μg/ml, and that serum α–tocopherol levels varied with age, geographical
area, type of population, occupation, socioeconomic status, and marital status. Furthermore, they found that serum α-tocopherol levels were positively correlated with serum cholesterol and serum vitamin A in both sexes and with body mass index and serum selenium in men. They concluded that the level of serum α-tocopherol, which is associated with the dietary intake of vitamin E, is dependent upon living conditions.

In view of the variability of tocopherol content in blood serum/plasma, it is necessary to develop more reliable procedures for assessing long-term vitamin E intake status rather than continuing to rely on serum/plasma tocopherol analysis. As mentioned above, measurement of tocopherol content in tissues, such as adipose biopsy samples (Rautalahti et al., 1990), is conceivably a more reliable index of vitamin E status than that of serum/plasma. However, ethical and other considerations prevent the routine use of this technique for large population studies.

B. DIETARY ASSESSMENT

Accurate dietary intake is difficult to assess. Unless properly controlled and complete, assessment of dietary nutrient intake has limited value.

1. Interview methods

Measurement of dietary intake is inherently error prone. For example, individuals' long-term intake of a given diet, food, or nutrient is difficult to assess accurately because their diets may have varied substantially over time. Well-trained persons and well-prepared questionnaires are required to perform the task properly. Of particular concern in the dietary assessment of vitamin E is that the consumption of some of the richest sources of tocopherols, e.g., soybean, sunflower, and cottonseed oils and products made from them such as margarine, is difficult to quantify by dietary history interviews (Bertram, 1987). Also, increased use of fat substitutes may complicate the assessment of dietary intake of fats and vitamin E by conventional interviewing techniques. Potential sources of errors in the use of interviews for obtaining food/nutrient intake information include a) estimation of only recent intake rather than earlier dietary intake which may be more relevant to disease etiology, b) reliance on limited food lists, and c) errors in subject estimation of food–intake frequency and size of food portion eaten.

Cooperation and compliance of subjects are needed in order to obtain reliable information concerning vitamin E intake and other relevant data. These constitute the biggest problems associated with intervention studies as there is no easy and reliable method for assessing whether subjects took vitamin E supplements as instructed.

2. Food composition tables

One approach to a more reliable measurement of dietary intake has been the use of food–frequency questionnaires (Willett et al., 1983). However, the accuracy of this type of information also depends on the reliability of information in the food composition data used. Tocopherol content of foods is highly variable depending upon genetic, seasonal, processing, storage, and other factors. Another concern is the reliability of information about tocopherol content of various foods. The majority of information concerning tocopherol contents of various foods was obtained in the past using colorimetric, rather than high performance liquid chromatographic techniques. Furthermore, food processing and storage and culinary practices can significantly influence the destruction of tocopherols present in the food.
Despite the inherent errors involved in dietary measurement, nutritional epidemiological studies can provide useful information when a quality dietary assessment method is used. However, it should be noted that vitamin E is functionally interrelated to a number of nutrients including polyunsaturated lipids, β-carotene, selenium, and ascorbic acid (Chow, 1988, 1991; Machlin, 1991). The variable nature of human requirements for vitamin E due to changes in consumption of fats and oils has been reported (Horwitt, 1974). Thus, to identify ultimately the cancer risk associated with low levels of vitamin E, the status of other interacting nutrients needs to be assessed in combination with vitamin E.
IV. VITAMIN E AND HUMAN CANCER

In recent years, the possibility that a higher intake of micronutrients, including vitamin E, may reduce the risk of certain human cancers has received considerable attention. This review deals only with recent reports concerning the possible role of vitamin E in the etiology and prevention of human cancers.

Epidemiological study designs can be classified according to the method of obtaining data: simple observation (non-experimental studies) or observation after some type of intervention (experimental studies) (Rogers and Longnecker, 1988). Intervention studies (clinical trials) are, in theory, the best and least likely to be biased methods to assess diet and cancer relationships. However, clinical trials are frequently not feasible for practical, financial, and ethical reasons. Follow-up and case-control studies are the two non-experimental study designs most commonly used in nutritional epidemiology. Data obtained with geographic correlation studies are less powerful and less useful than other non-experimental studies (Rogers and Longnecker, 1988). A review of the extant literature since 1987, organized by organ site, on the relationship of vitamin E and cancer follows. Summaries of cited human studies may be found in Appendix Table 8.

A. HEAD AND NECK CANCER

Drozdz et al. (1989) fluorometrically measured serum samples for vitamins A and E from 22 newly diagnosed cases of larynx cancer, 16 patients with nonmalignant laryngeal diseases, and 16 patients with other nonmalignant diseases, including cardiovascular disease and hernia, in Poland. Fasted serum samples were stored at -40°C for less than 2 weeks. The levels of vitamin A, but not vitamin E, were lower in cases than either control groups. There were no community-based controls nor was the hospital control group matched with cases for age, sex, or socioeconomic factors.

Gridley et al. (1990) conducted a population-based multicenter case-control study to assess the relationship between diet and oral and pharyngeal cancer among Afro-Americans. Cases were 248 pathologically-confirmed-incident patients with oral and pharyngeal cancer from the population-based cancer registries of Atlanta, Georgia, New Jersey, Los Angeles, and Santa Clara and San Mateo counties of California. Cancers of the tongue and pharynx and other oral cancers were included, but not cancers of the lip, salivary glands, or nasopharynx. Controls were chosen using random-digit-dialing (for those under age 65) and the Health Care Financing Administration roster (for those aged 65 years or older) and were matched on sex and age. Interviews were administered to obtain information on demographic variables, tobacco and alcohol use, diet, occupation, and medical history. Because of death or severe disability, next-of-kin interviews were obtained for 55 cases and 3 controls. Of the total subjects, 190 cases (142 males and 48 females) and 201 controls (139 males and 62 females) were included in the analysis. Information on portion size and nutrient indices were derived from the Second National Health and Nutrition Examination Survey (NHANES II) and from data of the U.S. Department of Agriculture. Food group and nutrient indices were categorized into quartiles based on sex-specific distributions of the control group. The association between dietary factors and oral cancer was assessed by the odds ratio as an estimate of relative risk. An increased intake of fruits and vegetables was found to be associated with decreased risk of oral cancer among both men and women. Risk was also found to decline in both sexes with an increase in consumption of vitamin C and fiber and in men only with increased consumption of carotene and vitamin E.

The role of nutrients and dietary factors in relation to esophageal cancer was examined in a large case-control study conducted in Calvados, France, a region having a relatively high incidence of the
disease (Tuyns et al., 1987). A total of 743 cases (704 males and 39 females) and 1975 controls (922 males and 1053 females) were interviewed about their usual food intake. The consumption of 40 food items was recorded, and intake of nutrients was derived from these data using food-composition tables. Relative risks for each nutrient or food item and the corresponding chi-square for association and for trend were computed over four levels of consumption. Higher intakes of vitamins E and C, along with fresh meat, citrus fruits and oils, were found to be associated with a reduction in risk of esophageal cancer. On the other hand, higher intake of retinol was found to be associated with increased risk of the cancer.

Van Helden et al. (1987) examined the relationship between nutritional status of vitamin E and certain other nutrients and risk of esophageal cancer in certain population groups in South Africa. Healthy subjects, who lived in the given area for 10 years or longer, were selected from areas known for high, intermediate, or low incidence of esophageal cancer. Plasma samples were kept at -20°C until analyzed.

In one study, the levels of serum vitamin E averaged 5.3 (n = 16), 4.3 (n = 21), and 3.8 (n = 18) μg/ml, respectively, for subjects from low-, intermediate-, and high-incidence areas. In another study conducted in a different region, the values were 8.7 (n = 24) and 6.1 (n = 27) μg/ml, respectively, for subjects in low- and high-incidence areas. The age of subjects (both sexes) ranged from 32–50 years. The results suggest that deficiency in vitamin E (vitamin A and B₁₂ were also studied) may play a role in the etiology of esophageal cancer. Whether dietary intake contributed to the difference in the serum level was not determined. Also, the levels of serum vitamin E from this study are lower than average values reported elsewhere (Machlin, 1989). In addition to storage conditions, genetic and other variables may account partly for the low values.

B. BREAST CANCER

Basu et al. (1989) measured the serum levels of vitamin E, vitamin A, β-carotene, and selenium in the sera of 30 breast cancer patients with advanced stage breast cancer with distal metastases, 29 patients with benign breast disease, and 30 healthy age-matched controls in Edmonton, Canada. All of the cancer patients were drug-free for 1 month prior to sampling. Serum samples were obtained from the National Cancer Institute serum bank. No significant differences were found for the levels of vitamins E and A, β-carotene, or selenium. All cancer patients were in advanced stages with metastases, which may have affected serum nutrient levels.

Gerber et al. (1989) estimated the intake of vitamin E, total lipids, total cholesterol, and fatty acids in 120 female patients, hospitalized with a first diagnosis of breast cancer and 109 female controls, admitted for neurologic syndromes of other than cardiovascular and tumoral origin or for lumbalgias or disc pathologies, in Montpellier, France. Subjects’ ages ranged from 25 to 65 years. The dietary history questionnaire covered 55 key food items in lipid and vitamin consumption and general overall nutritional habits. The weekly food consumption was converted to intake of nutrients by means of food composition tables. In addition to the fasting serum levels of vitamin E, total cholesterol, triglycerides, and fatty acids of patients and controls were measured. The results showed that while vitamin E intake was not significantly different, plasma levels of vitamin E were significantly higher in cases than in controls even after adjustment for serum cholesterol levels. In addition, plasma levels of the lipid peroxidation product, malondialdehyde were significantly lower than in controls. Given the potential interaction between neurological disorders, their treatment and nutritional intake, the appropriateness of this control group must be questioned.

In a related study, Gerber et al. (1990) investigated the nutritional factors related to breast cancer using a hospital-based case-control study in Milan, Italy and Montpellier, France. Vitamins A and E,
triglycerides, and cholesterol were measured in blood samples taken from interviewed subjects. Cases were 214 Italian and 103 French patients with primary carcinoma of the breast without metastasis. None of the cases had previously undergone therapy. Controls (215 in Milan and 103 in Montpellier) were hospital-based in both studies. No differences were found in vitamin A or E consumption in either population. Levels of serum cholesterol and plasma vitamin E were significantly higher in cases than in controls in both populations. The difference in plasma vitamin E was confirmed after adjustment for total cholesterol and triglycerides. Nutrient consumption and relevant blood markers were directly or partially correlated in both populations. The odds ratio values for the highest quartiles were 4.2 for plasma vitamin E, 0.56 for serum malondialdehyde, 1.9 for total lipids and 1.9 for cholesterol. As in the previous study there were no community-based controls. Furthermore, there were no comparisons reported between control groups to control for potential cultural differences. Partial data from this study have been published elsewhere (Gerber et al., 1988).

In another prospective study on breast cancer, Russell et al. (1988) collected blood samples from 5086 women, ages 6 to 88 years, resident in Guernsey, United Kingdom and stored them at −20°C until analyzed. During the subsequent 8-year period 30 women developed breast cancer. The levels of α-tocopherol and retinol from the sera of these women and 288 age-matched controls were then analyzed. No relationship was found between serum vitamin E (or retinol) level and subsequent development of breast cancer. While the follow-up time is long enough, the number of cases is relatively small in this study. Also, it is not known whether storage of samples at −20°C for 8 years will similarly affect the results of all serum samples. It is conceivable that the storage conditions contributed to the relatively low levels of vitamin E reported in controls and pre-cancer patients (6.2 μg/ml and 6.5 μg/ml, respectively).

Toniolo et al. (1989) studied the relationship between intake of vitamin E and risk of breast cancer in Italy. Cases were 250 breast cancer patients (free of metastases, except in regional lymph nodes). Controls were 499 women from the general population stratified by age and geographical area. All subjects interviewed were given food-frequency questionnaires structured by meals. Indigenous foods and recipes were added to the database. No significant difference in vitamin E intake was found between groups. Reduced risk was found to be associated with decreased intake of fat, especially saturated fat and animal protein.

Langeman et al. (1989) measured the levels of cholesterol, α-tocopherol, and γ-tocopherol in 64 breast tissue samples (neoplastic and non-neoplastic from the same patient) in Basel, Switzerland. Breast tissue samples were obtained from biopsies submitted for diagnosis. Tissue was stored at −80°C prior to analysis. The levels of α- and γ-tocopherols were not significantly different between neoplastic and non-neoplastic samples but were correlated with the percent fat in both types of tissue. The values of α-tocopherol ranged widely from 16 to 1439 (mean 288) nmol/g fresh weight in cancerous tissue and from 36 to 1439 (mean 275) nmol/g for non-neoplastic tissue. There were no data on dietary intake or other measures of nutritional status presented in this paper.

In a double-blind, placebo-controlled crossover trial conducted in Pretoria, South Africa, Meyer et al. (1990) examined the effect of vitamin E on the treatment of benign breast disease (fibrocystic disease) using mammography as an objective and sensitive parameter. The subjects were 105 women, ages 25 to 45, who had mammographic evidence of benign breast disease. They received dl-α-tocopheryl acetate (600 mg per day) or placebo for 3 months. Breast examinations and mammography were done after each 3-month treatment, at approximately the same phase of the patients' menstrual cycle. Although there were no difference in clinical exam scores, 37 (43 percent) out of 83 cases who completed the trial reported improvement and 16 versus 10 placebo cases had mammographic improvement after vitamin E. There was no report of dietary intake, supplement use, or biochemical measures of nutrient status. After 3 months of supplementation it was concluded that vitamin E had no beneficial effect on the treatment of benign breast cancer. While the risk of breast
cancer is increased in fibrocystic disease patients, it is not clear whether fibrocystic breast disease is a pre-cancerous state.

C. LUNG CANCER

Kok et al. (1987) measured the baseline levels of serum retinol, vitamin E, and cholesterol in 10,532 subjects in Rotterdam, the Netherlands. In the subsequent 9 years, 114 subjects died of cancer. Deaths in the first year of follow-up were excluded, as were eligible cases for which base-line data were incomplete or serum samples unavailable, leaving 69 cases (18 cases of lung cancer) for statistical analysis. Baseline serum micronutrient levels in these subjects were compared with levels in 138 controls who were matched for sex, age, and smoking status. The levels of vitamin E, but not of retinol, selenium, or cholesterol, were significantly lower in all cases than in controls. Risk analyses according to quintile comparisons showed a strong negative trend for vitamin E, suggesting an increased risk of all cancer associated with lower serum levels of vitamin E. The authors indicated that this was an ongoing study, and not much experimental detail was provided.

In a case-control study, LeGardeur et al. (1990) measured the serum levels of vitamins E, C, and A, carotenoids, total cholesterol, and retinol-binding protein from 59 newly diagnosed cases of lung cancer and 59 matched hospital controls in New Orleans. Controls were selected in a next-patient-encountered manner from the same hospital and matched to the index case by race, sex, age, and parish of residency. In addition, 31 community-based controls matched to the hospital controls were included for comparison. Nonfasting blood samples were collected from all subjects. Cases and hospital controls were given a structured interview to ascertain smoking habits and dietary intake patterns. Community controls were not interviewed.

There were more smokers in cases than controls, and the relative risk for smoking increased with the number of pack years of cigarettes smoked. Cases had significantly lower serum vitamin E and carotenoids than the controls. Adjustment for serum cholesterol levels reduced the case-control difference for serum vitamin E and carotenoid levels. The results suggest that serum vitamin E is associated with risk of lung cancer. The number of subjects employed in this study is relatively small, and composition of the hospital control group (34 percent with either cardiovascular disease or endocrine/metabolic disorders) make interpretation of this data difficult. See comments in Appendix Table for other concerns about this study.

Whether or not serum vitamin E and selenium concentrations are related to the incidence of lung cancer was studied in Sapporo, Japan (Miyamoto et al., 1987). The subjects were 115 children of 55 randomly selected cancer patients with primary lung cancer (squamous cell carcinoma, adenocarcinoma, small cell carcinoma, large cell carcinoma, and undefined lung cancer). Blood samples were collected from patients (28 males and 9 females), family members (54 males and 61 females), and 56 (28 males and 28 females) age-matched controls with no cancer history among their second degree relatives. Sera were stored at -70°C for less than 3 months prior to analysis.

Family members of cases were found to have a trend toward lower vitamin E levels. Serum vitamin E levels were significantly lower among family members of adenocarcinoma patients than the controls. Serum vitamin E levels were also significantly lower in lung cancer patients than in the controls. These findings suggest that there are familial factors in serum vitamin E among families of lung cancer patients. Similar results were found for serum selenium. The lower levels of serum vitamin E and selenium observed may also be related to common familial dietary habit and or hereditary factors in metabolizing vitamin E and selenium. Although many factors can influence blood levels of vitamin E (see Chapter III. A.), the mean value of serum vitamin E (14.1 μg/ml) in controls is much higher than the average values reported elsewhere.
D. GASTROINTESTINAL AND PANCREATIC CANCER

A case-control study in high- and low-risk areas was conducted to determine dietary factors and their contribution to the marked geographic variation in mortality from gastric cancer within Italy (Buiatti et al., 1990). Trained interviewers questioned 1016 gastric cancer patients and 1159 controls randomly selected from comparable sex and age strata of the same population. Usual frequency of intake and portion size in a 12-month period 2 years before the interview were assessed for 146 food and beverage items. Levels of α-tocopherol and other nutrients were estimated using food tables.

Risks of gastric cancer were found to vary significantly with estimated nutrient intake; however, risk was found to decrease in proportion to intake of α-tocopherol, β-carotene, ascorbic acid, and vegetable fat, and increase with increasing consumption of nitrates and protein. A 5-fold difference was found between those with a high intake of α-tocopherol/ascorbic acid and a low intake of protein/nitrite compounds when compared to those with a high intake of protein/nitrite compounds and a low intake of α-tocopherol/ascorbic acid. The study also showed that gastric cancer risk decreased with rising α-tocopherol intake in almost all ascorbic acid tertiles and vice versa. Similar results were found when they were calculated separately for males and females.

The findings of Buiatti et al. (1990) are consistent with the view that the ability of α-tocopherol and ascorbic acid to inhibit nitrosation may contribute to a reduced cancer risk of gastric cancer (Birt, 1986; Trickler and Shklar, 1987). This population study demonstrated an inverse relationship between the risk of gastric cancer and α-tocopherol intake. Further studies using biochemical assays and follow-up studies are needed to confirm the role of vitamin E in the prevention of gastric cancer in humans.

Charpil et al. (1989) examined the possible link between the risk of digestive cancer and serum levels of α-tocopherol, retinol, retinol-binding protein, and prealbumin in 70 patients with digestive cancer, 34 patients with colonic polyps, and 78 controls of both sexes, ages 31 to 94 years, from Marseille, France. Serum samples were stored at −18°C prior to analysis. Relative to controls, a significant decrease in serum levels of α-tocopherol, retinol, retinol-binding protein, and prealbumin was found in patients with digestive cancer, but not in the polyp group. Since retinol-binding protein and prealbumin levels (possible indicators of protein malnutrition) were decreased, the decreased levels of vitamins A and E observed in digestive cancer patients may be the result of malabsorption. Moreover, since no documentation of nutrient intake or clinical nutritional status was included, the possibility of a generalized malnutrition could not be ruled out. Control subjects were not matched with cases nor were they a pure community-based control group; a portion of control subjects were hospitalized for elective surgery.

Knekt et al. (1988b) studied the association between levels of α-tocopherol and selenium in serum and subsequent risk of gastrointestinal cancer in a longitudinal case-control study based on 36,265 initially cancer-free Finnish men and women from 25 population groups, ages 15–99 years. Serum levels of α-tocopherol and selenium at entry into the study were measured in stored samples of 150 incident gastrointestinal cancer cases diagnosed during a 6–10 year follow-up and from 276 controls who were matched for sex, age, and place of residence.

Subjects with low serum levels of α-tocopherol or selenium had a higher subsequent risk of cancer of the upper gastrointestinal tract (esophagus and stomach). This association persisted among men after adjustment of various confounding factors and after excluding those with cancer diagnosed during the first two years of follow-up. The relative risk of cancer among those who fell in the lowest quintile of serum α-tocopherol was 2.2 compared with those in the higher quintiles. Serum levels of α-tocopherol or selenium in general were not inversely related to colorectal cancer risk. It should be noted that serum samples were stored at −20°C for an average of 13 years.
During a follow-up of 6 to 10 years, 453 cancers were diagnosed among 21,172 Finnish men (Knekt et al., 1988c). The serum levels of \( \alpha \)-tocopherol were measured in stored serum samples from these men and 841 controls matched for municipality and age. The mean levels of serum \( \alpha \)-tocopherol among all cancer cases and controls were 8.02 and 8.28 mg/L, respectively. Significantly lower \( \alpha \)-tocopherol levels were found only in patients with pancreatic cancer. The relative risk of cancer in persons in the two highest quintiles of serum \( \alpha \)-tocopherol was lower in comparison with those in the three lowest quintiles. The association was strongest for the combined group of cancers unrelated to smoking and varied between subgroups of the study population as well as between different cancers. The association persisted when adjusted for serum cholesterol, vitamin A, selenium, and various confounding factors.

When all 36,265 Finnish participants were considered together, cancer of all sites was diagnosed in 766 persons during the 8-year follow-up period (Knekt et al., 1991). The levels of serum \( \alpha \)-tocopherol were determined from these cancer patients and 1419 matched control subjects. Individuals with low \( \alpha \)-tocopherol had about a 1.5-fold risk of cancer compared with those with a higher level. The strength of the association between serum \( \alpha \)-tocopherol level and cancer risk varied for different cancer sites and was strongest for some gastrointestinal cancers and for the combined group of cancer unrelated to smoking. The relative risk of cancer for those with a low level of serum \( \alpha \)-tocopherol was greater than 2.0 compared to those with a higher level of serum \( \alpha \)-tocopherol for different sites of gastrointestinal cancer (stomach, pancreas, and colorectum), with the exception of colorectal cancer among men. Since vitamin E deficiency is associated with malabsorption syndromes of various etiologies (Bieri and Farrell, 1976; Machlin, 1991), and gastrointestinal cancer can lead to malabsorption, the lower vitamin E status of patients with gastrointestinal cancer may be a consequence of the disease rather than the cause.

Burney et al. (1989) measured levels of \( \alpha \)-tocopherol, selenium, total carotenoids, \( \beta \)-carotene, lycopene, retinol, and retinol-binding protein in frozen sera (\(-70^\circ\)C) from 22 cases of cancer of the pancreas and 44 matched control subjects drawn from a larger pool of residents of Washington County, Maryland. In addition to matching for race and age, subjects were matched for the time between the last meal and blood sampling. The cases had a higher, though not statistically significant, mean level of serum vitamin E than the controls. Yet, the authors concluded that "low levels of serum vitamin E appear to have a protective effect, but a chance association between vitamin E and cancer of the pancreas could not reasonably be excluded." The authors also commented that the interpretation of the data is attenuated by the small sample size and long storage time.

E. COLORECTAL CANCER

DeCosse et al. (1989) studied the effects of wheat fiber and vitamins C and E on rectal polyps in patients with familial adenomatous polyposis in New York. The initial study population was comprised of 72 adult patients (average age 35 years) who had a total colectomy and ileorectal anastomosis at least 1 year before entry into the trial. After 3 months on placebo, 58 patients were randomly divided into groups and received daily either a) 8 capsules of a lactose placebo and 2.2 g of a low-fiber supplement (control group, 10 males and 12 females); b) 4 g of ascorbic acid, 400 mg of dl-\( \alpha \)-tocopherol and 2.2 g of the low-fiber supplement (vitamin group, 5 males and 11 females); or c) both vitamins plus 22.5 g of a high-fiber supplement (high-fiber group, 6 males and 14 females). Over the four years of the trial, each participant underwent proctosigmoidoscopy every 3 months for a total of 18 examinations.

Data obtained suggested an inhibitory effect on benign large bowel neoplasia by high fiber intake, but not by vitamin supplementation. Since the high fiber group also received vitamin supplements, the possibility of an interactive effect can not be excluded. Several issues confound the interpretation of
this study: 1) vitamin status was not assessed biochemically at baseline or during the trial; 2) because of the combined use of vitamins, an analysis of independent effects or interactions of individual vitamins was not possible; and 3) there was no control of dietary intake or reporting of previous or concurrent vitamin supplementation.

Freudenheim et al. (1990) conducted a case-control study of diet and rectal cancer in three counties in western New York. Cases were single, primary adenocarcinomas age 40 years or older with no previous history of cancer; controls were matched to cases on age, sex, and neighborhood, and selected by a standardized protocol. A total of 277 case-control pairs of males and 145 case-control pairs of females were interviewed about usual quantity and frequency of consumption of 129 food items. Intake of vitamin E and other nutrients was determined using U.S. Department of Agriculture food composition data and other food composition data. It was determined that higher vitamin E intake was not associated with lower risk of rectal cancer, while vitamin C, carotenoids, and fiber intake were. There were strong associations of risk with increased intakes of total kilocalories and fat and somewhat weaker associations with carbohydrates, protein, and iron. Increasing intake of retinol was found to be associated with increased risk in this study.

McKeown-Eyssen et al. (1988) conducted a double-blind randomized trial to examine the effects of vitamins C and E on the rate of recurrence of colorectal polyps, a presumed precursor for colorectal cancer. Two hundred male patients (age unknown) who had at least one polyp in the colon or rectum, identified by colonoscopic examination at two Toronto hospitals, were selected for the study. All participated in a brief dietary inquiry and were asked to discontinue their own vitamin supplement, if any, for the duration of the study. Participants were randomized to receive a supplement of vitamin E (400 mg α-dl-tocopherol) and vitamin C (400 mg ascorbic acid) or lactose placebo over a period of 2 years. A random urine sample was collected to assess the compliance with vitamin supplementation.

One hundred thirty-seven patients completed the study. Polyps were observed in the second colonoscopy in 41.4 percent of 70 subjects on vitamin supplements and in 50.7 percent of 67 subjects on placebos. Further examination of the pathology of polyps and analysis of polyp recurrence data suggests that any reduction in the rate of polyp recurrence associated with vitamin supplementation is small. The effect of vitamin E per se could not be assessed because all experimental subjects received both vitamins C and E. Also, the compliance of patients with the vitamin supplementation is difficult to assess as there was no biochemical assessment of vitamin status either at baseline or during the trial.

F. CERVICAL/OVARIAN CANCER

Cuzick et al. (1990) measured levels of vitamins A and E in sera of young women aged 16-40 participating in a case-control study of cervical intraepithelial neoplasia (CIN) carried out in London between 1984 and 1988. Cases were histologically classified from biopsy material as CIN I (n = 110), CIN II (n = 103), or CIN III (n = 284). Controls were randomly selected either among the patients of general practitioners (n = 206) or among women attending family planning clinics (n = 206). Women with CIN I lesions were similar to the controls with respect to most epidemiological factors whereas women with CIN III demonstrated all the major risk factors for invasive cervical cancer. Blood samples were collected from 68 percent of the controls and 86 percent of the cases. Serum levels of vitamins A and E were measured on age-stratified random samples composed of 45 controls, 30 cases of CIN I and 40 cases of CIN III.

The mean values of vitamin E, but not vitamin A, decreased from controls to CIN I to CIN III. Also, there was a significant trend for the estimates of the odds ratio for the risk of CIN I and CIN III for quintiles of vitamin E, but not vitamin A. Adjustments for the confounding effects of sexual behavior,
smoking habits, and use of oral contraceptives slightly strengthened the relationship with CIN III but had no effect on CIN I. The number of samples analyzed for serum vitamins A and E in each group is small. Also, no information regarding vitamin intake and supplementation of subjects was reported.

Heinonen et al. (1987) measured serum concentrations of retinol, α-tocopherol, and total carotene in 88 women with gynecological cancer (9 vulvar, 15 cervical, 36 endometrial, and 26 ovarian carcinomas) and 51 gynecological patients receiving treatment for abnormal bleeding, uterine fibromas or genital prolapse in Tampere, Finland. None of the patients received vitamin A or E supplementation, but no dietary intake data were collected. No significant differences were found in the serum levels of α-tocopherol between cancer cases and controls. There were no healthy community-based controls. Cases were in varying clinical conditions and were not matched with controls.

Palan et al. (1991) studied the relationship of plasma levels of α-tocopherol and β-carotene with incidence of uterine and cervical dysplasias and cancer in a cross-sectional sampling of 116 women in New York City. Of the 116 subjects, 36 had negative cytology, normal colposcopy, were not using oral contraceptives and had no gynecologic dysfunction (control group, average age 28.4). Eighty women had abnormal cytology and were referred for colposcopy. Of these, 27 had no significant pathology (No CIN, age 25.1). Forty-three were histopathologically graded and stratified as CIN I and CIN II (n = 31, age 27.4) or CIN III and carcinoma-in-situ (n = 12, age 30.7). The remaining 10 patients had pathologic evidence of cervical cancer (age 43.2). A blood sample was obtained from each subject prior to any therapeutic intervention.

An inverse association between plasma levels of both α-tocopherol and β-carotene and increasingly severe graded cervical pathology was reported. The effect of vitamin E was independent of smoking status. The findings suggest that vitamin E may play a role in the pathogenesis of cervical intraepithelial lesions and cervical cancer. The number of subjects in each group, however, is relatively small, and groups were not matched properly. Also, the plasma levels (ranging from 4.1 mg/L in the cancer group to 8.56 mg/L for controls) of α-tocopherol are lower than reported elsewhere.

Verreault et al. (1989) conducted a population-based, case-control study to assess the relation of dietary intake of vitamin E, as well as vitamins A and C and folic acid, to the risk of cervical carcinoma. Cases were 189 women diagnosed with cervical carcinoma in the Seattle area. Controls were 227 subjects selected using random digit dialing. Dietary intake during the year preceding diagnosis was assessed by using a 66-item food-frequency questionnaire. Mean daily intakes of nutrients were computed using standard portion sizes. The contents of vitamin E and other nutrients were estimated from food-composition tables. After adjustment for known risk factors, estimated consumption of vitamin E was related to the risk of cervical cancer. High vitamin E intake was related to a reduced risk, and the risk for women in the highest quartile was only one-third for those in the first quartile. Intake of retinol and folic acid was not found to be related to the risk of cervical cancer. This is the first report suggesting a protective effect of dietary vitamin E against invasive cervical cancer.

In the same Finnish study reported earlier (Knekt et al., 1988a), 313 of 15,093 female participants were diagnosed with cancer during the 8-year follow-up period (Knekt, 1988). Serum α-tocopherol levels of these cases were compared with 578 controls, matched for municipality and age. An inverse relation was observed between α-tocopherol levels and risk for cancer, even if the cancer cases of the first two years of follow-up were excluded. Women in the 3 lowest quintiles for α-tocopherol levels compared to those with highest values had a 1.6-fold risk of cancer when adjusted for possible confounding factors. It was suggested that a low level of α-tocopherol in general strongly predicted epithelial cancers while carrying an only slightly elevated risk of cancers in reproductive organs exposed to estrogens.
G. OTHER SITES

Helzlsouer et al. (1989) studied the association between the development of bladder cancer and serum levels of α-tocopherol and other micronutrients in Washington County, Maryland. Serum samples from 25,802 participants, aged 11–98, were collected in 1974 and stored at −70°C. In the subsequent 12-year period, 35 cases of bladder cancer developed among the participants. Comparisons of earlier serum levels among cases and two age and race–matched controls for each case showed that selenium, but not α-tocopherol, was significantly lower among cases than controls.

In another analysis of the Washington County, Maryland study no significant associations were observed for serum tocopherol levels and risk of prostate cancer (Hsing et al., 1990). Cases were 103 men who developed prostate cancer during the subsequent 15 years and control subjects matched for age, sex (all male), and race (all Caucasian).

Stryker et al. (1990) examined the relationship between the risk of malignant melanoma and dietary intake and plasma levels of retinol, carotenoids, and α-tocopherol. Cases (n = 204, 96 males and 108 females) and controls (n = 248, 96 males and 152 females) were patients age 18 years or older making their first visit to a Boston dermatology subspecialty clinic for pigmented lesions. Intakes of nutrients were estimated using a semiquantitative 118-item food–frequency questionnaire, a write-in section for other foods, and questions on the use of vitamin and mineral supplements, and on type of fats used for baking and frying.

The levels of plasma retinol, α-tocopherol, lycopene, α-carotene, and β-carotene were not different between cases and controls. Controls, however, had a significantly higher intake of vitamin E, not counting vitamin supplementation. No significantly different associations with malignant melanoma were observed for higher plasma levels of lycopene, retinol or α-carotene in logistic regression analyses after control for age, sex, plasma lipids, and known constitutional risk factors (hair color and ability to tan). Intake of vitamin E from food alone displayed a trend of decreasing risk with increasing intake (p = 0.003). The odds ratio comparing the highest with the lowest quintile of vitamin E intake from food was 0.7 (p = 0.4) for plasma α-tocopherol and total vitamin E intake. Controls were clinic–based and were not matched with cases.

Kanematsu et al. (1989) measured the levels of vitamin A and vitamin E in plasma and human hepatocellular carcinoma and adjacent liver parenchymal tissues from 26 patients (21 men and 5 women) with an average age of 57.4 years (ranging from 36 to 71 years) in Fukuoka, Japan. Tissue samples were obtained from resected specimens, and morphologically characterized by microscopic examination. Histological examination showed that 19 had cirrhosis, 4 had fibrosis, and 1 had chronic active hepatitis. The other two had no evidence of cirrhosis, fibrosis, or hepatitis. Samples were stored at −80°C prior to analysis. The level of vitamin A, but not vitamin E, was significantly decreased in hepatocellular carcinoma compared to normal liver tissues. Plasma levels of vitamin E (mean = 0.46 mg/dL) were lower than the normal values. However, no control samples were available for comparison.

H. COMBINED SITE STUDIES

Rougereau et al. (1987) studied the relationship between fat–soluble vitamins and cancer localization in cancer patients in France. The subjects were hospitalized patients, 464 males and 604 females, age 20 to 65 years. Controls were 527 healthy males and 653 females age 20 to 53 years. Fasted blood samples were drawn before surgical treatment, chemo– or radio–therapy and were analyzed within 24 hours. Serum samples from 880 controls were also analyzed for the levels of vitamin A, β-carotene,
α-tocopherol and carcinomedin \((1\text{-keto-24-methyl-25-hydroxycholecalciferol})\). A statistical multidimensional analysis of data led to five separate groups of cancer types. Within each group, alterations of vitamin spectra were the same as controls but were significantly different between groups. All these groups are statistically different from the reference group. There was an inverse association of vitamin E level with cancers of the lung, gastrointestinal tract, and nervous systems, but not for hormonally related cancers. The authors suggest that carcinogenesis may alter fat-soluble vitamin metabolism and that altered vitamin metabolism may be involved in the carcinogenic process.

Rougereau et al. (1988) subsequently examined and followed the serum levels of carcinomedin, vitamin A, β-carotene and α-tocopherol of 42 subjects with various cancers (stomach, esophagus, breast, ovaries, uterus, etc.) for up to 38 months. The patients of both sexes were between 38 and 66 years of age and were in varying therapeutic situations. The follow-up period extended from the initial clinical diagnosis of the tumor or from its preoperative stage up to the end of treatment, remission, or death. Controls were 23 cancer patients treated with chemotherapy and or radiotherapy, for whom the investigators had no knowledge of the clinical data. Patients whose serum vitamin A and α-tocopherol concentrations remained within normal limits survived longer than those with lower concentrations. The number of subjects in this study is small and the subjects (including designated controls) were in varying clinical and therapeutic situations.

From the same Washington County, Maryland survey reported earlier (Burney et al., 1989; Hsing et al., 1990), prediagnostic serum samples from 436 cancer cases representing 9 primary sites and 765 matched control subjects were also analyzed for vitamin E levels (Comstock et al., 1991; Schober et al., 1987). Higher serum vitamin E levels were found to associate with a reduced risk of the cancer of the lung, but not of colon, rectum, pancreas, melanoma, basal cell of skin, breast, prostate, or bladder.

In a prospective follow-up study conducted in Basel, Switzerland, Gey et al. (1987) measured the plasma levels of vitamin E and several micronutrients in approximately 3000 healthy male volunteers (mean age 51 years). The plasma analyses were performed immediately to avoid vitamin E destruction due to storage. The mortality (268 subjects or 9 percent of the population) was registered during the subsequent 7 years. A comparison of the baseline level of case subjects with all survivors \((n = 2707)\) was made. Cholesterol and triglyceride-standardized vitamin E at 30 μM (12.9 mg/L) was used as the lowest tertile of cancer cases to estimate the relative risk adjusted for age and smoking. The absolute baseline plasma level of age-standardized mean α-tocopherol was significantly lower in subjects who later died of all types of cancers and of combined gastrointestinal cancers.

Twelve years after blood samples were taken from 2974 healthy men in Basel, 553 of the subjects died. Among these, 204 died from cancer, including 68 with bronchus cancer and 37 with gastrointestinal cancer (Stahelin et al., 1989, 1991). A comparison of the mean vitamin E values among the different groups considered revealed no significant differences. By contrast, vitamin A, β-carotene and vitamin C were found to be associated with cancer mortality. The use of different statistical models may be partly responsible for the differing results in the 7-year versus 12-year follow-up studies. Unlike most other studies, fasting plasma levels of vitamin E were analyzed soon after sample collection. Thus the problem of oxidative destruction of vitamin E associated with long-term storage was avoided.

To investigate whether vitamin E status is related to future incidence of cancers, Wald et al. (1987) conducted a prospective study in London. Blood samples were collected from about 22,000 men ages 35–64 and sera stored at −40°C. During the subsequent 3–10 years, 271 men were identified as having developed cancer. The concentration of vitamin E was measured in serum samples obtained from these cases and from 533 control subjects matched for age, smoking history, and serum storage duration.
The mean vitamin E level was not significantly different between the cancer subjects and controls. However, the mean level of vitamin E in the cancer subjects, diagnosed within a year from the date of blood collection, was significantly lower than controls. For subjects whose cancers were diagnosed one or more years after blood collection, the difference was not statistically significant either for all cancers or for cancers of six sites (lung, colon and rectum, stomach, bladder, central nervous system, and skin) considered separately. The authors concluded that the low vitamin E levels observed in these subjects were a metabolic consequence of tumor development, rather than a precursor, of the cancer.

Connett et al. (1989) evaluated the baseline serum levels of β-carotene, total carotenoid, vitamins A and E, and retinol-binding protein of 156 men who died of cancer and 311 controls individually matched for age, smoking status, randomization group, date of randomization, and clinical center. The subjects were from a non-blinded, randomized trial of 12,866 men at risk of coronary heart disease, aged 35 to 57 years at baseline. Blood specimens were stored at −50° to −70°C for over 4 years. Except for β-carotene, serum levels of retinols, α-tocopherol, and retinol-binding protein were not found to differ significantly between cases and controls and were not related to any cancer site.
V. SUMMARY AND CONCLUSIONS

Many recent studies have attempted to relate serum or plasma levels of vitamin E, as well as intake of vitamin E, to the subsequent development of various cancers. Some of the studies have found that low levels of serum/plasma vitamin E or low dietary intake of the vitamin correlated with elevated risk of certain cancers; others have found no such correlations. In general, there are more studies suggesting a positive correlation between higher risk of cancers and lower intake or serum/plasma levels of vitamin E than those that do not find this association. Although several studies reported higher blood levels of vitamin E in cases than controls (Burney et al., 1989; Gerber et al., 1989, 1990), there have been no reports of a correlation between higher intake of vitamin E and higher risk of cancer. Currently, eight clinical intervention trials for vitamin E are in progress (Knekt, 1991). The completion of these large-scale studies is needed before the effectiveness of vitamin E in chemopreventive trials can be generalized. To date, no information obtained from intervention studies designed specifically to study the efficacy of vitamin E is available.

It should be pointed out that assessment of serum/plasma levels of vitamin E in cancer patients, especially those with gastrointestinal cancer, is not a specific indicator of vitamin E intake, as the results obtained may be the consequence, rather than a contributing factor, of the disease. Also, measurement of plasma/serum tocopherol levels, which is used almost exclusively in nutritional epidemiological studies, is not a reliable means for assessing long-term vitamin E status. Furthermore, many factors, such as a) handling and storage conditions and length of storage of plasma/serum samples, b) control of blood lipids and use of fasted versus fed samples, c) methods of vitamin E measurement, d) interviewing techniques, and e) quality of data in food-composition table regarding tocopherol content, can significantly compromise the accuracy and reliability of information obtained.

With regard to the relation of vitamin E and cancer risk, the Surgeon General's Report on Nutrition and Health (U.S. Department of Health and Human Services, 1988) concluded that "Although some studies suggest it has a protective effect, in human studies no link was reported between vitamin E levels and risk of cancer when incidence rates at all sites were combined." The Report of the Committee on Diet and Health (National Research Council, 1989a) concluded that "vitamin E intake is in itself not related to overall risk of cancer, but that low serum levels of vitamin E coupled with low selenium may increase the risk of at least some cancer such as breast and lung cancer." The latest information available still is not sufficient to make a definite conclusion concerning the relationship between vitamin E intake and risk of human cancers.

More studies, especially well-designed, large-scale observational studies and intervention trials using human populations in various circumstances, are needed to establish the possible anticarcinogenic effect of vitamin E in humans. Also, a better understanding of the mechanisms by which vitamin E protects against free-radical-induced lipid peroxidation tissue injury, as well as the possible role of vitamin E in malignant transformation, tumor cell proliferation, immune defense system competence, and precancerous lesions, is necessary to evaluate the possible role of vitamin E in preventing human cancer.
VI. BIBLIOGRAPHY*


*This bibliography contains all reference citations that are either in the text or the tables or both.


APPENDIX

CRITERIA FOR INCLUSION OF ARTICLES IN APPENDIX TABLE

Articles in peer-reviewed journals related to the topic of this review were selected primarily on the basis of date and content. In general, papers appearing in 1987 or thereafter were included, provided that they presented original data from studies in humans. Certain items tabulated for the sake of completeness may not have been cited in the body of the text if their weight or relevance did not add significantly to development of the author's argument. Reviews have not been listed except as they included new data or useful meta-analyses.
<table>
<thead>
<tr>
<th>Study</th>
<th>Type/Location</th>
<th>Subject # &amp; Description</th>
<th>Methods</th>
<th>Results</th>
<th>Comments</th>
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<tr>
<td>de Vries et al., 1990</td>
<td>Cross-sectional Netherlands</td>
<td>71 cases of squamous cell cancer of the head and neck (HNC-I), with only a single tumor. 17 cases HNC with at least 1 additional tumor (HNC-II).</td>
<td>Serum levels of vitamin A, vitamin E, and β-carotene were measured.</td>
<td>Statistically significant differences between groups for serum vitamin A and vitamin E levels (HNC-I&gt;HNC-II). No difference between groups for β-carotene.</td>
<td>No diet, no documentation of disease history, no supplementation data, no control group. No description of analytical methods, no time period for sampling to analysis. No demographics, no control for smoking, alcohol, or any other confounding risk factors.</td>
</tr>
<tr>
<td>Drozdz et al., 1989</td>
<td>Case-control Poland</td>
<td>22 newly diagnosed cases of larynx cancer 18 patients with nonmalignant laryngeal disease 16 patients with other nonmalignant diseases including CVD or hernia.</td>
<td>Overnight-fasted serum samples were collected and stored at −40°C for no more than 2 wk. Serum vitamins A and E were measured fluorometrically.</td>
<td>There was no difference in levels of vitamin E in any of the group comparisons. Mean levels of vitamin A were lower in cases than either control groups.</td>
<td>Inappropriate control groups. No diet data or report on supplement use. No matching of groups for age, sex, SES.</td>
</tr>
<tr>
<td>Gridley et al., 1990</td>
<td>Case-control Four regions: New Jersey, Atlanta, Los Angeles, Santa Clara and San Mateo, California</td>
<td>190 cases of oral (and pharynx) cancer (142 male, 48 female) including cancer of tongue and pharynx and other oral cancers, excluding cancers of the lip, salivary gland, or nasopharynx 201 population-based controls (139 male, 62 female) matched for age and sex. All subjects were Afro-Americans.</td>
<td>All subjects (or next-of-kins in those cases who were too ill) were given a structured interview to get data on tobacco and alcohol use, diet (61-item food-frequency questionnaire), medical history, occupation, and demographics. Reference period for food was normal intake during adulthood. Intakes were adjusted for seasonal variations in availability. Vitamin supplement usage was collected but did not affect outcomes.</td>
<td>1 risk in 5 associated with carotene and vitamin E. 1 intake of fruits and vegetables was associated with a decreased risk for oral cancer across sexes, although effect was stronger for female. Similar decline in risk associated with vitamin C and fiber.</td>
<td>No biochemical, reliance of retrospective diet data, no data on time period between diagnosis and participation (cases obtained from a cancer registry). The study not designed to address specific nutrients. Mean or median intakes for nutrients not reported. No direct comparison to Caucasian population sample.</td>
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<td>Study</td>
<td>Type/Location</td>
<td>Subject # &amp; Description</td>
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<tr>
<td>Tuyns et al., 1987</td>
<td>Case-control France</td>
<td>743 cases (704 case and 39 healthy) of esophageal cancer; 1975 controls from the same geographical region</td>
<td>All subjects interviewed about usual food intake with 40-item food-frequency questionnaire. Risk analysis was done heavy vs light consumers adjusted for age, two levels of alcohol and tobacco consumption, and residence (rural vs urban).</td>
<td>Significant association between vitamin E intake and relative risk. Higher intakes associated with significantly decreased risk. Cases consumed fewer proteins of animal origin and more proteins of vegetable origin and had a higher intake of sugars and starches of vegetable origin. Cases had a lower P:S ratio -- oils associated with decreased risk.</td>
<td>No biochemistry. No control for time between diagnosis and study. No documentation of medications or other treatment. No data on medical or family health history. Hard to determine environmental from genetic effects.</td>
</tr>
<tr>
<td>van Helden et al., 1987</td>
<td>Cross sectional South Africa</td>
<td>Study I: 63 subjects from 3 incidence areas, high, intermediate, low based on incidence of esophageal cancer (EC). Study II: 77 subjects from 2 incidence areas</td>
<td>Study I: Healthy subjects from 3 rural areas, high, intermediate, low based on incidence of EC. Samples collected during dry season. Study II: Healthy subjects from 2 areas high and low incidence of EC. Samples collected during wet season. Blood samples were collected from individuals with no overt clinical signs of deficiency. Serum frozen at -20°C. All samples were treated uniformly in terms of duration of storage. Vitamin E measured by HPLC.</td>
<td>Study I: Levels of vitamin A and E and red cell folate were significantly different. For each nutrient the highest concentrations were found in the low risk group. All concentrations were without exception in the lower range of clinically acceptable levels. This was especially true for vitamin E. Study II: Significant differences between high and low incidence groups for vitamins A, E, and B12 and red cell folate. Mean level of vitamin E was lowest.</td>
<td>No diet data, no matching for age, sex or demographics. No data on tobacco use, medical or family health history, occupation, or other potential environmental or genetic factors.</td>
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## APPENDIX TABLE. VITAMIN E AND BREAST CANCER

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<th>Study</th>
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<th>Subject # &amp; Description</th>
<th>Methods</th>
<th>Results</th>
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<tr>
<td>Banu et al., 1989</td>
<td>Case-control</td>
<td>89 subjects: 30 w/advanced stage breast cancer (BC) w/distant metastases, 29 w/ benign breast disease, 30 healthy age-matched controls.</td>
<td>All of the BC patients were drug-free for 1 mo prior to sampling. Serum samples were obtained from NCI serum bank. Analysis were blinded as to subject category. Vitamin E measured by HPLC with reverse phase column and UV detection.</td>
<td>No significant difference between groups for vitamin E. Levels of all nutrients studied (vitamin E, A, β-carotene and selenium) were lower in the BC group than in controls, although not significantly.</td>
<td>Although BC group was drug free there was no mention of supplements in any group. Age was the only matching variable. No dietary intake data or nutritional history. No clinical nutrition data. All BC patients were in advanced stage and metastasizing therefore effects may have been secondary to disease.</td>
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<tr>
<td>Gerber et al., 1989</td>
<td>Case-control</td>
<td>120 cases of breast cancer 100 controls hospitalized for non-malignant and non-CVD related neurological disorders. Recruitment over a 4-yr period (1982-86).</td>
<td>Subjects given a structured interview containing SES data, menopausal status, reproductive and health history, and food-frequency questionnaire (for 55 items). Subjects were asked about duration of nutritional habits and changes, if any, of the diet in the previous yr. Fasting blood samples were drawn the day after admission to hospital and stored at -18°C for unknown duration. Vitamin E measured by HPLC.</td>
<td>No significant differences in intake between groups. Plasma total cholesterol (TC) and vitamin E were higher in cases than controls. Differences in vitamin E were greater in premenopausal women as was the ratio vitamin E/TC (which was not significant in postmenopausal patients). Plasma vitamin E was significantly correlated with safflower oil intake in all subjects. Dietary vitamin E (adjusted for age and TC) was significantly associated with plasma vitamin E in both groups. Other significant findings relate to evidence of lower lipid peroxidation in cases than controls.</td>
<td>Possibly inappropriate control group (all were suffering neurological or spinal problems; no medication history was reported). Although stage of disease was known there was no analysis using this variable. Duration of sample storage not given. Relevant time frame for dietary information was not clear and retrospective.</td>
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<tr>
<td>Gerber et al., 1990</td>
<td>Case-control</td>
<td>317 cases of primary non-metastasizing breast cancer (214 Italy, 103 France) 318 hospital-based controls (215 Italy, 103 France)</td>
<td>Blood sampling and interview same as Gerber et al., 1989. Additional assays for retinol, β-carotene, vitamin C and riboflavin. The Italian sample was asked about all foods eaten; whereas the French group was only asked about key lipid and vitamin-rich foods. Reference period was the previous yr unless diet had changed, in which case subjects were asked about the previous 12 mo.</td>
<td>No difference in lipid soluble vitamin intake between groups. There was a higher serum level of cholesterol and plasma vitamin E in cases compared to controls of both groups. A significant difference was found in total fat and cholesterol between cases and controls of both groups; in the French groups there was a difference in saturated and mono-unsaturated fat intake. Increased risk associated with dietary cholesterol, total dietary lipids, plasma vitamin E, and serum zinc (only measured in Italian sample).</td>
<td>No community-based controls. No comparison of controls to each other or to community standards. Blood levels were correlated with past intake (data based on long retrospective period). Differences in quantification of diet records between 2 study sites. Blood assays were blinded and all vitamin assays were performed at the same lab. Storage time was not given.</td>
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<td>Study</td>
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<tr>
<td>Meyer et al., 1990</td>
<td>Intervention (randomized double-blind placebo-controlled crossover design) South Africa</td>
<td>166 cases with mammographic evidence of benign breast disease (31.6% had benign tumors, 23.8% had ductal hyperplasia, 10.1% had benign tumors and fibroadenosis, and 16.2% had fibrocystic disease).</td>
<td>Data collected on menstrual and reproductive history, dietary, and smoking habits, and family history of breast disease. Cases received either 800 mg α-tocopherol acetate or placebo for 3 mo, returned for exam and switched. Exams done by same examiner.</td>
<td>83 cases completed the trial. 37 reported subjective improvement while on vitamin E, 19 while on placebo. No difference in clinical exam score. 16 cases in vitamin E group showed mammographic improvements compared to 10 in placebo.</td>
<td>No baseline or trial diet data reported, no reported previous supplement use, no biochemical data, and no matching for age, or demographics. While the number of cases showing subjective and clinical improvement were greater while on vitamin E, it did not approach statistical significance due to small sample size. Whether those who did worse while on placebo got better on vitamin E or vice versa was not discussed.</td>
</tr>
<tr>
<td>Russell et al., 1988</td>
<td>Case-control England</td>
<td>30 cases of breast cancer (BC) 283 controls matched for age and menopausal status. Subjects selected from a pool of samples from 5086 volunteer 8 collected over an 8-yr period (1977-85).</td>
<td>Serum samples collected and stored at -20°C until analyzed by HPLC.</td>
<td>No differences between controls and BC cases for vitamins E, A, or B12.</td>
<td>No diet or supplement usage data nor matching for smoking or SES. No risk analysis only descriptive statistics. Long storage time at -20°C.</td>
</tr>
<tr>
<td>Toniolo et al., 1989</td>
<td>Case-control Italy</td>
<td>250 cases of breast cancer (free of metastases, except in regional lymph nodes) Controls were 469 % from general population stratified by age (≥10 yr) and geographical area.</td>
<td>All subjects interviewed (unblinded) were given modified food-frequency questionnaire structured by meals. Indigenous foods and recipes were added to the database. General demographic data was obtained from electoral rolls. Interview data included SES data, health and reproductive history.</td>
<td>No difference in vitamin E intake between groups. Reduced risk was associated with decreased intakes of fat especially saturated fat and animal protein.</td>
<td>Not blinded, no biochemical, long period of time between diagnosis or treatment and study (on average 7.8 mo after diagnosis). Retrospective diet data not necessarily indicative of data prior to diagnosis. Smoking histories not reported.</td>
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### APPENDIX TABLE: VITAMIN E AND LUNG CANCER

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<th>Study</th>
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<tr>
<td>Kok et al., 1987</td>
<td>Nested case-control Netherlands</td>
<td>69 cases of cancer death; 18 cases of lung cancer; 138 controls matched for age, sex, and smoking status. Subjects selected from a cohort of 10,032 subjects.</td>
<td>Baseline blood samples analyzed for serum α-tocopherol, β-carotene, retinol, selenium, and cholesterol.</td>
<td>There was no effect of vitamin E levels on risk of lung cancer; however vitamin E levels were inversely associated with risk of all cancers. Lung cancer cases had a 5% lower level of vitamin E than controls; however, this difference was not statistically significant. There were no associations between levels of other nutrients and cancer either total or lung.</td>
<td>Little detail of methodology given as this report is a letter to the editor. Small sample size, especially of the lung cancer cases. No diet data</td>
</tr>
<tr>
<td>LeGardeur et al., 1990</td>
<td>Case-control Louisiana</td>
<td>59 cases of newly diagnosed lung cancer (LC) 59 hospitalized controls (HC) matched for sex, race, age (within 5 yr), and county of residence. 31 community controls (CC) non-hospitalized matched to hospital controls for age, race, and sex.</td>
<td>Non-fasting blood samples collected from all subjects. Hospitalized cases and controls were given a structured interview about smoking habits and dietary intake. Community controls were not interviewed. Serum analyses were performed within a mo of blood collection. Vitamin E was measured colorimetrically.</td>
<td>Mean serum levels of carotenoids, vitamin E, and total cholesterol for LC cases were significantly lower than HC. Although reported as no difference, HC subjects had significantly lower levels of vitamin C and vitamin E than CC. Cholesterol adjusted serum levels of vitamin E were still significantly lower in LC cases than HC.</td>
<td>No diet data reported. No questionnaire data for CC group. The CC group was compared to HC group to test for appropriateness of HC as controls for LC group. Text reported no difference. Data in table indicated significant differences in the major dependent variables vitamin C and E. LC and HC group were not matched for smoking history. In addition the nature of the illnesses (e.g.,26% CHD, 14% metabolic endocrine or nutritional disorders) of the HC group also made it an inappropriate control. No comparisons between CC group and LC cases, although the LC cases did have lower levels of vitamin E and C and retinol and carotenoids. Given the inappropriateness of the controls and poor matching, the use of a paired t-test must be questioned. Poorly designed study.</td>
</tr>
<tr>
<td>Miyamoto et al., 1987</td>
<td>Cross-sectional Japan</td>
<td>115 children of 65 patients with primary lung cancer (CLC). 56 age-matched controls with no cancer (NLC) among relatives. 37 lung cancer patients (LC) who had not received any treatment.</td>
<td>Venous blood samples collected and stored at -70°C; all samples analyzed within 3 mo of collection. Vitamin E determined by high speed liquid chromatography.</td>
<td>Serum alpha tocopherol levels were significantly lower in CLC group than NLC group. The difference was more pronounced in children of adenocarcinoma patients. The LC group had lower levels than either the NLC or CLC groups. There were no association between sex or smoking habits and serum vitamin E.</td>
<td>No diet data or reference to supplement use. No matching for SES factors. Samples not fasted.</td>
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<td>Study</td>
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<td>Subject # &amp; Description</td>
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<tr>
<td>Buinetti et al., 1990.</td>
<td>Case-control</td>
<td>1016 patients with gastric cancer (GC) 1160 age- and sex-matched controls randomly selected from same area.</td>
<td>Subjects given a questionnaire covering demographics, SES, smoking, medical, occupational, family, and dietary history. Food frequency covered the 2 yr prior to the interview.</td>
<td>Increased GC risk with increasing intakes of protein, starch, and nitrites. No change in risk trend related to intake of fat, carbohydrates, cholesterol, fiber, calcium, alcohol, retinol, and nitrates. Lower GC risk with vitamins C and E and β-carotene. Risk decreased proportionally with increased intakes of vitamins C and E but not retinol or β-carotene. In a stepwise multiple regression protein, vitamins E and C were selected for the final model.</td>
<td>No clinical nutrition data reported, the study relied on retrospective data, neither duration of illness nor severity were documented. Reliance on various different databases from different countries for dietary analysis. Use of vitamin supplements was surveyed in an earlier pilot study and found not to be a contributing factor in this population. Most of the dietary vitamin consumed by these groups was associated with olive oil (23% of control intake).</td>
</tr>
<tr>
<td>Burney et al., 1989.</td>
<td>Case-control</td>
<td>22 cases of pancreatic cancer (PC); 44 controls matched for race, sex, and hr between the blood sampling and last meal. The 2 control subjects nearest in age to a case were selected.</td>
<td>Subjects were drawn from the larger pool of residents who had given blood samples during the period of Sept.-Nov. 1974. Samples were frozen at -70°C until assayed for retinol, total carotenoids, β-carotene, lycopene, and α-tocopherol by HPLC.</td>
<td>No differences between groups for smoking history, education, or marital status. There were no significant differences in any measures except lycopene and selenium which were both lower in cases. There was a protective although not statistically significant effect of low levels of vitamin E.</td>
<td>No diet or supplement use data, no medical history, or information about time of onset of PC. Storage of serum for 12 yr can result in invalid results.</td>
</tr>
<tr>
<td>Chariot et al., 1989.</td>
<td>Case-control</td>
<td>208 subjects 70 cases with digestive cancer (DC) 34 patients with colonic polyps 78 healthy controls</td>
<td>12-hr fasted blood samples were drawn from hospitalized cases before chemical, surgical, or radiological therapy. Samples were drawn from polyp and control groups just after hospitalization. Retinol and vitamin E assayed via HPLC. Other measures included RBP and prealbumin (TTR).</td>
<td>Retinol, RBP, TTR, and vitamin E were significantly lower in cases than controls. There were no differences between polyp group and controls. Lower carrier proteins presumed to be indicative of protein malnutrition.</td>
<td>Stage or type of cancer not documented. No dietary intake data or supplement use reported. Aside from a statement about a lack of &quot;deanitrition&quot; in the control groups, there was no documentation about clinical nutrition status. Sample storage time was not given. There was no matching for sex, or SES.</td>
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<td>Study</td>
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<tr>
<td>Knekt et al., 1998b</td>
<td>Case-control Finland</td>
<td>150 cases with primary gastrointestinal cancer (esophagus, stomach, duodenum, colon, and rectum) 278 controls matched for age, sex, and place of residence Subjects drawn from a pool of 36,263 survey participants Cases identified over a variable time period of 5–9 yr</td>
<td>Baseline questionnaire included info about occupation, drug use, medical history, and smoking habits. Body mass index was used to describe obesity. Serum samples were collected and stored at −20°C for between 11–15 yr before analysis of selenium, vitamin E, and vitamin A. Alpha-tocopherol was quantified by HPLC.</td>
<td>Mean vitamin E and selenium were lower in cases than controls, especially when data was partitioned by location in GI tract. Upper GI cancers (stomach and esophagus) were associated with lower vitamin E and Se levels with case status being significantly lower than control status for Se. There were no interactions between serum Se and serum vitamin E and cancer. High Se levels were protective against upper GI cancer at both high and low levels of vitamin E. The relative risk for upper GI cancer adjusted for cholesterol and smoking was higher for both low vitamin E and Se. In general, there was no association between serum Se and vitamin E and colorectal cancer. However, colorectal cancer risk was higher for Se with low vitamin E levels.</td>
<td>No diet data, supplement use data, or matching for SES or seasonal variations in food supply and time of blood sampling. Long storage time. Aside from removing those who were diagnosed within 2 yr of sampling, there was no control for time to diagnosis of cancer.</td>
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<td>Study</td>
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<tr>
<td>DeCosse et al., 1989.</td>
<td>Intervention-randomized,</td>
<td>69 patients with</td>
<td>Control group: 8 caps placebo (lactose)/d + 2.2 g of low fiber Vitamin</td>
<td>Subject groups were comparable in demographics, median time since</td>
<td>No blood levels of the vitamins were reported for baseline or during the trial. No data on dietary vitamin intakes at baseline or during trial were reported. Because of combined use of vitamins, an analysis of independent effects or interactions of individual vitamins was not possible.</td>
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<td>double-blind, placebo-</td>
<td>familial adenomatous</td>
<td>group: 4g vitamin C/d + 400 mg vitamin E/d + 2.2 g of low fiber. Fiber</td>
<td>coilotomy median intake of fiber at baseline and prior supplementation</td>
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<td>controlled New York, NY</td>
<td>polyps drawn from an</td>
<td>group: both vitamins + 22.6 g of high fiber/d. 3-mo placebo period, 4-yr</td>
<td>with vitamin C and E. There were no discernible effects for the vitamins.</td>
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<td>initial pool of 72 who</td>
<td>trial. All groups received 30 mg vitamin C, 2,000 IU vitamin A and</td>
<td>The high fiber group had the stronger benefit especially during the middle years of the trial. Compliance for all groups decreased over the course of the trial.</td>
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<td>had total colectomy and</td>
<td>equivalent amounts of several other vitamins and minerals (about 30% RDA).</td>
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<td>ileorectal anastomosis 1</td>
<td>Patients had 18 examinations over test period, and completed a 3-d diet</td>
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<td>yr prior to the study.</td>
<td>diary and a food-frequency questionnaire for each visit.</td>
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<tr>
<td>Freudenheim</td>
<td>Case-control</td>
<td>422 cases of rectal</td>
<td>Subjects given a 2.5 hr interview with a food-frequency questionnaire.</td>
<td>No association between intake of vitamin E and risk. Decreased risk with increasing intake of carotenoids, vitamin C, and dietary fiber from vegetables. Increased risk with increasing intakes of calories, fat, carbohydrate, and iron. Reliance on retrospective food frequency interviews No data on use of supplements or stage of disease (except that &quot;only relatively alert, healthy subjects could tolerate the 2.6 hr. interview&quot;). Well conceived study.</td>
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<tr>
<td>et al., 1989.</td>
<td>New York, NY</td>
<td>cancer (277 F, 145 M)</td>
<td>Reference period previous year for controls and for cases 1 yr prior to</td>
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<td>422 sex-, race-, age-</td>
<td>the onset of symptoms. Other information included smoking and alcohol use,</td>
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<td>neighborhood-matched</td>
<td>occupational and health histories, seasonality of intake, preparation, and</td>
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<td>controls</td>
<td>food storage.</td>
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<td>McKeeown-Eysen</td>
<td>Intervention (randomized</td>
<td>185 cases with at least</td>
<td>Data included a 24-item food-frequency questionnaire, demographics,</td>
<td>The 2 groups were matched for all parameters except that the vitamin</td>
<td>No biochemical data either at baseline or after trial. Insufficient power due to small sample size. Because of the design, the study could not distinguish differences in effect of individual vitamins.</td>
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<td>et al., 1989.</td>
<td>double-blind trial)</td>
<td>1 polyp in the colon or</td>
<td>smoking status, bowel habits, previous polyp history, and vitamin</td>
<td>group had more members who used vitamin C supplements prior to trial.</td>
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<td></td>
<td>Toronto, Canada</td>
<td>rectum. 90 received</td>
<td>supplement use. Subjests randomized to 400 mg vitamin E and C or lactose</td>
<td>There were no differences in food-frequency items. 127 subjects (79%)</td>
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<td>vitamins at baseline. 89</td>
<td>placebo once and re-examined (blinded). Compliance tested with random</td>
<td>completed the trial. Polyps found in 41.4% of vitamin group and 50.7% of</td>
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<td>received placebo. All</td>
<td>urine samples.</td>
<td>placebo.</td>
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### APPENDIX TABLE. VITAMIN E AND CERVICAL/OVARIAN CANCER

<table>
<thead>
<tr>
<th>Study</th>
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<th>Subject # &amp; Description</th>
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<tr>
<td>Cuzick et al., 1990</td>
<td>Case-control</td>
<td>46 controls 30 cases of Cervical Intraepithelial neoplasia I (CIN I) 40 cases CIN III Subjects chosen from a pool of 110 CIN I, 284 CIN III, and 833 controls involved in a larger study Serum samples were randomly selected from an age-stratified sample.</td>
<td>Sera were analyzed blindly for vitamins A and E by HPLC. Samples were stored for an unspecified period at an unspecified temperature.</td>
<td>The mean levels of serum vitamin E showed a significant decreasing trend lower in cases (CIN III and I) than controls and III were less than I. Significant trends were found on vitamin E levels for both CIN I and III with higher levels being protective. This trend was strengthened when adjustments were made for smoking, sexual behavior, and use of oral contraceptives.</td>
<td>No diet data or reported use of supplements. No matching for SES.</td>
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<td>Heinonen et al., 1987</td>
<td>Case-control</td>
<td>88 cases of gynecologic cancer (9 vulvar, 15 cervical, 33 endometrial, 29 ovarian) of various clinical stages 31 controls receiving treatment for abnormal bleeding, uterine fibromas, or genital prolapse.</td>
<td>Blood samples were collected the day before surgery or treatment in all subjects and stored at -70°C. α-tocopherol, total carotenoids, and retinol were measured by HPLC.</td>
<td>No significant differences were found in the serum levels of the vitamins and carotenoids in patients with vulvar, cervical, or endometrial cancer compared to the controls. Cases with ovarian cancer had a significantly lower mean serum level of retinol than the controls, while carotenoids and vitamin E levels were similar in both groups.</td>
<td>No community-based controls No diet Groups were not matched for age, or other potential risk factors, i.e., smoking, demographics. Fasting status of subjects was not documented.</td>
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<tr>
<td>Pinal et al., 1991</td>
<td>Cross-sectional</td>
<td>10 cases of cervical cancer 36 controls with no problems 27 with abnormal cytology but no cancer (No CIN) 43 were graded histopathologically (CIN I-III)</td>
<td>Samples collected prior to treatment α-carotene, retinol, and α-tocopherol were measured immediately by HPLC.</td>
<td>Mean levels of vitamins A and E were significantly reduced in all cases of dysplasia and cancer. Inverse association between nutrient levels and severity of dysplasia.</td>
<td>No diet, no supplement data, no demographics, no SES, small sample size. Control group may have been self-selected and more health conscious.</td>
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<td>Verrenult et al., 1989</td>
<td>Case-control</td>
<td>189 cases of cervical cancer</td>
<td>Subjects were given a 35-minute structured interview with questions about demographics, health and reproductive history, smoking history, height and weight (self reported), and sexual activity. 66-item food-frequency questionnaire. Questions about vitamin supplement use. Data analysis adjustment variables were education, smoking, frequency of PAP smears, use of contraceptives, age at first intercourse, number of lifetime sexual partners, and previous history of cervical-vaginal infections.</td>
<td>High vitamin E intake was significantly associated with lower risk. Use of vitamin A and E supplements was associated with slight reduction in risk. After adjustment for known risk factors, frequent consumption of dark green or yellow vegetables and fruit juices was related to lower risk. There was a significant inverse relationship between vitamin C intake and risk.</td>
<td>Use of retrospective diet assessment (referring to a period several yr prior to interview). The average delay between the reference date (time of telephone contact) and interview was 2.8 yr in cases and 2.7 yr in controls. No biochemical data</td>
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<td>Heizlauer et al., 1989</td>
<td>Case-control</td>
<td>35 cases of bladder cancer 70 controls (2/case) matched for nearest age, sex, race, and within 2-hr interval of blood sampling and last meal. Sample pool was 20,000 residents of Washington Co, MD. Cases were identified over a 11-yr period (1970-80).</td>
<td>Serum samples were collected in 1974 and stored at -70°C as part of a blood banking project for cancer research. All participants were given a questionnaire that included demographics, smoking history, medication use, and vitamin supplement use (with special reference to the 48 hr prior to blood sampling). Serum retinol, carotenoids, and vitamin E measured by HPLC.</td>
<td>Cases had lower mean nutrient levels of all nutrients than controls. There was a significant association between vitamin E levels and supplement use, but not for any other nutrient. There were no significant differences in prediagnostic levels of any nutrients except selenium which was lower in cases. There was no difference in risk by tertiles for any serum nutrient level except selenium. Serum α-tocopherol levels were non-significantly lower in cases.</td>
<td>Controls were more likely to have used supplements. There was no analysis of the vitamin E and selenium relationship. Similarly, there was no testing for interactions between any of the nutrients studied. Aside from supplement data, no dietary data was collected. Long storage time between collection and analysis. Overall sample pool characteristics were biased towards middle-aged, white, better educated, married &amp;.</td>
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<td>Hsing et al., 1990</td>
<td>Case-control</td>
<td>103 cases prostate cancer 103 controls matched for race (all white), age, and sex (all 70) Subjects drawn from same pool as Heizlauer et al., 1989 (see above)</td>
<td>Same as Heizlauer et al., 1989 (see above) except that 38 cases and 30 control samples were analyzed at a different lab as part of a pilot study. The remaining 140 samples (70/70) were analyzed as above. Interlaboratory variations ranged from 3% for retinol to 11% for β-carotene. None reported for α-tocopherol.</td>
<td>No differences or associations in vitamin E levels between cases and controls.</td>
<td>See Heizlauer et al., 1989 above.</td>
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<td>Stryker et al., 1990</td>
<td>Case-control</td>
<td>244 cases of malignant melanoma 243 control patients who were making first visit to clinic</td>
<td>Data collected about diet (116-item food-frequency questionnaire), use of vitamin and mineral supplements, type of fats used for cooking, medical history, constitutional and lifestyle factors, demographic data, pigmentation characteristics, and past medical history. Fasting serum samples collected and stored at -70°C for up to 6 mo. Vitamin E was measured by HPLC.</td>
<td>No differences in descriptive or risk trend analyses between groups. Some of the higher levels of α-tocopherol were associated with a decreased risk of melanoma (non-significantly). Intake of vitamin E from food alone was significantly associated with a trend of decreased risk with increased intake.</td>
<td>Possibly inappropriate controls as all subjects were patients in skin clinic. Controls may have been more health conscious. Analyses were adjusted for age and sex but no control for SES. Subjects in case group included patients who knew their diagnosis before the study began.</td>
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<td>Kanematsu et al., 1989</td>
<td>Cross-sectional Japan</td>
<td>26 patients (21 ¥ and 5 ¥) consecutively admitted for hepatic resection</td>
<td>Fasting AM blood samples were drawn and assessed for plasma levels of vitamin A (retinol) and E (presumably α-tocopherol), retinol binding protein (RBP), and prealbumin (PA). Tissue concentrations of vitamins A and E were measured in resection samples. In HCC samples comparisons were made between malignant hepatic tumor and adjacent &quot;normal&quot; parenchymal tissue.</td>
<td>No difference in vitamin E or cellular RBP levels. Statistically significant difference in levels of retinol between tumor and adjacent &quot;normal&quot; cells. There was no correlation between blood and tissue vitamin A levels. Low levels of retinol in tumor tissue not related to availability of cellular RBP.</td>
<td>Not a nutrition study — no diet, no control comparisons. No comparisons reported between HCC cases and those without cancer. Within-subject comparisons are of questionable value because of the appropriateness of the adjacent tissue in cancer patients as a control specimen.</td>
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### APPENDIX TABLE. VITAMIN E AND CANCER -- COMBINED SITE STUDIES

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<tr>
<td>Comstock et al., 1991</td>
<td>Case-control Maryland</td>
<td>438 cases with cancer (distribution of cases over various sites). 765 controls matched by age, race, sex, no blood was donated, and time since last meal. Subjects were selected from a pool of 25,620 residents who donated blood samples during a 4-yr period in 1974.</td>
<td>Serum was stored at ~70°C, thawed, and refrozen after aliquoting. Carotenoids and vitamin E assayed by HPLC. Sample sets included cases before and after diagnosis and 2 matched controls.</td>
<td>Cases were distributed as follows: 103 prostate, 99 lung cancer, 72 colon cancer, 34 rectum cancer, 22 pancreatic, 30 breast cancer, 35 bladder, 21 nasal and 26 melanoma. Prediagnostic serum vitamin E was lower in cases than controls for colon, rectum, lung, prostate, and bladder cancers. Only lung cancer showed a significant dose-response trend in a protective direction. The odds ratios for 4 different cell types of lung cancer were all increased for low serum vitamin E, although not significantly (presumably due to small n's).</td>
<td>No diet history, intake or supplement use data were reported. Samples were stored for about 10 yr prior to analysis. Samples were thawed and refrozen prior to analysis. All subjects were drawn from a pool from the same geographical location. No matching for SES.</td>
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<tr>
<td>Conn et al., 1989</td>
<td>Nested case-control Minnesota</td>
<td>158 cases of cancer deaths 311 controls matched for age, smoking status, randomization group, date of randomization, and clinical center. Subjects drawn from a pool of 12,866 subjects at high risk for coronary heart disease involved in an intervention trial (MRPIT). Subject selection was by risk status for CHD: smoking, diastolic BP, serum cholesterol at initial screening. Subjects then seen twice. Bloods collected at second visit and stored at ~50°C to ~70°C. Matched triads were analyzed within 3 mo of each other. Average duration of sample storage was not reported. The cancer cases (deaths) occurred over a 10-yr period (1973-1983). On the third visit all subjects completed a 24-hr dietary recall. Serum vitamin analyses was by HPLC.</td>
<td>Serum levels of α-tocopherol were not related to cancer of any site.</td>
<td>This was a prospective cohort study of CHD from which data on cancer was extracted. All subjects were at risk for CHD, 63% were smokers, therefore the generalizability of these results is suspect. There was a potential impact of storage time on outcomes. Questionable reliability of a single 24-hr dietary recall. Many of the &quot;controls&quot; may have been in early stages of cancer (7 died of cancer after the cutoff date for inclusion).</td>
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<td>Gey et al., 1987</td>
<td>Prospective cohort study Basel, Switzerland</td>
<td>3000 healthy ♀, 208 deaths over 7-yr period 2707 controls (survivors) 35% of total deaths (182) were from cancer</td>
<td>Samples collected over a 2-yr period (1971–73) Plasma analyzed immediately (no storage) Comparisons were adjusted for age and cigarette smoking.</td>
<td>Descriptive statistics demonstrated significantly lower levels of vitamin E in cancer group as a whole when compared with survivors. Significantly lower absolute levels of vitamin E in combined gastrointestinal cancer group than in survivors. Trend analysis indicated that lower levels of vitamin E (and β-carotene, vitamin A) were associated with increased risk of death from all cancer.</td>
<td>No diet data, no control for seasonal variations in intake of antioxidant rich foods, supplement use, or medications at time of sampling</td>
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<td>Knekt et al., 1988c</td>
<td>Case-control Finland</td>
<td>463 cases of cancer of all sites 941 controls drawn from the same municipality as cases and matched for age, time of baseline exam, and duration of samples storage time Subjects selected from a cohort of 21,172 ♀ involved in a longitudinal study</td>
<td>All subjects answered baseline questions about occupation, use of drugs, previous and current illnesses, and smoking habits. All subjects were asked not to eat, drink, or void for at least 4 hr prior to the initial exam. Serum samples were analyzed for content of α-tocopherol, retinol, β-carotene, retinol-binding protein, and selenium. Serum cholesterol and hemato-crit were also determined. Subjects were also asked about supplement use.</td>
<td>High serum α-tocopherol was associated with a 1 risk for cancer. The association was strongest for the combined groups of cancers unrelated to smoking (all cancers other than lip, oral cavity, and pharynx, respiratory organs, and urinary bladder).</td>
<td>No diet data or matching for SES Long storage time Aside from removing those who were diagnosed within 2 yr of sampling, there was no control for time to diagnosis of cancer.</td>
</tr>
<tr>
<td>Knekt et al., 1991</td>
<td>Case-control Finland</td>
<td>768 cases of cancer of all sites 1419 controls matched for age, sex, and duration of storage Subjects from same pool as Knekt et al., 1998 (see above)</td>
<td>Same as above</td>
<td>Mean serum vitamin E levels were significantly lower in cases than controls. Subjects with low level of vitamin E had about 1.5–fold risk of cancer compared to controls. There was no association with lip, oral and pharynx, lung, or bladder cancers, or hormone-related cancers (breast, ovary, endometrium, and prostate). 2 with low vitamin E and low Se had a 3X higher risk of hormone-related cancer.</td>
<td>Same as above</td>
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<td>Stühhlin et al., 1989</td>
<td>Case-control</td>
<td>Basel, Switzerland 204 cases of death from all cancer Controls were from the remainder of the sample pool of 2974♂</td>
<td>Fasted serum samples were collected and immediately analyzed. Descriptive statistics included analysis of covariance for age and smoking. Risk analysis used the lower quartile as high-risk group. Adjustments also made for cholesterol and triglycerides to derive a lipid-independent estimate of vitamin levels.</td>
<td>There were no differences between groups for serum vitamin E levels. Vitamin A, C, and β-carotene were associated with cancer mortality either by site or with overall cancer mortality. In risk analysis only for bronchial cancer was there a insignificant tendency toward increased risk for low vitamin E levels. Subjects with low cholesterol levels were found to be at significantly increased risk for cancer death.</td>
<td>No diet, no supplement use data, no matching for SES. See comment for Gey et al. (1987) above.</td>
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<td>Wald et al., 1987</td>
<td>Case-control</td>
<td>London, England 271 cases of cancer 633 controls with no cancer matched for age, duration of storage of serum sample, smoking status and habits (type), and duration of smoking habit. Subjects derived from pool of 22,000 ♂ aged 33-64 who donated serum during period 1975-1982</td>
<td>Serum collected and stored at −40°C Vitamin E measured by HPLC Samples were tested in 4 series: 2 in 1981, 1 in '83 and 1 in '85. Cases always run with same matched-control pair</td>
<td>No difference in mean levels between cases and matched controls. The mean levels of vitamin E of cases diagnosed &lt;1 yr after blood collection was significantly lower than controls. For cases diagnosed &gt;1 yr after collection there were no significant differences between total cases or cases by site.</td>
<td>No diet nor supplement use data No matching for SES No data on health history of controls Statistical analyses based on paired t-test of differences in levels between cases and controls. This may not be an appropriate test for between-subject differences in a heterogeneous population sample.</td>
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